

The effects of allethrin and prallethrin on plasma, whole blood and urine in human volunteers

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Abstract

The effect of allethrin and prallethrin based mosquito repellent pyrethroids on human whole Blood, urine levels of *chrysanthemum dicarboxylic acid* (CDCA), the chief degradatory product of pyrethroid catabolism are maintained significantly and subject to renal clearance and metabolism, and plasma hormones gonadotropins (FSH and LH), thyroid hormones (T3 and T4) and testosterone were investigated. Humans were chronically exposed to 7-10 years continuously for 8-10 hours per day by inhalation of these compounds exposure enter into circulation and changes in whole blood, plasma and urine. A significant decrease in the concentrations of thyroxine and testosterone with no change in the levels of LH, FSH and triiodothyroxine.

Keywords: Pyrethroids; chrysanthemum dicarboxylic acid; testosterone; FSH and LH; T3 and T4; allethrin; prallethrin;



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1. Introduction

Pyrethroids are the widely used insecticides due to their potential insecticidal activity in India and other countries to get protection against mosquitoes and other insects for various domestic and agricultural purposes (Yoshio et al., 1999; Kakko et al., 2003; Moya-Quiles et al., 1995; Narendra et al., 2007; 2008a;b). Over the half of world population have been using pyrethroid insecticides which may account for more than 25% of the insecticide market of the industrial countries in 90's and their demand/use is increasing now in these countries (Casida and Quistab, 1998; Timothy et al., 2005), as prevalence of mosquitoes and other insects are more in many endemic parts of the world. Initially, these pyrethroids were thought to be highly toxic to insects and less toxic to humans (Shaw and Chadwick, 1998). Now pyrethroid induced

neurotoxicity and other toxic effects ranging from whole body tremors to convulsions and death are well documented (Timothy et al., 2005; Herendorf et al., 2004; He et al., 1989; Dorman and Beasley, 1991; Soderlund et al., 2002). There is few published data available so far on the effects of pyrethroids on humans, and now slowly the facts related to their toxicity are coming into light. The fatality with pyrethroids in India has been reported to be 12.5 to 25% (Pankaj and Prahlad, 2004). Allethrin and prallethrin are among the most widely used pyrethroid insecticides. Allethrin poisoning can be much frequent due to its easy accessibility as mosquito repellent and/or insecticidal sprays etc (Sinha et al., 1995) and often reports of pyrethroid poisoning in India are evident (Mishra and Singh, 2003; Ganga and Rajarajeswari, 2001). However, it is clear that no relevant data on chronic effects exist in open scientific literature related to pyrethroid toxicity in humans and animals (Pankaj and Prahlad, 2004; Kolaczinski and Curtis, 2004). Since these pyrethroids are used routinely and/or regularly as mosquito repellents and/or through agricultural/gardening sprays exposing people continuously to the inhalation of these compounds for longer durations, their inevitable chronic use aroused a concern among public now, which formed the basis for the design of the present study. The purpose of the present study is two fold; First, to detect the changes in plasma hormones of human volunteers exposed to regular use of allethrin and prallethrin, and second to understand the role and status of whole blood and urinary levels of *chrysanthemum dicarboxylic acid* (CDCA) in such users of allethrin and prallethrin.

2. Subjects for study

The volunteers were using either Jet[®] mosquito repellent coils or mats, both from Godrej Sara Lee Ltd., Mumbai, India. The coils are composed of (w/w) 0.1% *d-trans* allethrin, 52.9% wood flour, 35% coconut shell powder, 12% starch, and the mats contained (w/w) 1.6% *d-trans* prallethrin and 98.4% relevant ingredients as indicated by the manufacturers. Release of the pyrethroid insecticide is either by burning the coil or placing the mat in the commercially available electric devices. All the subjects were known to get exposed to allethrin or prallethrin for at least 8h/day but not 10h/day, and the subjects had no known history of exposure to any other similar pyrethroids. Three groups, each group consisting of 24 male volunteers aged between 35-45 years, included in the present study were: Group I, controls who did not use mosquito repellents; Group II, allethrin exposed subjects; Group III prallethrin exposed subjects. All the volunteers were well explained about the experimentation and their written consent was obtained. This study was approved by the institutional ethical committee. Blood samples from over night fasted subjects were used for the study. All the volunteers in the present study were free from any other chronic disease or illness, and, were teetotalers with no smoking habit and free from use of any tranquillizers, drugs and anaesthetics.

2.1. Determination of CDCA from blood and urine

Preparation of stock solutions

Stock solutions of different pyrethroid insecticides are prepared separately using trace analytical grade acetone. Subsequently diluted the stock solutions using hexane and prepared the working standard solutions. An Artic 380 deep freezer supplied by Froilabo, Meyzieue, France with auto-matic temperature recorder and display facility is used for storing the stock solutions and the samples at $-40 \pm 2^{\circ}\text{C}$.

Collection of blood samples

About 5 ml of blood was collected from over night fasted subjects for the experimental purpose. Informed consent was obtained from all the donors. All the heparinized blood samples are stored at $-40 \pm 2^{\circ}\text{C}$ until analysis.

Extraction of residues from the whole blood

Prepared a mixture of two different concentrations of pyrethroids, allethrin and prallethrin. Control blood samples are spiked at different concentration levels to give 1, 10, 50, 100, 200, 400, 600 and 1000 ng/ml of pyrethroids in blood. Quantitatively 1 ml of blood sample was taken and added 20 µl of γ -BHC or gamma benzene hexachloride as internal standard. Mixed well and extracted with 5 ml of hexane and acetone (8:2, v/v) mixture by vigorous shaking for 10 min. Allowed for 10 min to settle. Collected the supernatant. The sample was again re extracted. Combined the extracts and centrifuged for 5 min at 5000 rpm. Collected the supernatant in a graduated test tube and concentrated to 0.5 ml at 40°C under gentle stream of nitrogen in a hood. Final volume was made up to 1 ml using hexane as solvent. Standard and sample are recorded. At the defined conditions injected the representative standard and samples in to the GC-MS and analyzed.

2.2. Pyrethroid metabolites

To get information about the amount of internal pyrethrum body burden following spraying or use of a mats and electric vaporizer in houses for insect control, the CDCA concentration were investigated in urine samples of exposed subjects before and directly after the 1-day lasting exposure. The exposure level in the air was not measured.

Urine over night samples were collected in polyethylene bottles from allethrin and prallethrin users. The pyrethrin metabolite CDCA was determined in each urine sample as well as the creatinine concentrations. In all cases, urine was collected in polyethylene bottles and than stored at -20°C until analysis. All subjects gave their informed content after they had received both verbal and written information with respect to the aim of the study and its execution. The study design for the elimination study was approved by an Ethical Committee.

2.3. Sample preparation

Two milliliter urine is transferred in a screw cap test tube. Twenty microlitres of the internal standard solution (2-phenoxybenzoic acid, 10 µg/l) and 500 µl concentrated hydrochloric acid is added. The test tubes are covered with screw caps and hydrolysed at 100°C in a block heater for 2 h. Three milliliters *tert*-butyl methyl ether is added to the cold sample and than the urine samples are shaken vigorously for 5 min, then centrifuged for 5 min at 2000 X g. The organic layer is separated in a new screw cap test tube. Again *tert*-butyl methyl ether (2 ml) is added followed by 5 min of shaking and centrifugation (5 min at 2000 X g). The organic layers are combined in a new screw cap test tube, the lower urine phases are discarded. The organic layer is dried under a gentle stream of nitrogen. The residue is dissolved in 250 µl acetonitrile.

For derivatization, 30 µl of 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) and 20 µl of *N,N*-diisopropylcarbodiimide (DIC) is added. The solution is slightly mixed for 10 min at room temperature. The derivatization with HFIP works only in water free samples. Therefore, it is important to separate *tert*-butyl methyl ether carefully from the lower water phase. One millilitre of 1 M sodiumhydrogencarbonate solution and 250 µl iso-octane is added. The test tubes are covered and vigorously mixed for the extraction. The sample is then centrifuged for 5 min at 2000 X g for phase separation. The iso-octane phase is finally transferred to a micro vial.

2.4. Gas chromatography-mass spectrometry

One millilitre of the sample is injected into a HP 5890II gas chromatograph, equipped with a CTC A 200S autosampler and a Rtx65 column (30 m x 0.25 mm x 0.25µm) and coupled with a Micromass AutoSpec Ultima.

2.5. Hormones

Serum testosterone was estimated by direct immunoenzymatic method using reagent kit (Equipar Diagnostic-Italy), and luteinizing hormone (LH) and Follicle stimulating hormone (FSH) were estimated by

microplate immunoenzymetric reagent kit (Monobind, INC.USA) in biomerius. T4 and T3 by enzyme immunoassay (EIA) kit method using commercial kits from Biomerica Inc., California USA.

2.6. Statistical analysis

Data were analysed for significant difference ($P \leq 0.05$) among values of control, experimental (Group II and Group III) samples using Duncan's Multiple Range (DMR) test (Megharaj et al., 1999).

3. Results and Discussion

Allethrin and prallethrin are the most commonly used pyrethroid-insecticides to get protection from mosquitoes and other insects. These compounds chiefly affect selective membrane transport processes and cellular metabolism causing toxicity in targeted insects and non-target mammals. Though acute pyrethroid toxicity is common and well known, limited information is available on chronic toxicity of these pyrethroids in humans and experimental animals. Actual effects/events related to pyrethroid toxicity and/or their precise mechanisms related to chronic use of these compounds are not clear. Upon prolonged exposure due to inhalation, allethrin and prallethrin are released as active ingredients. When these repellent-containing formulations are used, they enter into circulation continuously and are distributed to all tissues, and accumulate in several tissues causing effective damage. Blood levels of *chrysanthemum dicarboxylic acid* (CDCA), the chief degradatory product of pyrethroid catabolism are maintained significantly and subject to renal clearance and metabolism.

Biomembranes are the known targets of these lipophilic pyrethroids. Enhanced oxidative stress, decreased antioxidant status and involvement of NO have been implicated in chronic toxicity of pyrethroids. Pyrethroid-membrane interactions are responsible for several characteristic effects. Therefore the present study is designed to investigate the biochemical events and mechanisms associated with the membranes of RBCs, platelets and other relevant plasma, red cell and platelet parameters in human subjects exposed to allethrin and prallethrin inhalation.

Biochemical parameters related to plasma profile and blood:

Table 1 shows base line characteristics of study subjects of three groups viz., the controls I, allethrin users II and prallethrin users III. Mean ages of the chosen participants and other relevant details pertaining to their environment and exposure to pyrethroids Furthermore the concentration of CDCA in blood and urine are furnished in the Table 1 and Figure 1 and 2. It is surprising to note the presence of low concentrations of CDCA in blood of normals (who do not use mosquito repellent pyrethroids) when compared to the subjects using allethrin and prallethrin containing mosquito repellents. Allethrin and prallethrin users contained CDCA (0.58 ng/ml and 0.53 ng/ml) in blood and (0.98 µg/l urine and 0.93 µg/l urine) in urine respectively.

Effect of mosquito repellent use on plasma levels of gonadotropins, thyroid hormones and testosterone.

Data on the concentrations of hormones in plasma from control, allethrin and prallethrin exposed subjects presented in Table 2 show levels of plasma hormones gonadotropins (FSH and LH), thyroid hormones (T3 and T4) and testosterone. Data reveal a decrease in the concentrations of thyroxine and testosterone with no change in the levels of LH, FSH and triiodothyroxine.

Table 1. Some relevant details of the subjects of the study.

	Groups		
	Controls	Allethrin Users	Prallethrin Users
Age & Sex	43±2 Male	44±3 Male	45±1 Male
Number	24	24	24
Monthly Income	~12,000	~12,000	~12,000
Village/Town/City	Town	Town	Town
No. of people in household	5	4	5
Duration of exposure/day	-----	8 hours/day	8 hours/day
Brand of mosquito repellent used	-----	Jet® coils	All out® liquid vaporizers
Composition of mosquito repellent pyrethroid and other ingredients	-----	0.1% (w/w) d- <i>trans</i> allethrin, 52.9% wood flour, 35% coconut shell power, 12% starch.	1.6% d- <i>trans</i> prallethrin and 98.4% relevant ingredients
Dietary details	Non-Veg -10 Veg - 14	Non-Veg -12 Veg - 12	Non-Veg -11 Veg - 13
Concentration of CDCA in blood (ng/ml)	0.12±0.04 ^a	0.58±0.09 ^b	0.53±0.05 ^b
Concentration of CDCA in urine (µg/l urine)	0.20±0.05 ^a	0.98±0.09 ^b	0.93±0.12 ^b

Table 2. Effect of mosquito repellent pyrethroid use on plasma concentrations of Testosterone, Gonadotropins and Thyroid hormones.

Parameter	Groups		
	Controls	Allethrin Users	Prallethrin Users
Testosterone (ng/ml)	7.51±0.07 ^a	7.09±0.04 ^b	7.18±0.03 ^b

Leutenizing hormone (mIU/ml)	5.61±0.10 ^a	5.53±0.06 ^a	5.61±0.04 ^a
Follicle Stimulation hormone (mIU/ml)	8.16±0.06 ^a	8.15±0.03 ^a	8.20±0.03 ^a
Triiodothyroxine (T3) (ng/dl)	7.56±0.06 ^a	7.54±0.03 ^a	7.44±0.04 ^a
Thyroxine (T4) (ng/dl)	112.37±1.62 ^a	102.67±0.95 ^b	98.08±0.55 ^b

Values are expressed as Mean±SEM, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's Multiple Range (DMR) test. n=12.

Figure 1. Mosquito repellent pyrethroid concentrations in whole blood.

1-gamma benzene hexachloride (internal standard), 2,3-*cis* and *trans* isomers of allethrin users, 4-prallethrin users.

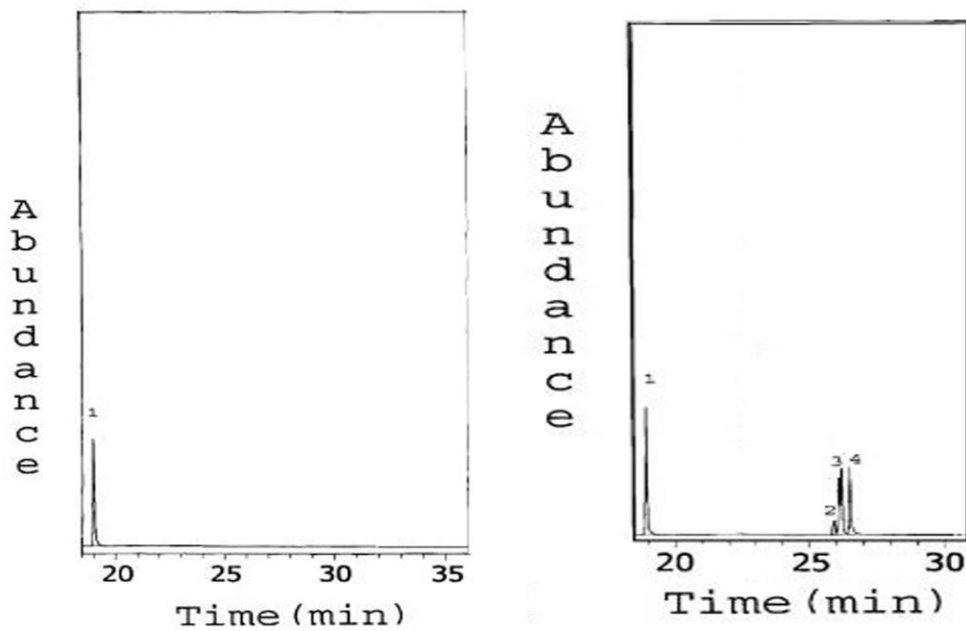
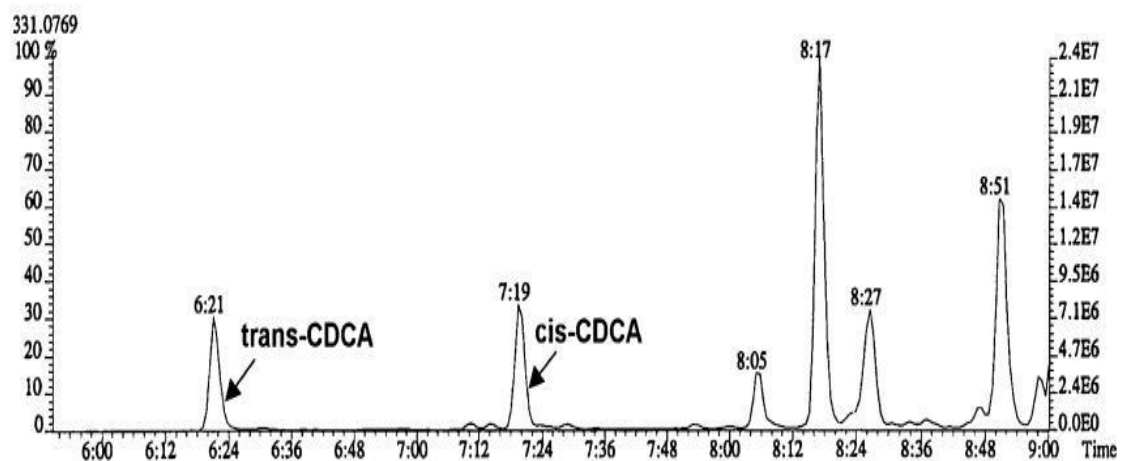


Figure 2. Mosquito repellent pyrethroid concentrations in urine.



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