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Antioxidant Diet as Protective Agents Against Biochemical Perturbation Effects Induced by Cypermethrin on Lipids and Protein Fractions as Well as Kidneys Function of Blood Rat

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Abstract: The toxic influences of technical and formulated cypermethrin (synthetic pyrethroid pesticide) and the attenuation treatments of mixture of dried guava and wheat germ (1:1) as an antioxidant diet (AD) which contains large amounts of vitamins C and E on albino rats metabolism were studied. Rats (60) were randomly divided into 10 groups: group I served as control rats (NC), groups II (normal rats) was fed on the antioxidant diet (AD). The other 48 rats (8 groups) were induced by the pesticide. Formulated (FC) and technical (TC) cypermethrin twentieth of the LD_{50} sublethal doses (1/20 LD_{50}) were applied orally and dermally every 48 hours for 90 days. Four groups of them fed on normal diet and the other 4 groups were fed on the present antioxidant diet. The levels of biochemical markers of blood (glucose, lipid profile, protein fractions and kidneys function) were determined. In connection, blood glucose levels were increased by the induction of both forms of cypermethrin (formulated and technical) and the highest level was observed when rats orally ingested formulated cypermethrin but the lowest effect was detected in rats inducted dermally technical cypermethrin. In intoxicated rats, the elevated blood sugar was accompanied with decrease in plasma total soluble protein and its fractions (albumin and globulin) as well as the ratio of albumin/globulin. The same trend was found in lipid profile (total lipid, cholesterol, triglyceride and phospholipids) which showed a significant decrease in these parameters. Also, the pesticide intoxication altered blood levels of lipoprotein fractions and atherogenic index (AI) where HDL-C and VLDL-C contents were decreased, but LDL-C and AI were increased. In addition, the kidneys function was changed in the cypermethrin intoxicated rats, which either technical or formulated cypermethrin administration elevated the blood content of uric acid, urea and creatinine (highly significant) which was induced orally or dermally. The highest effect was found in case of orally formulated cypermethrin but the lowest effect was detected in the administration of the dermally technical one. The treatment of antioxidant diet (normal diet supplemented with 20% dried guava and wheat germ) significantly reduced the toxic effects of cypermethrin induction of the intoxicated animals, in which blood glucose level was reduced, but total soluble protein, albumin and globulin as well as albumin/globulin ratio were improved and increased and reached near to those of the normal healthy control. Lipid profile (total lipids, cholesterol, triglycerides and phospholipids) levels were alleviated by antioxidant diet feeding which increased nearly normal control animals. In case of the lipoprotein fractions, the antioxidant diet treatments improved their contents in blood of intoxicated rats. HDL-C and VLDL-C levels were increased, but the levels of LDL-C and AI were decreased under the same conditions. On the other hand, Kidneys function was also improved by antioxidant diet feeding for cypermethrin intoxicated rats. The levels of uric acid, urea and creatinine of the intoxicated rats were decreased by feeding on the antioxidant diet. No significant changes in the above parameters were observed in the normal healthy rats fed on the present antioxidant diet compared with normal control. In conclusion, antioxidant vitamins compounds of guava and wheat germ (vitamins C and E respectively) could be used as an alleviated and improved treatment for the harmful influences in cypermethrin intoxicated animals.

Key words: Cypermethrin; Guava; Wheat germ; Lipids fractions; protein fractions; kidneys function.

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INTRODUCTION

Cypermethrin has been considered one of the synthetic pyrethroids insecticide family which is widely used throughout the world as a wide – spectrum insecticide for numerous crops and also indoor pest control in the public health sector and housing (Casida *et al.*, 1983). However, organochlorine, organophosphorus insecticides and carbamates were extensively used in the pest to improve agricultural production. Synthetic pyrethroid insecticides are now being substituted for pest control and increased production. These chemicals have been considered potentially toxic to mammals (Muthuviveganandavel *et al.*, 2008). Because cypermethrin is one of a series of relatively photostable pyrethroid insecticides, it is also suitable for its use in the field (Elliott, 1976). The biological studies of cypermethrin have shown that it inhibits liver ATPase activity and causes necrosis, inflammation and cytoplasmic hypertrophy (with intracytoplasmic droplets) in hepatocytes (Aldana *et al.*, 2001) cypermethrin used for control of a wide range of insects, especially Lepidoptera, but also Coleoptera, Diptera, Hemiptera and other classes, those are in fruit (including citrus) vines, vegetables such as potatoes, cucurbits, lettuce, capsicums, tomatoes, cereals, such as maize, soya beans, cotton, coffee, cocoa, rice, pecans, oil seed rape, beet, ornamentals etc. It controls flies and other insects in animal houses such as mosquitoes, cockroaches, houseflies and other insect pest public health. Also used as an animal ectoparasiticide (Tomlin, 2000). Due to their high efficacy, easy biodegradability and low toxicity to birds and mammals (Kale *et al.*, 1999), Synthetic pyrethroids (especially cypermethrin) are chosen over organochlorine, organophosphorus and carbamate insecticides (Fetoui *et al.*, 2008). The effect of sublethal doses of cypermethrin on blood biochemistry (protein, lipids, glucose and kidneys function) in rats had not been studied earlier. However, the present investigations therefore aims at studying the harmful effect of cypermethrin on level of glucose, protein and its fraction, lipids profile, lipoprotein fraction and kidneys function (uric acid, urea and creatinine) of albino rat blood.

MATERIALS AND METHODS

Pesticide Materials:

Cypermethrin [(R,S) α-cyano-3-phenoxybenzyl (1R,S)-cis, trans-3-(2,2-dichorovinyl)-2, 2-dimethylcyclopropane carboxylate)] technical (90% a.i) and formulated (25% E.C.) was provided from Central Agriculture Pesticides Laboratory, Agriculture Research Center, Giza, Egypt, and used in the present studies (cypermethrin LD_{50} = 250 mg/Kg b.w. orally and LD_{50} = 5000 mg/Kg b.w. dermally for rat) (Tomlin, 2000).

Plant Materials:

Guava fruits purchased from a local market, Cairo, Egypt. The fruits were washed, sliced, freeze-dried and pulverized with a blender to a fine powder and kept until use.

Wheat germ was procured 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.

Animals and Experimental Design:

Sixty healthy adult male albino rats (*Rattus Norvegicus*) Sprague Dewley strain, each weighing 120 ± 5g 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 adjusted at 25 ± 2ºC with relative humidity of ≈57% and 12-hour light-dark cycle) for two weeks (adaptation period) in their cages prior to the experiment for acclimatization. During this period, the rats were fed on normal diet consisting 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 excess of water and diet. They (60 rats) were divided into 10 groups (6 rats each). Five groups were fed on the normal diet and the other five groups were fed on the antioxidant diet (80% normal diet + 20% of mixture of dried guava and wheat germ 1:1 sources of vitamin C and E). The first group was served as normal healthy control (NC), the 2nd group rats were fed on the antioxidant diet (AD), the 3rd and 5th groups were ingested respectively with the sublethal dose of cypermethrin which was 1/20 of oral LD_{50} of either technical (TC) or formulated (FC) insecticide and fed on the normal diet. The same treatments were done in the 4th and 6th groups respectively but these animals were fed on the antioxidant 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 cypermethrin which was 1/20 of the dermal LD_{50} of either technical or formulated cypermethrin respectively. Dermal dose was applied on dorsal skin shaved area of 2 × 2 cm on the back of the dermally treated rats which were shaved with care not to abrade the shin as described by Abou-Zeid *et al.* (1993). The technical and formulated cypermethrin were used without any additives for dermal induction. The same treatments were done in the 8^{th} and 10^{th} groups respectively but their animals were fed on the antioxidant diet. For oral ingestion, the dose was emulsified with 0.5 ml distilled water. One dose was inducted every 48 hours during the experimental period (3 months) either for dermal or oral administration of both forms of cypermethrin. Normal diet or the antioxidant diet and water were supplied *ad libitum*.

The animals were killed by decapitation at the end of the experimental period (3 months). Blood was collected. Serum was separated by centrifugation at 2500 rpm at 37°C for 15 min which used to determine glucose, total soluble protein and its fractions, total lipids and the lipids profile and kidneys functions.

Biochemical Analysis:

Serum glucose was determined according to Trinder (1969), total soluble proteins and albumin were determined according to Henary (1964) and Doumas *et al.* (1971) respectively but globulin was calculated by difference between total protein and albumin. Total lipids, cholesterol, triglycerides and phospholipids (Lipids profile) were determined according to Joseph *et al.* (1972), Chaurchami *et al.* (1959), Young and Pestaner (1975) and Ketes (1972) respectively.

The lipoprotein fractions (HDL-C and LDL-C) were determined according to Grove (1979) and Wieland and Seidel (1983) respectively. VLDL-C and Atherogenic Index (AI) were calculated according to Lee and Niemann (1996) as in the following equations:

$$\text{VLDL-C} = \frac{\text{Triglycerides}}{5}$$

$$\text{Atherogenic Index (AI)} = \frac{\text{Total cholesterol} - \text{HDL-C}}{\text{HDL-C}}$$

Serum uric acid, urea and creatinine contents were determined according to Barham and Trinder (1972), Fawcett and Scott (1960) and Bartels and Bohmer (1971) respectively.

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).

RESULTS AND DISCUSSION

Pyrethroid insecticides are one of the most important classes of pesticides, which have been used in many purposes. Acceptability of these substances for utilization has been hindered by problems involving assessment of its net benefits. Indeed, literature revealed few reports on the hypointensive effect of natural antioxidants on the pyrethroid pesticides toxicity which dealt with metabolic changes and other side effects after its utilization. The present studies investigated the influences of an antioxidant diet (including guava and wheat germ in mixture with the normal diet) on the harmful effects of cypermethrin (pyrethroid pesticide) by determination of serum various biochemical parameters.

As shown in Table (1) blood glucose levels significantly increased by cypermethrin induction in all intoxicated animals relative to normal control, but the formulated cypermethrin ingestion orally produced the highest elevation in blood sugar, also the ingestion of technical pesticide orally showed significant increase in blood glucose but less than the formulated one. Similar results were obtained by dermal induction of formulated cypermethrin into rats. The lowest effect was observed in case of the technical insecticide induced dermally. Feeding on the present antioxidant diet improved and readjusted blood glucose level around that of normal control. In contrast, serum total soluble protein of intoxicated rats was reduced under the effects of cypermethrin administration. Herein orally formulated pesticide treatment showed the highest effect, but the

lowest ones detected in case of dermally technical cypermethrin induction. The same trend was observed for serum protein fractions (albumin and globulin), in which formulated cypermethrin administration decreased the serum contents of albumin or globulin as well as their ratio (albumin/globulin ratio) relative to those of normal healthy control. The treatments with antioxidant diet feeding attenuated the disturbed effect of the pesticide on serum protein and its fractions, in which the toxic effects of cypermethrin was inhibited by antioxidant diet treatment. The influences observed on serum glucose, protein, albumin and globulin may be due to chronic chemical exposure (Manna *et al.*, 2005).

Table 1: The blood content of glucose, total protein, albumin, globulin and albumin/ globulin ratio of normal and cypermethrin intoxicated rats fed on normal and antioxidant diets

Treatment	Glucose (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Alb/Glob ratio
NC	89.5±5.24^{e}	6.66±0.50	4.44±0.31^{a}	2.22±0.24^{ab}	2.04
NC+AD	93.0±6.42^{e}	7.00±0.46^{a}	4.65±0.29^{a}	2.35±0.22^{a}	2.05
Oral					
TC	119.0±6.36^{abc}	4.47±0.31^{c}	2.66±0.21^{de}	1.81±0.24^{d}	1.57
TC+AD	112.2±6.91^{bcd}	5.48±0.25^{b}	3.41±0.25^{b}	2.07±0.27^{abcd}	1.75
FC	125.8±8.30^{a}	4.01±0.25^{d}	2.11±0.20^{f}	1.90±0.10^{cd}	1.14
FC+AD	113.0±6.72^{bcd}	5.24±0.24^{b}	3.11±0.22^{c}	2.13±0.25^{abc}	1.55
Dermal					
TC	116.5±5.43^{bcd}	4.79±0.37^{c}	2.89±0.19^{cd}	1.90±0.22^{cd}	1.60
TC+AD	109.5±6.16^{d}	5.63±0.29^{b}	3.44±0.26^{b}	2.03±0.24^{bcd}	1.76
FC	120.3±7.61^{ab}	4.64±0.27^{c}	2.47±0.18^{e}	1.99±0.26^{bcd}	1.27
FC+AD	110.8±6.88^{cd}	5.50±0.30^{b}	3.43±0.33^{b}	2.07±0.30^{abcd}	1.76
LSD	7.72	0.390	0.289	0.277	

Values are mean ± SD of 6 rats per group, SD = standard deviation.
Means with the same superscript letters within the same column are not significantly different (P<0.05).
NC=Normal control, AD=antioxidant diet, TC=Technical cypermethrin, FC=Formulated cypermethrin

The results presented in Table (2) pointed out the serum lipid profile of normal and intoxicated rats either treated or untreated with antioxidant diet. Cypermethrin as a pyrethroid insecticide significantly decreased the serum total lipids of albino rats relative to normal control. Similar results were observed in case of serum lipids profile the contents of blood cholesterol, triglycerides and phospholipids of the experimental animals were also reduced significantly under the toxic effects of both forms of cypermethrin either by oral ingestion or dermal induction. The formulated pesticide observed more reduction in lipids profile than that of the technical one, also oral ingestion of cypermethrin was more effective than the dermal induction. In contrast, when the intoxicated rats were fed on the present antioxidant diet, the reduced values of lipid profile constituents were improved and returned around those of normal healthy control.

Table 2: The blood content of total lipids, triglycerides, cholesterol and phospholipids of normal and cypermethrin intoxicated rats fed on normal and antioxidant diets

	Blood lipid fractions (mg/dl)			
Treatment	Total lipids	Total cholesterol	Total triglycerides	Total phospholipids
NC	801±46.52^{a}	125±6.85	389±22.67^{ab}	156±8.60^{a}
NC+AD	811±53.52^{a}	124±7.39^{a}	400±28.74^{a}	154±9.70^{a}
Oral				
TC	653±41.67^{ef}	110±6.21^{d}	323±18.06^{efg}	127±11.58^{de}
TC+AD	730±45.28^{bcd}	115±5.91^{bcd}	360±15.65^{cd}	141±8.49^{bc}
FC	633±44.00^{f}	102±5.09^{e}	310±15.23^{fg}	120±10.87^{e}
FC+AD	687±49.58^{cdef}	112±5.29^{cd}	342±17.36^{de}	136±11.62^{bcd}
Dermal				
TC	710±53.97^{bcde}	117±6..42^{bcd}	303±15.11^{g}	138±10.30^{bcd}
TC+AD	772±42.73^{ab}	121±4.71^{ab}	333±15.96^{ef}	148±9.27^{ab}
FC	680±39.04^{def}	115±5.16^{bcd}	340±16.62^{de}	130±8.73^{cde}
FC+AD	741±38.52^{bc}	118±4.05^{abc}	370±22.34^{bc}	142±9.45^{bc}
LSD	53.02	6.72	22.13	11.51

Values are mean ± SD of 6 rats per group, SD = standard deviation.
Means with the same superscript letters within the same column are not significantly different (P<0.05).
NC=Normal control, AD=antioxidant diet, TC=Technical cypermethrin, FC=Formulated cypermethrin

In connection, serum lipoprotein fractions contents were altered under the effects of technical and formulated cypermethrin induction in albino rats. The serum LDL-C and AI values were significantly increased

in the animals administrated either technical or formulated cypermethrin, but the values of HDL-C and VLDL-C were significantly decreased under the same conditions (Table 3). These disturbed values of intoxicated rats blood were improved by antioxidant treatment with feeding on the mixture of guava and wheat germ with normal diet (antioxidant diet) and the values readjusted nearly to those of normal control. Blood lipids were found to be roughly proportionate to the clinical severity of the renal complication of the diseases (Keiding *et al.*, 1952). Therefore, although there may be some relation between the level of lipid substances and the presence of diseases, the estimation of total blood lipids is of no real value in the diagnosis of the renal disorders. So it was of great importance to estimate renal function by the classical methods which includes the determination of blood urea and uric acid to evaluate the disorders which may be occurred as a result of the pesticide ingestion and to determine to what extent the administration of the antioxidant diet was treated to improve such disorders.

Table 3: The blood content of HDL-C, LDL-C, VLDL-C (lipoprotein fractions) and atherogenic index of normal and cypermethrin intoxicated rats fed on normal and antioxidant diets

Treatment	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	Atherogenic index
NC	44.60±3.26^{a}	36.20±1.94^{f}	77.80±4.53^{ab}	1.81
NC+AD	45.00±2.83^{a}	35.98±2.25^{f}	79.97±5.75^{a}	1.76
Oral				
TC	33.10±2.01^{d}	44.10±2.84^{bc}	64.60±3.61^{efg}	2.32
TC+AD	39.68±2.12^{bc}	40.97±2.74^{cd}	71.97±3.13^{cd}	1.89
FC	30.10±1.80^{e}	48.40±3.25^{a}	62.07±3.05^{fg}	2.40
FC+AD	38.31±1.99^{c}	40.26±2.96^{d}	68.43±3.47^{de}	1.92
Dermal				
TC	34.20±2.42^{d}	46.02±3.16^{ab}	60.53±3.02^{g}	2.42
TC+AD	42.30±2.93^{ab}	38.10±2.73^{def}	66.63±3.19^{ef}	1.86
FC	32.93±2.25^{de}	43.78±3.17^{bc}	68.03±3.32^{de}	2.50
FC+AD	40.00±3.11^{bc}	39.50±2.49^{d}	73.97±4.74^{bc}	1.95
LSD	2.91	3.22	4.46	

Values are mean ± SD of 6 rats per group, SD = standard deviation.
Means with the same superscript letters within the same column are not significantly different (P<0.05).
NC=Normal control, AD=antioxidant diet, TC=Technical cypermethrin, FC=Formulated cypermethrin

The effects of the antioxidant diet on the kidneys function of normal and cypermethrin intoxicated albino rats were shown in Table (4). The results of serum uric acid, urea and creatinine contents were statistically analyzed. The finding observed that the induction of both technical and formulated cypermethrin orally and dermally elevated the serum contents of uric acid, urea and creatinine, in which the increased values were nearly and more than twice the values of those of normal healthy control. The treatment with the present antioxidant diet by feeding for intoxicated animals caused significant reductions in the levels of the three parameters of kidneys function. It means that the present antioxidant diet attenuated the perturbation effects of both technical and formulated cypermethrin intoxicated albino rats.

Table 4: The blood content of uric acid, urea and creatinine (kidneys function) of normal and cypermethrin intoxicated rats fed on normal and antioxidant diets

Treatment	Uric acid (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
NC	4.12±0.29^{f}	18.21±1.13^{f}	0.84±0.05^{f}
NC+AD	4.00±0.30^{f}	18.01±1.47^{f}	0.83±0.07^{f}
Oral			
TC	8.88±0.38^{b}	45.00±3.74^{b}	1.78±0.08^{b}
TC+AD	6.40±0.51^{d}	31.00±2.77^{d}	1.31±0.07^{de}
FC	9.98±0.58^{a}	49.77±3.79^{a}	2.00±0.11^{a}
FC+AD	6.88±0.42^{d}	33.33±2.00^{d}	1.39±0.12^{d}
Dermal			
TC	7.97±0.55^{c}	40.23±2.62^{c}	1.63±0.09^{c}
TC+AD	5.66±0.39^{e}	27.39±1.84^{e}	1.20±0.07^{e}
FC	8.79±0.61^{b}	44.73±2.71^{b}	1.80±0.12^{b}
FC+AD	6.49±0.54^{d}	32.73±2.44^{d}	1.30±0.07^{de}
LSD	0.54	3.00	0.10

Values are mean ± SD of 6 rats per group, SD = standard deviation.
Means with the same superscript letters within the same column are not significantly different (P<0.05).
NC=Normal control, AD=antioxidant diet, TC=Technical cypermethrin, FC=Formulated cypermethrin

In general, it should be emphasized that literature background has no available data about the present items which were investigated in this work. The present results indicated that the toxicity of the different treatments of cypermethrin administration can be arranged in the following increased order:
Orally formulated > orally technical ≥ dermally formulated > dermally technical

The present results (Table 1) showed that cypermethrin induction elevated blood glucose levels in intoxicated rats. These are in agreement with the results of Manna *et al.*, (2004a,b and 2005), they found that a-cypermethrin and deltamethrine (synthetic pyrethroid insecticides) significantly increased blood glucose levels. Also, our previous findings (Abdel-Rahim, 2007) and Pournourmohammadi, *et al.* (2005) showed that blood glucose content was elevated by the organophosphorus pesticide induction. These may be due to the pesticide subchronic exposure affects rat hepatic gluconeogenesis and glycogenolysis as well as inducing hyperglycemia (Abdollahi *et al.* 2004) and pesticides may be influenced muscle glycogenolysis and glycolysis as well as secretion of insulin from pancreas, which all may explain diabetic potential of cypermethrin (Manna *et al.*, 2004a,b and 2005).

In case of total soluble protein of blood and its fractions, Table (1) observed the blood contents of total protein, albumin and globulin were reduced by the induction of cypermethrin, and the ratio of albumin / globulin was also decreased. These results are in agreement with the results of Aldana *et al.* (2001) who found that the total serum proteins and albumin contents were decreased by cypermethrin induction.

The authors suggested that the results of alterations in blood protein corresponded to hepatotoxic effects of pesticide. Cypermethrin is hydrophobic compound which results in its low free concentration in blood. It is transported to the target organs through partitioning into blood lipid, and binding to blood protein. Albumin is the important transport protein, which can bind with many kinds of pesticides (including pyrethroids). The binding to albumin will effectively decrease the concentration of free pesticides, benefit their metabolic modification and transport them to the disposal site, alleviating the corresponding toxicity (Silva *et al.*, 2004 and Sulkowska *et al.*, 2004) and significantly affect the distribution, metabolism and excretion of pesticide (Gao *et al.* 2004). These bindings of exogenous agents (pyrethroids) could affect physiological functions of blood proteins through altering its conformation (Cui *et al.*, 2006). The present observed data of globulin support and reinforce the notion that pyrethroid pesticides induce stress – like symptoms and suggest globulins are necessary for suppression effects of pesticide on innate immune response (Righi *et al.*, 2008).

The decrease in blood total lipids, cholesterol, triglycerides and phospholipids in intoxicated rats after 90 days treatment with low dose of cypermethrin indicated degenerative changes (Table 2). The alterations observed in the present work of lipid profile and lipoproteins (Table 3) indicate that they can be important markers of hepatocyte structure and thus in liver and kidneys functions, which are in agreement with the observation of Malaguarnera *et al.* (1996), Aldana *et al.* (2001) and Crow *et al.* (2007), they reported that the cypermethrin intoxicated animals showed a decrease in total serum cholesterol and triglycerides. Also, the previous study (Abdel-Rahim, 2007) with chlorpyrifos and other organophosphorous pesticide (John *et al.*, 2001) supported the present findings that pesticide inductions reduced total lipids and cholesterol of intoxicated rats blood these may be related to lipases in blood and other tissues such as adipose tissue, that may contribute to pyrethroid metabolism but have not been adequately studied (Crow *et al.*, 2007). In addition, pancreatic lipase, secreted into the lumen of the small intestine to facilitate the hydrolysis of triglycerides and cholesterol esters (Hui and Howles, 2002) may also contribute to pyrethroid metabolism following oral or dermal exposures. The toxicity of insecticides (Pyrethroids or organophosphorous) vary with structures (WHO, 1991), these xenobiotics are known to have a strong affinity for interaction with membrane phospholipids, cholesterol and other lipid fractions. There are also evidences that oxygen free radical formation can be a factor in the toxicity effects of pyrethroids. The important target of creative oxygen – induced injury is lipid peroxidation and peroxidation of phospholipids and other lipid profile not only alters lipid milieu and structural and functional integrity of cell membrane but also affects the activities of various membrane bound enzymes (Muthuviveganandavel *et al.*, 2008 and Manna *et al.*, 2005).

The decreasing influences of cypermethrin induction on HDL and VLDL were observed in intoxicated rats blood, but LDL and atherogenic index (AI) a rather erratic profile (increasing) with cypermethrin induction (Table 3). These results are paralleled with the present findings of albumin, which is an important transport protein and can reversibly bind with lipids. Table (1) showed that cypermethrin induction decreased blood albumin level, this related to the decreased HDL (50% of HDL is protein) and VLDL lipoproteins blood levels. In contrast, the elevation of LDL under the same conditions may be due to that LDL is synthesized from VLDL by enzymes in plasma (LDL–synthetase complex), also there is an important enzyme in plasma called lipoprotein lipase which hydrolyses triglycerides and has the effect of reducing VLDL and HDL to smaller fragments (Campbell and Smith, 1982). As regarding to the atherogenic index (AI) proposed as a marker of

atherogenicity it is increased high risk for coronary heart disease. AI recorded significant increase as compared to normal control in the intoxicated rats. These results are good evidence to harmful effect of cypermethrin induction in rats (Lee and Niemann, 1996).

The effects of cypermethrin induction on rat kidneys function were shown in Table (4). The results of blood uric acid, urea and creatinine contents observed that the pesticide induction caused highly significant increases at normal control. Uric acid, urea and creatinine contents of intoxicated rats were more than twice the value of normal healthy control. These data are in agreement with those of Muthuviveganandavel *et al.* (2008) and Manna *et al.* (2005). They reported that cypermethrin induction altered the kidneys function of albino rats, which correlated with histopathological changes.

As mentioned, animals were induced either orally or dermally with either formulated or technical cypermethrin. A sub-lethal dose equal to 1/20 LD_{50} was administrated at 48-hours interval. At the end of the experimental period (90 days), the results showed that cypermethrin induction produced oxidative stress in the intoxicated rats, which was paralleled with several alteration in liver and kidneys function explained as well as blood lipid profile, glucose and protein fractions as previous explained. Inspection of data in Tables 1, 2, 3 and 4 showed either technical or formulated cypermethrin induced by oral or dermal, exhibited significant effects on the all studied blood parameters. The present results observed that the harmful effects of cypermethrin were more pronounced in case of formulated form particularly when ingested orally. It means that, the formulated cypermethrin induced greater effects than the technical one, and the oral of administration, expectedly resulted in higher effect than dermal one. These are in agreement with those obtained by Nasuti *et al.* (2003) and Manna *et al.* (2005). They reported that the vehicle has a great influence on the cypermethrin toxicity, probably by influencing absorption. Also our previous results on organophosphate pesticides (Abdel-Rahim and Abdel-Rahim, 2007 and 2008) found similar results, in which the toxicity effects of the different treatments of cypermethrin can be arranged in the following increasing order:

Technical dermal < formulated dermal ≤ technical oral < formulated oral

However to be ready for application against pests, certain additives and inducers are added to the technical pesticide to improve its performance and such ingredients may posses undesirable or adverse effect. The present results ascertained this assumption because the formulated pesticide produced high pattern of effect on different examined parameters compared with the technical one, and this result is in full agreement with those Nasuti *et al.* (2003), they reported that the remarkable effect of formulated pyrethroids could be explained by considering its chemical characteristics, which let it penetrate the cell more easily. Such findings may lead us to conclude that the additives induce in such a case a synergistic effect to the technical cypermethrin and the toxicity may increase the adverse effect of the pesticide to non-target organism. The present findings may be due to that cypermethrin induced oxidative stress (Kale *et al.*, 1999) and as a hydrophobic compound, it may accumulate in cell membranes and disturb membrane structure (Michelangeli *et al.*, 1990). Aerobic organisms generate superoxide anion radicals ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\cdot}$) as a result of the oxidative stress metabolism (Gabbiarelli *et al.*, 2002). Oxidative stress is defined as a disruption of the prooxidant-antioxidant balance in favour of the former leading to potential damage. It is a result of one of the three factors: An increase in reactive oxygen species (ROS), an impairment of antioxidant defense system or an insufficient capacity to repair oxidative damage. Damage induced by ROS induces alterations of cellular macromolecules such as lipids, DNA and protein which eventually can lead to cell death (Jalili *et al.*, 2007) Furthermore, according to WHO (1991) cypermethrin toxicity might be due to the release of cyanohydrins which were unstable under physiological conditions and further decomposed to cyanides and aldehydes which in turn could act as a source of free radical (Fetoui *et al.*, 2008)

Regarding the alleviation effects of the antioxidant diet on cypermethrin toxicity, results showed that, when intoxicated animals were feeding on the diet of guava and wheat germ (sources of vitamin C and E) all studied parameters (Tables 1, 2, 3 and 4) of intoxicated rats were readjusted and improved to around those of normal healthy control, in which the increased blood glucose, uric acid, urea, creatinine, LDL and AI were reduced but values were still more than those of normal control. In contrast, the decreased values of total protein, albumin, globulin, albumin/globulin ratio, total lipids, cholesterol, triglycerides, phospholipids, HDL and VLDL were elevated but the values were still less than those of normal healthy control. These ameliorated parameter by the present antioxidant diet.

Guava (*Psidium guajava*) fruit has many of antioxidant agents. These included vitamin: C, A, B_1 and B_2, also mineral: K, Fe, Zn and P while contained by 227 mg, 66 IU, 0.05 mg, 0.05 mg, 225 mg, 1.0 mg, 0.26 mg and 44 mg/100g respectively of edible portion which contains 89.9% water. Guava also contains several polyphenols and flavonoides compounds which act as good antioxidant agents (Nutrition Institute, 1996 and

Cheng and Yang, 1983). In case of wheat germ (*Tricicum Vulgare*), it has 9.5% lipids containing 350 mg tocopherol, since it is the main source of vitamin E (Jensen and Marten, 1983 and Syvaojo *et al.*, 1986). It has high contents of Zn, Fe, K and P, protein, dietary fiber, carotenoids, carbohydrate and vitamins (Ibrahim *et al.*, 1990 and Pennington, 1989). It means that, the present antioxidant diet contains a large amount of vitamin C and E. In connection, Nasuti *et al.* (2003) and Prasanthi *et al.*, (2005) reported that oxidative damage induced by pyrethroids might be due to their lipophilicity, whereby they could penetrate easily to the cell membrane (and more easily by formulations of the pesticide) and cause membrane lipid peroxidation. The Co-administration of vitamin C attenuated the *in vivo* effect of cypermethrin by scavenging or neutralizing reactive oxygen species (ROS). These indicated that vitamin C might have a beneficial role in lowering cypermethrin toxicity (Giray *et al.*, 2001 and Grajeda-Cota *et al.*, 2004). Also, vitamin E can prevent lipid peroxidation *in vivo*. This vitamin does not form a covalent bond with free radicals, it is suggested that vitamin E acts by blocking the formation of lipoperoxides by blocking free radical attack (Aldana *et al.*, 2001). Nakagawa *et al.* (1991) have demonstrated that vitamin C with vitamin E function in coordinated manner against the oxidative stress of pesticides. The two vitamins are most important among many low molecular weight compounds which can act as biological antioxidant. Ascorbate is capable of reducing a variety of oxidative compounds, especially free radical. Vitamin C is an electron donor. When it donates its two high – energy electrons to scavenge free radicals, much of the resulting dehydroascorbate is reduced to ascorbate and therefore can be used repeatedly. α-Tocopherol is the primary radical scavenger in biological membranes and is continuously regenerated at the expense of ascorbate and GSH oxidation, in which ascorbate and GSH being regenerated by NADH and NADPH respectively in the presence of relevant reductases (Lukaszewicz-Hussain and Moniuszko-Jakoniuk, 2003). When vitamin C is present with vitamin E in an aqueous environment, efficiently inhibits *in vitro* lipid peroxidation due to a combination of direct radical interception (where aqueous radical are involved) and interaction with α-tocopherol as a co-antioxidant. The generation of α-tocopherol from α-tocopheryl radical by ascorbate, with concomitant generation of the ascorbyl radical, is well established (Sharma and Buettner, 1993). However, *in vivo* cellular location of each compound may be an important factor in conferring protection against oxidative stress (Verma *et al.*, 2007). Also, the other antioxidant agents of guava and wheat germ diet such as vitamin A and other vitamins as well as minerals especially Zn and also polyphenols and flavoniodes compound…etc act as good antioxidant agents with vitamin E and C of the present antioxidant diet.

The present results concluded that cypermethrin produced oxidative stress which increases lipid peroxidation and reduced antioxidative systems against the pesticide toxicity. The feeding on vitamin C plus vitamin E and other antioxidant agents of the antioxidant diet of guava and wheat germ considerably alleviated the toxic effects of cypermethrin oxidative stress on albino intoxicated rats.

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