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Preventive Action of Ascorbic Acid and β-Carotene from Beta-Cyfluthrin Insecticide Toxicity on Rats

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Abstract: The present study investigated the protective potential of ascorbic acid and β-carotene supplementation in intoxication rats by pyrethroid betacyfluthrin insecticide. Salivation and diarrhea was observed in betacyfluthrin treated-rats. Significant increase in the level of total protein, albumin, bilirubin, urea, creatinine, cholesterol and triglycerides in treated rats with insecticides associated toxicity as compared to untreated animals. Co-administration of β-carotene and ascorbic acid significant improvement in the concentrations of all parameters was seen. In the recovery period, most of the vital parameters amounts reached normal level pointing towards degradations of betacyfluthrin in the treated –rats. Generally, results revealed the highest total residues of betacyfluthrin were found in stomach followed by liver (4.53 and 3.7 ppm, respectively), while the lowest were detected in spleen (0.392 ppm). The total amounts of this insecticide detected in kidney and fats were 1.47 and 1.37 ppm. On the other hand, our results revealed that ascorbic acid and β-carotene didn't effect on betacyfluthrin residues in different organs. Consequently, it seems that an antioxidant agents may be useful in decreasing the adverse effects of exposure to different toxicants compounds or pollutants.
Key words: Betacyfluthrin, β carotene, Ascorbic acid, Antioxidant.

Introduction

Cyfluthrin is a synthetic pyrethroid used widely in agriculture against Lepidoptera, Coleoptera, Hemiptera and Homoptera on cotton, fruits, vegetables, cereals and other crops, also it used in human hygiene[1]. Following its first use in 1987 in the USA[2], the environmentalist estimated its toxic effects and found that it had genotoxic and cytotoxic effects on human lymphocytes[3], genotoxic effects on human mucosa epithelium cells. In addition to all known biochemical changes, pyrethroids may also stimulate the production of reactive oxygen species (ROS) and result in oxidative damage in aquatic organisms and animals erythrocytes. These molecules are very effective in causing damage to enzymes, lipids, proteins, carbohydrates, and nucleic acids[4]. ROSs are produced in the body during oxygen metabolism, and living organisms develop different defense mechanisms in order to protect themselves from oxidative stress. Under normal conditions, ROS and antioxidant systems are in balance. The imbalance between prooxidant and antioxidant systems leads to oxidative stress. Oxidative stress and resulting damage to essential cell components caused by oxygen-free radicals are generally considered a serious mechanism. Although more than 600 different compounds have been characterized, until now β-carotene is the most potent one of the antioxidants[5]. β-carotene exhibits antioxidant activity by suppressing singlet oxygen, scavenging peroxide radicals, and directly reacting with peroxy radicals, thus stabilizing membrane lipids from free radical attack[6]. While, vitamin C is a water soluble antioxidant agent that accumulates in the brains of mammals more than any other tissue. Babies brain has its accumulation more than adults12. There are many reports about the reduction of DNA damage probability and preventing of cancer and

heart disease by vitamin C because of antioxidant properties[7,8]. It is well known that the liver is the main organ for the detoxification of xenobiotic, and antioxidants are able to decrease the sensitivity of kidney in oxidative protection. It is also well known that toxic materials are metabolized in the liver and they are ejaculated through the kidney[9]. Therefore, the present work aims to investigate the ameliorative effect of β-carotene and ascorbic acid on the oxidative stress changes induced by administration of betacyfluthrin insecticide.

Materials and Methods

Albino rats *Rattus norvegicus* var. albinos (weighing approximately 150 – 170 g) which were used as material in this study and obtained from the Animal House, National Research Centre (NRC), Dokki, Cairo, Egypt. The animals were housed in plastic cages and allowed to adjust to the new environment for 7 days before starting the experiment. Rats were fed standard food pellets and tap water *ad libitum*. The rats were housed at 25 ± 2 ºC and daily dark / light cycle. The design of the study was in accordance with the ethical guidance prescribed by NRC. Animals were randomly divided into four groups of ten rats each. Rats in 1st group served as control and given only standard pellet diet and corn oil. The 2nd group was given $^1/_{10}$ LD_{50} of betacyfluthrin insecticide (LD_{50} = 380 mg / kg b.w., according to Anonymous [10]) day after two days during one month, while 3rd and 4th groups were given $^1/_{10}$ LD_{50} of tested insecticide plus β-carotene or ascorbic acid (10 and 20 mg / kg, b.w. according to Matos *et al.* [11] and Hussein *et al*.,[12], respectively). All rats were weighed at pretreatment, 5th, 10th dose and after 2 weeks at the last dose (recovery period).

Biochemical analysis:

At 5th, 10th doses and 2 weeks recovery periods, blood samples were taken from retro-orbital venous plexus, placed into sterile tubes and centrifuged at 3500 rpm for 20 min to separate the serum. Biochemical parameters (total protein, albumin, bilirubin, urea, creatinine, cholesterol and triglycerides) were determined, and analyzed spectrophotmetrically using kits purchased from Bio-Marieux Company France. Using automated clinical chemistry analyzed Olympus Au 4oo Analyzer.

Determination of betacyfluthrin residues:

Extraction and clean-up:

All the solvents were of analytical grade and glass distilled before use. The extraction and clean-up procedure for betacyfluthrin was done according to the method of Vermuri *et al*.[13]. 1g of liver, kidney, stomach, spleen and fats was homogenized with 10 ml acetone: hexane (1:9) and was filtered. The filtrate was partitioned after adding with saturated NaCl and Dichloromethane. The extract was cleaned up with florosil column eluting with hexane. The elute evaporated to dryness for Gas Chromatography analysis.

Estimation:

The Betacyfluthrin sample were analyzed on (Shimadzu) GC-2010 equipped with fused silica capillary column using 63Ni electron-capture detector (ECD). Operating conditions were: Column temperature program: initially 200 ˚C for 2 min, increase at 3◦C/min to 240 ˚C hold for 10 min, Total programmers is 25.33 min; injection volume: 1 µl nitrogen flow rate 0.93 ml/min and makeup 25 ml/min with split ratio 1:10; using carrier gas (N2) 99.5%; Injector port temperature 260 ˚C; detector temperature 300 ˚C. Retention time of Betacyfluthrin is 14.5 min.

Results and Discussion

Effects on body weight:

Data presented in Table 1 revealed that there is no significant changes in body weight gain of treated rats after 5th and 10th doses comparison with untreated rats, except in betacyfluthrin treated rats their weight decreased after ten doses (205 g). After two weeks (recovery periods) at the last dose, significant decrease was noticed in body weight of treated rats by pyrithroid insecticide (significant decrease), while significant increase in weight of treated-rats by insecticide plus ascorbic acid, but no significant change in weight of treated -rats by insecticide plus β- carotene as compared with control.

Table (1): Effect of tested chemicals on body weight of rats (g)

Periods Treatments	Pre-treatment	After 5 doses	After 10 doses	After recovery periods
Betacyfluthrin	200 a	205 a	205 b	205 c
Betacyfluthrin + Ascorbic acid	200 a	213.3 a	223.3 a	233.3 a
Betacyfluthrin + β Carotine	205 a	210 a	216.7 ab	220 b
Control	196 a	210 a	213.3 ab	216.7 b
LSD 5 %	7.016	20.994	12.788	11.41

(According to Duncan test) Letters means the significant differences between treatments and control.

Table (2): Toxic symptoms in male albino rats which received tested compounds during the experiment period.

Compound	Signs of toxicity	Animal mortality
Control	Nil	Nil
Betacyfluthrin	Salivation and diarrhea.	Two animals were died; one at 2nd dose and one at 7th dose.
Betacyfluthrin+ ascorbic acid	Salivation and lacrimation	One animal was died at 3rd dose.
Betacyfluthrin + β-carotene	Increased activity and lacrimation	One animal was died at 3rd dose.

Clinical sings:

During the experiment, animals from all groups were checked daily for any signs of toxicity (Table 2). Betacyfluthrin treated rats caused salivation, diarrhea and two animals died at the 2nd dose and one at 7th dose. These results may be due to improper assimilation or metabolism of feed due to enteritis which shown as diarrhea and hepatic lesion[14, 15]. Slight choreoathetosis and hypersensitivity were noticed in rats dosed with betacyfluthrin followed ascorbic acid or β-carotene about 4-5 hours after dosing and these signs gradually declined after next few hours, but one rat was died at the 3rd dose in each treatment.

Table 3: Effect of oral administration of betacyfluthrin alone and in combinations with β-carotene and ascorbic acid on total protein, albumin and bilirubin in rats

Parameters	Total Protein			Albumin			Bilirubin		
Treatments	5 doses	10 doses	2 weeks	5 Doses	10 doses	2 weeks	5 doses	10 doses	2 weeks
Betacyfluthrin	8.6 a (+1.4)	8.64a (+3.2)	8.58 a (+5.9)	2.35 a (+1.7)	2.31a (+17.9)	2.35a (+30.6)	3.38a (+7.6)	3.39a (+11.9)	3.36a (+14.7)
Betacyfluthrin + Ascorbic acid	8.43b (-0.6)	8.4b (+0.36)	8.42 b (+4.0)	2.26 b (-2.2)	2.21b (+12.8)	2.16 b (+20.0)	3.26ab (+3.8)	3.07c (+1.3)	3.05b (+4.1)
Betacyfluthrin + β-carotine	8.62a (+1.7)	8.56a (+2.3)	8.54 ab (+5.4)	2.34 a (+1.3)	2.25b (+14.8)	2.11b (+17.2)	3.12 c (-0.64)	3.2 b (+5.6)	2.98 b (+1.7)
Control	8.48b	8.37b	8.1c	2.31 a	1.96 c	1.8 c	3.14bc	3.03c	2.93b
LSD 5 %	0.087	0.083	0.146	0.05	0.061	0.074	0.12	0.057	0.128

(According to Duncan test) Letters means the significant differences between treatments and control. Each figure between brackets represents the percentage of content as check.

Effects on liver functions:

Blood is a sensitive index of the physiological changes of an animal to any environmental pollutants and it is well known that toxic stress of any nature would show conspicuous and significant changes in the haematological parameters. The data of this study revealed that betacyfluthrin caused significant increase in total protein (Table 3) amount rather than control (1.4, 3.2 and 5.9 % above normal level, after 5th, 10th and recovery periods, respectively). The same trend was happened when insecticide followed by β carotene, while ascorbic acid doesn't show significant enhancement. Results showed also, negligible changes in albumin levels

of rats that received 5 doses of betacyfluthrin alone or accompanied by both ascorbic acid and β carotene (+1.7, -2.2 and + 1.3 %, respectively). Both antioxidants were not effective, after 10th dose and after recovery periods significant increase in albumin was observed in all treatments as compared with untreated rats and between each other. Treatment with betacyfluthrin resulted in a significant increase in bilirubin levels after 5th, 10th dose and recovery period (7.6, 11.9 and 14.9 % above the normal level, respectively) as compared with untreated rats. However, treatment with ascorbic acid and β-catotene to insecticide-treated rats led to a significant decrease of bilirubin concentration. Consequently, no significant changes in bilirubin levels in treated rats by betacyfluthrin followed by both antioxidants as compared with control, except in case of treated animals by insecticide plus β-carotene at 10th dose. Regarding to total serum protein is the majority of serum proteins which are synthesized in the liver, so used as an indicator of liver impairment. Yang and Chen [16] reported that malathion treated –rats caused significant reduction in total protein, albumin and globulin level values but showed no significant change in albumin/globulin ratio. These results may be due to disturbance in protein synthesis in the liver due to hepatocytes dysfunction[17] whom mentioned that reduction of serum albumin with impaired synthetic function of the liver.

Effects on kidney functions:

The kidney damage was evaluated by the measurement of serum levels of urea and creatinine. The extend of kidney damage sustained following exposure to $^{1}/_{10}$ LD_{50} of betacyfluthrin is shown in Table 4. A significant increase in both parameters was noticed in the serum of insecticide-treated animals (after 5th, 10th dose and recovery periods) than those of untreated. Following treatment with ascorbic acid and β-carotene significant improvement in the level of both parameters was seen. The urea concentration was significant decreased in treated rats by betacyfluthrin plus ascorbic acid and β-carotene (2.3 and 8.1 % above the normal level) at the end of recovery periods as compared with those in insecticide-treated rats alone. While, no significant changes after recovery period in creatinine concentration as compared with untreated animals except, in case of betacyfluthrin plus β-carotene (-9.7 % below the normal level). In this context, data obtained by Elzoghby *et al*. [15], showed that significant increase in uric acid and creatinine in malathion treated-rats, while in malathion plus vitamin C or malathion plus green tea there was significant decrease in both parameters as compared with untreated animals. Similar effects were observed by Bhushan *et al*.[18] who reported that most of the values (AST, ALT, glycerides, total proteins, cholesterol and bilirubin) reached to normal level during the recovery periods pointing towards degradation of cypermethrin in the treated animals.

Effects on lipids profile:

Our results revealed that betacyfluthrin caused significant increase in cholesterol and triglycerides concentrations at 5th and 10th doses. These vital parameters did not return to the normal level after recovery period (2 weeks). The highest amount was happened in cholesterol level after 10th dose and after 5th dose in triglycerides (27.5 and 112.8 % above the normal level). Following administration of ascorbic acid and β-carotene showed fluctuated pattern of change in both vital biochemical components in rats, showing the pattern of decrease – increase – normal – decrease level than normal. Accordingly, both antioxidant improvement in the level of cholesterol and triglycerides when compared with insecticide-treated rats. Ascorbic acid caused decrease in both vital parameters level and reach below the normal level (3.5 and 0.3 %) at the end of recovery period. These results are in agreement with those found by Nisar *et al*.,[19] who reported that treatment of chlorpyriphos and lead treated rats with vitamin C significantly improved some of altered oxidative stress parameters revealing the protective effect of this vitamin C against oxidative stress induced by chlorpyriphos and lead. On the contrary, data obtained by Elzoghby *et al*.,[15] showed significant decrease in total protein, albumin and globulin levels in malathion treated rats, while, in malathion plus vitamin C and malathion plus green tea treated groups there was no significant changes when compared with control.

Table (4): Effect of oral administration of betacyfluthrin alone and in combinations with β-carotene and ascorbic acid on urea, creatinine, cholesterol and triglycerides in rats

Parameter / Treatments	Urea			Creatinine			Cholesterol			Triglycerides		
	5 doses	10 doses	2 weeks	5 doses	10 doses	2 weeks	5 doses	10 doses	2 weeks	5 doses	10 doses	2 weeks
Betacyfluthrin	55.13a (+30.9)	57.38a (+19.0)	74.8a (+39.8)	0.68a (+19.3)	0.71a (+14.5)	0.7 a (+12.9)	78.8a (+15.9)	88.6a (+27.5)	72.1 b (+7.1)	167.6a (+112.8)	127.7a (+61.9)	93.5a (+20.6)
Betacyfluthrin + Ascorbic acid	57.0a (+35.4)	56.4a (+17.0)	54.75c (+2.3)	0.69a (+21.1)	0.62b (0.0)	0.66 a (+6.5)	78.3a (+15.1)	71.5b (+2.9)	64.95d (-3.5)	123.9b (+57.3)	120.4b (+52.6)	77.3b (-0.3)
Betacyfluthrin + β-carotine	45.05b (+7.0)	57.0a (+18.3)	58.2b (+8.1)	0.63ab (+10.5)	0.72a (+16.1)	0.56 b (-9.7)	70.95b (+4.3)	72.8b (+4.7)	74.3a (+10.4)	102.4c (+30.0)	117.0b (+48.3)	93.3a (+20.4)
Control	42.11c	48.2b	53.5c	0.57b	0.62b	0.62ab	68.0b	69.5b	67.3c	78.75d	78.9c	77.5b
LSD 5 %	2.57	1.54	2.27	0.086	0.037	0.075	6.54	4.92	1.59	16.87	3.66	9.65

(According to Duncan test) Letters means the significant differences between treatments and control. Each figure between brackets represents the percentage of content as check.

Effect of ascorbic acid and β-carotene on insecticide residues in some organs:

Table (5): Effects of ascorbic acid and β-carotene on betacyfluthrin residues in some organs of rats

Organs Treatments	Liver (ppm)			Kidney (ppm)			Stomach (ppm)			Fats (ppm)			Spleen (ppm)			Total
	5th doses	10th doses	Recovery period	5th doses	10th doses	Recovery period	5th doses	10th doses	Recovery period	5th doses	10th doses	Recovery period	5th doses	10th doses	Recovery period	
Betacyfluthrin	0.4	0.63	0.14	0.11	0.27	0.08	0.63	0.83	ND	0.17	0.22	0.06	ND	0.11	ND	3.65
Betacyfluthrin + ascorbic acid	0.35	0.72	0.16	0.14	0.23	0.1	0.72	0.87	ND	0.2	0.31	0.06	0.08	0.08	ND	4.02
Betacyfluthrin + β carotene	0.41	0.68	0.21	0.16	0.31	0.07	0.69	0.79	ND	0.13	0.18	0.04	0.05	0.072	ND	3.792
Total in organs (ppm)	3.7			1.47			4.53			1.37			0.392			11.462

Data in Table 5 showed the highest amounts were detected in stomach after the 10th dose in all treatments(0.83, 0.87 and 0.79 ppm) followed by in liver (0.63, 0.72 and 0.68 ppm) after the same dose. Generally, results revealed the highest total residues of betacyfluthrin were found in stomach (Fig.1) followed by liver (4.53 and 3.7 ppm, respectively), while the lowest were detected in spleen (0.392 ppm). The total amounts of this insecticide detected in kidney and fats were 1.47 and 1.37 ppm. On the other hand, our results revealed that ascorbic acid and β-carotene didn't effect on betacyfluthrin residues in different organs (Fig. 2), the detected residues was high in treated rats by insecticide plus ascorbic acid (4.02 ppm) followed by insecticide plus β-carotene (3.792 ppm) than that treated by insecticide alone (3.65 ppm). Also, the residues detected after 10th dose was higher than its found after 5th dose in all organs , these amounts were decreased during recovery periods. In this context, no residues were founded in stomach and spleen after recovery periods. Similar results were obtained by Shalaby *et al.* [20] who reported that thiamethoxam residues in stomach are high (2.17 and 3.78 ppm) followed by fats (1.7 and 3.03 ppm) and brain (1.6 and 2.78 ppm) after 5 and 10 days of treatment; while, residues in kidney and liver are low. But no residues were detected in the kidney at the end of recovery period. Also, Aioub and Hegab [21] reported that the amount of methomyl residues in the liver, kidney, heart and spleen ranged from 0.33 to 1.22 ppm after the first dosing, as expected, the liver contained the highest amount.

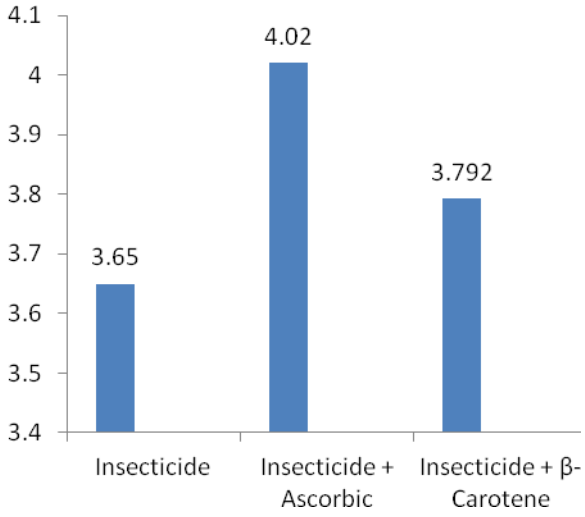

Figure1: Effect of ascorbic acid and β-carotene on betacyfluthrin residues

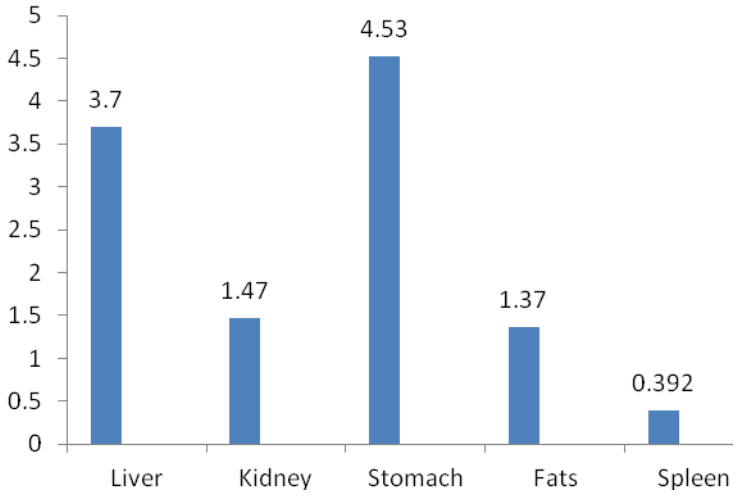

Figure 2: Total betacyfluthrin residues in different organs of treated rats

Generally, this study shows that ascorbic acid and β-carotene has a protective effect on betacyfluthrin induced oxidative liver and kidney damage. Consequently, it seems that an antioxidant agents may be useful in decreasing the side effects of exposure to different toxicants compounds or pollutants.

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