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Comparative Toxicity of Cypermethrin Following Oral and Dermal Routes in Guinea Pigs and the Ameliorative Effect of Ascorbic Acid.

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ABSTRACT

The effect of repeated environmental intoxication and exposure to cypermethrin was studied in the guinea pig. The sub-acute effect was examined on hematological, some biochemical and tissue changes. Twenty four healthy guinea pigs of either sex weighing (30-40g) were divided into six equal groups. Some of the animals were given Vitamin C pretreatment and exposed to Cypermethrin via oral and dermal routes for six weeks. Long term cypermethrin exposure did not cause significant change in body weights of the guinea pigs. It significantly ($p<0.05$) decreased the packed cell volume, haemoglobin concentration, red blood cell and lymphocyte counts. Decreased the white blood cell counts and also, increased neutrophil counts. There was slight increase in the enzymes Aspartate aminotransferase, Alanine aminotransferase and Blood urea nitrogen activities. Other changes in serum profiles were insignificant between treatment group and untreated control groups ($p<0.05$). There was thickening of alveolar septae in the lungs, moderate hydropic change in the liver, coagulation necrosis of renal tubular epithelium and neuronal necrosis in the cypermethrin exposed animals. Repeated cypermethrin exposure induced cellular injury with commensurate tissue changes.

Keywords: Cypermethrin, Environment, Repeated, Sub-acute, Toxicity, Wildlife

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INTRODUCTION

The introduction of novel, more toxic and rapidly disseminating pesticides into the environment has necessitated an accurate identification of possible potential hazards to human, wildlife health and non-target species. Pesticides are compounds that kill pests and include insecticides, rodenticides, herbicides, fungicides and fumigants [1]. Although these toxic chemicals have become an integral part of the ecosystem, many of them remain extremely toxic to mammals and other non-target creatures [2].

Cypermethrin (CYP) is a synthetic pesticide (pyrethroid) which has been in use for more than a decade, because agricultural utilization of natural pyrethrins is limited due to their low photostability, whereas synthetic pyrethroid of the second and third generations are photostable and highly effective against broad spectrum of insects [3]. CYP is used not only as ectoparasiticide in animals but also employed extensively as insecticide in agriculture and public health programmes [4]. These widespread uses may have led to their contamination in the food chain and the environment [1]. Despite pyrethroids having a wide mammalian-insect toxicity ratio they are capable of exerting toxicopathological changes upon sub-acute or chronic exposure (5). A significant amount of work is being undertaken on monitoring toxicants including pesticides, metals and other chemicals in water and food materials in the environment [6]. Vitamin C (ascorbic acid) is a nutritional supplement; it helps to boost immunity and promotes wound healing.

The present study has been undertaken to examine effect of sub-acute exposure to cypermethrin on hematological, some biochemical enzymes and tissue changes in guinea pig model and also study ameliorative effect of vitamin supplement in toxication.

MATERIALS AND METHODS

Animals and Experimental design

Twenty four healthy guinea pigs of either sex weighing (30-40g) were divided into six equal groups consisting of 6 animals each (table 1).

Table 1: Experimental design on repeated intoxication of cypermethrin

I	4	RO	CYPERMETHRIN 1.25%
II	4	RO + supplements	
III	4	DE	
IV	4	DE + supplements	
V	4	Supplements only	CONTROL
VI	4	Nothing/blank	

All the experimental animals were kept under laboratory conditions (temperature: 24±1.0^{o}C and humidity: 60±5%) for acclimatization for two weeks. They were given pesticide free pellet feed and drinking water *ad libitum*. The experimental protocol met the institutional guidelines (University of Ibadan) on the proper care and use of animals in laboratory research and the study was approved by the institutional animal ethics committee.

Pesticide

Technical grade á-cypermethrin (á-CP w/v 99%, Gharda Chemicals Ltd. Mumbai).

Cypermethrin exposure

Experimental groups were exposed to cypermethrin for six weeks. Group I- cypermethrin repeated oral (CYRO), II- cypermethrin repeated oral and vitamin C (CYROVC), III- Cypermethrin dermal exposure (CYD), IV- Cypermethrin dermal with Vitamin C (CYDVC), V- Control blank with vitamin C (CBVC), and VI- Control blank (CB). Administered 0.16ml of the pesticide based on estimated sub lethal dosage of 50mg/kg body weight [7,8]. Vitamin C was administered in 1ppm.

Sensory stimulation was quantified by counting the number of times each animal turned to lick or bite its treated flank in preference to the untreated flank. Skin stimulation was observed during a 2-h period at all dose levels except the lowest [9].

The animals were fasted overnight and anesthetized in chloroform chamber. Blood was collected from the ocular sinus using capillary tube into plain sample bottles and bottles containing 1mg of anticoagulant EDTA (Ethylene Diamine Tetra Acetate) from each of the animals across the groups. The blood was used for the hematological profile as described by Benjamin [10] and serum biochemical analysis- Aspartate transaminase (AST), Alanine transaminase (ALT) by colorimetric method [11], and Alkaline phosphatase (ALP), Gamma glutamyl transferase activity (GGT), Blood urea nitrogen (BUN) and Creatinine [12].

Representative animals from each group were necropsied. Tissues from the lung, liver and kidney and brain were collected, immersed in 10% buffered formalin and processed routinely for histopathology [13,14].

Microscopic evaluation of tissue changes was done using light microscope (CX21).

Statistical Analysis

All values expressed were as mean ± SEM. Statistical analysis was done using SPSS 16 program. The statistical significance of differences between the two means was assessed by one way ANOVA. P values < 0.05 were considered to be significant.

RESULTS

Long term CYP exposure in the guinea pigs did not cause significant change in the body weights.

CYP significantly (*p<0.05)* decreased the PCV, HB, RBC and LYM in CYRO (group I) only, decreased the WBC in both CYRO and CYD (group III). Also, it increased NEUT in CYRO (table 2).

Table 2: Hematological values of guinea pigs exposed cypermethrin and Vit C supplements

Group	PCV	HB	RBC	WBC (10^3)	PLT (10^4)	LYM (10^3)	NEUT (10^3)	MON (10^3)	EOS (10^3)	MCV	MCHC
I	35.75±3.64*	11.95±1.24*	5.84±0.52*	3.75±0.45*	5.98±0.86	2.01±0.20	1.57±0.26	0.08±0.02	0.09±0.03	55.9±6.1	33.4±0.3
II	44.75±3.09	15.13±0.99	7.77±0.55	5.01±1.02	8.85±2.79	2.81±0.40	2.04±0.54*	0.16±0.03	0.09±0.03	57.6±0.5	33.8±0.4
III	43.50±2.02	14.65±.49	7.14±0.23	3.75±0.29*	9.20±0.12	2.66±0.52	0.86±0.25	0.86±0.43	0.09±0.03	60.8±0.9	33.7±0.4
IV	46.75±2.14	15.73±0.68	7.98±0.51	4.33±0.95	8.20±0.12	3.15±0.61	1.00±0.40	0.08±0.02	0.04±0.02	58.8±1.2	33.7±0.4
V	47.25±1.93	15.85±0.66	8.20±0.34*	5.44±0.59	9.60±0.85	4.02±0.41	1.20±0.24	0.08±0.02	0.13±0.03	57.7±0.6	33.6±0.3
VI	40.00±3.34	13.25±1.19	6.86±0.58	4.80±0.99	4.80±1.21	3.73±0.90	0.91±0.27	0.05±0.03	0.09±0.04	58.4±1.8	33.1±0.3

*Values with superscript are significant at 0.05.

Packed Cell Volume- PCV (%), Haemoglobin Concentration-Hb (g/dl), Red Blood Cell-RBC (*10^3/ µL), White Blood Cell-WBC (*10^3/ µL), Platelet Count-Plate (*10^5/ µL), Lymphocytes-Lympho, Neutrophils-Neutro, Monocytes-Mono, Eosinophils-Esino, Mean Cell Volume-MCV (fl), Mean Cell Haemoglobin Concentration-MCHC (pg)

CYP slightly increased the enzymes AST, ALT and BUN activities in CYRO (group I). Other changes in serum profiles were insignificant between treatment group and untreated control groups (p<0.05) (Table 3).

Table 3: Serum biochemical values of guinea pigs exposed cypermethrin and Vit C supplements

Group	Weight kg		AST	ALT	GGT	BUN	CREAT
	Before	After					
I	0.32±0.02	0.3±0.0	44.7±1.2*	39.3±1.3*	2.1±0.3	26.8±0.8*	1.9±0.1
II	0.40±0.0	0.40±0.0	43.0±1.1	36.3±0.5	1.0±0.2*	24.3±0.3	1.1±0.1
III	0.30±0.0	0.3±0.0	43.0±1.0	38.0±1.0	1.6±0.9	23.5±0.5	0.8±0.1
IV	0.30±0.0	0.29±0.03	42.8±2.5	37.8±2.6	1.8±0.5	23.8±1.0	1.2±0.1
V	0.25±0.03	0.25± 0.1	41.7±1.2	36.7±3.7	1.9±0.5	24.7±0.3	1.3±0.1
VI	0.25±0.03	0.25±0.03	42.0±0.5	36.0±3.2	1.6±0.4	24.0±1.2	1.2±0.1

*Values with superscript are significant at 0.05.

AST- Aspartate Amino transferase (U/l), ALT-Alanine Aminotransferase (U/l), Gamma glutamyl transferase –GGT (U/l), Urea- Blood Urea Nitrogen (mg/dl), Creat-Creatinine (mg/dl).

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There was extensive thickening of alveolar septae characterized by mononuclear and phagocytic infiltrates in the interstitial spaces and ectasia of alveolar spaces in the lungs (plate 1). There was a moderate hydropic change in the liver. The kidney showed coagulation necrosis of renal tubular epithelium (plate 2) and there was neuronal necrosis (pink, angulated) in the cypermethrin only exposed groups (plate 3).

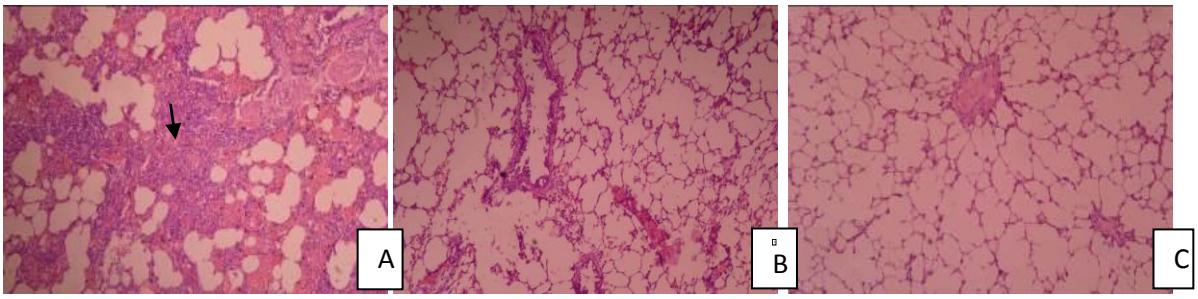

Plate 1-lungs: A- thickened interstitium with mononuclear infiltrates (arrow) in CYRO. B & C normal histological appearance of lungs in CYDVC and CB. HE x100

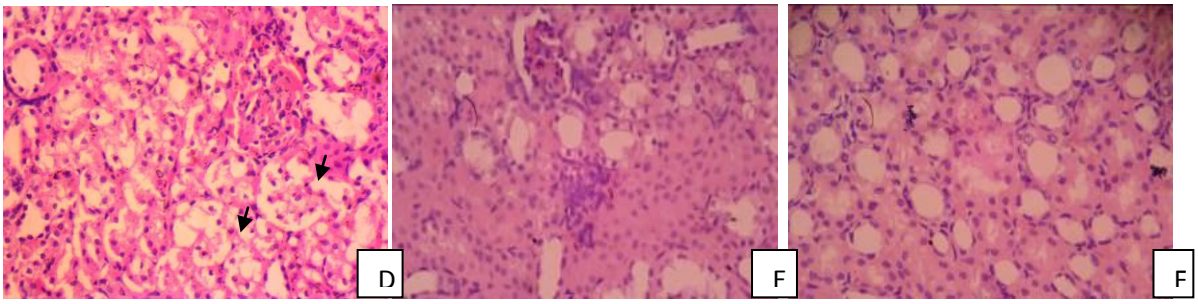

Plate 2- kidney: D- tubular epithelia necrosis (arrow) in CYRO. E & F are normal from CYDVC & CB. HE x100

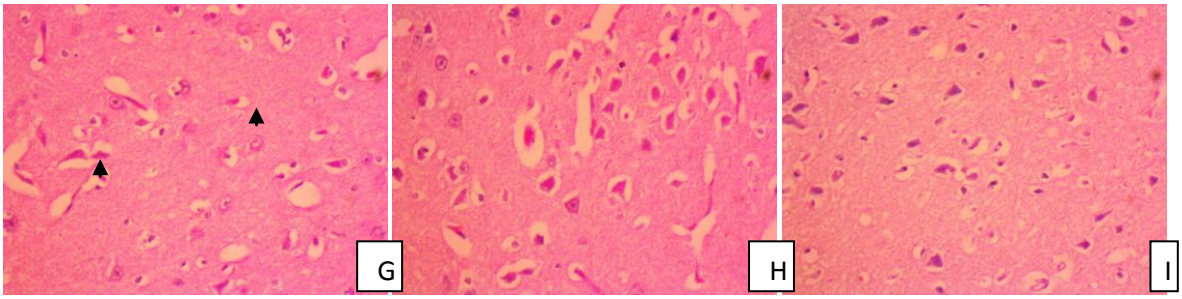

Plate 3- Brain: G- neuronal cell necrosis (arrow) in CYRO. H & I show normal neurons from vitamin C supplemented and control groups.

Vitamin supplements improved the PCV, HB, RBC, WBC and platelet values in the CYRO and CYD (groups I & III).

DISCUSSION

Synthetic pyrethroids represent one quarter of the insecticides used in the agriculture all over the world. This belongs to diverse class of potent, broad spectrum insecticides used to control insect pests in animals, agriculture, households, and stored products [15].

Cypermethrin is known to undergo metabolism through the cytochrome P450 microsomal system resulting in oxidative stress. Consequently CYP led to depletion of glycogen level, CAT and superoxide dismutase activity [16]. The acute LD50 value for α-cypermethrin in DMSO is reported to be 145mg/kg bwt. This possibly explains the degenerative changes and increased enzyme activities observed in this study.

Increased total protein in the developing muscle in newly hatched chick (Gallus domesticus) was reported as due to the use of sublethal dose of permethrin [17]. Cypermethrin was found to suppress serum

globulins, gamma globulins and specific haemagglutination-inhibition (HI) and ELISA antibodies in chickens (18). This is very much likened to the decreased WBC observed in the cypermethrin only exposed groups.

AST is normally found in a diversity of tissues including liver, heart, muscle, kidney, and brain. It is released into serum when any one of these tissues is damaged. ALT is, by contrast, normally found largely concentrated in liver and is released into the bloodstream as the result of liver injury. With higher doses of permethrin (80 and 120 mg/kg), an increase in alanine aminotransferase (ALT), aspartate amino transferase (AST) and blood glucose levels was observed [19]. Pyrethroid related-sensory irritation in the respiratory tract has been studied by nose only exposure studies in mice and rats.

In conclusion, repeated cypermethrin exposure induced cellular injury with commensurate tissue changes. Vitamin C supplementation had good ameliorative effect. Even though cypermethrin exposure was equally toxic, the oral route showed more toxicity. Cypermethrin exposure and intoxication of animals in the environment may contribute to morbidity and ecotoxicity.

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