

BIOCHEMICAL AND HISTOLOGICAL CHANGES IN RAT LIVER CAUSED BY CYPERMETHRIN AND BETA-CYFLUTHRIN

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Received in July 2012
CrossChecked in July 2012
Accepted in February 2013

Cypermethrin and beta-cyfluthrin are two most widely used multipurpose pyrethroids. After determining their oral LD₅₀ (416.98 mg kg⁻¹ and 354.8 mg kg⁻¹ body weight, respectively), we assessed their hepatotoxicity in Wistar rats following acute (0.1 LD₅₀ for 1 day) and sub-acute (0.1 LD₅₀ for 7, 14, 21 or 28 days) poisoning. The assessment was based on hepatic marker enzymes AST, ALT, LDH, ALP, glycogen, total proteins, total lipids, cholesterol, free fatty acids, and phospholipids. AST, ALT, LDH, total lipids, cholesterol, phospholipids, and free fatty acids in hepatic homogenate increased following pyrethroid stress. In contrast, hepatic proteins, glycogen, and ALP activity decreased due to lysis of structural proteins and leakage of enzymes into the blood stream. Biochemical data were consistent with histological alterations (cytoplasmic vacuolisation, nuclear polymorphism, eccentric nucleus, karyolysis, karyorrhexis, and sinusoidal dilation). Comparatively greater hepatocellular damage was noted in beta-cyfluthrin than in cypermethrin-treated rats, which is probably related to the fluorine atom in beta-cyfluthrin.

KEY WORDS: *albino rat, hepatotoxicity, histopathology, marker enzymes*

Pesticides are the most effective means of pest eradication all over the world, but their use has reached an alarming rate due to a number of adverse effects on non-target organisms (1-3). For the last few decades, pyrethroid pesticides have strengthened their place in the pesticide market for several uses. This enhanced use, however, affects more and more non-target species (4, 5). The situation has got even worse due to continuous growth of chemical and pesticide industry (6, 7). Research & development units of multinational companies keep synthesising new cyanoderivatives to counteract genetically modified, resistant pest species. Modifying pesticide structure and activity is the demand of the day.

The aim of this study was to establish hepatic toxicity of the most common, new-generation, type II pyrethroids cypermethrin and beta-cyfluthrin in Wistar rats.

MATERIALS AND METHODS

Experimental animals

The study included 75 eight-week old female Wistar rats from an inbred colony weighing (110±20) g and receiving standard rat pellet feed and water *ad libitum*. The rats were divided in three groups (cypermethrin, beta-cyfluthrin, and control), which were further divided in five sub-groups of five rats

each receiving either compound for 1 day (acute dose) or for 7, 14, 21, and 28 days (sub-acute doses, see Table 1). Controls were kept in identical conditions, but did not receive pesticide treatment.

The experiment was approved by the Ethics Committee of Dr B. R. Ambedkar University Department of Zoology, Agra, India.

Experimental compounds

Technical-grade cypermethrin and beta-cyfluthrin (95 % purity) were obtained from Bayer India Ltd., Mumbai, and their acute oral LD₅₀ was calculated as 416.98 mg kg⁻¹ and 354.8 mg kg⁻¹ body weight (b.w.), respectively, based on earlier research (3, 8, 9).

Dose administration and tissue collection

The animals were receiving pyrethroids orally. The acute, one-day dose of cypermethrin was 41.70 mg kg⁻¹ b.w. and of beta-cyfluthrin 35.48 mg kg⁻¹ b.w. (0.1 LD₅₀), while sub-acute doses were obtained by dividing the acute dose by the number of treatment days (Table 1).

The rats were killed the day after the last day of treatment, their liver excised immediately, placed in physiological saline (pH 7.4), mechanically homogenised, and then processed as per standard protocols for biochemistry tests, including aminotransferases (ALT and AST), ALP, LDH, hepatic glycogen, total proteins, total lipids, cholesterol, phospholipids, and free fatty acids following procedures described elsewhere (10-18). These biochemical tests were performed using related diagnostic kits and an automatic biochemistry analyser (Erba Diagnostics Mannheim GmbH, Germany).

Liver histology

Liver was fixed in Carnoy's fixative for five hours (19), washed, dehydrated, and embedded in paraffin wax (56 °C melting point). It was then cut in 5 µm sections and stained with haematoxylin and eosin to inspect for histoarchitectural changes using a Motic microscope at 400x and 1000x magnification (Motic Optical Ltd., China) (20, 21).

Statistical analysis

Biochemistry data were analysed for difference in means using the SPSS version 11.5 for Windows ANOVA, followed by Dunnett's test.

RESULTS

Biochemical changes

Rats treated with either cypermethrin or beta-cyfluthrin showed a significant increase in aminotransferase (AST and ALT) and dehydrogenase (LDH) activity and a decrease in hepatic phosphatases (ALP). They also showed an increase in liver total lipids, cholesterol, phospholipids, and free fatty acids and a drop in glycogen and total protein levels. These changes were more pronounced in rats treated with beta-cyfluthrin than with cypermethrin (Tables 2-11).

Histopathological liver changes

Histopathological examination of the liver from treated animals revealed various cellular and lobular abnormalities, including intralobular vein (ILV) membrane dilation, presence of hepatocytes in ILV, cytoplasmic vacuolisation, multinuclear cells, nuclear polymorphisms, nuclear vacuolisation, hepatocyte membrane damage, nuclear division, nuclear eccentricity, pyknosis, necrosis, and karyorrhexis.

These abnormalities were more pronounced in animals receiving beta-cyfluthrin acutely and sub-acutely (Figure 1a-v).

DISCUSSION

Mammalian liver, by virtue of its unique relationship with the gastrointestinal tract and its role in xenobiotic-metabolism, is a target organ of xenobiotic stress. Disturbed liver homeostasis under such stress is sufficient to alter normal body physiology of any organism (22). Liver is a hub for protein synthesis, regulating cell functions such as maintenance of cellular rigidity, flow management of material through cell membranes, catalysis of an extraordinary range of chemical reactions, regulation of metabolic concentration, and arrangement of nuclear material to control gene function (23, 24). Protein depletion observed in the present study due to the lysis of structural proteins is evident histologically as hepatocellular membrane damage, caused by the interference of experimental compounds and their toxic metabolic intermediates (3, 25-27).

Elevated ALT and AST in the present study point toward active utilisation of amino acids in energy-

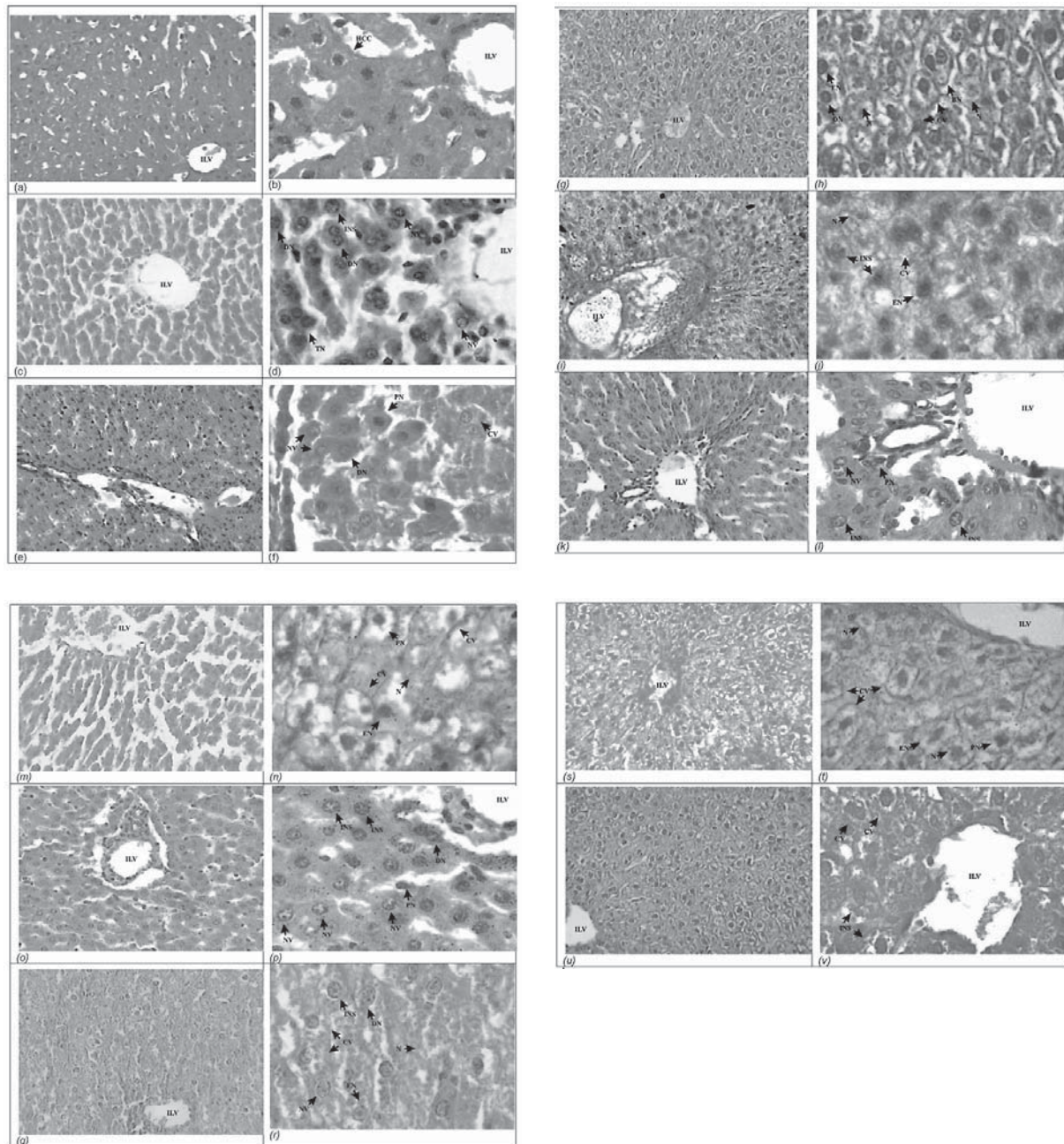


Figure 1a to 1v Histoarchitecture of Wistar rat liver; (a) control albino rat (400x); (b) control albino rat (1000x); (c) after acute (1-day) cypermethrin treatment (400x); (d) after acute (1-day) cypermethrin treatment (1000x); (e) after sub-acute (7-day) cypermethrin treatment (400x); after sub-acute (7-day) cypermethrin treatment (1000x); (g) after sub-acute (14-day) cypermethrin treatment (400x); after sub-acute (14-day) cypermethrin treatment (1000x); (i) after sub-acute (21-day) cypermethrin treatment (400x); (j) after sub-acute (21-day) cypermethrin treatment (1000x); (k) after sub-acute (28-day) cypermethrin treatment (400x); (l) after sub-acute (28ds) cypermethrin treatment (1000x); (m) after acute (1-day) beta-cyfluthrin treatment (400x); (n) after acute (1-day) beta-cyfluthrin treatment (1000x); (o) after sub-acute (7-day) beta-cyfluthrin treatment (400x); (p) after sub-acute (7-day) beta-cyfluthrin treatment (1000x); (q) after sub-acute (14-day) beta-cyfluthrin treatment (400x); (r) after sub-acute (14-day) beta-cyfluthrin treatment (1000x); (s) after sub-acute (21-day) beta-cyfluthrin treatment (400x); (t) after sub-acute (21-day) beta-cyfluthrin treatment (1000x); (u) after sub-acute (28-day) beta-cyfluthrin treatment (400x); (v) after sub-acute (28-day) beta-cyfluthrin treatment (1000x)

ILV = intralobular vein, HCC = hepatic cord cells, INS = irregular nuclear shapes, NV = nuclear vacuolization, DN = dividing nucleus, TN = trinucleate condition, PN = pycnotic nucleus, CV = cytoplasmic vacuolization, N = necrosis, ES = eccentric nucleus

Table 1 Oral administration of cypermethrin and beta-cyfluthrin as per treatment schedule

Days of treatment	Dose / mg kg ⁻¹ day ⁻¹	
	Cypermethrin	Beta-cyfluthrin
1	41.70	35.48
7	5.96	5.07
14	2.98	2.53
21	1.99	1.69
28	1.50	1.27

Table 2 Liver ALT (U L⁻¹) following acute and sub-acute cypermethrin and beta-cyfluthrin treatment

Type of dose	Days of treatment	Type of treatment	Dose / mg kg ⁻¹ day ⁻¹	Hepatic ALT Mean ± SD	F-value
Acute	1	Control	---	323.95±2.17	37.19***
		Cypermethrin	41.70	352.01±3.62***	
		Beta-cyfluthrin	35.48	359.29±3.20***	
Sub acute	7	Control	---	318.50±2.40	30.43***
		Cypermethrin	5.96	346.24±3.23***	
		Beta-cyfluthrin	5.07	351.08±3.76***	
	14	Control	---	319.54±3.04	17.22***
		Cypermethrin	2.98	344.78±4.15***	
		Beta-cyfluthrin	2.53	347.39±3.85***	
Sub acute	21	Control	---	322.82±1.73	9.50**
		Cypermethrin	1.99	339.91±4.00**	
		Beta-cyfluthrin	1.69	342.24±4.06**	
	28	Control	---	320.05±2.44	8.39**
		Cypermethrin	1.50	331.09±2.41*	
		Beta-cyfluthrin	1.27	335.91±3.45**	

*=*p*<0.05, **=*p*<0.01, ***=*p*<0.001 vs. control**Table 3** Liver AST (U L⁻¹) following acute and sub-acute cypermethrin and beta-cyfluthrin treatment

Type of dose	Days of treatment	Type of treatment	Dose / mg kg ⁻¹ day ⁻¹	Hepatic AST Mean ± SD	F-value
Acute	1	Control	---	267.48±3.38	22.64***
		Cypermethrin	41.70	296.33±4.88***	
		Beta-cyfluthrin	35.48	302.33±3.27***	
Sub acute	7	Control	---	272.03±2.56	17.22***
		Cypermethrin	5.96	298.42±5.14**	
		Beta-cyfluthrin	5.07	303.71±4.15***	
	14	Control	---	270.73±3.37	13.71***
		Cypermethrin	2.98	294.46±4.31**	
		Beta-cyfluthrin	2.53	298.97±4.52***	
Sub acute	21	Control	---	272.52±2.92	8.47**
		Cypermethrin	1.99	290.19±4.99*	
		Beta-cyfluthrin	1.69	297.34±4.96**	
	28	Control	---	268.58±3.79	6.40*
		Cypermethrin	1.50	280.53±4.82 ^{NS}	
		Beta-cyfluthrin	1.27	291.70±5.01**	

*=*p*<0.05, **=*p*<0.01, ***=*p*<0.001 vs. control

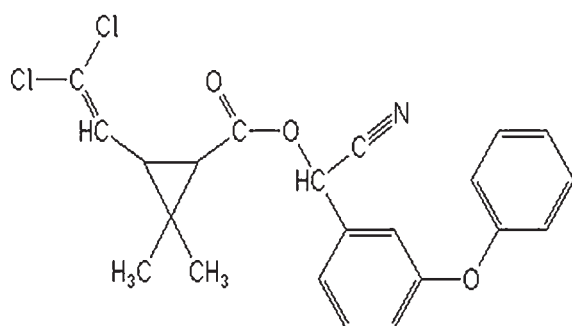


Figure 2 Structure of cypermethrin

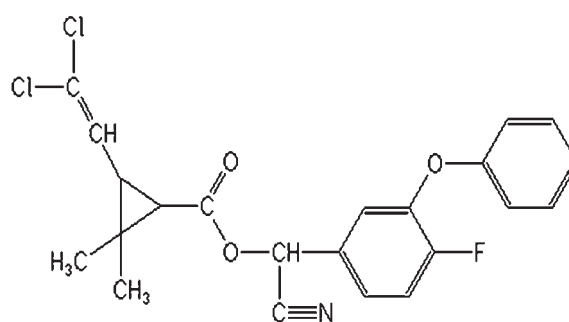


Figure 3 Structure of beta-cyfluthrin

Table 4 Liver ALP ($U L^{-1}$) following acute and sub-acute cypermethrin and beta-cyfluthrin treatment

Type of dose	Days of treatment	Type of treatment	Dose / $mg \cdot kg^{-1} \cdot day^{-1}$	Hepatic ALP Mean \pm SD	F-value
Acute	1	Control	---	527.31 \pm 3.81	
		Cypermethrin	41.70	503.99 \pm 4.18**	13.26***
		Beta-cyfluthrin	35.48	497.98 \pm 4.72***	
Sub acute	7	Control	---	526.64 \pm 4.41	
		Cypermethrin	5.96	504.31 \pm 3.95**	12.01**
		Beta-cyfluthrin	5.07	500.88 \pm 3.72**	
	14	Control	---	526.23 \pm 3.22	
		Cypermethrin	2.98	512.81 \pm 2.36**	12.59**
		Beta-cyfluthrin	2.53	508.46 \pm 2.12**	
21	Control	---	526.42 \pm 3.20		
	Cypermethrin	1.99	513.54 \pm 3.37*	8.32**	
	Beta-cyfluthrin	1.69	508.06 \pm 3.23**		
28	Control	---	523.88 \pm 5.06		
	Cypermethrin	1.50	508.83 \pm 3.27*	7.03**	
	Beta-cyfluthrin	1.27	502.43 \pm 3.94**		

*= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$ vs. control

Table 5 Liver LDH ($U L^{-1}$) following acute and sub-acute cypermethrin and beta-cyfluthrin treatment

Type of dose	Days of treatment	Type of treatment	Dose / $mg \cdot kg^{-1} \cdot day^{-1}$	Hepatic LDH Mean \pm SD	F-value
Acute	1	Control	---	709.46 \pm 2.51	
		Cypermethrin	41.70	730.71 \pm 3.45***	23.12***
		Beta-cyfluthrin	35.48	738.20 \pm 3.26***	
Sub acute	7	Control	---	710.51 \pm 3.56	
		Cypermethrin	5.96	729.94 \pm 3.39**	14.08***
		Beta-cyfluthrin	5.07	736.96 \pm 3.98***	
	14	Control	---	706.01 \pm 3.58	
		Cypermethrin	2.98	726.12 \pm 3.64**	12.63**
		Beta-cyfluthrin	2.53	732.70 \pm 4.46***	
21	Control	---	711.67 \pm 3.57		
	Cypermethrin	1.99	722.62 \pm 3.02 ^{NS}	8.11**	
	Beta-cyfluthrin	1.69	730.11 \pm 3.16**		
28	Control	---	711.92 \pm 3.94		
	Cypermethrin	1.50	716.74 \pm 3.12 ^{NS}	2.25 ^{NS}	
	Beta-cyfluthrin	1.27	722.04 \pm 3.00 ^{NS}		

*= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$ vs. control

Table 6 Liver glycogen (mg g⁻¹) following acute and sub-acute cypermethrin and beta-cyfluthrin treatment

Type of dose	Days of treatment	Type of treatment	Dose / mg·kg ⁻¹ day ⁻¹	Hepatic glycogen Mean ± SD	F-value
Acute	1	Control	---	6.36±0.06	15.31***
		Cypermethrin	41.70	5.95±0.07**	
		Beta-cyfluthrin	35.48	5.83±0.08***	
	7	Control	---	6.37±0.05	16.46***
		Cypermethrin	5.96	6.03±0.05**	
		Beta-cyfluthrin	5.07	5.96±0.05***	
Sub acute	14	Control	---	6.35±0.04	8.41**
		Cypermethrin	2.98	6.12±0.05*	
		Beta-cyfluthrin	2.53	6.08±0.05**	
	21	Control	---	6.40±0.04	6.54*
		Cypermethrin	1.99	6.24±0.04*	
		Beta-cyfluthrin	1.69	6.19±0.05**	
28	Control	---	6.34±0.05	1.05 ^{NS}	
	Cypermethrin	1.50	6.26±0.05 ^{NS}		
	Beta-cyfluthrin	1.27	6.25±0.05 ^{NS}		

*=p<0.05, **=p<0.01, ***=p<0.001 vs. control

Table 7 Liver total proteins (µg mL⁻¹) following acute and sub-acute cypermethrin and beta-cyfluthrin treatment

Type of dose	Days of treatment	Type of treatment	Dose / mg·kg ⁻¹ day ⁻¹	Hepatic total proteins Mean ± SD	F-value
Acute	1	Control	---	111.8±2.08	51.44***
		Cypermethrin	41.70	78.8±3.56***	
		Beta-cyfluthrin	35.48	70.6±3.28***	
	7	Control	---	109±2.51	24.59***
		Cypermethrin	5.96	81.6±3.61***	
		Beta-cyfluthrin	5.07	78.4±3.89***	
Sub acute	14	Control	---	109.2±1.93	21.98***
		Cypermethrin	2.98	91.63±3.19**	
		Beta-cyfluthrin	2.53	84.6±2.84***	
	21	Control	---	107.8±2.52	10.81**
		Cypermethrin	1.99	95.8±3.06*	
		Beta-cyfluthrin	1.69	89.8±2.76**	
28	Control	---	108.8±3.06	9.36**	
	Cypermethrin	1.50	96.6±2.34*		
	Beta-cyfluthrin	1.27	92.4±2.91**		

*=p<0.05, **=p<0.01, ***=p<0.001 vs. control

yielding metabolic processes such as gluconeogenesis. Aminotransferases are sensitive inductors of hepatocellular damage under oxidative stress caused by xenobiotics, which histologically presented as cytoplasmic vacuolisation, karyolysis, and karyorrhexis in this study. The increased activity of hepatic aminotransferases in our study reflects genetic abnormality in their production in order to overcome pyrethroid-induced oxidative stress (28-34).

The lower hepatic ALP may also be a consequence of cell membrane damage. ALP is an important

hepatocyte lysosomal enzyme with a crucial role in the metabolism and biosynthesis of energy macromolecules for different cellular functions in the liver, as it catalyses the splitting of phosphoric esters. Membrane damage in the present study might have caused leakage of this enzyme from hepatocytes into the blood stream. As a result, normal hepatocellular functions stopped, leading to pathological changes such as pyknosis and necrosis (33, 35-37).

Hepatic LDH is an important oxidative enzyme in carbohydrate metabolism and it catalyses the conversion

Table 8 Liver total lipids(mg g⁻¹ tissue) following acute and sub-acute cypermethrin and beta-cyfluthrin treatment

Type of dose	Days of treatment	Type of treatment	Dose / mg·kg ⁻¹ day ⁻¹	Hepatic total lipids	F-value
				Mean ± SD	
Acute	1	Control	---	47.11±1.63	3.77 ^{**}
		Cypermethrin	41.70	52.80±2.39 ^{NS}	
		Beta-cyfluthrin	35.48	55.17±2.31*	
Sub acute	7	Control	---	46.81±1.43	8.63 ^{**}
		Cypermethrin	5.96	59.27±3.31*	
		Beta-cyfluthrin	5.07	63.40±3.60 ^{**}	
	14	Control	---	47.36±1.95	10.89 ^{**}
		Cypermethrin	2.98	60.13±2.67 ^{**}	
		Beta-cyfluthrin	2.53	62.89±2.82 ^{**}	
	21	Control	---	47.63±2.54	4.82*
		Cypermethrin	1.99	59.07±3.87 ^{**}	
		Beta-cyfluthrin	1.69	62.18±3.89*	
28	Control	---	47.79±2.96	3.50 ^{**}	
	Cypermethrin	1.50	54.92±3.11 ^{NS}		
	Beta-cyfluthrin	1.27	59.42±3.33*		

*=p<0.05, **=p<0.01, ***=p<0.001 vs. control

Table 9 Liver cholesterol (mg per 100 mL) following acute and sub-acute cypermethrin and beta-cyfluthrin treatment

Type of dose	Days of treatment	Type of treatment	Dose / mg·kg ⁻¹ day ⁻¹	Hepatic cholesterol	F-value
				Mean ± SD	
Acute	1	Control	---	100.91±3.59	8.27 ^{**}
		Cypermethrin	41.70	121.92±5.24*	
		Beta-cyfluthrin	35.48	127.49±5.57 ^{**}	
Sub acute	7	Control	---	102.74±3.61	8.93 ^{**}
		Cypermethrin	5.96	126.20±6.01*	
		Beta-cyfluthrin	5.07	133.80±6.18 ^{**}	
	14	Control	---	102.60±2.94	21.39 ^{***}
		Cypermethrin	2.98	131.39±5.12 ^{**}	
		Beta-cyfluthrin	2.53	146.62±5.93 ^{***}	
21	Control	---	104.26±2.01	6.57*	
	Cypermethrin	1.99	115.19±3.56*		
	Beta-cyfluthrin	1.69	119.60±3.43 ^{**}		
28	Control	---	101.10±2.59	5.52*	
	Cypermethrin	1.50	108.78±2.89 ^{NS}		
	Beta-cyfluthrin	1.27	113.86±2.71*		

*=p<0.05, **=p<0.01, ***=p<0.001 vs. control

of pyruvate into lactate. Its enhanced activity under pesticide stress in the present study was caused by hypoxic conditions that shifted normal aerobic respiration towards anaerobic (5, 29, 33, 38).

The drop in hepatic glycogen was a consequence of abruptly increased catabolism to meet higher pyrethroid-induced energy demands. Undoubtedly, the hypoxic condition is responsible for incomplete energy output through glycolysis and Krebs's cycle. Hypoxia may be responsible for necrotic lesions (26-27, 39, 40-41).

Increased lipogenesis reflects abnormal carbohydrate metabolism. It led to excessive conversion of pyruvate to free fatty acid. Increased cholesterol is likely to have substantially contributed to the total lipid levels in treated rats (39, 42) and may have played a role in the significant increase in phospholipid content and abnormal ALP (37, 39, 42, 43).

Our findings suggest that both pesticides strongly disrupt normal hepatic function in rats. Hepatotoxic properties of cypermethrin have already been described in mice (44). The major finding of our

Table 10 Liver phospholipids (mg mL⁻¹) following acute and sub-acute cypermethrin and beta-cyfluthrin treatment

Type of dose	Days of treatment	Type of treatment	Dose / mg kg ⁻¹ day ⁻¹	Hepatic phospholipids Mean ± SD	F-value
Acute	1	Control	---	1.77±0.02	24.56***
		Cypermethrin	41.70	1.96±0.03***	
		Beta-cyfluthrin	35.48	2.01±0.03**	
Sub acute	7	Control	---	1.77±0.02	15.82***
		Cypermethrin	5.96	1.95±0.03**	
		Beta-cyfluthrin	5.07	2.00±0.04**	
	14	Control	---	1.79±0.02	15.30***
		Cypermethrin	2.98	1.90±0.02**	
		Beta-cyfluthrin	2.53	1.97±0.03**	
21	Control	---	1.79±0.02	5.26*	
	Cypermethrin	1.99	1.88±0.03*		
	Beta-cyfluthrin	1.69	1.90±0.03*		
28	Control	---	1.78±0.02	1.51 ^{NS}	
	Cypermethrin	1.50	1.81±0.02 ^{NS}		
	Beta-cyfluthrin	1.27	1.84±0.03 ^{NS}		

*= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$ vs. control**Table 11** Liver free fatty acids (mg g⁻¹) following acute and sub-acute cypermethrin and beta-cyfluthrin treatment

Type of dose	Days of treatment	Type of treatment	Dose / mg kg ⁻¹ day ⁻¹	Hepatic free fatty acids Mean ± SD	F-value
Acute	1	Control	---	0.75±0.01	22.00***
		Cypermethrin	41.70	0.93±0.03**	
		Beta-cyfluthrin	35.48	1.01±0.04***	
Sub acute	7	Control	---	0.77±0.01	11.76**
		Cypermethrin	5.96	0.88±0.03**	
		Beta-cyfluthrin	5.07	0.91±0.02**	
	14	Control	---	0.74±0.01	6.15*
		Cypermethrin	2.98	0.81±0.02*	
		Beta-cyfluthrin	2.53	0.83±0.03*	
21	Control	---	0.74±0.01	4.77 ^{NS}	
	Cypermethrin	1.99	0.79±0.02 ^{NS}		
	Beta-cyfluthrin	1.69	0.83±0.03*		
28	Control	---	0.75±0.01	1.01 ^{NS}	
	Cypermethrin	1.50	0.78±0.02 ^{NS}		
	Beta-cyfluthrin	1.27	0.78±0.02 ^{NS}		

*= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$ vs. control

experiment is that beta-cyfluthrin has a greater hepatotoxic potential than cypermethrin. The difference in toxicity between the two stems from differences in their structure, that is, to the presence of a fluorine atom in beta-cyfluthrin (Figures 2 and 3). Fluorinated hydrocarbons undergo limited biotransformation and can affect cell enzymes, cell-cell communication, membrane transport, and energy production (45). The increased toxic potential of fluorine is due to its unique chemical properties. The

fluorine atom has a Van der Waals radius of 1.35 Å, which is similar to oxygen (1.40 Å) and which makes fluorine isosterically similar to the hydroxyl group with which it shares some properties (46). In addition, fluorine has a higher electronegativity (4.0) than other halogens. Higher electronegativity strongly polarises the carbon-fluorine bond, making it difficult to break. This renders fluorinated hydrocarbons very stable and therefore more toxic (47).

Future studies could involve still higher mammalian groups with more changes at the level of side chains and groups, which would help to understand more complex structure-activity relationships.

REFERENCES

1. Yehia MAH, El-Banna SG, Okab AB. Diazinon toxicity affects histophysiological and biochemical parameters in rabbits. *Exp Toxicol Pathol* 2007;59:215-25.
2. Singh AK, Saxena PN, Sharma HN. Stress induced by beta-cyfluthrin, a type-2 pyrethroid, on brain biochemistry of albino rat (*Rattus norvegicus*). *Biol Med* 2009;1:74-86.
3. Bhushan B, Saxena N, Saxena PN. Beta-cyfluthrin induced histochemical alterations in the liver of albino rat. *Scand J Lab Anim Sci* 2010;37:61-6.
4. Rana N, Saxena N, Sharma HN, Saxena PN. Comparative genotoxicity of alpha-cyano pyrethroids on *Drosophila melanogaster*. *Entamon* 2008;33:135-8.
5. Fetoui H, Garoui EM, Zeghal N. Lambda-cyhalothrin-included biochemical and histopathological changes in the liver of rats: Ameliorative effect of ascorbic acid. *Exp Toxicol Pathol* 2009;61:189-96.
6. Khambay BPS. Pyrethroid insecticides. *Pestic Outlook* 2002;13:49-54.
7. Saxena PN, Tomar V. Assessment of comparative hemotoxicity of cybil and fenvalerate in *Rattus norvegicus*. *Bull Environ Contam Toxicol* 2003;70:839-46.
8. Finney DJ. Probit Analysis. 3rd ed. Cambridge: Cambridge University Press; 1971.
9. Singh VK, Saxena PN. Genotoxic potential of cypermethrin in mammalian haemopoietic system. *Him J Environ Zool* 2002;16:195-202.
10. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamyl oxalacetic acid glutamyl pyruvate transaminase. *Am J Clin Pathol* 1957;28:56-8.
11. Kind PRN, King EJ. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. *J Clin Pathol* 1954;7:322-6.
12. Commission Enzymologie de la Societ e Francaise de Biologie Clinique. Recommandations pour la mesure de la concentration catalytique de la lactate d eshydrog enase dans le s erum humain   + 30 C [Recommendations for the measurement of catalytic lactate dehydrogenase concentration in human serum at 30  C, in French]. *Ann Biol Clin* 1982;40:123-8.
13. Montgomery R. The determination of glycogen. *Arch Biochem Biophys* 1957;67:378-86.
14. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951;193:265-75.
15. Folch J, Lees M, Stanley GHS. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 1957;226:497-509.
16. Zlatkis A, Zac BB, Boy GJB. A colorimetric method for determination of cholesterol. *J Lab Clin Med* 1953;41:486-96.
17. Zilversmith DB, Davis AK, Memphis BS. Microdetermination of plasma phospholipids by trichloroacetic acid precipitation. *J Lab Clin Med* 1950;35:155-60.
18. Soloni F, Sardina LC. Colorimetric microdetermination of free fatty acids. *Clin Chem* 1973;19:419-29.
19. Gatenby JB, Beams HM. The Microtometist's Vade-Mecum. 11th ed. London: Churchill; 1950.
20. Humason GL. Animal Tissue Techniques. 4th ed. San Francisco (CA): Freeman and Company; 1979.
21. Pearse AGE. Histochemistry: Theoretical and Applied. Vol. I. London: Churchill Livingstone; 1980.
22. Hinton RH, Grasso P. Hepatotoxicity. In: Ballantyne B, Marrs T, Syversen T, editors. General and applied toxicology. Vol. 2. New York (NY): Groves Dictionaries Inc.; 2000. p. 853-92.
23. Tortora GJ, Grabowski SR. The digestive system. In: Principles of anatomy and physiology. 10th ed. New York (NY): John Wiley & Sons. Inc.; 2003. p. 851-903.
24. Lodish H, Berk A, Zipursky SL, Matsudaria P, Baltimore D, Darnell J. Molecular Cell Biology. 4th ed. New York (NY): W.H. Freeman and Company; 2000.
25. Sakr SA, Okdah YA, El-Abd SF. Gibberellin A₃ induced histological and histochemical alterations in the liver of albino rats. *Science Asia* 2003;29:327-31.
26. Sakr SA, Abdel Samei HA, Soliman ME. Exploring hepatotoxicity of benomyl: histological and histochemical study of albino rats. *J Med Sci* 2004;4:77-83.
27. Omotuyi IO, Oluyemi KA, Omofoma CO, Josiah SJ, Adesanya OA, Saalu LC. Cyfluthrin induced hepatotoxicity in rats. *Afr J Biotechnol* 2006;5:1909-12.
28. El-Tawil OS, Abdel-Rehman MS. The role of enzyme induction and inhibition on cypermethrin hepatotoxicity. *Pharmacol Res* 2001;44:33-40.
29. Kumar K, Saxena PN. Diazol induced liver transaminase and lactate dehydrogenase activity in female rats. *Proc Acad Environ Biol* 2001;9:131-3.
30. El-Demerdash FM, Yousef MI, Kedwany FS, Baghdadi HH. Role of alpha-tocopherol and beta-carotene in ameliorating the fenvalerate induced changes in oxidative stress, haematobiochemical parameters and serum quality of male rats. *J Environ Sci Health B* 2004;39:443-59.
31. Doneriya R, Saxena PN. Hepatopathological changes in pyrethroid toxicity in rats. *Rattus norvegicus*. *Indian J Environ Toxicol* 2004;14:86-91.
32. Ksheerasagar RL, Kaliwal BB. Histological and biochemical changes in the liver of albino mice on exposure to insecticide cabosulfan. *Caspian J Env Sci* 2006;4:67-70.
33. Arshad N, Shabbir G, Aleem S, Arshad M. Effect of  -Tocopherol on liver biochemistry of endosulfan intoxicated mice; A preliminary study. *Asian J Exp Sci* 2007;21:239-46.
34. Afshar S, Heidari R, Fashid AA, Ilkhanipour M. Oral toxicity of fenitrothion in wistar rats: A biochemical and histopathological study. *Int J Appl Environ Sci* 2008;3:185-93.
35. Singh VK, Saxena PN. Effect of cybil (cypermethrin 25 EC) and cybil-sevin (carbaryl 50 EC) combination on liver and serum phosphatases in wistar albino rats. *J Ecophysiol Occup Health* 2001;1:229-34.
36. Singh VK, Dixit P, Saxena PN. Cybil induced hepatobiochemical changes in wistar rats. *J Environ Biol* 2005;26:725-7.
37. Pereira C, Mapuskar K, Rao CV. Chronic toxicity of diethyl phthalate in male wistar rats a dose response study. *Regul Toxicol Pharmacol* 2006;45:169-77.

38. Kurutas EB, Doran F, Ciralik H. The effect of endosulfan on lactic dehydrogenase enzyme system in liver of *Mus musculus*: A histochemical study. *Eur J Gen Med* 2006;3:148-51.
39. Saxena PN, Doneriya R. Hepatobiochemical response in albino rat following oral administration of cybil and hafen. *Toxicol Int* 2004;11:23-6.
40. Manna S, Bhattacharya D, Mandal TK, Das S. Repeated dose toxicity of deltamethrin in rats. *Indian J Phrmacol* 2005;37:160-4.
41. Rezg R, Mornagui B, Kamoun A, El-Fazza S, Gharbi N. Effect of subchronic exposure to matathion on metabolic parameters in rats. *CR Biol* 2007;330:143-7.
42. Saxena PN, Kumar K. Assessment of liver lipid profile in female rats after diazot intoxication. *Indian J Environ Toxicol* 2001;11:71-2.
43. Parimala MD, Kaliwal BB. Dose-dependent estrous cycle ovarian follicles and biochemical content reversal in albino mice after exposure to mancozeb. *Caspian J Env Sci* 2005;3:118-31.
44. Đikić D, Mojsović-Ćuić A, Čupor I, Benković V, Horvat-Knežević A, Lisičić D, Oršolić N. Carbendazim combined with imazalil or cypermethrin potentiate DNA damage in hepatocytes of mice. *Hum Exp Toxicol* 2012;5:492-505.
45. Key BD, Howell RD, Criddle CS. Fluorinated organics in the biosphere. *Environ Sci Technol* 1997;31:2445-54.
46. Finar IL. *Organic Chemistry. Vol. I. The Fundamental Principles*. London: Longman Publishing Group; 1998.
47. Bhushan B. Comparative hepatotoxicity under stress of cypermethrin and beta-cyfluthrin in albino rat. [PhD thesis.]. Agra: Dr. B.R. Ambedkar University; 2011.

Sažetak

BIOKEMIJSKE I HISTOLOŠKE PROMJENE U JETRIMA ŠTAKORA UZROKOVANE CIPERMETRINOM I BETA-CIFLUTRINOM

Primjena piretroida cipermetrina i beta-ciflutrina veoma je raširena diljem svijeta. Nakon što smo odredili njihov LD_{50} ($416,98 \text{ mg kg}^{-1}$, odnosno $354,8 \text{ mg kg}^{-1}$ tjelesne mase) ispitali smo njihovu toksičnost u jetrima Wistar štakora koji su primili jednokratnu akutnu ($0,1 LD_{50}$), odnosno odgovarajuće subakutne doze pesticida ($0,1 LD_{50}$ kumulativno tijekom 7, 14, 21, odnosno 28 dana). Za markere toksičnosti uzeli smo jetrene enzime AST, ALT, LDH, ALP, glikogen, ukupne proteine, ukupne lipide, kolesterol, slobodne masne kiseline te fosfolipide. Razine AST-a, ALT-a, LDH-a, ukupnih lipida, kolesterola, fosfolipida i slobodnih masnih kiselina u homogenatu jetara bile su povišene u štakora izloženih piretroidima u odnosu na kontrolne štakore. S druge strane, razine proteina, glikogena i ALP-a bile su niže, vjerojatno zbog lize strukturnih proteina i curenja enzima u krvotok. Biokemijski nalazi potvrdili su histološke promjene na jetrima poput vakuolizacije citoplazme, polimorfizama jezgara, ekscentričnih jezgara, kariolize, karioreksije i sinusoidnih proširenja. Beta-ciflutrin se pritom pokazao toksičnijim od cipermetrina, što je vjerojatno povezano s prisutnosti atoma fluora u beta-ciflutrinu.

KLJUČNE RIJEČI: *enzimski markeri, hepatotoksičnost, histopatologija, Wistar štakori*

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