EFFECTS OF SIMULTANEOUS ADMINISTRATION OF CYPERMETHRIN AND CHLORPYRIFOS ON PHARMACOKINETIC AND BIOCHEMICAL PROFILES IN SWISS ALBINO MICE

Paul S[1], Kundu T[1], Dutta S[1], Khargharia S[2], Mandal T K[2], Chakraborty A K[2]
[1]Medical College, Kolkata,India, [2]West Bengal University of Animal and Fishery Sciences, 37, K.B. Sarani, Kolkata-700 037, West Bengal, India

Summary

To evaluate the blood and tissue concentration as well as biochemical parameters after administration of commonly used pesticides: cypermethrin, chlorpyrifos and co-administration of both in swiss albino mice.

Swiss albino mice of either sex were divided into 4 groups (n=18) Group II received Cypermethrin (25 mg/kg), group III received Chlorpyrifos (6mg/kg) and group IV received both Cypermethrin (25 mg/kg) and Chlorpyrifos (6mg/kg) orally daily for 21 days. Group I was considered as control. Six animals from each group were sacrificed on 7, 14 and 21 days post dosing and blood samples and tissues (muscle, liver, heart, brain and kidney) were collected. Concentration of both Cypermethrin and Chlorpyrifos were measured and biochemical parameters were analysed in all the blood samples. Cypermethrin and Chlorpyrifos ($\mu g\ gm^{-1}$) was estimated in individual tissues.

Both Cypermethrin and Chlorpyrifos were recovered from blood and different tissue samples from 7th day onwards**.** Concentration of pesticides was found to be lower in combined group compared to when co-administered in comparison to individual dosing at the end of study. Highest concentration was observed in the liver in all the treated groups. AST, ALT and blood glucose levels were increased considerably in treated groups while protein level was decreased.

The results indicate that concomitant administration of both cypermethrin and chlorpyrifos may have some pharmacokinetic type of drug interaction which influences both biochemical parameters as well as tissue concentrations favorably.

Corresponding Author:

Dr Sanjib Khargharia, MVSc, PhD
Scientist B, Clintox Bioservices
Plot 4B, Phase-1, SP Biotech Park
A P – 500078, India

Email: sanjibkharghoria@yahoo.com

Introduction

Chlorpyrifos is a potent organophosphorus (OP) insecticide widely used to control pests of soil, plants and animals [1]. The widespread agricultural and animal applications of organophosphorus pesticides are attributed to their low environmental persistence [2]. Despite their low persistence, OP exhibit toxicity to humans and animals and the presence of residues of such compounds in our food supply has raised the safety issue. Cypermethrin is a type II pyrethroid insecticide used in domestic animal infested by different arthropod parasites like mites, ticks, bots and grubs which causes economic loss of livestock producers. Now-a-days, a combination of chlorpyrifos and cypermethrin is extensively used in agricultural field for crop production and contaminated crops may cause damage to human beings due to consumption resulting possible health hazards. But literature on adverse effect caused by combination of these two insecticides in mice is scarcely available. Therefore the present work was undertaken to explore the effect of chlorpyrifos and cypermethrin alone and their combination on biochemical parameters and residual level in different tissues of mice.

Materials and Methods

Animals

Swiss albino mice of either sex weighing between 20-25 grams were used for this experiment. The mice were kept in polysulfone mice cages and maintained at a 12 h light/dark cycle. The temperature was maintained 22 -26^{o}C while the relative humidity was 50-60%. The experiment was conducted in the animal house of Department of Pharmacology, West Bengal University of Animal and Fishery Sciences, Mohanpur Campus. Mice were fed on standard pellet diet and provided filtered autoclaved *ad* libitum drinking water. The experiments were performed following approval by the Institutional Animal Ethics Committee.

Experimental protocols

The selected animals were divided into 4 groups (I, II, III and IV) each containing 18 animals (9 male and 9 female) and each group was subdivided into A, B C and D consisting of 6 animals (3 male and 3 female). Group II received cypermethrin (25 mg/kg), group III received chlorpyrifos (6mg/kg) and group IV received both Cypermethrin (25 mg/kg) and chlorpyrifos (6mg/kg). The insecticides were suspended in 1% aquous Tween 80 and administered orally daily by gavage with the help of a standard mice feeding needle to each mice of experimental groups. Group I was considered as control and received only the same volume of 1% aqueous Tween 80. Each mouse of both control and experimental groups was fasted for 16 h prior to the administration of insecticides in Tween 80 or Tween 80 alone. Eighteen animals at 3 batches containing 6 from each group were sacrificed under deep isoflurane anaesthesia on 7, 14 and 21 days post dosing and blood and tissues (muscle, liver, heart, brain and kidney) samples were collected from each mouse, blotted, weighed, and placed in sterilized plastic bottles capped and kept at -20ºC till further use. Blood samples of 6 mice from orbital plexus were collected, out of which 0.5 ml was utilized for estimation of insecticide and remaining was allowed to clot for serum separation and used for ALT, AST [3] glucose [4] and protein [5]. Concentration of both Cypermethrin and Chlorpyrifos was measured by HPLC after extraction and clean up with appropriate solvents.

Collection of Sample
Blood and tissues were collected from six animals of each group at 7, 14 and 21 days of experiment. Six animals of each group were considered as single replication and six such replication was considered.

Analysis of Chlorpyrifos
Method of Richard *et al* [6] was modified as for estimation of Chlorpyrifos. 0.5 ml blood or 0.5 gm of tissue was added to 5 ml of premixed mixture of acetonitrile and phosphate buffer at the ratio of 9:1, homogenized thoroughly and transferred to a centrifuge tube. It was then centrifuged at 5000 rpm for 10 min. The supernatant fluid was collected and sediment was mixed properly with 5 ml of premixed solution. Mixture was centrifuged again and the supernatant was collected with the previous one. The whole supernatant was reduced using rotary vacuum evaporator at 60°C and reconstituted with 0.5 ml of premixed solution and filtered through membrane filter. Reconstituted solution (20 µl) was injected to HPLC column. The retention time of Chlorpyrifos was found to be 14.4 min.

Analysis of Cypermetrhin
Tissue (0.5gm) or blood (0.5 ml) was added to 2 ml of acetonitrile (HPLC grade).Mixture was homogenized thoroughly and centrifuged at 2000 rpm for 10 min. The supernatant was collected and 2 ml of acetonitrile added again and the procedure repeated twice. Whole collected supernatant was reduced in volume using rotary vacuum evaporator and reconstituted with 0.5 ml of methanol (HPLC grade) and subjected to membrane filtration and injected 20 µl of sample to the HPLC injection port. Retention time of the drug was 2.45 min.

Instrument and Chromatographic Condition
Cypermethrin and Chlorpyrifos in blood and tissues were analysed on a HPLC system (SHIMADZU, SPD – M 10 A, JAPAN) fitted with binary pump (LC-20AT), diode array detector, sampler and data station. A 5 µ Luna Phenomenox (250 x 4.6 mm) C 18 (2) HPLC column was used. The mobile phase consisted of acetonitrile and water with a ratio of 50: 50 (V/V). The flow rate of mobile phase was 1.0 ml min^{-1} and the eluent was monitored with a diode array detector adjusted wave length at 273 nm. The chromatograms were integrated on a data station.

Statistical Analysis
Statistical analysis was carried out by one-way analysis of variance (ANOVA) and comparison between the control and experiment groups was done using the LSD test. $P<0.05$ was considered as significant by uisng SPSS 17.0

Results

Blood Insecticide Concentration
Mean blood concentration of Cypermethrin and Chlorpyriphos following consecutive daily oral administration in mice for 21 days has been presented in Table 1.
Cypermethrin was detected in blood on 7^{th} day in group II (0.33± 0.003 µg mL^{-1}) and group IV (0.13 ± 0.002 µg ml^{-1}), while Chlorpyrifos could not be detected in group III and IV animals on 7th day of experiment. Blood concentration of Cypermethrin was higher on 14 day compared to 7 day in groups II (0.35 ± 0.004 µg ml^{-1}) and IV (0.17 ± 0.003 µg ml^{-1}); blood chlorpyrifos level was detected on 14 day in both groups III (0.08 ± 0.008 µg ml^{-1}) and IV (0.06 ± 0.001µg ml^{-1}) which attained a maximum concentration on 21 day.

Table 1: Mean blood concentration of Cypermethrin and Chlorpyriphos (μg ml^{-1}) following consecutive daily oral administration in mice for 21 days (Mean of six replicates with SE)

Serial No	Groups		Blood conc. in (μg ml^{-1})		
			Day		
			7	14	21
1	II	CYP	$0.33^{a} \pm 0.003$	$0.35^{a} \pm 0.004$	$0.53^{b} \pm 0.006$
2	III	CPF	----	$0.08^{a} \pm 0.008$	$0.12^{a} \pm 0.003$
3	IV	CYP	$0.13^{a} \pm 0.002$	$0.17^{a} \pm 0.003$	$0.42^{b} \pm 0.021$
		CPF	----	$0.06^{a} \pm 0.001$	$0.10^{a} \pm 0.003$

Group II: Cypermethrin alone at 25 mg/kg, Group III: Chlorpyrifos alone at 6 mg/kg,
Group IV: Combination of Cypermethrin and Chlorpyrifos, CYP: Cypermethrin, CPF: Chlorpyrifos, BDL: Below detection limit, (-): Not available, Values with at least one similar superscripts do not vary significantly (a and b are different superscripts)

On the other hand blood concentration of Cypermethrin and Chlorpyrifos were higher on 21 day compared to 14 day in group II (0.53 ± 0.006 μg ml^{-1} Cypermethrin), III (0.12 ± 0.003 μg ml^{-1} Chlorpyrifos) and IV (0.42 ± 0.021 μg ml^{-1} for Cypermethrin, 0.10 ± 0.003 μg ml^{-1} for Chlorpyrifos).
It is interesting to note that concentration of both cypermethrin and Chlorpyrifos decreased in combined therapy compared to unitherapy. Further, Cypermethrin level was reduced significantly in presence of Chlorpyrifos.

Biochemical Parameters
Effects of Cypermethrin and Chlorpyrifos on blood biochemical profiles have been presented in Table 2. It is apparent from Table 2 that the values of ALT, AST and glucose were increased significantly *($p<0.05$)* in animals of groups II and III compared to control *($p<0.05$)* from seventh day onwards while there is a steady fall in protein in groups II and III. On the contrary the above values of ALT, AST and glucose were decreased in mice of group IV compared to groups II and III. This suggests that co-administration of cypermethrin and chlorpyrifos might have lesser effect on above biochemical parameters.

Tissue Concentration
Mean residual concentrations of Cypermethrin and Chlorpyrifos recovered from different tissues of mice are presented in Table 3.

Table 2: Effects of Cypermethrin and Chlorpyrifos on biochemical profiles following consecutive daily oral administration in swiss albino mice for 21 days (Mean of six replicates with SE)

Parameters	7day				14 day				21 day			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
ALT	22.86^{a} ±0.57	25.59^{b} ±0.27	26.02^{b} ±0.27	24.60^{b} ±0.43	24.39^{a} ±0.57	30.29^{b} ±0.6	31.35b ±1.13	29.66^{b} ±1.42	24.99^{a} ±0.33	33.36^{b} ±0.96	34.66^{b} ±0.84	34.18^{b} ±2.17
AST	62.97^{a} ±0.52	66.3^{b} ±1.35	66.01^{b} ±1.11	65.7^{b} ±1.32	62.87^{a} ±2.47	72.23^{b} ±2.12	74.56^{b} ±3.61	72.33^{b} ±1.52	62.49^{a} ±1.27	79.31^{b} ±3.21	82.31^{b} ±3.42	77.4^{b} ±1.82
GLU	52.63^{a} ±0.23	59.4^{b} ±1.13	62.77^{b} ±0.57	58.00^{b} ±1.5	52.55^{a} ±0.36	65.47^{b} ±1.56	67.83^{b} ±1.32	64.51^{b} ±1.8	53.01^{a} ±0.09	71.52^{b} ±2.58	75.46^{b} ±1.95	69.4^{ab} ±1.75
PRO	62.18^{a} ±0.54	62.9^{a} ±0.94	61.07^{a} ±1.31	66.2^{a} ±0.51	62.99^{a} ±0.51	57.16^{b} ±1.31	58.69^{b}± 2.46	62.87^{a} ±0.97	63.82^{a} ±0.64	53.92^{b} ±0.82	52.47^{b} ±2.80	62.29^{a} ±1.28

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GLU: Serum glucose, PRO: Protein, Group I: Control, Group II: Cypermethrin, Group III: Chlorpyrifos alone, Group IV: Cypermethin + Chlorpyrifos. Values with at least one similar superscripts do not vary significantly (a and b are different superscripts)

Table 3: Mean tissue concentration (µg gm^{1}) of Cypermethrin (@ 25 mg/kg b.w) and Chlorpyrifos (@6mg/kg b.w) following consecutive daily oral administration in swiss albino mice (Mean of six replicates with SE)

Tissue	Insecticide	Concentration (µg gm^{-1})								
		Day 7			Day 14			Day 21		
		Group			Group			Group		
		II	III	IV	II	III	IV	II	III	IV
Muscle	CYP	BDL	NA	BDL	0.11±0.002	NA	0.12±0.005	0.21±0.004	NA	0.11±0.005
	CPF	NA	BDL	BDL	NA	0.09±0.001	0.13±0.004	NA	0.14±0.01	0.09±0.005
Liver	CYP	0.42±0.02	NA	0.22±0.005	1.2±0.035	NA	0.61±0.01	1.12±0.012	NA	0.78±0.015
	CPF	NA	0.08±0.003	0.06±0.003	NA	0.53±0.014	0.19±0.01	NA	0.54±0.02	0.35±0.012
Heart	CYP	0.26±0.01	NA	0.09±0.003	0.37±0.014	NA	0.15±0.008	0.69±0.026	NA	0.14±0.01
	CPF	NA	BDL	BDL	NA	0.21±0.012	BDL	NA	0.38±0.01	BDL
Brain	CYP	BDL	NA	BDL	0.03±0.002	NA	0.07±0.002	0.05±0.002	NA	0.05±0.02
	CPF	NA	BDL	BDL	NA	BDL	BDL	NA	BDL	BDL
Kidneys	CYP	0.37±0.01	NA	0.42±0.01	0.47±0.008	NA	0.62±0.011	0.49±0.018	NA	0.49±0.02
	CPF	NA	BDL	0.08±0.003	NA	0.09±0.003	0.13±0.005	NA	0.45±0.01	0.38±0.08

CYP = Cypermethrin (25 mg/kg), CPF= Chlorpyrifos (6mg/kg), BDL= Below detection limit, NA= Not applicable

In Group II, Cypermethrin was detected in liver, heart and kidney on day 7, while it could also be recovered from muscle and brain on the day 14 and attained a maximum concentration on day 21 in liver (1.12 ± 0.012 μg gm^{-1}) followed by heart (0.69 ± 0.026 μg gm^{-1}) and kidneys (0.49 ± 0.018 μg gm^{-1}). Lowest concentration in brain was observed (0.05 ± 0.002 μg gm^{-1}).

Chlorpyrifos was detected only in the liver on day 7, but it could also be recovered from liver, heart and kidney on day 14 in group III. Maximum quantity of Chlorpyrifos was recovered on day 21 from liver (0.54 ± 0.02 μg gm^{-1}) followed by kidney (0.45 ± 0.01 μg gm^{-1}), heart (0.38 ± 0.01 μg gm^{-1}) and in muscle (0.14 ± 0.01 μg gm^{-1}).

In Group IV, Cypermethrin was detected in liver, heart and kidney on 7 day and also from muscle and brain on day 14 attaining a maximum concentration in liver (0.78 ± 0.015 μg gm^{-1}) followed by kidneys (0.69 ± 0.02μg gm^{-1}) and heart (0.14 ± 0.01 μg gm^{-1}) on 21 day. Chlorpyrifos was detected in liver and kidney on 7^{th} day, while it could also be recovered from muscle on 14^{th} day which attained a maximum concentration in kidney (0.38± 0.08 μg gm^{-1}) followed by liver (0.35 ± 0.012μg gm^{-1}) and muscle (0.09 ± 0.005 μg gm^{-1}). It is worth mentioning that Chlorpyrifos could not be recovered from brain at any point of time which suggests that the insecticide has poor penetrability ofcentra nervous system. The concentraton of Cypermethrin and Chlorpyrifos was decreased in combined therapy compared to unitherapy (Table 3).

Discussion

Blood concentrations of Cypermethrin on different days were decreased in presence of chlorpyrifos. Cypermethrin increased serum AST, ALT activity and glucose level and decreased protein level compared to control. Manna *et* al [7] also reported that α-Cypermethrin, the active component of Cypermethrin caused hyperglycaemia, hypoproteinamia and increased activity of serum AST and ALT in rats after repeated daily oral administration for 30 days. Biochemical parameters like serum AST, ALT and glucose level were decreased in co-administration of Chlorpyrifos and Cypermethrin compared to individual administration. Blood and tissue residue of Chlorpyrifos and Cypermethrin in some important organs reveal that level of both insecticides decreased compared to uni-administration. Both Cypermethrin and Chlorpyrifos undergo metabolism in liver through microsomal oxidation. Manna *et al* [7] showed that α-cypermethrin is inhibitor of microsomal enzyme system in rats which may increase concentration and persistence for a longtime. Koley *et al* [8] reported that Chlorpyrifosis also inhibitor of microsomal oxidation in rat and goat. Combination of Chlorpyrifos and Cypermethrin may cause indauction of microsomal oxidation resulting in rapid metabolism of both leading to lower concentration in blood and tissues of mice. But no such study was carried out in mice therefore further study is warranted with lower level of insecticides.

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