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Original article

Ameliorating effect of *Phoenix dactylifera* on lambda cyhalothrin induced biochemical, hematological and hepatopathological alterations in male wistar rats



Mani Ramadhas, Krishnan Palanisamy, Munisamy Sudhagar, Vinayagam Magendira Mani*

PG & Research Department of Biochemistry, Islamiah College (Autonomous), Vaniyambadi, Vellore District, Tamil Nadu 635751, India

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ABSTRACT

Lambda cyhalothrin (LTC) is a synthetic pyrethroid insecticide, widely used to control insect pests in agriculture, public health, and homes and gardens. In the present study, an attempt has been made to study the effect of lambda cyhalothrin on biochemical, hematological parameters and ameliorating effects of palm dates (*Phoenix dactylifera*) in male wistar rat. Adult male Wistar rats were divided into four different groups. Group I served as control; group II received lambda cyhalothrin at a dose of 8 mg/kg (1/10 LD50) dissolved in water for 21 days orally; group III received *P. dactylifera* (200 mg/kg BW for 21 days) orally; group IV *P. dactylifera* alone treated. LTC-induced liver toxicity was measured by the increased activities of serum hepatic marker enzymes like aspartate transaminase, alanine transaminase, alkaline phosphatase, lactate dehydrogenase, along with increased elevation of lipid peroxidation and reduction in the levels of enzymic and non-enzymic antioxidants levels. Lambda cyhalothrin exposure leads to adverse effects on hematological parameters including erythrocyte (RBCs) and leukocyte (WBCs) counts, hemoglobin concentration (Hb), hematocrit (Hct) and blood indices (MCV and MCH). However, treatment with *P. dactylifera* normalized the levels of hepatic markers, antioxidant and non-enzymic antioxidant, lipid peroxidation products and all the hematological parameters. These findings highlight the efficacy of *P. dactylifera* as protective effects against lambda cyhalothrin induced toxicity.

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1. Introduction

Lambda cyhalothrin (LTC) is a type II, α -cyano-pyrethroid and it has found extensive use to control a broad spectrum of insects and ectoparasites, including cockroaches, flies, lice, mosquitoes, and ticks in public, agriculture and animal health applications [1]. It is highly used in cotton plantation and in vegetable production. But, the widespread use of these substances has led to serious health problems including neurotoxic effects through voltage dependent sodium channels [2,3] chromosomal aberrations, genotoxicity, micronucleus formation in rat bone marrow cells, reproductive toxicity, cardiotoxicity and hepatotoxicity [4,5] on non-target organisms such as mammals, birds, and fishes. These compounds also interfere with many vital physiological functions and constitutently alter the levels of various biochemical and

hematological constituents [6,7]. Annually, more than 25–77 million poisoning cases and 0.22 million casualties due to pesticide poisoning have been reported [8]. Recent studies [9] reported that reactive oxygen species (ROS) are involved in LTC-induced neurotoxicity, hepatotoxicity and oxidative damages [10]. Hematological parameters including erythrocyte (RBCs) and leukocyte (WBCs) counts, hemoglobin concentration (Hb), hematocrit (Hct) and blood indices (MCV and MCH) are important for the assessment of various systemic functions and health of animals under various environmental conditions and most importantly, for diagnosis of drug or chemical induced toxicity [11,12]. Recent reports were shown pyrethroid exposure leads to hematotoxicity by significant alterations hematological findings [13,14]. The information suggests that hematological parameters could be used as potential biomarkers of pyrethroid insecticides toxicity. The liver was found to accumulate a greater concentration of pesticide metabolites since it is the major site of pyrethroid metabolism. Pesticides are known to increase the production of reactive oxygen species (ROS), which in turn generate oxidative stress in different tissues [15] leads to the central mechanism of pyrethroid toxicity [16]. Pyrethroid insecticides are hydrophobic molecules that bind

* Corresponding author. Tel.: +919486000227, +914174 232227;
 fax: +914174232876.

E-mail addresses: magendiramani@rediffmail.com, magivbio@gmail.com
 (V. Magendira Mani).

extensively to biological membranes, especially phospholipids bilayers [17], and they may damage membranes by inducing lipid peroxidation [18]. As observed pyrethroid metabolized in liver by hepatic xenobiotic metabolizing enzymes, cytochrome P450 monooxygenases [19] this metabolites affects liver mitochondria and leads to alterations of liver specific marker enzymes such as AST, ALT, LDH, ALP. Fruits and vegetables have been implicated in preventing or reducing the risk of coronary heart diseases [20], cancer [21] and other chronic liver diseases [22]. For these reasons, recommendations to increase the dietary intakes of fruits and vegetables have been suggested by many world authorities [23]. Date palm trees, *Phoenix dactylifera* L., are an important plantation crop for many countries and are still considered by many people in this part of the world as a staple food [24]. It could be used for generations to come due to its remarkable nutritional, health and economic and environmental benefits [25]. Date fruits are listed in folk remedies for treatment of various infectious diseases, atherosclerosis, diabetes, hypertension and cancer [26]. Numerous studies showed Date fruits have beneficial properties such as antiviral, antifungal, antioxidant, anti hyperlipidemic and hepatoprotective activity [27]. These are attributed to the rich contents of antioxidant in date fruit such as the coumaric acid and ferulic acid. Moreover, it contains flavonoids, sterols, procyanidins, carotenoids, anthocyanins, vitamins and minerals [28]. Hence, this study was interested to evaluate the toxic effects of lambda cyhalothrin probably through the generation of free radicals and the possible preventive effects of *P. dactylifera* by its antioxidant and free radicals scavenging activity.

2. Materials and methods

2.1. Chemicals

Lambda cyhalothrin commercial formulation (Decis, lambda cyhalothrin 5% EC denotes 5% of technical grade lambda cyhalothrin [w/w] in emulsifiable concentrate) was procured from Bayer crop science limited, Mumbai, India. All other chemicals and reagents used in this study were of analytical grade.

2.2. Animal model

Wistar male albino rats weighing between 200 g–240 g were housed in animal cages with food and water ad libitum. Six animals were housed per cage, and maintained on 12/12 h day and night cycle. The animals were fed with commercial pellet diet (Hindustan lever Ltd., Bangalore, India). The experiments were conducted according to ethical norms approved by the Ministry of Social Justice and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines.

2.3. *P. dactylifera* palm dates extract preparation

Fresh dates were collected from the local market. Fruit flesh was extracted two times with distilled water (1/10 w/v) by grinding with a mortar and pestle. It was centrifuged at 4 °C for 20 min at 4000 g and the supernatant was collected. We selected an aqueous extract because most of the antioxidant components in dates are extracted in water [28]. During the experience, the aqueous date fruit extract of was daily prepared and administrated to rats.

2.4. Experimental design

Toxicity of lambda cyhalothrin was evaluated by literature study and the LD50 of lambda cyhalothrin to wistar rats was found to be 80 mg/kg BW. Hence, this study 1/10 of LD50 value (8 mg/kg bw) was selected as sublethal dose.

Experimental animals were divided into four groups of six rats each as follows.

Group I – served control rats.

Group II – lambda cyhalothrin administered orally at a dose of 8 mg/kg (1/10 LD50) dissolved in water for 21 days.

Group III – animals were administered lambda cyhalothrin as in Group II, immediately followed by administration of *P. dactylifera* (200 mg/kg BW orally) dissolved in water for 21 days.

Group IV – animals received *P. dactylifera* (200 mg/kg BW orally) dissolved in water for 21 days.

After the experimental period, the animals were fasted overnight, anesthetized with sodium pentothal and blood collected from jugular vein for serum isolation and sacrificed by cervical decapitation. The liver tissue was excised immediately and a portion of the tissue was homogenized in 0.1 M Tris buffer, pH 7.4 and used for various biochemical assays.

2.5. Histological examination

A portion of the liver tissue was fixed in 10% neutral buffered formalin and embedded in paraffin wax for histological evaluation. Sections with thickness 5 μm were stained with hamatoxylin and eosin (H & E), examined under high power light microscope.

2.6. Biochemical assays

The collected serum was used to estimate antioxidant enzymes such as superoxide dismutase SOD [29], catalase CAT [30], glutathione peroxidase GPx [31], glutathione reductase GR [32], Glutathione-S-transferases GST [33] and non-enzymic antioxidants reduced glutathione GSH [34], vitamin C [35], vitamin E [36]. The liver tissue homogenate was used to measure aspartate transaminase [37], alanine transaminase [38], alkaline phosphatase [39], Lactate dehydrogenase (LDH), was measured by the method of [40]. Lipid peroxidation level was determined by measuring thio barbituric acid reactive substances (TBARS) according to the method of [41].

2.7. Hematological evaluation

The blood samples were collected into tubes containing EDTA and were immediately used for determination of hematological parameters. Total red blood cell (RBC) and white blood cell (WBC) counts were estimated according to the method of [42]. The percentage packed cell volume (PCV) was determined according to the hematocrit method [43] while the blood hemoglobin (Hb) concentration in all samples was estimated according to the cyanomethemoglobin method using Drabkin's reagent [43]. Mean cell volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated as outlined in [42]. Differential white blood cell counts were estimated using the method of [44].

2.8. Statistical method

All the results were expressed as mean ± SD for six rats in each group. All the grouped data were statistically evaluated with SPSS/12.0 software. Hypothesis testing method included one-way analysis of variance (ANOVA), followed by least significant difference (LSD) test; $P < 0.05$ was considered to indicate statistical significance.

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