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THE PROTECTIVE EFFECT OF ASCORBIC ACID AGAINST TOXICITY INDUCED BY CHLORPYRIFOS AND CYPERMETHRIN ON ACETYLCHOLINESTERASE, TRANSAMINASES AND PHOSPHATASES ACTIVITIES IN ALBINO RATS.

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ABSTRACT

The present study concerns the evaluation of chlorpyrifos, and cypermethrin toxicity on acetylcholinesterase, transaminases and phosphatases activities in male albino rats. In addition, the protective effect of ascorbic acid (Vitamine C) against the toxicity induced by the examined insecticides were studied. Results indicated a significant inhibition in the acetylcholinesterase (AChE) activity in serum, liver and brain within two days to 29.43, 36.96 and 26.43% by chlorpyrifos and to 58.34, 62.34 and 67.39% by cypermethrin in the three organs, respectively. Recovery of AChE was observed after 4 days from treatment by both insecticides. However, treament by ascorbic acid with chlorpyrifos reduced the inhibition, where the activity reached 48.46, 46.88 and 36.00%, while ascorbic acid with cypermethrin the activity reached 78.63, 71.25 and 75.24% in the three organs, respectively. The activities of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and acid phosphatase (AP) were significantly increased throughout experimental periods upon treatment by both insecticides. Ascorbic acid also depressed the elevated activities of AST, ALT ALP and AP upon treatment with the examined insecticides. The residues and coefficient of distribution (CDT) of the tested insecticides were determined in blood, brain, liver, kidney, heart and lung and the results were consistant with the remaining activities of the examined enzymes.

Keywords: Insecticides, Ascorbic acid, AChE, AST, ALT, ALP AP and Residues.

INTRODUCTION

There has been an increase in public concern about the environmental contamination by pesticides. Crop protection chemicals which are widely used in modern agriculture such as chlorpyrifos (organophosphorus) and cypermethrin (pyrethroid) insecticides.

Acetylcholinesterase plays a crucial role in cholinergic synaptic transmission in insect nervous system and it is also the target site of inhibition by organophosphorus insecticides (Hassall, 1990). Chlorpyrifos phosphorylates acetyl cholinesterase and inhibits its activity causing accumulation of acetylcholine at the nerve synapse leading to the disruption of the central nervous system (Gallo and Lawryk ,1991). Exposure of rats to sublethal dose of chlorpyrifos caused inhibition of brain and plasma cholinesterase activity (Pope and Chakraborti, 1992). Administration of sublethal doses of chlorpyrifos increased the activities of AP, ALP, ALT and AST ,10-300%, (Jabbar *et al.*,1994).

Cypermethrin is a synthetic pyrethroid belongs to a group of insecticides widely used around the world and posseses higher insecticidal activity with lower mammal toxicity than organophosphate and carbamate insecticides (Fina and Mostafa, 1992 and Kale *et al.*,1999). Hens treated with a single dose of cypermethrin (30 mg /kg body weight) showed a significant increase in the activities of aminotransferases (ALT and AST) and alkaline phosphatase in serum for 14 days especially during the early days (Mohamed and El-Sheamy,1988).

Ascrobic acid has an essential role as antioxidant and protective agent against toxic compounds (Venkaterman *et al.*, 1994). Vitamin C has a direct relationship with certain drug-metabolizing enzymes and alleviates some of the toxic effects of certain organophosphorus and pyrithriod compounds in animals (Chatterjee *et al.*,1981).

The present study aims to evaluate the protective effect of supplementing ascorbic acid against the toxicological consequences of acute administration of chlorpyrifos (organophosphorus) and cypermethrin (pyrethroid) insecticides. The activities of AST, ALT, ALP and AP in serum and AChE in serum, liver and brain tissues were determined upon treatments with the insecticides or ascorbic acid with the tested insecticides. In addition, accumulation of chlorpyrifos and cypermethrin in blood, brain, liver, kidney, heart and lung of rats were determined.

MATERIALS AND METHODS

The active ingredient of chlorpyrifos (94%) ,Dursban, *O,O*-diethyl *O*-(3,5,6 trichloro-2 pyridinyl) phosphorothioate and cypermethrin (90%) ,Cyperkill, (±) ل-cyano-3-phenoxybenzyl (±) -cis,trans-3-(2,2dichlorovinyl)-2,2 dimethylcyclopro- panecarboxylate were obtained from Bayer Co., Cairo Egypt and Sumitomo Chemical Co. (Osaka)-Japan, respectively. The oral LD50 of chlorpyrifos and cypermethrin were determined according to Finney (1952). The selected single oral doses of chlorpyrifos and cypermethrin were 45.75 and 62.50 mg /kg body weight, respectively which are 1/4 of LD50 of each insecticide. L-Ascorbic acid (vitamin C) was obtained from Memphis Co.Pharm. and Chemical Industries, Cairo Egypt.

Animals:

Three months old sexually mature male albino rats weighing 150 ± 10 gm were purchased from animal house colony, Giza, Egypt.The animals were maintained for one week on standard laboratory diet (16.04% protein, 3.63% fat; 4.01% fiber and water) for acclimation under the laboratory condition.

Experimental Design :

Animals were divided into six groups (10 rats / group) and each group was housed in glass cage (65x70x20 cm). Group one was kept untreated to serve as control. All other groups were treated orally with a single dose of each treament. Group 2 was given ascorbic acid (10 mg/kg body weight). Group 3 and 5 were supplemented with chlorpyrifos or cypermethrin (45.75 or 62.50 mg /kg.body weight), respectively. Each of group 4 and 6 were given the same dose of the insecticides with ascorbic acid(10 mg /kg body weight).

After 2, 4, 8 days from the treatment, blood, liver, brain, kidney, heart and lung were obtained from the sacrificed animals. At each interval three animals from each group were sacrificed without the use of anesthesia and blood was collected. Half of each blood sample was centrifuged to get the serum for enzyme assays. kidney, heart, lung and the second half of the whole blood were subjected to residual analysis. Brain and liver samples were divided into two parts for residual and biochemical analysis.

Biochemical analysis :

Brain and liver organs were homogenized immediately in a cold phosphate buffer, pH 7.2 (0.1 M), then centrifuged for 10 minutes in an Eppendorf centrifuge. Acetylcholinesterase (AChE) activity was determined in serum, liver and brain samples, using 0.1M phosphate buffer, pH 7.2 and acetylthiocholine iodide as substrate, according to the method of Ellman et al, (1961). Alanine transaminase (ALT) and aspartate transaminase (AST) were determined in serum according to the method described by Reitman and Frankel (1957). Alkaline phosphatase (ALP) was determined in serum samples using diethanolamine buffer, pH 9.8 and p-nitrophenylphosphate as substrate according to the method of Rec.Gscc (1972). Acid phosphatase (AP) was determined in serum according to the method of Szasz (1972).Total protein was assayed in liver, brain and serum according to the method of Bradford (1976) using bovine serum albumin as standard.

Residual analysis :

Organs were homogenized in CH2Cl2-THF (2:1) then centrifuged and extracted twice with the same solvent.The organic layer extracts of each sample were combined, dried over anhydrous MgSO4 and evaporated. The dry residues were redissolved in acetone and analyzed via 12A Shimadzu GC equipped with (FID).The glass column emplyed was packed with 3% silicon OV-101 on 60-80 mesh chromosorb Q. Deoxygenated nitrogen was used as a carrier gas at flowrate 15ml min-1. Injection temperature was 250 C° and oven temperature program was 200-230 C° (5 C° /min).

Statistical analysis:

The obtained results were computerized by the use of the statistical program SPSS 2000 and analyzed according to Selvin,(1996). Series of one way analysis of variance (ANOVA) were performed to compare means betwen the control and the treatments . LSD was calculated to show significant difference between means at 0.01 significance level.

RESULTS AND DISCUSSION

Results indicated that there was no significant changes in all parameters in the control group during the experimental period (8 days). Therefore, one set of the control data was listed that expresses the average activities during the whole period. Group 2 which treated with ascrobic acid did not show any significant difference in all tested parameters from control during the experiment.

Data in Table (1) and Fig (1) showed that chlorpyrifos or cypermethrin solely caused a significant inhibition in the specific activity of acetylcholinesterase (AChE) in serum, liver and brain throughout the experimental periods, where the maximum AChE inhibition was observed in

the three organs after 2 days . Begining AChE recovery in the examined organs was observed 4 days after treatment with both insecticides. Chlorpyrifos significantly inhibited AChE activity in serum to 29.43, 36.49 and 80.94% after 2, 4 and 8 days respectively; however, supplementing ascobic acid with insecticide reduced the inhibition, where the activity reached 48.46, 62.97 and 96.45% after the same periods, respectively.

Table (1): Effects of chlorpyrifos and cypermethrin individual or in combination with ascorbic acid on acetylcholinesetrase (AChE) of male rats.

Time (days) / Treatments	2 (days)	%	4 (days)	%	8 (days)	%
	S.A of AChE in Serum					
Control	28.17 ± 0.04 a	(100)	28.17 ± 0.04a	(100)	28.17 ± 0.04ab	(100)
Ascorbic acid	28.02 ± 0.02a	(99.47)	28.36 ±1.92a	(100.67)	28.61±1.57ab	(101.56)
Chlorpyrifos	8.29 ± 0.24 e	(29.43)	10.28 ± 0.19d	(36.49)	22.8 ± 0.4 c	(80.94)
Chlorpyrifos+ ascorbic acid	13.65 ± 0.46 d	(48.46)	17.74 ± 0.11c	(62.97)	27.17 ± 0.54b	(96.45)
Cypermethrin	16.43 ± 0.19 c	(58.34)	18.69 ± 0.46c	(66.34)	24.14 ± 0.14 c	(85.69)
Cypermethrin +ascorbic acid	22.15 ± 0.13 b	(78.63)	25.06 ± 0.45b	(88.96)	29.07 ± 0.07 a	(103.19)
LSD	0.58		2.08		1.74	
	S.A of AChE in Liver					
Control	44.24 ± 0.45 a	(100)	44.24 ± 0.45a	(100)	44.24 ± 0.45 a	(100)
Ascorbic acid	44.13 ± 0.5 a	(99.75)	43.72±0.02a	(98.82)	43.67 ± 1.2 a	(98.71)
Chlorpyrifos	16.35 ± 0.35e	(36.96)	22.92±0.18c	(51.81)	35.31 ± 0.01c	(79.81)
Chlorpyrifos + ascorbic acid	20.74 ± 0.02d	(46.88)	28.26±0.62bc	(63.88)	40.1 ± .98 b	(90.64)
Cypermethrin	27.58 ± 0.37c	(62.34)	32.62 ± 0.59 b	(73.73)	41.11 ± .11d	(92.92)
Cypermethrin +ascorbic acid	31.52 ±0.25b	(71.25)	33.77 ± 0.69b	(76.33)	43.1 ± 0.1a	(97.42)
LSD	0.89		5.88		1.65	
	S.A of AChE in Brain					
Control	88.37 ± 0.22 a	(100)	88.37 ± 0.22 a	(100)	88.37 ± 0.22 a	(100)
Ascorbic acid	87.50 ± 0.52 a	(99.02)	87.88 ± 0.34a	(99.45)	87.73 ± 0.65a	(99.28)
Chlorpyrifos	23.35 ± 0.92 e	(26.43)	33.96 ±0.5 e	(38.43)	72.85 ± 0.36e	(82.44)
Chlorpyrifos + ascorbic acid	31.80 ± 0.29 d	(36.00)	39.00 ± 0.25b	(44.13)	82.06 ± 1.21c	(92.86)
Cypermethrin	59.55 ± 0.13 c	(67.39)	63.40 ± 0.69c	(71.27)	76.45 ± 0.45d	(86.51)
Cypermethrin +ascorbic acid	66.46 ± 1.43 b	(75.24)	73.27 ± 0.27b	(82.91)	85.62 ± 0.04b	(96.89)
LSD	1.85		1.38		1.53	

S.A : specific activity (nmol /min /mg protein) ± SD (% remaining activity).
For each ANOVA, results sharing the different letters are significantly different ($P \leq 0.01$).
Results are means of three experiments.

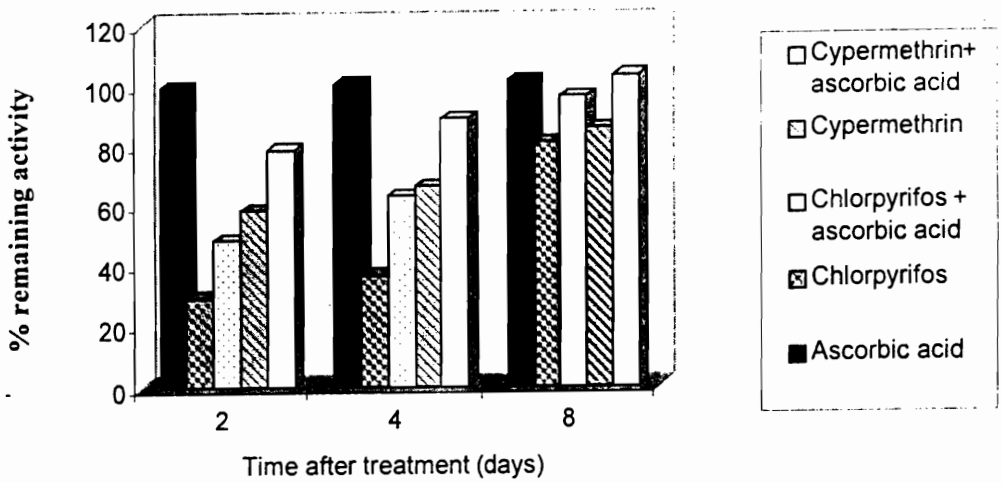

Fig. (1): Effect of chlorpyrifos and cypermethrin individaual or in combination with ascorbic acid on AchE activity in serum of male rats.

Table (1) showed the same protective effects of ascorbic acid in both liver and brain tissues. Similar results were obtained by Vodela and Dalvi (1995), where rats were given a single oral dose of 50 mg chlorpyrifos / kg body weight which significantly inhibited serum AChE. The severe inhibition of AChE activity after 2 days could be attributed to the formation of more toxic oxon metabolite phosphothioate of pesticides e.g (chlorpyrifos), which is rapidly eliminated and AChE activity started to be recovered (Nigg and Knaak, 2000).

Treatment with cypermethrin significantly dereased AChE activity in serum to 58.34, 66.34 and 85.69% ; whereas upon treatment with cypermethin and ascobic acid the activity changed significantly to 78.63, 88.96 and 103.19% after 2, 4 and 8 days, respectively. Table (1) also showed the same elevation in enzymes activities upon using ascrobic acid with such insecticide in both liver and brain. Malaviya *et al.* (1993) indicated that exposed female rats to cypermethrin caused significant decrease in AChE activity in rat brain 12 hours after treatment.

Data in Table (2) and Fig (2) revealed that oral administration with chlorpyrifos caused a gradually significant increase in serum aspartate transaminase (AST) and alanine transaminase (ALT) activities. AST activity increased to 166.13, 187.50 and 240.04%, while ALT acitivity elevated to 253.96, 293.58 and 402.44% after 2, 4 and 8 days, respectively.

Table (2): Effects of chlorpyrifos and cypermethrin individual or in combination with ascorbic acid on transaminases in serum of male rats.

Time (days) / Treatments	Activity of AST (U/L)		
	2 (days) %	4 (days) %	8 (days) %
Control	24.80 ± 3.36 c (100)	24.80=3.36 e (100)	24.80 ± 3.36 d (100)
Ascorbic acid	25.44 ± 2.67c(102.60)	25.02 ± 1.43 d (100.89)	24.49 ± 1.15 e(98.75)
Chlorpyrifos	41.20 ± 0.95 a(166.13)	46.50 ± 1.13 a 187.50)	59.53 ±1.12a(240.04)
Chlorpyrifos + ascorbic acid	32.99 ± 0.42 b(133.02)	33.42 ± 1.87 c (134.76)	42.29 ±1.20c(170.52)
Cypermethrin	30.98 ± .082b(124.92)	40.23 ± 2.05 b (162.22)	48.78 ±1.59b(196.69)
Cypermethrin + ascorbic acid	28.17± 0.88bc 113.59)	30.97 ± 1.81 c (124.88)	35.50 ±3.12d(143.15)
LSD	4.67	5.15	5.35
Activity of ALT (U/L)			
Control	21.33 ± 3.31 cd (100)	21.33 ± 3.31 d (100)	21.33 ± 3.31e (100)
Ascorbic acid.	19.47 ± 0.80 d (91.28)	21.56 ± 1.45 d 101.08)	20.60 ± 1.45e (96.58)
Chlorpyrifos	54.17 ± 1.24 a(253.96)	62.62 ± 2.32 a (293.58)	85.84 ± 2.99a402.44)
Chlorpyrifos + ascorbic acid	42.31 ± 2.35b(198.36)	50.44 ± 0.84 b (236.47)	61.67 ±0.81b(289.12)
Cypermethrin	36.12 ± 3.57b (169.34)	46.21 ± 1.06 b(216.64)	52.37 ±2.58c(245.52)
Cypermethrin + ascorbic acid	26.14 ± 2.51c(122.55)	32.70 ± 1.22 c(153.31)	39.18 ±1.27d(183.68)
LSD	6.25	4.70	5.67

AST and ALT activities were expressed as U /L ± SD (% remaining activity).
For each ANOVA, results sharing the different letters are significantly different ($P \leq 0.01$).
Results are means of three experiment.

On the other hand, ascorbic acid treatment with chlorpyrifos depressed the elevation in AST activity significantly to 133.02, 134.76 and 170.52% ; while the increase depressed in ALT activity significantly to 198.36, 236.47 and 289.12% after the same periods, respectively. These are in agreement with the previously mentioned results obtained by Jabbar *et al.* (1994). Ceron *et al.* (1995) stated that organophosphorus insecticides (OP) induced a significant increase in AST and ALT in serum. The authors pointed out that this observation indicates a toxic effect of the used insecticides (OP) on liver tissue causing hepatocyte damage and bile duct alternations. In addation, treament with cypermethrin significantly increased both AST activity to 124.92, 162.22 and 196.69% and ALT activity to 169.34, 216.64 and 245.52% after 2, 4 and 8 days, respectively. Furthermore, ascrobic acid with cypermethrin decreased the elevation in AST activity to 113.59, 124.88

and 143.15% and depressed the increase in ALT activity to 122.55, 153.31 and 183.68% after 2, 4 and 8 days, respectively. It should be noted that treament with ascorbic acid and cypermethrin did not show significant difference from control after two days. The increase in AST and ALT activities upon treatment with cypermethrin was in agreement with that reported by (Mohamed and El-Sheamy, 1988). These results were explained by causing degenerative changes and hypofunction of liver as an effect of insecticides on the hepatocytes, where cellular enzymes are released into the blood stream (Justin *et al.*, 1991).

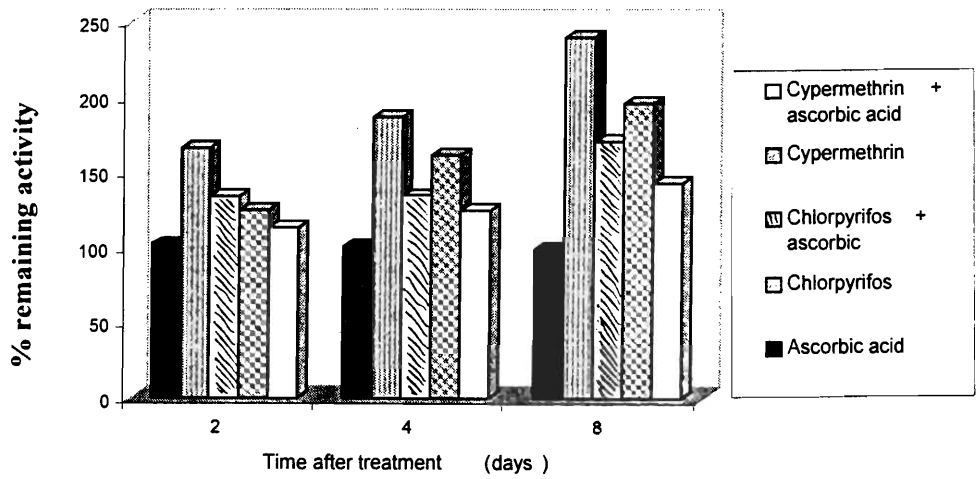

Fig. (2 a): Effect of chlorpyrifos and cypermethrin individaual or in combination with ascorbic acid on AST in serum of male rats.

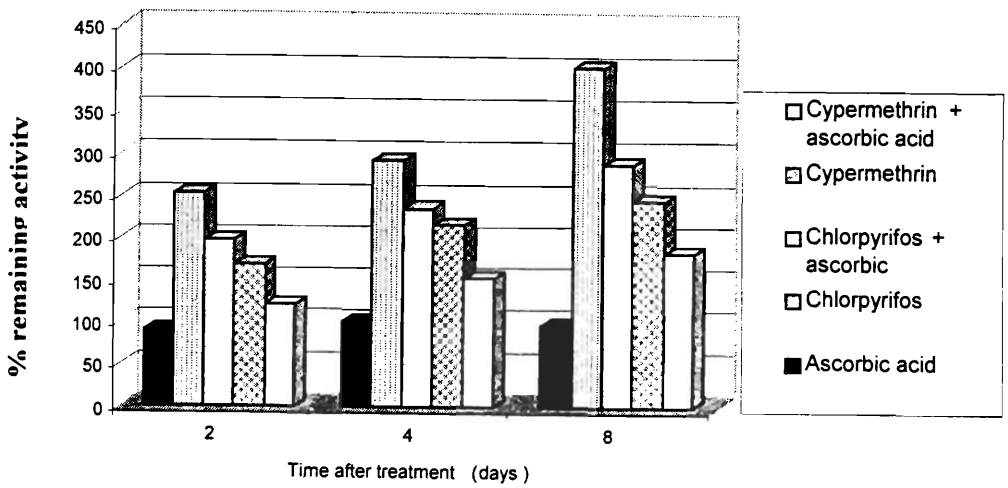

Fig. (2 b): Effect of chlorpyrifos and cypermethrin individaual or in combination with ascorbic acid on ALT in serum of male rats.

Table (3) and Fig (3) showed that administration of chlorpyrifos resulted in a significant increase in serum alkaline phosphatase (ALP) activity to 120.70, 134.62 and 140.32% and acid phosphatase (AP) activity to 253.01, 327.35 and 362.89%.

Table (3): Effects of chlorpyrifos and cypermethrin individual or in combination with ascorbic acid on phosphatases activites in serum of male rats.

Time (days) / Treatments	2 (days)	%	4 (days)	%	8 (days)	%
	Activity of ALP (U/L)					
Control	160.02±3.11c	(100)	160.02 ± 3.11d	(100)	160.02 ± 3.11 e	(100)
Ascorbic acid	161.50±1.08c	(100.92)	162.50 ± .69d	(101.55)	159.46 ± 1.27e	(99.65)
Chlorpyrifos	193.14±2.63a	(120.70)	215.42 ±3.13a	(134.62)	224.54±1.34a	(140.32)
Chlorpyrifos + ascorbic acid	182.64±1.25b	(114.02)	191.80 ±2.87b	(119.50)	198.26±2.75b	(123.90)
Cypermethrin	162.10±2.54c	(101.30)	169.67 ±1.19c	(106.03)	182.08 ± 2 c	(113.79)
Cypermethrin + ascorbic acid	159.04 ± 1.77c	(99.39)	161.71 ±3.50d	(101.06)	172.77±2.15d	(108.00)
LSD	5.48		6.59		5.51	
	Activity of AP (U/L)					
Control	4.15 ± 0.61 b	(100)	4.15 ± 0.61 e	(100)	4.15 ± 0.61 d	(100)
Ascorbic acid	4.5 ± 1.29 b	(108.43)	4.33 ± 0.72 e	(104.34)	4.60 ± 0.02 d	(100.00)
Chlorpyrifos	10.5 ± 1.29a	(253.01)	13.60 ± 0.6 a	(327.35)	15.06 ± 0.6 a	(362.89)
Chlorpyrifos + ascorbic acid	6.5 ± 0.50 b	(156.63)	8.12 ± 0.12bc	(195.63)	11.51 ± 0.02b	(277.35)
Cypermethrin	5.70 ± 0.61b	(137.35)	9.76 ± 1.72 b	(235.18)	10.6 ± 0.65 b	(255.42)
Cypermethrin + ascorbic acid	4.16 ± 0.12 b	(100.24)	6.41 ± 0.57 cd	(154.46)	7.05 ± 0.08 c	(169.88)
LSD	2.42		1.72		1.1	

Enzymes activities in serum were expressed U/L ± SD (% remaining activity). For each ANOVA, results sharing the different letters are significantly diffirent ($P \leq 0.01$).
Results are means of three experiments.

However, treatment with ascrobic acid and chlorpyrifos significantly reduced the increase in ALP activity reached 114.02, 119.50 and 123.90% and AP activity to 156.63,195.63 and 277.35% after 2, 4 and 8 days, respectively. The observed increases in ALP and AP activities by chlorpyrifos agreed with the results obtained by Jabbar *et al.* (1994). Deeb,(1996) also indicated that a single dose of organophosphorus insecticides caused an increase in AP in hens. Additionaly, cypermethrin caused a significant increase in ALP activity to 101.30,106.03 and113.79% and AP activity to137.35,235.18 and 255.42% after 2, 4 and 8 days, respectively. Treatment with ascorbic acid plus cypermethrin depressed the elevation in ALP activity significantly to 99.39, 101.06 and 108.00% and AP to 100.24,154.46 and169.88% after the same periods, respectively. It was observed that ALP was recovered i.e. not significant different from control after 2 and 4 days of ascrobic with cypermethrin treatment. In addition, AP activity was recovered after 2 days of the same treatment. The increase in ALP activity indicates that there is an impairment of liver function and abstruction of flow (Lykasova and Rabinovich, 1988).

It is important to note that the effects of both insecticids on the elevation of AST, ALT, ALP and AP activities were significantly depressed upon supplementing ascrobic acid with insecticids. Ascrobic acid also minimized the depression in AChE activity in serum,liver and brain upon using both insecticids. This finding is consistent with that obtained by Hashim and Kadry (2002) who found that ascrobic acid plus fenitrothion caused an increase in AChE activity. Ascorbic acid inhibits or repairs cell damage and regulates and support the immune system.

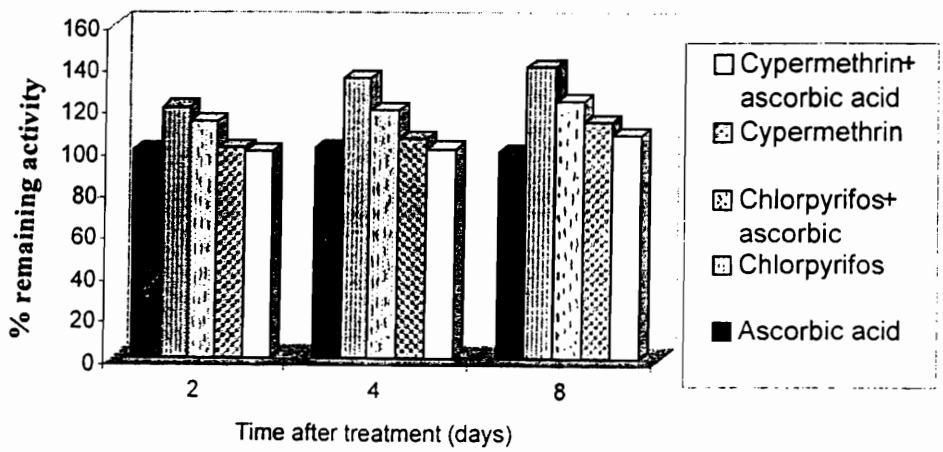

Fig. (3 a): Effect of chlorpyrifos and cypermethrin individaual or in combination with ascorbic acid on ALP in serum of male rats.

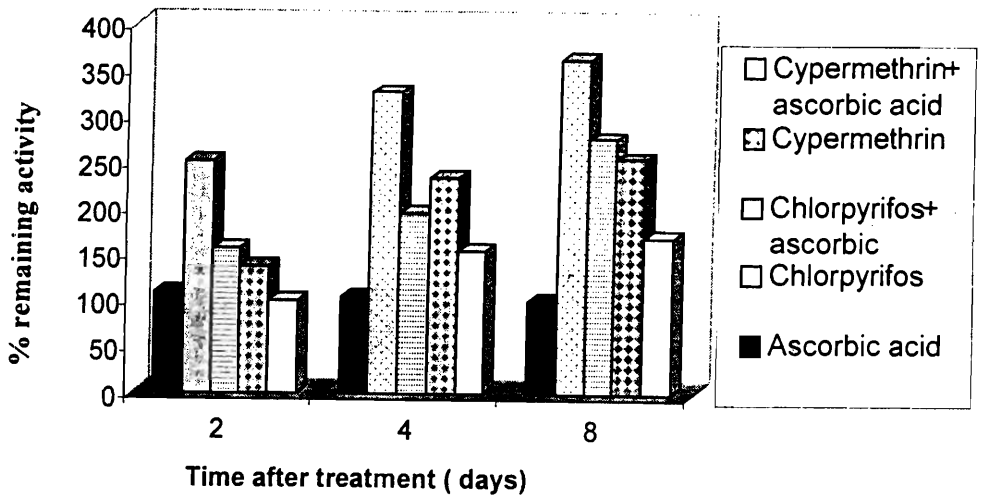

Fig (3 b): Effect of chlorpyrifos and cypermethrin individaual or in combination with ascorbic acid on AP in serum of male rats.

Table (4) shows the detected amounts (ppm) of chlorpyrifos and cypermrthrin in blood, brain, liver, kidney, heart, and lung.

It is very clear that 2 days after the oral adminstration the concentration of chlorpyrifos was very high in brain, liver and blood (460.41, 432.7 and 350.32 ppm), respectively compared with the detected residues in heart, kidney and lung (265.5, 223.82 and 72.45 ppm), respectively.

Rsults, as presented in Table (4) indicated that chlorpyrifos showed higher residue after two days in all organs(460.41-72.45 ppm) while the range decreased with time to 291.90-70.11 ppm after four days and 116.20-26.81 ppm after eight days. The percentage reduction of residues were 78.04 and 94.18% in brain, 50.00 and 74.25% in blood, 68.74 and 80.61% in liver, 35.86 and 48.08% in kidney, 16.57 and 87.76% in heart and 3.23 and 5.73% in lung after 4 and 8 days, respectively. This trend generally parallel to that of decreasing the inhibition in the specific activity of acetylcholinesterase (AChE) in serum, liver and brain observed during the same period.

On the other hand, cypermethrin showed higher residue after two days in blood (30.60 ppm) compared with the detected residues in brain, liver, kidney, heart and lung (6.04, 2.48, 9.70, 2.68 and 5.09 ppm), respectively . Four days after the oral administration, residues of insecticide in blood, brain, and kidney was decreased to12.20, 0.21 and 7.80 ppm, respectively and disappeared in liver, heart and lung. After eight days, residues did not detected in all organs.In this connection Fina and Mostafa (1992) and Kale *et al.* (1999) stated that cypermethrin has lower mammal toxicity than organophosphorus and carbamate insectisides.The previous results are consistant with that of the remaining activities obtained in the examined enzymes under study during the same period.

Table (4): Detected concentrations of chlorpyrifos and cypermethrin (ppm) in blood and different organs of male rats.

Time(days) / Parameters	Chlorpyrifos			Chlorpyrifos + ascorbic acid		
	2 (days)	4 (days) %	8 (days) %	2 (days)	4 (days) %	8 (days) %
Blood	350.32 -	175.21 (50.00)	90.19 (74.25)	370.12	160.30(56.69)	55.12 (85.11)
Brain	460.41 -	101.1 (78.04)	26.81 (94.18)	548.30	140.76 (74.33)	28.90 (94.73)
Liver	432.70 -	135.28 (68.74)	83.90 (80.61)	310.90	107.90 (65.29)	21.70 (93.02)
Kidney	223.82 -	143.56 (35.86)	116.20 (48.08)	160.20	149.86 (6.45)	53.35 (66.70)
Heart	265.5 -	291.9 (16.57)	40.40 (87.76)	144.90	64.40 (55.56)	65.50 (54.80)
Lung	72.45 -	70.11 (3.23)	68.30 (5.73)	120.90	75.92 (37.20)	26.30 (78.30)
	Cypermethrin			Cypermethrin + ascorbic acid		
Blood	30.60 -	12.20 (60.13)	N.D. -	25.31 -	8.70 (65.63)	N.D. -
Brain	6.04 -	0.21 (96.52)	N.D. -	4.07 -	N.D. -	N.D. -
Liver	2.48 -	N.D. -	N.D. -	2.21 -	N.D. -	N.D. -
Kidney	9.70 -	7.80 (19.59)	N.D. -	8.90 -	N.D. -	N.D. -
Heart	2.68 -	N.D. -	N.D. -	N.D. -	N.D. -	N.D. -
Lung	5.09 -	N.D. -	N.D. -	N.D. -	N.D. -	N.D. -

% relative to 2 days. **N.D. mean not detected.**

On the other hand, supplementing ascorbic acid with chlorpyrifos and cypermethrin did not directly affect on both insecticeds, but it lowered the adverse toxcity effects in these insecticeds on the examined enzymes activites during the same period. Ascorbic acid as antioxidant reduced the free radicals and acts as a primary defense against harmful radicals (Levine,1989). Furthermore, ascrobic acid as a reducing agent can eliminate the degenerative effect of the insecticides on the metabolic processes in hepatic cells as reported by Kang *et al.* (1998).

Each pesticide showed a specific pattern of affinity towards different tissues. To test the accumulation of pesticide in different tissues, the coefficient of distribution in tissue (CDT) was calculated by the formula:

$$CDT = [pesticide]_{tissue} / [pesticide]_{blood}$$

The CDT provides informations on the relative affinity of a compound for different tissues relative to blood (Repetto *et al.*, 1995); the CDT was not calculated when residue in blood was zero. The results encountered in Table (5) show that accumulation of chlorpyrifos (CDT > 1) might occur in brain and liver with CDT equal 1.31 and 1.21, after 2 days, respectively while a little accumulation in kidney, heart and lung was observed (CDT<1) at the same time. After 4 and 8 days data show a little accumulation in all organs excepet in kideny, where CDT was equal to 1.29 at 8 days while it was 1.67 in heart at 4 days. In the meantime, accumulation of cypermethrin was not deteceted in liver, heart and lung after 4 days and in all organs after 8 days. It should be noted that suppplementing ascrobic acid with insecticides did not show difference from insecticeds used alone at the same period.

Table (5): Coefficient of distribution (CDT) of the chlorpyrifos and cypermethrin residues in diffrerent organs of male rats.

Time (days) / Parameters	Chlorpyrifos			Chlorpyrifos + ascorbic acid		
	2(days)	4 (days)	8 (days)	2 (days)	4 (days)	8 (days)
Brain	1.31	0.58	0.30	1.48	0.88	0.52
Liver	1.21	0.77	0.93	0.84	0.67	0.39
Kidney	0.64	0.82	1.29	0.43	0.93	0.98
Heart	0.76	1.10	0.45	0.39	0.40	1.19
Lung	0.21	0.40	0.76	0.33	0.47	0.48
	Cypermethrin			Cypermethrin+ascorbic acid		
Brain	0.20	0.02	N.D.	0.16	N.D.	N.D.
Liver	0.08	N.D.	N.D.	0.09	N.D.	N.D.
Kidney	0.32	0.64	N.D.	0.35	N.D.	N.D.
Heart	0.09	N.D.	N.D.	N.D.	N.D.	N.D.
Lung	0.17	N.D.	N.D.	N.D.	N.D.	N.D.

N.D. mean not detected.

In conclusion, chlorpyrifos and cypermethrin caused depression in acetylcholinesterase (AChE) activity and elevation in the transaminases (AST, ALT) and phosphatases (ALP, AP) activities.Chlorpyrifos caused genral stronger effect on all tested enzymes than cypermethrin; however, ascrobic acid significantly improved all enzymes activities after treament with both insecticides. The residues and coefficient of distribution (CDT) of both insecticides determined in blood, brain, liver, kidney,heart and lung were gernerally parallel with the remaining activities of the tested enzymes.

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التأثير الوقائي لحمض الأسكوربيك ضد سمية الكلوربيرفوس والسيبرمثرين على نشاط إنزيمات الاسيتيل كولين استيريزوانزيمات الترانسفيريز والفوسفاتيز في الفئران البيضاء.

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الهدف من هذا البحث هو تقييم سمية كل من المبيد كلوربيرفوس والمبيد سيبرمثرين على نشا ط إنزيم الاسيتيل كولين استيريز فى كل من السيرم و الكبد والمخ ونشا ط إنزيمات الاسبارتات والالانين أمينو ترانسفيريز بالاضافة الى الفوسفاتيز القاعدى والفوسفاتيز الحامضى في السيرم وكذا دراسة التأثير الوقائى لحمض الاسكوربيك ضد سمية هذه المبيدات فى ذكور الفئران البيضاء.

كما تم تقدير متبقيات المبيدين وكذا معامل التوزيع فى الاعضاء المختلفة بعد ٢ , ٤، ٨ يوم من المعاملات تحت الدراسة.

وكانت النتائج كالتالي:

حدث أقصى انخفاض في نشا ط إنزيم الاسيتيل كولين استيريز فى كل من السيرم والكبد والمخ و كانت ٢٩,٤٣ ، ٣٦,٩٦ ، ٢٦,٤٣ % بعد ٢ يوم من المعا ملة بالمبيد الكلوربيرفوس كذلك كانت ٥٨,٣٤ ، ٦٢,٣٤ ، ٦٧,٣٩ % بعد نفس الفترة عند المعاملة بالمبيد سيبرمثرين في الأعضاء السابقة.

أدت المعاملة بحمض الاسكوربيك مع المبيد كلوربيرفوس حدوث تقليل في الانخفاض لنشا ط إنزيم الاسيتيل كولين استيريزواصبحت ٤٨,٤٦ ، ٤٦,٨٨ ، ٣٦,٠٠% وهو ما حدث ايضا بعد المعا ملةا بحمض الاسكوربيك مع المبيد سيبرمثرين و كانت ٧٨,٦٣ ، ٧١,٢٥ ، ٧٥,٢٤% في السيرم والكبد والمخ كما أدت المعا ملة بكل من المبيد كلوربيرفوس والمبيد سيبرمثرين الى حدوث زيادة فى نشاط انزيمات الاسبارتات والالانين أمينو ترانسفيريز وايضا حدث زيادة في نشا ط إنزيمات الفوسفاتيز القاعدي والفوسفاتيز الحامضى وذلك خلال فترة التجربة .

كما أدت المعاملة بحمض الاسكوربيك مع كل من المبيد كلوربيرفوس والمبيد سيبرمثرين إلى حدوث نقص في نشاط إنزيمات الاسبارتات والالانين أمينو ترانسفيريز وإنزيمات الفوساتيز القاعدى والفوسفاتيز الحامضى .

وجد ان المبيد الفوسفورى كلوربيرفوس قد تراكم بتركيز عالى بعد ٢ يوم من المعاملة فى الدم و كل الاعضاء (مخ - كبد - كلى - قلب والرئة) ثم بدأ يقل تدريجيا خلال فترة التجربة كما كان معامل التوزيع <١ عند نفس الفترة .

وجد ان المبيد سيبرمثرين كان اعلى تركيز له فى الدم عن باقى الاعضاء بعد ٢ يوم من المعاملة بينما حدث لة تلاشى من الاعضاء كلها بعد ٤ ، ٨ يوم وهذا يدل على ان المبيد سيبرمثرين اقل تاثير على الانزيمات من المبيد كلوربيرفوس .

لم يكن لحمض الاسكوربيك تاثير مباشر على المبيدين السابقين ولكنة قلل من التاثيرات الضارة للمبيدين كنتيجة لارتباطة بالشقوق الحرة الضارة الناتجة عن المبيدين ولذلك ينصح بتناول مضادات الاكسدة مثل حمض الاسكوربيك لكى يقلل من خطر سمية هذة المبيدات على الاعضاء الحيوية فى الانسان .