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Profenofos Toxicity on Cytochrome-C System and Energy Metabolites of the Respiration and Ameliorate these Effects by wheat Germ (α-Tocopherol Agent) in Intoxicated Albino Rats

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Abstract: Profenofos insecticide (1/25LD_{50} technical and formulation) was inducted orally and dermally into albino rat males to evaluate toxicity of both forms. The alleviation of their harmful effects was done with wheat germ diet (α-tocopherol agent). Experimental period was 12 weeks. Respiratory cytochromal–c-system (cyt.c, cyt-c-oxidase and succinate-cyt-c-reductase), energy metabolites (ATP, ADP and AMP) and myokinase activity in liver, kidneys and brain tissue were studied under profenofos toxicity. Profenofos forms toxicity changed energy metabolites amount, ATP content was increased, but ADP and AMP were decreased. In addition, cytochrome-c-system of mitochondria tissues was disturbed relative to control; cytochrome-c content of tested organs was reduced. Formulated profenofos effected more than technical, which may be due to adjuvant synergistic influences of formulation. Oral profenofos toxicity was more than dermal administration. Semi-modified wheat–germ diet (α-tocopheral source) used as an antioxidant agent feeding to attenuate the toxicity of both forms. Treatments of α-tochopheral improved the parameters of intoxicated rats. Energy system, myokinase activity and cytochrome-c of the respiratory system activities were improved nearly to control values. In conclusion, wheat-germ semi modified diet was a good source of antioxidant which reduced the profenofos toxicity in testing animals and improved both respiratory systems.

Key words: Insecticide Profenofos · Respiratory Cytochromal–C-System · Energy Metabolites · Intoxicated Albino Rats

INTRODUCTION

Organophosphorus insecticide profenofos is widely used for pest control in agriculture and to a lesser degree for indoor use, also for soil application to control termites [1-3]. This group of pesticides induced a wide range of toxicity and harms to animals, including acetylcholinesterase inhibition and the consequent accumulation of the neurotransmitter acetylcholine in synaptic junctions leads to excessive stimulation of postsynaptic cells leading to cholinergic toxicity [4]. Profenofos belongs to the phosphorothioate class of organophosphorus pesticides and gets metabolically activated to its corresponding oxygen analog and oxon in organ tissues. Transformation of profenofos is catalyzed by cytochrome P450 and associated enzyme system, which are present in microsomal membranes [5]. Profenofos administration induces an increased oxidative stress in the animal body systems. Certain enzyme reactivators, such as oximes, constitute the most important means of preventive treatment following exposures to organophosphorus insecticides in humans [6]. The possible protective roles of safer preventative compounds, offering the least amount of side effects are warranted to be explored. Some studies have suggested antioxidant agents as a beneficial agent during peroxidative damage [7-10]. Vitamin such as C, A and E have a number of biological activities such as immune stimulation and an alteration in metabolic activity of carcinogens, which can prevent genetic system changes by reducing nucleic acids (DNA and RNA) damage produced by the free radical of reactive oxygen metabolites [11, 12].

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In relation to the role of antioxidants in offering protection against oxidative stress and injuries, the present studies were aimed to comparative investigation between the influence of technical and formulation of profenofos insecticide (oral ingestion or dermal administration) on the levels of energy metabolites (ATP, ADP and AMP) and myokinase activity in the tissues of liver, kidneys and brain in albino rats, also, the respiratory system (cytochrome-c content and the activity of cytochrome-c- oxidase and succinate- cytochrome–c-reductase of mitochondria) in the same tissues. The biochemical influences of semi-modified wheat-germ diet (a source of vitamin E) as antioxidant and hypo-intensive agent against the harmful injuries induced by technical and formulated profenofos orally or dermally administrated into male albino rat were studied.

MATERIALS AND METHODS

Profenofos is *O*-4-bromo-2-chlorophenyl *O*-ethyl *S*-propyl phosphorothioate. The technical material (90% a.i) and formulation (72% E.C) were provided from Central Agricultural Pesticides Laboratory, ARC, Ministry of Agriculture, Dokki, Egypt, which obtained from Agrochem company, Alexandria, Egypt, which calls teliton 72%EC. The LD_{50} of profenofos for rat is 358 mg/kg body weight for oral ingestion but for dermal toxicity LD_{50} is 3300 mg/kg body weight [1].

Sixty health adult male albino rats, *Rattus norvegicus*, Sprague Dawley strain (weight range 100±10g) were obtained from the animal house of Nutrition Institute, Cairo. Animals were kept under normal healthy laboratory conditions for 10 days in their cages period for acclimatization. During the adaptation period, rats were fed on normal diets which consisted of 18.8% casein, 0.2% methionine, 10% cotton seed oil, 4% salt mixture, 1% vitamins mixture and 65% starch [13]. The animals were allowed free excess of water and diet. They were divided into 10 groups (6 rats each). The first group served as normal health control, the 2nd group fed on the semi-modified diet (85% normal diet +15% wheat germ). The 3rd group ingested sub-lethal dose of profenofos which was 1/25 of oral LD_{50} of technical insecticide, the 4th groups was the same of 3rd group but fed on the semi-modified (15% wheat germ 85% normal diet), the 5th group was used for the dermal sub-lethal administration dose of technical profenofos (1/25 dermal LD_{50}) which applied on dorsal skin shaved area of 2x2 cm. One day before dosing an area of 2x2 cm in the back of the dermally administered rats was shaved with care not to brada the shin. The shaved area was washed with acetone; the doses were then applied evenly and carefully on the shaved area on rat skin [14]. The 6th group was the same of the 5th group but fed on semi-modified wheat germ diet. The 7th group was orally ingested by the sub-lethal dose of teliton 72% EC (equal 1/25 of oral LD_{50} for rats). The 8th group was the same of 7th group but fed on the semi- modified-wheat germ diet. The 9th group was used for dermal sub-lethal dose of teliton 72% EC (equal 1/25 of dermal LD_{50} for rats) which applied like the same of group 5, the 10th group was the same of 9th group but fed on the semi-modified-wheat germ diet.

Technical profenofos and formulation were used without any additions for dermal induction, but for oral the doses were emulsified with 0.5 ml distilled water. One dose was inducing every 48 hours during the experimental period. The treatment took 12 weeks either for dermal or oral administration of technical profenofes or teliton 72% EC. The diet and water were supplied *ad libitum* for all groups during the experimental period.

At the end of the12 weeks (experimental period), animals were killed by decapitation and liver, kidneys and brain were dissected. Mitochondria of the three organs were prepared then emulsified with 1% Triton X-100 (3 ml) at 0°C for 30 min., mitochondria metabolites and enzymes were liberated and then assayed [15, 16].

Cytochrome-c content was determined according to Williams and Thorp method [17] also cytochrome–c-oxidase and succinate–cytochrome–c-reductase activities were determined according to the methods of Smith [18] and King [19], respectively for liver, kidneys and brain mitochondria. Energy system metabolites (ATP, ADP and AMP) were determined in the three organs tissue homogenates according to Lamprecht and Trautschold [20] for ATP and Adams [21] for ADP and AMP. Myokinase activity was determined according to the method described by Bergmeyer [22], total soluble protein content is clear homogenate was determined according to the method of Astawrov [16].

Statistical Analysis: Was achieved using the analysis of variance (t-test) as described by Snedecor and Cochran [23].

RESULTS AND DISCUSSION

The toxic effects of technical profenofos and its formulation (teliton 72%EC) and the ameliorated effects of the antioxidant agent (semi-modified wheat germ diet) as a good source of vitamin E on the profenofos toxicity for energy and cytochrome-c respiratory systems were investigated.

Table 1: Cytochrom - c contents in liver, kidneys and brain tissues of the experimental rats mitochondria % Relative to health control values are represented mean of 6 rats mean ± SD means ± SD in the column followed by same letter are not significantly different at $p < 0.05$

		Liver		Kidneys		Brain	
Insecticide Form	Treatments	µ mole/g tissue/min	%	µ mole/g tissue/min	%	µ mole/g tissue/min	%
Control	Health control	24.12 ± 1.63 [a]	100	28.02 ± 9.22 [a]	100	10.01 ± 1.00 a	100
	Control ± wheat germ diet	25.00 ± 2.00 [a]	104	28.51 ± 2.07 [a]	102	10.11 ± 0.84 a	101
Technical form	Oral profenofos	20.87 ± 1.63 [b]	87	23.16 ± 1.68 b	83	8.03 ± 0.53 b	80
	Oral profenofos ± wheat germ diet	22.00 ± 1.52 [a]	91	25.00 ± 2.11 ab	89	8.54 ± 0.61 b	85
	Dermal profenofos	21.11 ± 1.43 b	88	24.00 ± 1.97 b	86	8.42 ± 0.42 b	84
	Dermal profenofos ± wheat germ diet	22.32 ± 2.01 a	93	26.01 ± 2.00 b	86	9.00 ± 0.55 ab	90
formulation from	Oral teliton	19.00 ± 1.83 b	79	21.78 ± 1.73 b	78	7.89 ± 0.41 b	79
	Oral teliton ± wheat germ diet	21.00 ± 1.91 b	87	23.89 ± 1.70 b	85	8.32 ± 0.52 b	83
	Dermal teliton	21.00 ± 1.72 b	87	24.02 ± 1.99 b	86	8.02 ± 0.60 b	80
	Dermal teliton ± wheat germ diet	22.21 ± 2.03 a	92	26.36 ± 2.04 a	94	9.16 ± 0.63 a	92

Table 2: Cytochrom- c-oxidase and succinate cytochrome - c - reductase activities in liver, kidneys and brain tissues of the experimental rats mitochondria

		Cytochrome - C - oxidase activity						Succinate - cytochrome - C - reductate activity					
		Liver		Kidneys		Brain		Liver		Kidneys		Brain	
Insecticide Form	Treatments	µ mole/g tissue/min	%	µ mole/g tissue/min	%	µ mole/g tissue/min	%	µ mole/g tissue/min	%	µ mole/g tissue/min	%	µ mole/g tissue/min	%
Control	Health control	15.01 ± 1.01 [c]	100	13.11 ± 1.11 [b]	100	50.01 ± 3.32 b	100	2.40 ± 0.14 d	100	3.13 ± 0.21 d	100	36.01 ± 1.72 c	100
	Control + wheat germ diet	15.13 ± 1.12 [c]	101	13.21 ± 1.07 [b]	101	51.00 ± 2.97 b	102	2.47 ± 0.13 d	103	3.21 ± 0.19 d	103	36.56 ± 2.01 c	102
Technical form	Oral profenofos	18.00 ± 1.32 a	120	15.21 ± 1.22 a	116	58.41 ± 3.10 a	117	3.52 ± 0.16 b	147	4.11 ± 0.30 b	131	55.99 ± 2.34 a	155
	Oral profenofos + wheat germ diet	16.99 ± 1.27 b	113	14.62 ± 1.23 a	112	54.89 ± 3.03 a-b	110	3.01 ± 0.15 c	125	3.27 ± 0.27 d	104	44.44 ± 2.07 b	123
	Dermal profenofos	17.01 ± 1.42 b	113	14.82 ± 1.32 a	113	56.12 ± 2.89 a-b	112	3.21 ± 0.18 c	134	4.00 ± 0.30 b	128	45.72 ± 2.17 b	127
	Dermal profenofos + wheat germ diet	16.43 ± 1.34 b	109	14.00 ± 1.20 a-b	107	53.00 ± 3.20 a-b	106	2.88 ± 0.13 c	120	3.63 ± 0.27 c	116	38.14 ± 2.03 c	106
formulation from	Oral seliton	19.01 ± 1.27 a	127	16.00 ± 1.35 a	122	60.16 ± 3.79 a	120	4.07 ± 0.18 a	170	4.877 ± 0.27 a	156	59.41 ± 3.01 a	165
	Oral teliton + wheat germ diet	16.12 ± 1.28 b	107	13.74 ± 1.37 a-b	105	56.17 ± 3.36 a-b	112	3.00 ± 0.14 c	125	4.00 ± 0.30 b	128	42.88 ± 2.66 b	119
	Dermal teliton	17.61 ± 1.40 a	117	15.62 ± 1.26 a	119	59.00 ± 3.67 a	118	3.78 ± 0.18 b	158	4.20 ± 0.25 b	134	56.92 ± 3.61 a	158
	Dermal teliton + wheat germ diet	16.00 ± 1.51 b	107	13.96 ± 1.02 a-b	106	56.48 ± 3.24 a-b	113	2.73 ± 0.12 c-d	114	3.43 ± 0.22 c-d	110	40.07 ± 2.78 c-d	111

% Relative to health control values are represented mean of 6 rats mean ± SD means ± SD in the column followed by same letter are not significantly different at $p < 0.05$

Cytochrome-c is used as a good marker for mitochondrial synthesis and its turnover. Cytochrome-c levels in liver, kidneys and brain tissues were determined in the experimental animals at the end of 12 weeks experiment period. The decrease of cytochrome-c content under the toxicity of technical and formulation of profenofos either for dermally or orally administrations (Table 1) were noticed for intoxicated rats relative to control. It means that cytochrome-c was decomposed predominantly during the insecticide induction that cytochrome-c is extra mitochondrial membrane [24]. It should be a good marker of inner mitochondrial membrane turnover. The results of cytochrome-c suggested the hypothesis that organophosphorus pesticide leads to damage and destruct of 7-25% of mitochondria [4]. The results of profenofos toxicity on respiratory mitochondrial enzymes related to cytochrome-c was determined and shown in Table (2). Formulation profenofos significantly stimulated the activity of cytochrome-c-oxidase and succinate cytochrome-c-reductase. More than those of technical one, the stimulation was not due to an overall enhancement of the activity of respiration system, but it appeared to be due to the increase in dehydrogenases activity and the rate limiting step of the oxidation of metabolites as succinate [4].

These results are in agreement with another study [25] which showed that the content of cytochrome-c in organs tissues of male rats was decreased, but the respiratory enzyme activities were stimulated under the effect of malathion (organophosphorus pesticide) either in case of formulation or technical form. These were supported by succinate-cytochrome-c-reductase, which stimulated by the organophosphorus insecticide treatment, which includes the rate limiting step catalyzed by the primary dehydrogenase and the limited stimulation of the cytochrome-c-oxidase activity, as known to be far in excess of the overall rate of metabolites oxidation [26, 27].

Cellular oxidation and activities of several oxidoreductases stimulation have been reported in animal tissues [28]. Our findings pointed out those oxidative enzymes could result in an elevation in the rate of oxidation such as succinate and represent a

compensatory mechanism which overcome the initial lack of O_2 and provide the minimal energy requirement. Thus the specific stimulation in oxidative enzyme activity in liver, kidneys and brain tissues under the effect of pesticides could be due to animal physiological status [28, 29]. All alterations were shown that the insecticide formulation were more effective than those of technical ones. These may be due to the adjuvant which are added to technical pesticide and may increase the degree of absorption from the gastrointestinal tract or skin or oral or dermal administration one might add that the polarizing of the formulator molecules had a great effect on pesticide absorption rate and alter the physical properties of the technical pesticide [25, 10].

Cytochrome–c system (cyt-c, cyt.c oxidase and succi.cyt.c reductase) was connected with respiratory system of mitochondria, thus considered an important marker of mitochondrial biosynthesis and turnover [30]. The present results showed that cytochrome-c content in liver, kidneys and brain was decreased relative to control under the ingestion of profenofos pesticides. The reading on semi-modified wheat germ diet (source of vitamin E) attenuated the harmful of profenofos toxicity in intoxicated animals. Also, the stimulated activity of the both mitochondrial cytochrome-c-oxidase and succinate-cytochrome-c-reductase in the liver, kidneys and brain tissues was improved by semi-modified wheat germ diet feeding in intoxicated rats, but their values were still more than those of healthy normal control animals. It was reported that antioxidant agents alleviated the toxic influences of dimethoate either in formulation or technical form on body weight gain and thyroid function as well as blood sugar and liver glycogen and also on the organs weight ratio of intoxicated animals [10] which in agreement with our results. The redox enzymes could enhance metabolites oxidations such as succinate via cytochrome-c system which may be due to the physiological status of rats [24]. The co-administration of zinc into chlorpyrifos as an organophosphorus insecticide in intoxicated animals normalized the enzymatic activities of cytochrome P450, NADH-cytochrome C-reductase and NADH-cytochrome-c-reductase within normal range. Zinc supplementation is protective in the animals subjected to organophosphorus pesticide intoxication, as in markedly helps regulating the activities of key xenobiotic metabolizing enzymes in the condition of this group of insecticide toxicity, in addition, zinc-induced metallothionein levels and its antioxidant effects may be controlled to its biological protective role [4].

The respiratory chain, including cytochrome-c system is a structurally organized entity, since all of its components persist not in a dissolved state, but are other located in the inner mitochondrial membrane. Therefore, the release of energy under tissue respiration conditions occurs not as a single event, but proceeds through a number of successive stages. The released energy is stored in ATP phosphate bonds and is consumed in the biological cellular processes [30]. For these reasons, it must be studied the energy system (compounds related to ATP formation) under the present experimental conditions.

The influences of inducting technical and formulation of profenofos as toxic agents as well as semi-modified wheat germ diet as a source of vitamin E (anti-oxidative agents against the toxicity of profenofos on the energy- system) was investigated and the results as shown in Tables (3 and 4)

The ingestion orally or administration dermally of both forms of profenofos affected the energy system (amounts of ATP, ADP and AMP and myokinase activity) in rat liver, kidneys and brain tissues. The ATP content was elevated in all experimental animals relative to healthy control, the highest one was shown in rats ingested the formulation form of profenofos followed by those animals ingested technical ones which were nearly around to the rats administered dermally the formulation form of the studied pesticide, but the lowest effects were noticed for dermal technical profenofos administration. The contents of ADP and AMP in the same tissues under the same conditions were decreased relative to those of normal healthy control animals. The reduced values of ADP and AMP were nearly similar. These may be due to the high rate of ATP through trapping inorganic phosphate with AMP and then/or ADP to form ATP. Another study found that the formulation form of organophosphorus insecticide induction stimulated protein biosynthesis and cytochrome – enzymes –system more those of technical form [25] which in agreement with our results. Also, the present results are in parallel with another study of thyroid gland function [10], that the stimulation of this gland elevated the excretion of T4 and T3 which stimulated the formation of ATP [31].

It can be suggested that carbohydrate as a prime dietary source of energy supported the findings of ATP, ADP and AMP which showed that at any circumstance associated with the diminished availability of this source will accentuate utilization of fatty acids for the same purpose, which stimulated glycolytic catabolism to form

Table 3: ATP and ADP contents in liver, kidneys and brain tissues of the experimental rats % Relative to health control values are represented mean of 6 rats mean ± SD means ± SD in the column followed by same letter are not significantly different at p < 0.05

		ATP µ mole / g tissue						ADP µ mole / g tissue					
		Liver		Kidneys		Brain		Liver		Kidneys		Brain	
Insecticide Form	Treatments	µ mole/g tissue/min	%	µ mole/g tissue/min	%	µ mole/g tissue/min	%	µ mole/g tissue/min	%	µ mole/g tissue/min	%	µ mole/g tissue/min	%
Control	Health control	9.50 ± 0.63 [b]	100	0.82 ± 0.051 [d]	100	6.09 ± 0.42 c	100	0.149 ± 0.007 a	100	0.136 ± 0.007 a	100	0.550 ± 0.031 a	100
	Control ± wheat germ diet	9.57 ± 0.71 [b]	101	0.81 ± 0.042 d	99	6.11 ± 0.41 c	100	0.151 ± 0.009 a	101	0.138 ± 0.008 a	101	0.548 ± 0.029 a	100
Technical form	Oral profenofos	11.87 ± 0.92 [a]	125	1.32 ± 0.061 a	161	8.10 ± 0.53 a	133	0.060 ± 0.002 d	40	0.100 ± 0.006 b	74	0.381 ± 0.017 b	69
	Oral profenofos ± wheat germ diet	10.90 ± 0.64a-b	115	1.09 ± 0.054 b-c	133	7.87 ± 0.49 a	129	0.110 ± 0.004 d	74	0.109 ± 0.006 b	80	0.402 ± 0.028 b	73
	Dermal profenofos	11.61 ± 0.65 a	122	1.19 ± 0.049 b	145	8.00 ± 0.51 a	131	0.108 ± 0.005 b	73	0.059 ± 0.003 c	43	0.371 ± 0.018 b	69
	Dermal profenofos ± wheat germ diet	10.11 ± 0.70 b	106	1.00 ± 0.041 c	122	7.10 ± 0.48 b	117	0.112 ± 0.006 b	75	0.068 ± 0.004 c	50	0.400 ± 0.026 b	73
formulation from	Oral seliton	12.31 ± 0.75 a	130	1.19 ± 0.061 b	145	8.01 ± 0.51 a	132	0.116 ± 0.006 b	78	0.129 ± 0.007 a	95	0.490 ± 0.031 a	89
	Oral teliton ± wheat germ diet	11.00 ± 0.81 a-b	116	1.10 ± 0.058 b-c	134	7.90 ± 0.41 a	130	0.120 ± 0.006 c	54	0.132 ± 0.008 a	97	0.500 ± 0.036 a	91
	Dermal teliton	11.69 ± 0.69 a	123	1.20 ± 0.060 b	146	8.00 ± 0.39 a	131	0.100 ± 0.004 b-c	67	0.112 ± 0.006 b	82	0.402 ± 0.029 b	73
	Dermal teliton ± wheat germ diet	10.37 ± 0.61 b	109	1.03 ± 0.054 c	126	7.08 ± 0.42 b	116	0.114 ± 0.006 d	77	0.124 ± 0.007 a	91	0.516 ± 0.037 a	94

Table 4: AMP contents and myokinase activity in liver, kidneys and brain tissues of the experimental rats % Relative to health control values are represented mean of 6 rats mean ± SD means ± SD in the column followed by same letter are not significantly different at p < 0.05

		AMP µ mole / g tissue						Myokinase activity µ mole / g tissue					
		Liver		Kidneys		Brain		Liver		Kidneys		Brain	
Insecticide Form	Treatments	µ mole/g tissue/min	%	µ mole/g tissue/min	%	µ mole/g tissue/min	%	µ mole/g tissue/min	%	µ mole/g tissue/min	%	µ mole/g tissue/min	%
Control	Health control	0.629 ± 0.041 a	100	0.300 ± 0.017 a	100	0.271 ± 0.018 a	100	0.500 ± 0.031 a	100	0.220 ± 0.011 a	100	0.520 ± 0.032 a	100
	Control ± wheat germ diet	0.631 ± 0.037 a	100	0.301 ± 0.016 a	100	0.268 ± 0.017 a	99	0.502 ± 0.032 a	100	0.222 ± 0.015 a	101	0.513 ± 0.031 a	99
Technical form	Oral profenofos	0.452 ± 0.029 b	72	0.202 ± 0.011 b-c	67	0.167 ± 0.009 c	62	0.320 ± 0.017 b	64	0.151 ± 0.010 c	69	0.311 ± 0.021 b-c	60
	Oral profenofos ± wheat germ diet	0.486 ± 0.024 b	77	0.211 ± 0.015 b	70	0.183 ± 0.010 c	68	0.371 ± 0.020 b	74	0.160 ± 0.009 c	73	0.360 ± 0.019 b	69
	Dermal profenofos	0.391 ± 0.020 b	62	0.171 ± 0.014 c	57	0.130 ± 0.008 d	48	0.281 ± 0.014 c	56	0.120 ± 0.008 d	55	0.258 ± 0.017 c	50
	Dermal profenofos ± wheat germ diet	0.611 ± 0.031 a	97	0.180 ± 0.016 c	60	0.174 ± 0.008 c	64	0.331 ± 0.021 b	66	0.141 ± 0.011 c-d	64	0.320 ± 0.018 b	62
formulation from	Oral seliton	0.560 ± 0.037 a	98	0.233 ± 0.031 b	78	0.220 ± 0.016 b	81	0.346 ± 0.020 b	69	.161 ± 0.012 c	73	0.380 ± 0.016 b	73
	Oral teliton ± wheat germ diet	0.601 ± 0.028 a	96	0.236 ± 0.014 b	79	0.281 ± 0.012 a	104	0.401 ± 0.025 a-b	80	0.180 ± 0.010 b	82	0.400 ± 0.018 b	77
	Dermal teliton	0.482 ± 0.030 b	77	0.177 ± 0.010 c	59	0.200 ± 0.011 c	74	0.320 ± 0.016 b	64	0.132 ± 0.007 d	60	0.340 ± 0.015 b	65
	Dermal teliton ± wheat germ diet	0.573 ± 0.032 a	91	0.214 ± 0.016 b	71	0.234 ± 0.014 b	86	0.441 ± 0.030 a	88	0.157 ± 0.007 c	71	0.369 ± 0.020 b	71

pyruvic acid and then acetyl CoA led to accumulating ATP and creatinine storage of energy [28, 30]. In addition, protein biosynthesis rapidly utilized ATP which stimulated by an adenylate cyclase enzyme to produce cyclic AMP [24].

The significant inhibition of myokinase activity in all tissues of the intoxicated animals relative to normal healthy control group was noticed in the results of Table (4). These may be due to the plenteous ATP. Myokinase catalyzed the reaction between 2 of ADT to produce one ATP and one AMP molecule. This reaction was done after the complete utilization of ATP energy. Also, the amount of AMP may be due to cyclic AMP which produced by conversion of one ATP by the adenylate cyclase enzyme to produce cyclic AMP and ADP. The elevation of ATP values under the induction of the studied pesticides was mainly attributed to the effect of pesticide on the respiratory system as reported in the cytochrome-c results of the present work.

The maintenance of tissues as likely accomplished through is stimulated glycolytic process. The extent of coupling oxidation of phosphorylation evident in mitochondria, provided means by which the role of oxidation of foodstuffs by respiratory O_2 was regulated by the requirement of the cell for useful energy. The using of ATP to drive energy requiring the process of the cell automatically increased the available supply of ADP and inorganic phosphate, which in turn, become available to react to the coupling mechanism and permit respiration to proceed [24, 30].

In the intoxication condition by profenofos oxidative phosphorylation was stimulated due to the respiratory O_2 which stimulated the ATP formation [28]. The more effects of the formulation form of profenofos then technical one may be due to the formulators' adjutants which may cause synergism to the harmful of profenofos active ingredient. Such formulators are expected to affect the pesticide penetration distribution through the skin and retention in the body tissues and blood constituents are factors to determine the trend for their tissue deposition, partitioning and toxic kinetics [32].

Semi-modified wheat germ diet feeding into intoxicated male albino rats alleviated the profenofos toxicity. Wheat germ contains a large amount of α.

tochopherol [33]. Also, wheat germ has good values of minerals, including, Zn, Fe, K and P as well as protein, lipid, dietary fibers, carotenoids, carbohydrate and other vitamins [29, 34]. Vitamin E is thought to be an important chain breaking antioxidant, which plays an important role in various stages of carcinogenesis through its contribution to immune competence membrane and DNA repair as decreasing oxidative DNA damage, prevent the uncontrolled formation of free radicals and activated oxygen species or inhibit their reaction with biological structure [9].

Semi –modified wheat germ diet feeding to profenofos intoxicated rats accompanied by improvement the ATP contents in body tissues (Table 3) the level of ATP in liver and brain tissues was more than those of the kidneys. The values also of ADP and AMP were improved also and increased in the same tissues under the same conditions (Tables 3 and 4). In connection, the inhibition in the myokinase activity in the profenofos intoxicated animals was alleviated by the semi-modified wheat germ diet feeding as anti-oxidative agents. Different effects of profenofos in liver, kidneys and brain were noticed in intoxicated rats, thus the overall response varies from tissue to tissue. Vitamin E is a well-known antioxidant, when present in aqueous medium efficiently inhibits lipid peroxidation due to a combination of direct radical interception [9].

Profenofos as an organophosphorus insecticide is an anti-cholinesterase inhibitor. It covalently modified acetyl cholinesterase thus inhibiting its activity the protection of the enzyme activity in the organs tissue of intoxicated animals by organophosphorus pesticide is affected by pretreatment with antioxidant vitamins including vitamin E. Our results are confirmed with these phenomena on that treatment of an antioxidant agent (wheat germ) attenuated the profenofos-induction peroxidation. A study [35] showed that vitamin E depletion causes a marked increase in H_2O_2 production in skeletal muscle mitochondria to a lower extent of liver mitochondria, while its dietary supplementation reduced the rate of H_2O_2 production. Vitamin E is capable of regulating the generation of superoxide reduced mitochondrial generation of reactive oxygen metabolites not only attenuated oxidative stress but also modulate the expression and activation of signal transduction pathways and other redox sensitive modifiers. It may prevent the onset of enervative diseases or reduce the toxicity of oxidative stress generated by pesticide [36]. The protective effect of vitamin E from the enzyme`s inhibitory action of profenofos may be due vitamin E the binding of making them unavailable for phosphorylation [37]. Certain enzymes reactivators, such as oximes, constitute the most important means of preventive treatments following exposures to organophosphorus insecticides in human [6]. The protective roles of safer preventative compounds, offering the least amount of side effects are warranted to be explored. Some investigations have suggested zinc and vitamin E as beneficial agents during peroxidative damage [38, 39].

In conclusion, simultaneous administration of semi-modified profenofos diet as a good source of vitamin E and zinc as well as other antioxidant agents attenuated the harmful toxicity of profenofos in cytochrome-c respiratory system and ATP-energy system which play a good role in regulating the metabolizing enzymes of the xenobiotic in the intoxicated animal tissues.

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