

Protective effects of a wheat germ rich diet against the toxic influence of profenofos on rat tissue lipids and oxidative pentose phosphate shunt enzymes

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RESUMEN

**Efecto protector de dietas ricas en germen de trigo contra la influencia tóxica de profenofós en lípidos de tejido de rata y en enzimas de la vía de la pento-
sa fosfato.**

El efecto de formas técnicas o formuladas de profenofós en la fracción lipídica metabólica de hígado, cerebro y riñones así como la actividad de la glucosa-6-fosfato deshidrogenasa (G6PD) y 6-fosfogluconato deshidrogenasa (6PGD), que son consideradas enzimas relacionadas con los lípidos, fueron estudiadas. Ambas formas de profenofós fueron suministradas separadamente tanto por vía oral como cutánea a una dosis de 1/20 LD₅₀ durante 3 meses (una dosis cada 48 horas). Los lípidos totales y fracciones lipídicas (contenido de colesterol, triglicéridos y fosfolípidos) decrecieron en los tres órganos estudiados de ratas tratadas con profenofós tanto técnicos como formulado comparado con animales control. El mayor efecto fue observado en el caso de tratamientos orales con profenofós formulados, mientras que el más bajo fue detectado para el tratamiento cutáneo con profenofós técnicos. La misma tendencia fue encontrada en las actividades de G6PD y 6PGD asociadas con el metabolismo lipídico de tejidos de hígado, cerebro y riñón bajo las mismas condiciones. Por otra parte, animales tratados con profenofós y alimentados con una dieta rica en germen de trigo (como agente antioxidante) produjo una significativa mejora tanto en el contenido de las fracciones lipídicas como en la actividad enzimática. Es más, el efecto de dietas ricas en germen de trigo (fuente rica de α -tocoferol) reajustó y mejoró el trastorno metabólico del perfil lipídico de las fracciones en ratas tratadas con profenofós, así como su actividad enzimática relacionada (G6PD y 6PGD: vía oxidativa de las pento-
sas fosfato).

PALABRAS CLAVE: *Germen de trigo – Perfil lipídico – Profenofós – Ratas.*

SUMMARY

Protective effects of a wheat germ rich diet against the toxic influence of profenofos on rat tissue lipids and oxidative pentose phosphate shunt enzymes.

The effects of technical and formulated forms of profenofos on the metabolic lipid fractions of the liver, brain and kidneys as well as the activity of glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD), which consider lipid related enzymes, were studied. The two forms of profenofos were given separately either orally or by

dermal at doses of 1/20 LD₅₀ for 3 months (one dose every 48 h). Total lipids and lipid fractions (cholesterol, triglycerides and phospholipid contents) decreased in the three studied organ tissues either in technical or formulated profenofos-induced rats compared with normal control animals. The highest effect was observed in the case of orally formulated profenofos induction, and the lowest was detected for the dermal technical one. The same trend was found in the activities of G6PD and 6PGD associated with lipid metabolism in the liver, brain and kidney tissues under the same conditions. On other hand, the treatment of profenofos-induced animals by feeding a wheat germ rich diet (as antioxidant agent) produced significant improvements in both lipid fraction content and enzyme activity. In addition, the effects of the wheat germ rich diet (α -tocopherol rich source) readjusted and improved the disturbed metabolic fractions of the lipid profiles in the profenofos-induced rats as well as their related enzyme activities (G6PD and 6PGD: oxidative pentose phosphate shunt).

KEY-WORDS: *Lipids profile – Profenofos – Rats – Wheat germ.*

1. INTRODUCTION

Profenofos is employed as an organophosphorus insecticide in the keeping and growing of agricultural products. Organophosphate pesticides have been in use for over 50 years and their acute toxicity is relatively well understood, but chronic low-level exposure to these pesticides has been implicated as a causal factor in a variety of different forms of human ill-health (COT, 1999 and Ray and Richards, 2001). Organophosphorus insecticides (OPI) are widely used throughout the world for the control of agricultural and domestic insect pests because of their low persistence and easily biodegradable nature. The use of this class of insecticides has resulted in worldwide increases in food and fiber production, along with the control of major disease carrying vectors and structure damaging insect pests (Grover *et al.*, 2003). Contamination of food occurs from contact with pesticide-laden surfaces, thus increasing the potential for excess dietary exposure of children and adults (Rohrer *et al.*, 2003). Profenofos, as an organophosphorus insecticide, shows its effect directly by inhibiting the

acetylcholinesterase enzyme (USEPA, 2000). Profenofos affects the antioxidant system, which plays an important role in making xenobiotics entering the body ineffective. The basic reason for this effect is that profenofos is made with glutathione-S-transferase (GST) which is an essential enzyme in the detoxification of the antioxidant system. Decreasing the detoxification process leads to the accumulation of harmful metabolites and parallel to this, it inevitably causes cytotoxic and genotoxic incidents. Some changes observed in enzyme activities can sometimes be brought under control thanks to vitamins (Abdel-Rahman *et al.*, 2006; Verma *et al.*, 2007 and Goel *et al.*, 2005 and 2007).

Oral *et al.* (2006) and Verma *et al.* (2007) applied vitamin E, A and C for dichlorvos and chlorpyrifos toxicities in rats. Also, our previous studies applied these vitamins for chlorpyrifos, dimethoate and profenofos intoxicated rats to attenuate the pesticide perturbation in albino rats (Abdel-Rahim and Abdel-Rahim, 2007, 2008 and Abdel-Rahim 2009). The most detected tissues are cytosol and the membranes of many organs such as the brain and kidneys, but especially the liver (Hayes *et al.*, 2005). The involvement of oxidative stress following acute exposure to OPI has demonstrated unequivocally that lipid peroxidation is one of the molecular mechanisms involved in OPI-induced toxicity (Abdollahi *et al.*, 2004). Accordingly, interest has recently grown in the role and use of natural antioxidants as a strategy to prevent oxidative damage in various health disorders with oxidative stress (Coskum *et al.*, 2005). Natural antioxidants from plants are reported to provide substantial protection that slows down the process of oxidative damage caused by ROS (Jacob and Burri, 1996). Hence there has been growing interest in natural antioxidants of plant origin since they also find use as nutraceuticals due to their impact on the status of human health and disease prevention (Noguchi and Nikki, 2000).

Vitamin E (a fat soluble vitamin) is used to describe isomers of tocopherol and tocotrienol that qualitatively exhibit the biological activity of α -tocopherol. The antioxidant activity of tocopherols is well documented (Ohkatsu *et al.*, 2001). One physiological role of vitamin E is its ability to react with and quench free radicals in cell membranes and other lipid environments, thereby preventing polyunsaturated fatty acids (PUFA) from oxidation. Wheat germ, a by-product of the flour milling industry and the oil extracted from wheat germ are the richest known natural dietary sources of tocopherols (vitamin E) of plant origin (Piras *et al.*, 2009). Leenhardt *et al.* (2008) showed that wheat germ and wheat germ oil intake results in a rapid increase in the content of vitamin E in different rat tissues and a change in the intensity of lipid peroxidation processes.

The aim of the present study was to evaluate the biochemical perturbations induced by profenofos in rat lipid profiles as well as the oxidative pentose

phosphate shunt of liver, brain and kidneys tissues and to determine the protective effects of the administration of wheat germ rich diet (α -tocopherol and other antioxidant) against the above effects induced by profenofos.

2. MATERIALS AND METHODS

2.1. Materials

Profenofos (O-(4-bromo-2-chlorophenyl)-O-ethyl-S-propyl phosphorothioate) forms, technical (95% a.i) and formulated (72% E.C.) of LD₅₀: 358 mg/kg rat body weight for oral dose, and 3300mg/kg rat body weight for dermal dose (Tomlin, 2000) were provided by Central Agricultural Pesticides Laboratory, Agriculture Research Center, Dokki, Giza, Egypt.

Wheat germ was obtained from South Cairo and Giza flour Mills and Bakeries Company, Cairo, Egypt. It came as a dried crushed sample. The crushed wheat germ was pulverized with a blender to a fine powder and kept until use. Vitamin E was determined in wheat germ colorimetrically according to the method obtained by Fisher *et al.* (1964).

2.2. Animals and Experimental Design

Sixty healthy adult male albino rats (*Rattus Norvegicus*) Sprague Dawley strain each weighing about 120 ± 5 g were raised in the animal house of the Central Agricultural Pesticides Laboratory, Dokki, Giza, Egypt. The animals were kept under normal healthy laboratory conditions (temperature was set at $25 \pm 2^\circ\text{C}$ with relative humidity of $\approx 57\%$ and 12-hour light-dark cycle) for two weeks in their cages prior to the experiment for acclimatization. During this period, rats were fed a normal diet which consisted of casein 15%, cotton seed oil 10%, cellulose 5%, salt mixture 4%, vitamins mixture 1% and starch 65% (Lane-Peter and Pearson, 1971). Rats were allowed free diet and water (*ad libitum*). Rats were divided into 10 groups (6 rats each). Five groups were fed the normal diet and the other five groups were fed the normal diet supplemented with 20% wheat germ (wheat germ rich diet) as a source of α -Tocopherol. The first group was fed the normal diet without any supplementation which served as the normal control. The second group was fed the wheat germ rich diet. The 3rd and 5th groups were ingested respectively with a sublethal dose of either the technical or formulated profenofos (1/20 of oral LD₅₀) and fed the normal diet. The same treatments were done with the 4th and 6th groups respectively but these animals were fed the wheat germ rich diet (the oral doses of the pesticide were ingested through a stomach tube). The 7th and 9th groups were treated with the dermal sublethal dose of technical or formulated profenofos (1/20 of dermal LD₅₀) respectively, and fed the normal diet. The

same treatments were done with the 8th and 10th groups respectively but these animals were fed the wheat germ rich diet.

For the dermal treatment, the application of dorsal skin was as follows: one day before dosing, an area of 2 × 2 cm on the back of the dermally treated rats was shaved with care not to abrade the skin. The shaved area was washed with acetone. The doses were then applied evenly and carefully on the shaved area skin (Abou-Zeid *et al.*, 1993). Technical or formulated profenofos was used without any additives for dermal treatment. For oral ingestion the pesticide was emulsified with 0.5 ml distilled water. One dose was inducted every 48 hours during the experimental period (three months), either for oral or dermal administration of both forms of profenofos. Diets (normal diet or wheat germ rich diet) and water were supplied *ad libitum*.

At the end of experimental period (three months), the rats were killed by decapitation. Liver, brain and kidneys of all experimental animals were removed and chilled until analysis. The three organs were homogenated separately before analysis.

2.3. Biochemical Analysis

Total lipids, cholesterol, triglycerides and phospholipid contents in the homogenate of the studied organ tissues were determined by the methods of Joseph *et al.* (1972), Chaurchami *et al.* (1959), Young and Pestaner (1975) and Ketes (1972), respectively. Total soluble proteins in the homogenates were determined according to Bradford (1976) methods. The activity of glucose-6-phosphate dehydrogenase (G6PD: EC. 1.1.1.49) and 6-phosphogluconate dehydrogenase (6PGD: EC. 1.1.1.43) was determined in the homogenates of the different organs tissues according to the method of Glock and McLean (1953).

2.4. Statistical Analysis

All data are expressed as mean ± standard deviation. The data was analyzed by the analysis of variance (ANOVA). Testing of mean values in different groups was done by Duncan's multiple range test. SPSS 10 window version was used for the statistical analysis (Middle Brooks, 1977).

3. RESULTS AND DISCUSSION

The effects of different forms of profenofos pesticide induction into adult male albino rats on total lipids, cholesterol, triglycerides and phospholipid contents in the liver, brain and kidneys were determined and the results of normal and profenofos-induced animals are recorded in Tables 1 to 4. In connection, the activity of G6PD and 6PGD (oxidative pentose phosphate shunt enzymes) was also determined in

the three organ tissues under the same conditions as shown in Tables 5 and 6. In the case of the wheat germ (containing 27.38mg vitamin E/100g) rich diet feeding for profenofos-induced rats, the lipids profile and also the activity of G6PD and 6PGD of liver, kidneys and brain were carried out to evaluate the hypointensive influences of wheat germ used as an antagonistic agent on the effect of profenofos.

The present results (Tables 1 and 2) showed that both forms of profenofos (technical and formulated) inductions lowered total lipids and total cholesterol contents in the subjected rat tissues relative to that of the normal control animals. The formulated pesticide induced orally was more effective than the other induction, but the induction of the technical formulation dermally had the lowest effects. Among the organs studied, the kidneys were characterized by the highest decreases in total lipid and cholesterol contents but for the liver and brain, the total lipid and cholesterol contents showed the same trend and decreased to slightly lower than the kidneys of profenofos-induced animals under the same conditions. The feeding of the wheat germ rich diet improved the values of the total lipid contents of the liver, brain and kidney tissues of profenofos-induced animals and returned these values to around that of the normal value of the healthy control. The same trend was observed in the case of total cholesterol content of the same organs tissues, feeding the wheat germ rich diet decreased the harmful effects of different forms of profenofos and the content of total cholesterol was alleviated, in the liver, brain and kidney tissues.

In addition, both forms of profenofos (technical and formulated) separately induced by dermal or orally showed significant decreases in total triglycerides and phospholipid contents of the three examined tissues (Tables 3 and 4). However, the kidneys were also characterized by a relatively higher decrease than those of the liver and brain for triglyceride contents under the same conditions in profenofos-induced animals; while the brain was characterized by a high decrease in phospholipid content. On the other hand, the relative percent decreases of the above constituents (total lipids, cholesterol, triglycerides and phospholipids) in the other organs produced nearly the same figures. These decreased values were improved by feeding the wheat germ rich diet. This means that wheat germ (mainly α -tocopherol antioxidant) reduced the harmful effects of the present pesticide in the three organ tissues.

In connection, Tables 5 and 6 show that significant inhibition had occurred in the G6PD and 6PGD activities of the liver, kidneys and brain tissues affected by profenofos induction. It is of interest to note that the liver was characterized by higher G6PD activity than the other organs during the experimental period; whereas that of the kidneys showed the highest inhibition compared to those of liver and brain relative to the control. The

Table 1
The total lipid contents in different organ tissues of the experimental male albino rats

Treatments	Total lipids (g/100g)					
	Liver		Brain		Kidneys	
	Values \pm SD	%	Values \pm SD	%	Values \pm SD	%
Normal healthy control	3.99 \pm 0.17 ^a	100	4.35 \pm 0.20 ^a	100	3.61 \pm 0.14 ^a	100
Normal healthy + wheat germ	4.01 \pm 0.19 ^a	101	4.40 \pm 0.21 ^a	101	3.59 \pm 0.14 ^a	99
<i>Oral</i>						
Technical P	3.21 \pm 0.14 ^b	80	3.46 \pm 0.16 ^c	80	2.80 \pm 0.13 ^c	78
Technical + wheat germ	3.81 \pm 0.15 ^a	95	4.11 \pm 0.17 ^{ab}	94	3.40 \pm 0.12 ^a	94
Formulated P	2.88 \pm 0.19 ^c	72	3.00 \pm 0.13 ^d	69	2.52 \pm 0.11 ^d	70
Formulated P + Wheat germ	3.70 \pm 0.18 ^a	93	4.00 \pm 0.12 ^b	92	3.14 \pm 0.10 ^b	87
<i>Dermal</i>						
Technical P	3.80 \pm 0.14 ^a	95	3.71 \pm 0.18 ^c	85	2.86 \pm 0.10 ^c	79
Technical P + wheat germ	3.91 \pm 0.17 ^a	98	4.24 \pm 0.19 ^{ab}	97	3.44 \pm 0.12 ^a	95
Formulated P	3.19 \pm 0.13 ^b	80	3.50 \pm 0.16 ^c	80	3.00 \pm 0.13 ^{bc}	83
Formulated P + wheat germ	3.82 \pm 0.14 ^a	96	4.10 \pm 0.17 ^{ab}	94	3.60 \pm 0.14 ^a	100
LSD	0.276		0.292		0.208	

P = Pesticide (Profenofos). % relative to normal healthy control. Values are expressed as means \pm SD (n = 6). Values with different superscript letters within the same column are significantly different ($P < 0.05$).

activity of 6PGD had shown a similar trend to G6PD in the three organ tissues under the same conditions. However, profenofos inhibited the activity of G6PD and 6PGD in tissue homogenates either in the form of technical or formulated. Among

the organs studied, the kidneys were characterized by the highest G6PD and 6PGD inhibition activities but the liver and brain had lower inhibition than the kidneys under the induced effects of profenofos. The inhibited activities of G6PD and 6PGD of the

Table 2
The total cholesterol content in different organ tissues of the experimental male albino rats

Treatments	Total cholesterol (mg/100g)					
	Liver		Brain		Kidneys	
	Values \pm SD	%	Values \pm SD	%	Values \pm SD	%
Normal healthy control	250 \pm 20 ^a	100	1821 \pm 117 ^a	100	331 \pm 30 ^a	100
Normal healthy + wheat germ	256 \pm 21 ^a	102	1827 \pm 125 ^a	100	330 \pm 28 ^a	100
<i>Oral</i>						
Technical P	210 \pm 19 ^{bc}	84	1585 \pm 110 ^{bc}	87	271 \pm 21 ^{cde}	82
Technical + wheat germ	231 \pm 14 ^{abc}	92	1771 \pm 132 ^{ab}	97	301 \pm 14 ^{abc}	91
Formulated P	201 \pm 13 ^c	80	1501 \pm 101 ^c	82	240 \pm 11 ^e	73
Formulated P + Wheat germ	225 \pm 17 ^{abc}	90	1750 \pm 124 ^{ab}	96	294 \pm 13 ^{bcd}	89
<i>Dermal</i>						
Technical P	215 \pm 19 ^{bc}	86	1594 \pm 108 ^{bc}	88	301 \pm 15 ^{abc}	91
Technical P + wheat germ	240 \pm 20 ^{ab}	96	1800 \pm 111 ^{ab}	99	319 \pm 14 ^{ab}	96
Formulated P	211 \pm 18 ^{bc}	84	1580 \pm 119 ^{bc}	87	267 \pm 12 ^{de}	81
Formulated P + wheat germ	234 \pm 21 ^{abc}	94	1785 \pm 125 ^{ab}	98	300 \pm 12 ^{abc}	91
LSD	31.36		200.67		30.55	

P = Pesticide (Profenofos). % relative to normal healthy control. Values are expressed as means \pm SD (n = 6). Values with different superscript letters within the same column are significantly different ($P < 0.05$).

Table 3
The total triglyceride contents in different organ tissues of the experimental male albino rats

Treatments	Total triglycerides (g/100g)					
	Liver		Brain		Kidneys	
	Values \pm SD	%	Values \pm SD	%	Values \pm SD	%
Normal healthy control	2.00 \pm 0.17 ^a	100	2.14 \pm 0.17 ^{ab}	100	1.86 \pm 0.13 ^a	100
Normal healthy + wheat germ	2.07 \pm 0.24 ^a	104	2.20 \pm 0.16 ^a	103	1.88 \pm 0.14 ^a	101
<i>Oral</i>						
Technical P	1.61 \pm 0.11 ^{bcd}	81	1.80 \pm 0.12 ^{cd}	84	1.41 \pm 0.11 ^{cd}	76
Technical + wheat germ	1.91 \pm 0.10 ^a	96	2.01 \pm 0.17 ^{abcd}	94	1.71 \pm 0.12 ^{ab}	92
Formulated P	1.52 \pm 0.12 ^d	76	1.51 \pm 0.10 ^e	71	1.27 \pm 0.10 ^d	68
Formulated P + Wheat germ	1.84 \pm 0.14 ^{ab}	92	1.98 \pm 0.14 ^{abcd}	93	1.58 \pm 0.13 ^{bc}	85
<i>Dermal</i>						
Technical P	1.80 \pm 0.12 ^{abc}	90	1.86 \pm 0.13 ^{bcd}	87	1.50 \pm 0.12 ^{bc}	81
Technical P + wheat germ	1.92 \pm 0.11 ^a	96	2.13 \pm 0.16 ^{ab}	100	1.72 \pm 0.11 ^{ab}	92
Formulated P	1.58 \pm 0.13 ^{cd}	79	1.76 \pm 0.14 ^{de}	82	1.43 \pm 0.13 ^{cd}	77
Formulated P + wheat germ	1.89 \pm 0.12 ^a	95	2.06 \pm 0.16 ^{abc}	96	1.81 \pm 0.12 ^a	97
LSD	0.239		0.255		0.203	

P = Pesticide (Profenofos). % relative to normal healthy control. Values are expressed as means \pm SD (n = 6). Values with different superscript letters within the same column are significantly different (P < 0.05).

profenofos-induced rats were ameliorated by feeding the wheat germ rich diet. The results clearly show that both forms (technical and formulated) of profenofos ingestion had a disturbed effect on the activity of oxidative pentose phosphate shunt

enzymes (G6PD and 6PGD) in the three rat organ tissues of the profenofos-induced animals, but the wheat germ rich diet improved these effects.

The present results are in agreement with Lukaszewicz-Hussain and Moniuszko-Jakoniuk

Table 4
The total phospholipid contents in different organ tissues of the experimental male albino rats

Treatments	Total phospholipids (g/100g)					
	Liver		Brain		Kidneys	
	Values \pm SD	%	Values \pm SD	%	Values \pm SD	%
Normal healthy control	1.00 \pm 0.08 ^a	100	1.19 \pm 0.11 ^a	100	0.87 \pm 0.05 ^a	100
Normal healthy + wheat germ	1.02 \pm 0.10 ^a	102	1.20 \pm 0.10 ^a	101	0.90 \pm 0.05 ^a	103
<i>Oral</i>						
Technical P	0.80 \pm 0.06 ^{cd}	80	0.91 \pm 0.07 ^{cd}	76	0.71 \pm 0.04 ^{cd}	82
Technical + wheat germ	0.91 \pm 0.08 ^{abc}	91	1.00 \pm 0.06 ^{bcd}	84	0.85 \pm 0.05 ^{ab}	98
Formulated P	0.77 \pm 0.04 ^d	77	0.73 \pm 0.04 ^e	61	0.64 \pm 0.04 ^d	74
Formulated P + Wheat germ	0.86 \pm 0.06 ^{bcd}	86	0.98 \pm 0.06 ^{bcd}	82	0.77 \pm 0.07 ^{bc}	89
<i>Dermal</i>						
Technical P	0.90 \pm 0.07 ^{abcd}	90	0.94 \pm 0.07 ^{cd}	79	0.74 \pm 0.06 ^c	85
Technical P + wheat germ	0.95 \pm 0.08 ^{ab}	95	1.08 \pm 0.06 ^{ab}	91	0.87 \pm 0.05 ^a	100
Formulated P	0.80 \pm 0.06 ^{cd}	80	0.88 \pm 0.05 ^d	74	0.71 \pm 0.06 ^{cd}	82
Formulated P + wheat germ	0.93 \pm 0.06 ^{abc}	93	1.02 \pm 0.07 ^{bc}	86	0.86 \pm 0.06 ^{ab}	99
LSD	0.120		0.119		0.092	

P = Pesticide (Profenofos). % relative to normal healthy control. Values are expressed as means \pm SD (n = 6). Values with different superscript letters within the same column are significantly different (P < 0.05).

Table 5
The glucose-6-phosphate dehydrogenase (G6PD) activity in different organ tissues of the experimental male albino rats

Treatments	G6PD activity ($\mu\text{mol of NADPH.H}^+/\text{min/mg protein}$)					
	Liver		Brain		Kidneys	
	Values \pm SD	%	Values \pm SD	%	Values \pm SD	%
Normal healthy control	98.0 \pm 5.11 ^{ab}	100	71 \pm 4.17 ^a	100	30.1 \pm 2.06 ^{ab}	100
Normal healthy+wheat germ	100.3 \pm 7.35 ^a	102	71 \pm 3.61 ^a	100	31.0 \pm 2.00 ^a	103
<i>Oral</i>						
Technical P	78.9 \pm 4.20 ^{de}	81	57.0 \pm 3.91 ^{de}	80	21.8 \pm 1.56 ^e	72
Technical+wheat germ	91.0 \pm 5.77 ^{abc}	93	66.0 \pm 4.83 ^{abc}	93	27.2 \pm 1.99 ^{bcd}	90
Formulated P	71.2 \pm 3.91 ^e	73	50.5 \pm 3.00 ^e	71	18.4 \pm 0.99 ^f	61
Formulated P+Wheat germ	88.1 \pm 6.61 ^{bcd}	90	63.3 \pm 3.82 ^{bcd}	89	24.1 \pm 1.60 ^{de}	80
<i>Dermal</i>						
Technical P	80.9 \pm 6.54 ^{cde}	83	63.1 \pm 4.01 ^{bcd}	89	25.1 \pm 1.91 ^{cd}	83
Technical P+wheat germ	93.1 \pm 7.26 ^{ab}	95	69.3 \pm 4.51 ^{ab}	98	28.3 \pm 1.76 ^{ab}	94
Formulated P	75.4 \pm 4.32 ^e	77	59.0 \pm 3.61 ^{cd}	83	24.2 \pm 1.32 ^{de}	80
Formulated P+wheat germ	92.1 \pm 6.90 ^{ab}	94	65.4 \pm 4.54 ^{abc}	92	27.3 \pm 1.04 ^{bc}	91
LSD	10.10		6.87		2.87	

P = Pesticide (Profenofos). % relative to normal healthy control. Values are expressed as means \pm SD (n = 6). Values with different superscript letters within the same column are significantly different ($P < 0.05$).

(2003) and our previous findings (Abdel-Rahim, 2009 and Abdel-Rahim and Abdel-Rahim, 2008) which reported that respiratory mitochondria enzymes and glycolysis systems were changed by the ingestion of profenofos in rats but these effects

were improved and treated with the feeding of an antioxidant diet. Also, Abdel-Rahim *et al.* (2009) reported that the blood total lipids, cholesterol, triglycerides and phospholipid contents were reduced in cypermethrin insecticide intoxicated

Table 6
The 6-phosphogluconate dehydrogenase (6PGD) activity in different organ tissues of the experimental male albino rats

Treatments	6PGD activity ($\mu\text{mol of NADPH.H}^+/\text{min/mg protein}$)					
	Liver		Brain		Kidneys	
	Values \pm SD	%	Values \pm SD	%	Values \pm SD	%
Normal healthy control	42.8 \pm 2.30 ^a	100	80.2 \pm 5.11 ^a	100	33.2 \pm 2.03 ^a	100
Normal healthy+wheat germ	43.7 \pm 2.17 ^a	102	80.8 \pm 4.54 ^a	101	33.4 \pm 1.52 ^a	100
<i>Oral</i>						
Technical P	35.0 \pm 2.00 ^{bc}	82	62.9 \pm 4.37 ^{cd}	78	25.4 \pm 1.60 ^a	76
Technical+wheat germ	40.9 \pm 3.12 ^a	96	75.2 \pm 4.73 ^{ab}	94	29.1 \pm 1.90 ^a	88
Formulated P	27.82 \pm 1.91 ^d	65	59.0 \pm 4.76 ^d	74	21.9 \pm 1.40 ^a	66
Formulated P+Wheat germ	40.43 \pm 3.20 ^a	94	72.1 \pm 4.56 ^{ab}	90	26.3 \pm 1.98 ^a	79
<i>Dermal</i>						
Technical P	39.2 \pm 2.02 ^{ab}	92	70.5 \pm 5.05 ^{bc}	88	28.8 \pm 1.95 ^a	87
Technical P+wheat germ	42.1 \pm 3.48 ^a	98	76.7 \pm 4.61 ^{ab}	96	29.7 \pm 1.68 ^a	90
Formulated P	34.0 \pm 2.03 ^c	79	61.8 \pm 5.01 ^d	77	25.1 \pm 1.37 ^a	76
Formulated P+wheat germ	41.1 \pm 2.90 ^a	96	74.0 \pm 5.07 ^{ab}	92	27.2 \pm 2.12 ^a	82
LSD	4.39		8.15		3.02	

P = Pesticide (Profenofos). % relative to normal healthy control. Values are expressed as means \pm SD (n = 6). Values with different superscript letters within the same column are significantly different ($P < 0.05$).

rats, but these effects were improved by feeding an antioxidant diet (guava and wheat germ).

G6PD and 6PGD were considered the fundamental enzymes of pentose phosphate pathway activity. The primary results of the pathway are: the generation of reducing equivalents, in the form of NADPH (accounting for approximately 60% NADPH production), used in reductive biosynthesis reactions within cells (e.g. fatty acid synthesis) and production of ribose-5-phosphate (R5P), used in the synthesis of nucleotides and nucleic acids (Kruger and von Schaewen, 2003). The inhibition of nucleotide biosynthesis, i.e. formation of ribose component and the phosphorylation of ribonucleotides inhibited protein biosynthesis (Abdel-Rahim and Abdel-Rahim, 2007 and Abdel-Rahim, 2009). The obtained results of the present experiment may suggest that the relationship between the pentose phosphate pathway and fatty acids causing triglyceride biosynthesis (i.e. the using of NADPH. H^+ in the reduction system of cell fatty acid synthesis) disappeared and produced NADPH. H^+ from the pentose phosphate pathway was utilized for energy production instead of the normal pathway. This may be due to the fact that the ingestion of the insecticides produced hypoxia for intoxicated animals (Lukaszewicz-Hussain, 2001 and Lukaszewicz-Hussain and Moniuszko-Jakoniuk, 2003).

The present results are in agreement with those of Karaoz *et al.* (2002); Kaur and Dhanju (2005); Abdel-Rahman *et al.* (2006) and Kamath *et al.* (2008), who showed that organophosphorus insecticide intoxications have been reported to potentiate the biological activity in the lipid profiles of the animal tissues. Although the liver is the main site of metabolic activation of toxic chemicals, recent attention has been paid to extrahepatic organs which are often targets for xenobiotics. In addition to the liver, the brain and kidneys are involved in the metabolism of xenobiotics and contribute to the overall toxic profile of a toxicant although typically much less than the liver (Mahboob *et al.*, 2004). In connection with the treatments with antioxidant agents such as vitamins (α -tocopherol: Vit. E), attenuated xenobiotic induced biochemical cell perturbation has several ways to alleviate the effect of the oxidative stress produced by pesticides, either by repairing the damage (lipid peroxidation by-products) or by directly reducing the pro-oxidative state via enzymatic and non-enzymatic antioxidants. Non-enzymatic (vitamin E, A and C etc) and enzymatic (superoxide dismutase, glutathione peroxidase and catalase etc) antioxidants have been shown to scavenge free radicals (Stephen *et al.*, 1997 and Karaoz *et al.*, 2002). Wheat germ contains the highest tocopherol content of all vegetable oil, and also the highest content of α -tocopherol, which represents around 60% of the total content (Piras *et al.*, 2009). Also, wheat germ has high contents of minerals including Zn, Fe, K and P as well as protein, lipid, dietary fibers, carotenoids, carbohydrate

and other vitamins (Pennington, 1989 and Ibrahim *et al.*, 1990). Vitamins A and E are most important among many low molecular weight compounds which can act as biological antioxidants (Karaoz *et al.*, 2002; Lukaszewicz-Hussain and Moniuszko-Jakoniuk, 2003 and Abdel-Rahim and Abdel-Rahim, 2007). Recent findings indicate that the toxic manifestation induced by organophosphorus insecticides may be associated with the enhanced production of reactive oxygen species (ROS), which gives an explanation for why ROS is proposed to be caused by a mechanism in which the xenobiotic toxicant and pathological conditions may produce oxidative stress and induce various tissue damage (Dwivedi *et al.*, 1998 and Abdel-Rahman *et al.*, 2006).

ROS may interact with cellular protein, lipid and DNA causing alterations in the target cell function. Acute oxidative injury may produce selective cell death and a compensatory increase in cell proliferation (Stephen *et al.*, 1997 and Klaunig *et al.*, 1998). Thus, the administration of wheat germ rich diets (containing vitamins E and A and other antioxidants) was shown to protect various biomolecules, such as DNA, membrane lipids and cytosolic proteins from oxidative damage induced by oxygen-derived free radicals (Karaoz *et al.*, 2002; Goel *et al.*, 2007 and Verma *et al.*, 2007).

The decrease in lipids and lipid fractions in the liver, brain and kidneys of rats after long-term induction with 1/20 LD₅₀ of profenofos indicated degenerative changes. All organophosphorus pesticides (OP) are lipophilic and these environmental xenobiotics are known to have a strong affinity for interaction with membrane phospholipids, and the phospholipid component of the biomembrane is believed to be the site of action of OP insecticides with the formation of ROS; and one of the targets of creative oxygen-induced injury is lipid peroxidation. Thus, in addition to acetylcholinesterase being the principal mode of action of OP pesticides, increased lipid peroxidation and altered enzyme activity have also been implicated in mediating OP toxicity in animals (Kaur and Dhanju, 2005).

In connection with oxidative pentose phosphate shunt G6PD and 6PGD are the key enzymes of this pathway which is responsible for the generation of NADPH. H^+ . These enzymes play essential roles in the regulation of oxidative stress by regulating NADPH. H^+ level, the main intracellular reductant. For this reason, both enzymes are required for the antioxidant defence system (Salvemini *et al.*, 1999 and Zhang *et al.*, 2000). G6PD and 6PGD catalyze the first steps in the pentose phosphate pathway, oxidizing glucose-6-phosphate to 6-phosphogluconate, which is then oxidized to 3-keto 6-phosphogluconate, reducing 2 molecules of NADP⁺ to NADPH. H^+ (Murray *et al.*, 2006). The NADPH, in turn, maintains the supply of reduced glutathione in the cells which is used to mop up free radicals that cause oxidative damage (Mehta *et al.*, 2000). In the case of genetic G6PD and 6PGD deficiency, or

other circumstances where NADPH.H^+ is in short supply, the reduction of G-S-S-G is impaired; there is a deficiency of GSH and hemolytic anemia (Parke and Piotrowski, 1996). It has been recently suggested that the primary physiological role of G6PD in mammalian cells is the defense against oxidative stress injury. The formation of GSH from its oxidized form (G-S-S-G) is dependent on NADPH.H^+ produced by the pentose phosphate pathway. This pathway can be activated in response to GSH depletion (Salvemini *et al.*, 1999 and Karaoz *et al.*, 2002). As is reported by some authors the activities of G6PD, 6PGD and glutathione peroxidase (GPx) are linked in their capacity to prevent peroxidative tissue damage from oxidants. GPx converts toxic lipid hydroperoxides and H_2O_2 using reducing equivalents generated by the oxidative shunt of the pentose phosphate pathway (Bus *et al.*, 1978; Frei *et al.*, 1989 and Lukaszewicz-Hussain and Moniuszko-Jakoniuk, 2003).

4. CONCLUSIONS

The present results suggest that profenofos decreases the lipid profile and G6PD and 6PGD activities which may cause depletion of NADPH.H^+ , which is the main intracellular reductant; but that feeding a wheat germ rich diet (rich source of vitamin E) considerably reduces the effects of profenofos on the liver, brain and kidney tissues of profenofos-induced rats. Generally, profenofos caused extensive biochemical injury. Such effects were relevant to the amount of dose given (Abdel-Rahman *et al.*, 2006), which were alleviated by the antioxidant agents of the wheat germ rich diet.

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