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RESEARCH ARTICLE

INDUCED EFFECT OF LAMBDA CYHALOTHRIN ON BIOCHEMICAL PARAMETERS OF PROTEIN METABOLISM IN SELECTED TISSUES OF ALBINO MICE

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ABSTRACT

Pyrethroids are synthetic chemical analogues of pyrethrins, which are naturally occurring insecticidal compounds produced in the flowers of chrysanthemums (*Chrysanthemum cinerariaefolium*). Lambda-cyhalothrin is a pyrethroid insecticide. Insecticidal products containing pyrethroids have been widely used to control insect pests in agriculture, public health, and homes and gardens. With regard to effectiveness and toxicity, this compound appears to be the first-choice insecticide used than organochlorines, organophosphates and carbamates. It is highly used in the cotton plantation, in vegetable production and to control a wide range of insect pests in a variety of crops. The present study is aimed to evaluate the effect of Lambda cyhalothrin on biochemical parameters such as Total Proteins, FAA, Protease, GDH, Ammonia and Urea in selected tissues Intestine and Testis. To assess the effect of Lambda-cyhalothrin, mice were gavaged orally for 10, 20 and 30 consecutive days with sub lethal doses of Lambda (1/5th of LD50 4.8 mg/kg bw). This caused significant decrease in total proteins and increase in FAA, protease, glutamate dehydrogenase, ammonia and urea in Intestine and Testis when compared to the controls.

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INTRODUCTION

A review of the toxicological literature reveals that exposure of chemical can produce unexpected effects (Feron *et al.,* 1995). According to Waliszewski *et al.,* (2003), Aronson *et al.,* (2000) and Abdul (2003) most pesticides may enter into the food channels and cause physiological damage. A number of recent clinical studies revealed that most of the pesticides and chemicals could alter the immune system (Barcarolli and Martinez, 2004; Thangavel *et al.,* 2004; Chen *et al.,* 2004). λ-cyhalothrin is an insecticide registered by the U.S. Environmental Protection Agency (EPA) in 1988 (Pesticide Factsheet, 1988). Lambda is a pyrethroid insecticide. Pyrethroids are synthetic chemical analogues of pyrethrins, which are naturally occurring insecticidal compounds produced in the flowers of chrysanthemums (*Chrysanthemum cinerariaefolium*). The insecticides used earlier are now getting replaced by the recently developed fourth-generation broad spectrum synthetic pyrethroids like cypermethrin and Lambda cyhalothrin, as they are more effective for the purpose. Insecticidal products containing pyrethroids have been widely used to control insect pests in agriculture, public health, and homes and gardens (Amweg and Weston 2005; Oros and Werner 2005). Lambda cyhalothrin has been observed to exert significant genotoxic and cytotoxic effects on human lymphocytes cultured in vitro (Naravaneni and Jamil, 2005), a dose dependent chromosomal aberration in mice (Chauhan *et al.,* 2005). The sub letha lexposure of pyrethroids has produced several changes in energy metabolism of rats and mice. The present study is under taken to evaluate the toxic effects of Lambda cyhalothrin pesticide during the exposure time periods on the protein metabolism in intestine and testis.

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MATERIALS AND METHODS

Chemical Substance

Lambda cyhalothrin, a synthetic pyrethroid has been considered for this toxicology study. The effective dose 4.8 mg/kg/day given orally in corn oil vehicle for 10, 20 and 30 days below their acute LD_{50} level of intoxication according to their body weight. The mouse oral LD_{50} for Lambda cyhalothrin is 24 mg/kg body weight.

Experimental Animal

The toxicological studies have been done on albino mice which are closely related to human beings and easy to handle

in laboratory. Albino mice of 30±5g were selected from an inbred colony for the experimentation. The mice were maintained in the laboratory. They were housed in separate cages containing sterile paddy husk as the bedding material. The animals were provided with standard pellet and water throughout the study. The mice were maintained under normal day/ night schedule (12L: 12 D) at room temperature 26˚C ± 1˚C. The control and experimental animals after a stipulated period (*i.e.* on 11th, 21st and 31st day) were sacrificed and the tissues were quickly isolated, cleaned in physiological saline and processed immediately for microscopic analysis under ice-cold conditions. The tissues were also quickly isolated and were kept in deep freezer at -80°C and used for biochemical analysis.

Biochemical studies

Biochemical studies such as Protein levels were estimated by the method of Lowry *et al*., (1951); Free amino acid content was estimated by the method of Moore and Stein (1954) as described by Colowick and Kaplan (1957). Protease activity was estimated by the method of Moore and Stein (1954); GDH activity levels were estimated following method of Lee and Lardy (1965); Ammonia was estimated by the method of Berg Meyer (1965); Urea content was estimated by the method of diacetyl monoxime as described by Natelson (1971).

Statistical analysis

Statistical significance between the control and experimental data were subjected to analysis of variance (ANOVA) together with Dunnett's test ($P<0.05$).

RESULTS AND DISCUSSION

Oral administration of Lambda cyhalothrin caused significant decrease in Total Protein levels and increase in FAA, Protease, GDH, Ammonia and Urea. Protein metabolism consists of, essentially transformation of amino acids which are more readily absorbed from the intestine to portal blood to be conveyed to the liver. Some proteins can be synthesized in the body from amino acids ingested in food as proteins, while number of them is synthesized from amino acids not present in the diet. The enzymes which are involved in protein metabolism were altered significantly. The increase in activity of deaminases reveals the breakdown of nucleotides to yield excess energy to overcome the toxic pressure. Exposure to sublethal doses of Lambda cyhalothrin caused significant ($p < 0.05$) time and dose dependent reduction in the levels of total protein. According to Nelson and Cox (2005), Harper (2005) and Sathyanarayana (2005), the physiological activity of animal was indicated by the metabolic status of proteins. The depletion of protein fraction in Intestine and Testis tissues may have been due to their degradation and possible utilization for metabolic purposes. Increase of free amino acid levels were the result of breakdown of protein for energy and impaired incorporation of amino acids in protein synthesis. The toxicants may affect the hormonal balance which could directly or indirectly affect the tissue protein levels (Singh *et al.,* 1996; Morthy and Priyamvada, 1982; Khilare and Wagh, 1988). Several authors reported decreased total protein content in different animal models under pesticidal toxicity, such as in fishes *(Labeo rohita)* treated with organophosphate compound triazophos (Abdul Naveed *et al.,* 2010), quinalphos (Das and Mukherjee, 2000), in albino rat treated with hexachlorophene (Suhasini *et al.,* 2006). Kaur *et al.,* (2003) reported decreased total protein content in rats serum exposed to inhaled dimethylformamide for 13 weeks. The enhanced FAA levels may be channelled for energy synthesis and other metabolic reactions (Kovacs and Seglen, 1981). The elevated FAA levels are utilized for energy production by feeding them as keto acids into the TCA cycle through aminotransferases to contribute energy needs during toxic stress. Increases in free amino acid levels were the result of breakdown of protein for energy and impaired incorporation of amino acids in protein synthesis (Singh *et al.,* 1996).

The increase in FAA levels of tissues indicates stepped up proteases activities and fixation of ammonia into keto acids (Srinivasa murthy *et al.,* 1986; Ali, 2003). This higher level of FAA can also be attributed to the decreased utilization of amino acids and is also suggestive of its involvement in the maintenance of osmotic and acid base balance (Moorthy *et al,* 1984). Tissue specific increase in protease activity observed at multiple doses of Lambda cyhalothrin were clearly reflected in the breakdown of proteins. Under proteolysis, enhanced breakdown dominates over synthesis. While in the case of anabolic process, increased synthesis dominates the protein breakdown (Murray *et al.,* 2007). Moreover, histopathological damage and hydro mineral imbalance during pesticide stress has been reported to account for the elevated protease activity (Moorthy *et al.,* 1984). The Lambda cyhalothrin pesticide cause increased in GDH activity in the tissues during initial periods of exposure. The important function of GDH is that the amino group of most amino acids is transferred to α-ketoglutarate to produce glutamate. The increased GDH activity may indicate increased rapid utilization of amino acids (Nelson and Cox, 2005; Sathyanarayana, 2005). The oxidation of glutamate in the Kreb's cycle leads to increased energy though small (Narasimha and Ramana, 1985). GDH catalyzes the reversible reaction of oxidative deamination of glutamate to α-ketoglutarate and ammonia and plays an important role in the catabolism and biosynthesis of amino acid (Murray *et al.,* 2007). The elevation observed in the GDH activity indicates its contribution to enhanced ammonia levels and glutamate oxidation during cypermethrin toxicity. Increased free amino acid levels and their subsequent transamination results in greater production of glutamate thus increasing the intracellular availability of substrate, glutamate for consequent oxidative deamination reaction through GDH. Besides elevation in transaminases and GDH helps in supplying keto acids to the TCA cycle in order to compensate the energy crisis in different tissues during Lambda cyhalothrin toxicity.

GDH activity may be the factors for the enhanced ammonia level in the present study as function of Lambda cyhalothrin stress. Because of its high toxicity ammonia may bring about a shift from aerobiosis to anaerobiosis. Brandt *et al.* (1983) reported large amounts of ammonia accumulate in the tissues when amino acids are deaminated. In the present study elevated activities of protease, transaminase reactions and increased deamination reactions (GDH) support the augmented ammonia levels during Lambda cyhalothrin toxicosis. Ammonia cannot be stored for longer period of time in the body as it leads to endogenous ammonotoxicity. The reduction in ammonia content suggests that the ammonia might have been converted into non-toxic compounds, glutamine and urea in Lambda cyhalothrin. From these observations made in the mice under Lambda cyhalothrin intoxication, it is concluded that the changes are dependent on the dose of pesticide. High

dose caused more damage to the physiological, biochemical activities of the mice. Hence, there appeared an irrecoverable loss to the biochemical integrity of the cells due to Lambda cyhalothrin stress.

Table 1. Changes in Total Protein content in different tissues of control and Lambda cyhalothrin treated albino mice (mg/gm wet wt of tissue)

Tissues	Control	10 Days	20 Days	30 Days
Intestine				
Mean	100.222	92.338	80.406	65.314
S D	±2.5184	±1.9884	±1.2599	±1.7668
PC		(-7.8665)	(-19.772)	(-34.8306)
Testis				
Mean	70.306	65.446	59.538	48.598
S D	±2.1328	±1.4210	±1.6065	±1.5518
PC		(-6.912)	(-15.315)	(-30.876)

Values are mean of six individual observations
±SD-Standard Deviation; PC - Percent Change over control

One Way Anova

Source of Variation	DF	Intestine	Testis
		Mean Squares	Mean Squares
Between Groups	3	1386.919*	524.625*
Within Groups	20	2.715	2.452
Total	23		

All the values are Significant at P<0.05

Table 2. Changes in Free Amino Acid content in different tissues of control and Lambda cyhalothrin treated albino mice (µ moles of tyrosine /gm wet wt of tissue)

Tissues	Control	10 Days	20 Days	30 Days
Intestine				
Mean	12.930	14.627	16.133	17.348
S D	±1.4201	±0.7805	±0.9003	±1.3175
PC		(13.124)	(24.711)	(34.168)
Testis				
Mean	9.328	10.058	11.115	12.065
S D	±1.1218	±1.4157	±0.9313	±1.3569
PC		(7.825)	(19.157)	(29.341)

Values are mean of six individual observations
±SD-Standard Deviation; PC - Percent Change over control

One Way Anova

Source of Variation	DF	Intestine	Testis
		Mean Squares	Mean Squares
Between Groups	3	21.902*	8.630*
Within Groups	20	1.293	1.493
Total	23		

All the values are Significant at P<0.05

Table 3. Changes in Protease activity (µ moles of tyrosine/mg protein/hr) level in different tissues of control and Lambda cyhalothrin treated albino mice

Tissues	Control	10 Days	20 Days	30 Days
Intestine				
Mean	0.630	0.676	0.706	0.750
S D	±0.0113	±0.0138	±0.0120	±0.0159
PC		(7.301)	(12.063)	(19.841)
Testis				
Mean	0.539	0.570	0.589	0.632
S D	±0.0190	±0.0170	±0.0180	±0.0192
PC		(5.751)	(9.276)	(17.254)

Values are mean of six individual observations
±SD-Standard Deviation; PC - Percent Change over control

One Way Anova

Source of Variation	DF	Intestine	Testis
		Mean Squares	Mean Squares
Between Groups	3	0.015*	0.00896*
Within Groups	20	0.00015	0.00035
Total	23		

All the values are Significant at P<0.05

Table 4. Changes in Glutamate dehydrogenase (µ moles of formazon formed/mg protein/hr) levels in different tissues of control and Lambda cyhalothrin treated albino mice

Tissues	Control	10 Days	20 Days	30 Days
Intestine				
Mean	0.118	0.127	0.135	0.146
S D	±0.0041	±0.0174	±0.0045	±0.0101
PC		(7.627)	(14.406)	(23.728)
Testis				
Mean	0.114	0.119	0.126	0.134
S D	±0.0045	±0.0052	±0.0054	±0.0056
PC		(4.385)	(10.526)	(17.543)

Values are mean of six individual observations
±SD-Standard Deviation; PC - Percent Change over control

One Way Anova

Source of Variation	DF	Intestine	Testis
		Mean Squares	Mean Squares
Between Groups	3	0.0028*	0.000173*
Within Groups	20	0.00104	0.000025
Total	23		

All the values are Significant at P<0.05

Table 5. Changes in Ammonia levels (µ moles of ammonia/gm wet wt of tissue) in different tissues of control and Lambda cyhalothrin treated albino mice

Tissues	Control	10 Days	20 Days	30 Days
Intestine				
Mean	2.115	2.218	2.276	2.318
S D	±0.060	±0.0783	±0.101	±0.301
PC		(4.869)	(7.612)	(9.598)
Testis				
Mean	1.080	1.117	1.138	1.162
S D	±0.0867	±0.0459	±0.0263	±0.0712
PC		(3.425)	(5.370)	(7.592)

Values are mean of six individual observations
±SD-Standard Deviation; PC - Percent Change over control

One Way Anova

Source of Variation	DF	Intestine	Testis
		Mean Squares	Mean Squares
Between Groups	3	0.047*	0.007*
Within Groups	20	0.00378	0.004
Total	23		

All the values are Significant at P<0.05

Table 6. Changes in Urea levels (µ moles of urea /gm wet wt of tissue) in different tissues of control and Lambda cyhalothrin treated albino mice

Tissues	Control	10 Days	20 Days	30 Days
Intestine				
Mean	0.483	0.502	0.533	0.553
S D	±0.0466	±0.0762	±0.0820	±0.0184
PC		(3.933)	(10.351)	(14.492)
Testis				
Mean	0.235	0.242	0.251	0.258
S D	±0.0038	±0.0062	±0.0037	±0.0091
PC		(2.978)	(6.808)	(9.787)

Values are mean of six individual observations
±SD-Standard Deviation; PC - Percent Change over control

One Way Anova

Source of Variation	DF	Intestine	Testis
		Mean Squares	Mean Squares
Between Groups	3	0.006	0.001*
Within Groups	20	0.004	0.0004
Total	23		

All the values are Significant at P<0.05

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