



Research Article

Cuticular Biochemistry: Lambda-Cyhalothrin Induced Alterations in Mutant *Drosophila melanogaster*

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Abstract: Derivatives of natural pyrethrum, synthetic pyrethroids, are well-established neurotoxins. However, they do interfere with the functioning of metabolic processes; the most important of these is chitin metabolism, a key process in the development of insects. Type II synthetic pyrethroid, lambda-cyhalothrin, when orally fed to *Drosophila melanogaster* revealed its efficacy in chitin synthesis modulation. Total proteins, glucosamine, N-acetylglucosamine, chitinase activity and chitin content exhibit significant changes in the final developmental stage, the adults. A reduction in chitin synthesis is suggestive of interference in polymerization process which is a must for cuticle formation. Involvement of lambda-cyhalothrin in chitin synthesis has been sought to be an additional mode of action, other than its neurotoxic nature.

Keywords: Insect, Cuticle, Pyrethroid.

1. Introduction

Insects are the most important creatures in the wide class of pests. They destroy our crops and cause food scarcity to human beings. Many insecticides have been used to control these mighty creatures from the categories of organochlorines, organophosphates, carbamates, and pyrethroids. These insecticides prominently affect their physiology and biochemistry, results in a negative effect on their population. At present pyrethroids are in vogue as they are almost safe to human beings and used in houses for insect control. Pyrethroids are well known for their neurotoxic action (Wouters and Bercken, 1980; Soderlund and Knipple, 2003) since the day of their inception. They are in vogue due to their quick effects. However, in long treatment practices, they may alter other metabolic processes of insect body like energy metabolism and synthesis of chitin. Chitin is a linear polymer of N-acetylglucosamine and is the most abundant biopolymer of amino sugar. Its mechanical strength provides support to structural elements in insects and is also present in the peritrophic membrane of insect gut. The importance of chitin for insect survival reflects the need of those insecticides which interfere with this crucial process of insect life. Glucose being the precursor of

chitin subsequently changes to fructose followed by glucosamine and N-acetylglucosamine and lastly polymerizes into chitin. Chitin synthesis is regulated by chitin synthase and chitinase enzymes which help in polymerization and degradation respectively. *Drosophila melanogaster* was used as a model insect in present study due to their short generation time as 10 days at 25°C, known culture medium, production of a large number of progeny required for statistical accuracy and possessing only four pairs of chromosomes. An attempt has been made to assess modulation in chitin synthesis by the experimental compound, lambda-cyhalothrin in mutant *Drosophila melanogaster*.

2. Materials

Adult white mutant *Drosophila melanogaster* has been used as a model insect, reared and maintained at 25°C temperature and 50% relative humidity in Toxicology Laboratory, Department of Zoology, Dr. B.R. Ambedkar University, Agra. The test compound lambda-cyhalothrin was obtained from Bayer India Ltd. Most of other chemicals were purchased from Sigma and Qualigence.

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2.1 Experimental protocol

In the present study, males and females of white mutant *Drosophila melanogaster* were crossed for each biochemical estimation, consisting of six sets for statistical accuracy, first three as control sets (UT♀ X UT♂) and another three as treated sets (T♀ x T♂). Control sets were free from treatment, while the rest were treated with lambda-cyhalothrin. Estimation of various biochemical parameters leading to chitin synthesis in the adults of white mutant of *Drosophila melanogaster* for control as well as treated sets has been done to observe effect of lambda-cyhalothrin on chitin metabolism.

3. Methods

3.1 LC₅₀ determination

The experimental flies were divided into five sets, each set consisting of randomly selected 10 individuals. The stock solution of test compound was prepared in acetone and serially diluted to 8µl, 4µl, 2µl, 1µl, and 0.5µl per 100ml food. Same amount of diluent was given to the control groups and the flies were released in culture bottles. The mortality and survival number of flies were recorded for each dose in the respective bottles after 48 hours. Moribund flies were considered as dead. The collected data were analyzed statistically by log-dose/probit regression line method (Finney, 1971).

3.2 Biochemical estimations

Estimation of total proteins, hexosamines (glucosamine and N-acetylglucosamine), chitinase enzyme activity and chitin content in the whole body of adults of white mutant *Drosophila* were done by the method of Lowry *et al.*, (1951); Gardell (1958); Reissig *et al.*, (1955) and Hackman (1954) respectively.

3.3 Statistical analysis

The observations were analyzed using Student's t-test at various significance levels (Fisher and Yates, 1950).

4. Results and Discussion

LC₅₀ value for white mutant *Drosophila melanogaster* was 2.12 µl/100ml of food and the

sublethal concentration for experiment was 0.2 µl/100ml of food.

The data obtained from biochemical estimations has been treated statistically. Total proteins, glucosamine, N-acetylglucosamine and chitinase enzyme levels were increased, while the chitin content was decreased simultaneously after sublethal concentration of lambda-cyhalothrin (Table 1).

Biochemical components of an organism may vary within a range according to its physiological state. Under stress condition, the biochemical components may lie beyond these limits. Chitinase activity has been increased after treatment which induces degradation of chitin into its simpler units i.e. glucosamine and N-acetylglucosamine (Kramer *et al.*, 1995) as revealed by their increased level in *Drosophila melanogaster*. Total protein content has been increased (Tufail and Shakoori, 1992; Verma and Nath, 1995) in the present study due to breakdown of chitin-protein complex in *Drosophila* integument by the action of protease and chitinase enzymes collectively ((Karaiyan and Thangaraj, 1999). The chitin content has been decreased (Muzzarelli, 1974; Hajjar, 1979; Saxena and Kumar, 1981) following lambda-cyhalothrin treatment, reveals its chitin synthesis modulatory action in *Drosophila melanogaster*. Cuticle is degraded by moulting fluid enzymes in a planned stepwise manner. The active moulting fluid has been observed to penetrate the layers of chitin and protein complex (Brookhart and Kramer, 1990). This fluid contains two major enzymes, one, the protease which unmask the cuticular chitin for the action of chitinase and the two, chitinase which act on the unmasked cuticular chitin to degrade it into simpler units, glucosamine and N-acetylglucosamine (Karaiyan and Thangaraj, 1999). However, it is also possible that the enzyme chitin synthase which helps in amino sugar polymerization, decreases following treatment with lambda-cyhalothrin, resulting in decreased chitin content as chitin synthase and chitinase are the main enzymes to regulate chitin synthesis (Merzendorfer and Zimoch, 2003). The possibility of involvement of chitin synthase cannot be ruled out and has been considered an important regulatory step.

It is thus concluded that lambda-cyhalothrin, a type II synthetic pyrethroid has the potentiality to modulate chitin synthesis.

Table 1. Biochemical alterations in cuticular components of white mutant *Drosophila melanogaster* following lambda-cyhalothrin intoxication.

| S. No. | Parameters | Control set (Mean ± S.Em.) | Treated set (Mean ± S.Em.) |
|--------|-----------------------------|-------------------------------|-------------------------------|
| 1. | Total proteins (mg/dl) | 13.13 ± 2.76 | 16.26 ± 3.70* |
| 2. | Glucosamine (mg/dl) | 1.23 ± 0.086 | 1.6 ± 0.173* |
| 3. | N-acetylglucosamine (mg/dl) | 4.63 ± 0.29 | 5.6 ± 0.34* |
| 4. | Chitinase activity (Unit) | 6.28 ± 0.32 | 8.01 ± 0.153*** |
| 5. | Chitin content (mg/gm) | 1.053 ± 0.014 | 0.9 ± 0.089*** |

S.Em. – Standard Error of Mean; *(P>0.05); **(P<0.05); ***(P<0.01)

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Abbreviations used:

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|---------------------------|---------------------|--------------------|-------------------|---------------|
| 1. °C – degree centigrade | 2. % - percent | 3. UT – untreated | 4. T – treated | 5. ♂ - male |
| 6. ♀ - female | 7. µl – micro liter | 8. ml – milliliter | 9. dl – deciliter | 10. gm – gram |