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Assessment of DNA Degradation in Lymphocytes of Albino Rat (*Rattus norvegicus*) under Lambda Cyhalothrin Stress

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Abstract: The use of pyrethroids is increasing, because of their low mammalian toxicity but high insecticidal activity. The application of pyrethroids, which is often carried out without adequate expert knowledge, may lead to high pesticide residues, often combined with health hazards for the people concerned. Objective of the present study is to evaluate Genotoxic potential of lambda-cyhalothrin in albino rat lymphocytes on the basis of qualitative and quantitative analysis. In the present study nucleic acids have been found to be increased significantly in all treatments, while non-significant (DNA) and significant increase (RNA) has been observed in recovery group. The increase in nucleic acids contents in the present investigation have been correlated with increased number of blood cells especially W.B.Cs. Further, the increase in nucleic acids content in blood is also correlated with the stimulated growth of cellular protein and RNA in animal under pyrethroid stress. Genomic DNA does not show any kind of DNA damage like degradation or fragmentation except in 30ds sub acute study in which severe DNA degradation has been observed. These present changes are indicative of possibility of the experimental compound to travel to the extent of higher levels of food chains and warrant its indiscriminate application.

Key words: Lambda-cyhalothrin · DNA · RNA · Albino rat

INTRODUCTION

Synthetic pyrethroids are biological, physical and chemical agents used to kill organisms, which are harmful to human beings. Pesticides might be incorporated into plant tissue and food grains and as a result of this they enter into food chain and accumulate at various tropic levels after each generation through biomagnifications. Such pesticides are a menace, in a sense that they get entry into the mammalian body and cause alterations in various cytological, biochemical and physiological processes leading to serious complications. Of the several pyrethroids, those having cyno-group, are known to possess clastogenic activity [1-4]. LCT being a third generation pesticide contains α-cyno group and is available in a number of formulations [5]. Due to its rapid metabolism and excretion its toxicity for mammal at present is quite low, however it may create problems in non-target species in future if applied indiscriminately. The present study is aimed to observe the possible effect of the experimental pyrethroid on albino rats as they are easily reared in laboratory conditions and their resemblance to higher mammalian groups with regard to their physiology cannot be denied. The peripheral blood lymphocytes have been selected, as they are specific body defense system and have the capability to disturb the physiological processes of body [6], which are governed at molecular level through synthesis of proteins, whose message for formation is coded in the nucleic acids of cells. The ample data are available on the clastogenic potential of synthetic pyrethroids including LCT [4]. The qualitative (gel banding pattern assay) and quantitative (Nucleic acid estimation) analysis is further carried out to evaluate the genotoxic potential of LCT.

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The *in vivo* genotoxicity of LCT has been evaluated by assessing the ability of the pyrethroid to damage DNA by using microgel electrophoresis assay. The qualitative and quantitative analysis of nucleic acids was proposed as a useful parameter for assessing the genotoxic properties of environmental pollutants [7].

MATERIALS AND METHODS

Test Compound: Lambda cyhalothrin (LCT), a non-systemic pyrethroid insecticide with the trade name 'Karate' chemical name (R+S)-α-cyano-3-(phenoxyphenyl) methyl-(1S+1R)-cis-3-(2-2chloro-3,3,3-trifluoroprop-1-enyl)-2-2-dimethyl-cyclopropane-carboxylate (CAS no. 91465-08-06), of 98 % purity with contact and stomach action and repellent properties was procured from Zeneca-ICI Agro Chemicals, Chennai (India), for the present study.

Maintenance and Feeding of Experimental Albino Rats: The experimental albino rats (*Rattus norvegicus* [Berkenhout]), procured from inbred colony were acclimated for one month to the laboratory conditions (temperature. 25±0.5°C, relative humidity 60±5% and photoperiod 12 hr/day) before using them for the experiment. Adult male and female rats of almost equal size and weight were kept in the polypropylene cages and cleaned regularly to avoid any infection or undesirable odour in the laboratory. Each cage was equipped with a metallic food plate and water bottle. The albino rats were offered fresh feed daily throughout the experimentation on Gold Mohar rat and mice feed, manufactured by Hindustan Lever Ltd., India at regular interval and water was provided *ad libitum*.

Selection of Individuals: The LD_{50} data were analyzed statistically by log dose/probit regression line method [8]. Oral LD_{50} of male and female rats was found to be 75.85-mg/kg body weight and 56.695-mg/kg body weight respectively. For the experimentation individuals selected randomly irrespective of sex because on comparing percent mortality of male and female rats non significant change ($p > 0.05$) (Figure 1) could be revealed.

Five healthy adult albino rats (6-8 weeks of age, with average body weight of 150-200 g) were selected randomly for test, control and recovery studies; sacrificed after 1, 2, 15, 30 and 45 (recovery) days for the collection of blood in the present investigation. Each rat was assigned a number for convenience prior to experimentation. All the rats of the experimental sets were given doses of LCT orally with the help of gavage tube and those of control sets equal amount of vehicle i.e. ground nut oil.

Selection of Dose: Test agent-An oral dose of 18 mg/kg body weight for acute (1d and 2ds) treatment, while for sub acute treatment, 1/30th of acute dose was given for 30 days i.e. 0.6 mg/kg body weight/day by gavage tube. The recovery group did not receive any dose after 30 days of sub acute treatment till day 45.

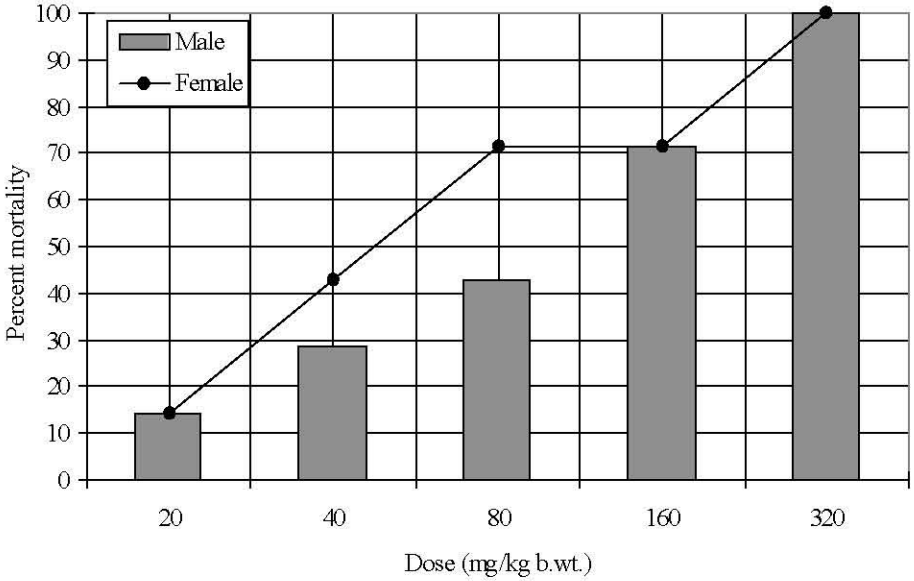

Fig. 1: Graph showing comparision of percent mortality of LCT in male and female albino rats after oral treatment

Negative Control-Negative controls consisting of vehicle (groundnut oil) were treated per Os with all treatment groups.

Collection of Blood Samples: The rats were autopsied in the early morning hours (7-8 AM), anaesthetized with chloroform and were placed in dissecting tray with their ventral side upwards. The blood samples were obtained with the help of 5.0 ml disposable syringe, fitted with the hypodermic needle (22 swg) directly from the ventricle of the heart of the dissected albino rats. The collected blood was transferred immediately into plain sterilized centrifuge tubes for the separation of lymphocytes and quantitative studies of nucleic acids.

Quantification of Nucleic Acids (DNA and RNA): The nucleic acid content (separately for DNA and RNA) were estimated by the well established method [9,10]. Lymphocytes were separated from the whole blood by Ficoll-paque method [11,12], homogenized with ice cold 10% trichloroacetic acid and were centrifuged at 3000 rpm. They were resuspended and recentrifuged. The precipitate was suspended in ethanol-ether mixture and centrifuged. Sodium hydroxide was added to the precipitate, mixed well and was left for eighteen hours at 37°C. The supernatant containing protein and RNA was separated after centrifugation from precipitate containing protein and DNA. The supernatant and precipitate was used for RNA, DNA estimation respectively.

Gel Banding Pattern Analysis: Banding pattern analysis was accomplished in three steps. First of all lymphocytes were separated from whole blood [11,12] *vide supra*, DNA was then isolated from lymphocytes and the so isolated DNA was then used for gel banding pattern analysis [13].

RESULTS AND DISCUSSION

In the present investigation Nucleic acids (DNA, RNA) have been found to be increased significantly in all treatments, while non-significant and significant increase has been observed in Recovery Group of albino rats for DNA and RNA respectively (Fig. 2)

The increase of DNA content in the present investigation may be correlated with increased number of leucocytes as earlier observed in rats, mice, rats, albino rats following cypermethrin, supercypermethrin, cybil, tetramethrin toxicity [14-17] respectively. It is a well-known fact that the amount of DNA in the cells is constant in an individual cell; any increase in DNA content should therefore accompany the increase in number of cells in body [18].

DNA carries genetic information into specific mRNA and then translated into proteins that determine the phenotype [19]. DNA mediates the synthesis of nucleic acid in the cells would likely to affect the protein content of cells in body [20]. Alteration in rate of protein synthesis under the physiological circumstances of body results in change of protein concentration.

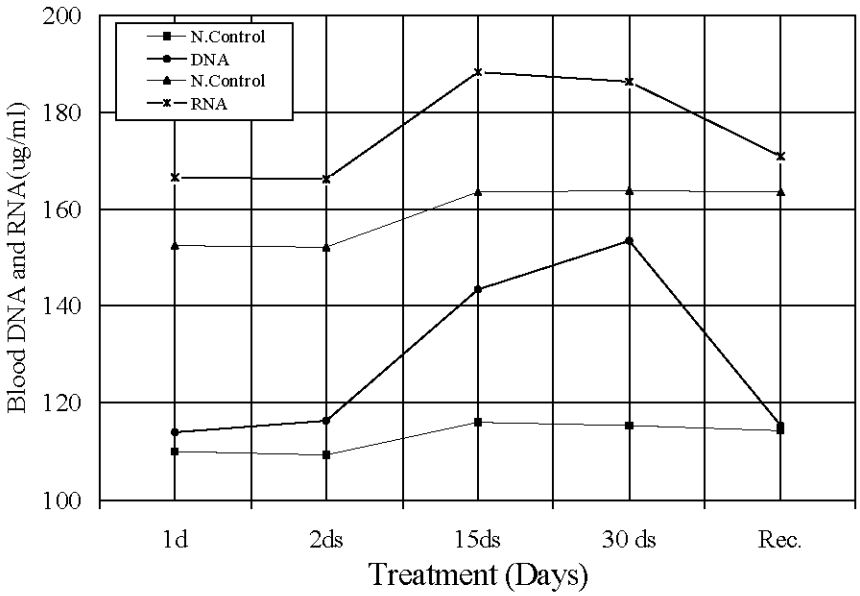

Fig. 2: Graph showing amount of DNA and RNA in the blood of albino rat after LCT intoxication and in recovery

The amount of protein and the amount of DNA seems to be directly correlated. Further, the increase of serum total proteins [3] also suggests increase of DNA content. The increase of RNA content (*vide infra*) is again in affirmation with the increase of DNA content.

The increase of ACTH [21] may also be considered responsible for increase of DNA content in the present investigation because the principal effect of ACTH is to stimulate adenyl cyclase in cell membrane [6]. This activated adenyl cyclase promotes the DNA replication in the cell [22] to elevate the DNA. Similar mechanism may hold true to elevate DNA content in the present investigation.

The non-significant increase in Recovery Group shows that animal overcomes the toxic effect if they don't receive xenobiotic substances because the new lymphocytes are synthesized regularly and the old one get degraded.

The increase in RNA content in blood is associated with increase in DNA (*vide supra*) and total proteins. Earlier correlation between stimulated growth of cellular protein and RNA in animal under pesticide stress has been documented [23,24]. Increase in DNA directed RNA synthesis in the microsome of mice treated with permethrin [25] and increase in rat RNA polymerase activity on insecticide treatment, which is responsible for RNA synthesis [26] also observed earlier. Similar increase in RNA polymerase activity in cellular component of blood may be responsible for the increase in RNA content in blood after LCT intoxication in the present investigation.

Genomic DNA does not show any kind of DNA damage like degradation or fragmentation because DNA is almost alike to control and seems to be present entirely in the well of gel during acute treatment of LCT, while in 15ds sub acute treatment slight degradation of genomic DNA has been observed as compared to control. In 30ds sub acute study severe DNA degradation observed in form of a white seamier on gel in comparison to control but DNA of recovery studies does not show any kind of degradation and entire DNA seems to be present in well as is seen in control.

DNA degradation observed in the present investigation may perhaps be due to reactive oxygen species beacuse that reactive oxygen species are involved in the toxicity of various pesticides [27]. Further, mixture of pesticides has been documented inducing oxidative damage to DNA through the production of reactive oxygen radicals [28]. Similarly LCT may generate reactive oxygen species during its metabolism and produce oxidative stress in rat [29,30]. During metabolism of pesticides electrophilic epoxide produced [31], which has a tendency to react with electron rich moieties in the DNA and give rise to DNA damage. Similar mechanism may probably hold true for DNA damage by LCT in the present investigation.

Pesticide induced metabolic intermediate (epoxide) so formed get inactivated by the microsomal epoxide hydrolase (mEH; EC 3.3.2.3) [32]. Further, in the recovery group since access of LCT has been restricted, formation of epoxide could not have been possible and which is evident in gel banding pattern analysis revealing no DNA degradation. Lambda-cyhalothrin possesses potential to induce cytogenetic changes [3] in lymphocytes, which are mostly used in defense and cell immunity. These changes inform of DNA banding pattern are indicative of possibility of the experimental compound to approach the higher trophic which must properly taken care of in order to minimize their status as potential future mutagen.

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