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Serum biochemical responses under stress of cypermethrin in albino rat

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Abstract

Toxicity due to acute (1 day) and subchronic (7, 14, 21 and 28 days) orally administrated doses of cypermethrin in albino rats was evaluated using serum biochemical parameters (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, total lipids, phospholipids, glycerides, total proteins, cholesterol and bilirubin). The parameters had significantly higher values in treated rats with cypermethrin-associated toxicity. One more set was run simultaneously for 22 more days following 28 days of subchronic cypermethrin intoxication to evaluate possible recovery. In the recovery period, most of the values reached normal level pointing towards degradation of cypermethrin in the treated animals.

Key words: albino rats, blood biochemistry, cypermethrin, pyrethroid toxicity.
Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase.

Introduction

Synthetic pesticides have become an integral component of various pest eradication programmes for modern farming, various vector borne diseases and household pests. Pyrethroid pesticides represent a major class of very effective multipurpose chemicals, accounting for about 30% of global insecticidal market. Newly designed analogues have been synthesized and launched periodically, boosted up with enhanced potential against pre-existing pests, and those that have become resistant (Bian et at. 2004; Abdou et al. 2009; Assayed et al. 2010; Bhushan et al. 2010; El-Magd et al. 2011; Adjrah et al. 2013).

Pyrethroid pesticides are broadly divided into type I and II, depending upon absence and presence of an alpha-cyano group, respectively, and their produced behavioural changes (Sayim et al. 2005; Spencer et al. 2005; Wolansky et al. 2006; Saka et al. 2011). Pyrethroids are considered as comparatively safe pesticides, but their increased utility due to enhanced toxic potential and easy biodegradability, necessitate non-target toxicity assessment (Aldana et al. 2001; Salama et al. 2005; Addy-Orduna et al. 2011). Cypermethrin is a promising type II pyrethroid pesticide, used widely in developing and undeveloped nations for almost every aspect of pest control, either alone or in combination (Singh, Saxena 2001). Cypermethrin is a universally used pesticide, and therefore has maximum chance of accumulating in various food chains and thus imparting related toxicity (Anwar 2004; Yavasoglu et al. 2006; Rana et al. 2008; Grewal et al. 2009; Muthuviveganandavel et al. 2011; Bhushan et al. 2013; Sangha et al. 2013).

The aim of the present study was to evaluate toxicity of cypermethrin on albino rats, by estimation of serum total lipids, phospholipids, glycerides, total proteins, cholesterol, total bilirubin and the enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH). In addition, the study was extended up to concomitant recovery phase of twenty two more days following twenty eight days of subchronic cypermethrin exposure, to assess possible recovery from intoxication.

Materials and methods

The present study was conducted with 72 male albino rats, *Rattus norvegicus* (Berkenhout), of an inbreed colony, acclimatized for two weeks to laboratory conditions prior to dose administration. The animals were maintained under standard conditions of light and temperature, provided standard rat pellet feed and water ad libitum throughout the study.

Technical grade cypermethrin (95% purity), obtained from Rallis Indian Ltd., Mumbai, was used as experimental compound. Its calculated LD_{50} was 433.6 mg kg^{-1} b.wt. (Finney 1971; Pande 2001). After acclimatization, albino rats were divided into six sets, one corresponding to acute treatment (300 mg kg^{-1} b.wt. of cypermethrin dissolved in groundnut oil for 1 day through oral route by gavage tube) and four sets corresponding to subchronic doses (10.7 mg kg^{-1} b.wt. of cypermethrin dissolved in groundnut oil for 7,

14, 21 and 28 days respectively through oral route by gavage tube) and last group for recovery evaluation. Animals corresponding to the recovery set were orally administrated 10.7 mg kg^{-1} b.wt. cypermethrin through oral route by gavage tube for 28 days initially and then were kept without the dose for the next 22 days. Control sets corresponding to each treatment set and containing an equal number of rats i.e. six, were used simultaneously. The control rats were orally administered groundnut oil only.

Experimental albino rats were sacrificed after predetermined time intervals, blood samples were collected from ventricle into test tubes, mixed with EDTA, kept for 30 min at room temperature and centrifuged at 3000 rpm for 20 min to separate out serum. The following biochemical parameters were analyzed in serum: total serum lipids (Folch et al. 1957), serum triglycerides (McGowan 1980), serum phospholipids (Zilversenith et al. 1950), cholesterol (Wybenga et al. 1970), total bilirubin (Malloy, Evelyn 1937), total proteins (Dumas et al. 1971), alanine aninotransferase (ALT), aspartate aminotransferase (AST)(Reitman, Frankel 1957), alkaline phosphatase (Kind, Kin 1954) and lactate dehydrogenase (King 1965). Significant differences in values between control and treatment groups were determined using Student's *t*-test (Fisher, Yates 1963).

Results

All of the considered biochemical components significantly increased following cypermethrin intoxication in comparison to control values. Serum total lipids were found to increase significantly, up to maximum level following subchronic (14 days) cypermethrin intoxication, but the levels normalized in the 22-day recovery period (Table 1). Serum triglyceride concentration slightly increased following sub chronic (28 days) cypermethrin intoxication, but reached the normal level in the experimental recovery phase (Table 2). There was an increase in serum phospholipid concentration following acute (1 day) and subchronic (28 days) cypermethrin intoxication, but the difference with the level after 22-day recovery was non-significant (Table 3). Serum cholesterol showed an increasing trend for all cypermethrin doses: the maximum difference was in the case of subchronic exposures, and concentration also reached a normal level in the recovery phase (Table 4). Serum bilirubin concentration increased significantly in subchronically cypermethrin-treated rats, but differed non-significantly in rats of the acute and recovery sets (Table 5). Serum total protein concentration showed significantly increasing trend in all cypermethrin-treated sets, and reached a normal level in the recovery period (Table 6).

A significant increase of serum AST activity was observed in all cypermethrin-treated rats, but the value normalised in the recovery period (Table 7). Serum ALT values were significantly increased in cypermethrin-treated rats, maximum in the case of subchronic intoxication, and normal after recovery (Table 8). There was an increase in serum ALP activity following all doses of cypermethrin intoxication, maximum in the case of sub chronic doses; the levels reached those of control rats after 22 days of recovery (Table 9). LDH activity in serum of subchronically cypermrthin-treated rats significantly increased in comparison to control rats, but the difference was non-significant after 22 days of recovery (Table 10).

Discussion

Blood is an easily available fluid that can be used as an important diagnostic tool to assess toxicity of any xenobiotic, including pyrethroid pesticides. Almost every living tissue is exposed to this fluid for exchange of material. Blood is essential for survival. Therefore, alteration of any

Table 1. Effect of cypermethrin on serum total lipids (mg dL^{-1}) in *Rattus norvegicus*. Control were given groundnut oil only. Recovery group were given oil (11) and cypermethrin (12) treatment for 28 days only and effects were assessed after 60 days. Number of rats analyzed for every treatment were six

No.	Treatment	Treatment duration (days)	Dose (mg kg^{-1} b.w.)	Range	Mean ± SE	Change (%)	Significance level
1	Control	1	-	220.7 – 262.3	245.2 ± 2.09		
2	Acute	1	300	215.0 – 273.2	248.9 ± 1.23	+1.5	$P > 0.05$
3	Control	7	-	225.1 – 261.6	249.3 ± 0.91		
4	Subchronic	7	10.7	250.5 – 261.3	256.0 ± 1.85	+2.7	$P > 0.05$
5	Control	14	-	212.0 – 264.7	247.9 ± 2.39		
6	Subchronic	14	10.7	233.2 – 285.7	259.1 ± 2.11	+4.5	$P < 0.01$
7	Control	21	-	214.5 – 264.7	248.3 ± 1.47		
8	Subchronic	21	10.7	221.3 – 278.5	254.1 ± 1.21	+2.3	$P < 0.05$
9	Control	28	-	216.6 – 264.7	249.4 ± 1.30		
10	Subchronic	28	10.7	231.0 – 262.4	256.2 ± 1.86	+2.7	$P < 0.05$
11	Recovery control	28 (60)	-	209.6 – 259.5	243.0 ± 1.72		
12	Recovery treated	28 (60)	10.7	212.3 – 261.7	246.8 ± 1.60	+1.4	$P > 0.05$

Table 2. Effect of cypermethrin on serum triglyceride level (mg dL^{-1}) in *Rattus norvegicus*. Control were given groundnut oil only. Recovery group were given oil (11) and cypermethrin (12) treatment for 28 days only and effects were assessed after 60 days. Number of rats analyzed for every treatment were six

No.	Treatment	Treatment duration (days)	Dose (mg kg^{-1} b.w.)	Range	Mean ± SE	Change (%)	Significance level
1	Control	1	–	52.3 – 81.4	67.2 ± 1.54		
2	Acute	1	300	60.5 – 73.1	65.6 ± 2.04	–2.4	$P > 0.05$
3	Control	7	–	40.5 – 94.3	67.1 ± 0.38		
4	Subchronic	7	10.7	53.0 – 86.2	69.4 ± 1.96	+3.4	$P > 0.05$
5	Control	14	–	60.1 – 76.2	70.1 ± 2.19		
6	Subchronic	14	10.7	57.2 – 89.5	72.0 ± 1.98	+2.7	$P > 0.05$
7	Control	21	–	56.8 – 95.7	71.5 ± 1.61		
8	Subchronic	21	10.7	68.4 – 99.1	74.2 ± 1.82	+3.8	$P > 0.05$
9	Control	28	–	65.2 – 87.8	68.5 ± 1.11		
10	Subchronic	28	10.7	58.5 – 77.8	73.6 ± 1.29	+7.4	$P < 0.05$
11	Recovery control	28 (60)	–	63.3 – 74.0	68.8 ± 1.95		
12	Recovery treated	28 (60)	10.7	55.1 – 93.4	70.4 ± 1.69	+2.3	$P > 0.05$

Table 3. Effect of cypermethrin on serum phospholipid level (mg dL^{-1}) in *Rattus norvegicus*. Control were given groundnut oil only. Recovery group were given oil (11) and cypermethrin (12) treatment for 28 days only and effects were assessed after 60 days. Number of rats analyzed for every treatment were six

No.	Treatment	Treatment duration (days)	Dose (mg kg^{-1} b.w.)	Range	Mean ± SE	Change (%)	Significance level
1	Control	1	–	105.2 – 131.5	117.8 ± 1.0		
2	Acute	1	300	99.3 – 133.4	121.3 ± 0.7	+3.00	$P < 0.05$
3	Control	7	–	109.6-– 153.1	122.3 ± 1.0		
4	Subchronic	7	10.7	112.5 – 137.1	124.8 ± 0.8	+2.0	$P < 0.05$
5	Control	14	–	100.0 – 136.2	123.4 ± 1.0		
6	Subchronic	14	10.7	94.2 – 168.5	125.1 ± 0.9	+1.4	$P > 0.05$
7	Control	21	–	97.8 – 155.0	123.9 ± 1.4		
8	Subchronic	21	10.7	103.2 – 149.8	125.8 ± 1.0	+1.5	$P > 0.05$
9	Control	28	–	105.5 – 132.4	119.1 ± 1.2		
10	Subchronic	28	10.7	110.0 – 148.4	123.3 ± 1.2	+3.5	$P < 0.05$
11	Recovery control	28 (60)	–	121.2 – 127.9	124.3 ± 1.2		
12	Recovery treated	28 (60)	10.7	100.2 – 146.7	123.6 ± 0.8	+0.6	$P > 0.05$

component of blood can be assessed by evaluating serum biochemistry, provided the appropriate parameters are considered (Stonard, Evans 1999; Fetoui et al. 2008).

Cypermethrin induced toxicity manifested its activity by elevated activity of enzymes AST, ALT, ALP and LDH along with those of other molecules in serum of cypermethrin intoxicated rats.

ALT activity is related to general hepatocellular and AST to mitochondrial damage. Increased aminotransferase (AST and ALT) activity in serum reflects hepatocellular damage under cypermethrin stress, leading to leakage of these enzymes into general circulation (Manna et al. 2004; Attia, Nasr 2009; Prakash et al. 2009; El-Magd et al. 2011; Nair et al. 2011; Bhushan et al. 2013). Further, liver is a prime organ associated with xenobiotic metabolism (Hinton and Grasso 2000). Perhaps, production of metabolically toxic intermediates capable of causing hepatocellular damage occurs during processing of cypermethrin in liver, causing respective leakage of these enzymes in blood (Anadon et al. 1995; Bhushan et al. 2010).

Increase in activity of serum alkaline phosphatase (ALP) in the present study can be attributed initially to some patho-physiological changes in liver as a consequence of pesticide intoxication, probably due to damage in membrane permeability of hepatocytes, resulting in leakage of this enzyme into the blood stream, as cypermethrin is capable of altering normal hepatocellular architecture (Gupta et al. 1991; Yavasoglu et al. 2006; Yadav 2010; Bhushan et al. 2013). Further, ALP is excreted through liver via bile juice; any obstruction in biliary tract (cholestasis)

Table 4. Effect of cypermethrin on serum cholesterol level (mg dL^{-1}) in *Rattus norvegicus*. Control were given groundnut oil only. Recovery group were given oil (11) and cypermethrin (12) treatment for 28 days only and effects were assessed after 60 days. Number of rats analyzed for every treatment were six

No.	Treatment	Treatment duration (days)	Dose (mg kg^{-1} b.w.)	Range	Mean ± SE	Change (%)	Significance level
1	Control	1	–	198.2 – 153.4	120.2 ± 0.8		
2	Acute	1	300	100.0 – 159.3	124.7 ± 1.6	+3.7	$P < 0.05$
3	Control	7	–	95.0 – 127.2	113.5 ± 1.2		
4	Subchronic	7	10.7	105.3 – 133.5	118.3 ± 1.3	+4.2	$P < 0.01$
5	Control	14	–	195.2 – 141.0	118.2 ± 0.9		
6	Subchronic	14	10.7	101.3 – 147.2	124.5 ± 1.1	+5.3	$P < 0.01$
7	Control	21	–	106.7 – 129.0	113.3 ± 2.3		
8	Subchronic	21	10.7	102.5 – 158.7	125.1 ± 1.0	+10.4	$P < 0.01$
9	Control	28	–	122.1 – 128.5	125.5 ± 1.0		
10	Subchronic	28	10.7	125.4 – 160.1	143.4 ± 2.5	+14.3	$P < 0.01$
11	Recovery control	28 (60)	–	106.2 – 132.3	119.4 ± 1.1		
12	Recovery treated	28 (60)	10.7	109.5 – 129.3	117.2 ± 1.0	–1.84	$P > 0.05$

Table 5. Effect of cypermethrin on serum bilirubin level (mg dL^{-1}) in *Rattus norvegicus*. Control were given groundnut oil only. Recovery group were given oil (11) and cypermethrin (12) treatment for 28 days only and effects were assessed after 60 days. Number of rats analyzed for every treatment were six

No.	Treatment	Treatment duration (days)	Dose (mg kg^{-1} b.w.)	Range	Mean ± SE	Change (%)	Significance level
1	Control	1	–	0.3 – 0.7	0.4 ± 0.06		
2	Acute	1	300	0.4 – 0.8	0.5 ± 0.07	+25.0	$P > 0.05$
3	Control	7	–	0.4 – 0.8	0.6 ± 0.05		
4	Subchronic	7	10.7	0.6 – 0.8	0.7 ± 0.14	+16.7	$P > 0.05$
5	Control	14	–	0.3 – 0.6	0.5 ± 0.05		
6	Subchronic	14	10.7	0.7 – 0.9	0.7 ± 0.03	+40.0	$P < 0.01$
7	Control	21	–	0.3 – 0.7	0.4 ± 0.06		
8	Subchronic	21	10.7	0.8 – 1.2	0.9 ± 0.06	+125.0	$P < 0.01$
9	Control	28	–	0.4 – 0.6	0.5 ± 0.04		
10	Subchronic	28	10.7	0.9 – 1.3	1.1 ± 0.07	+120.0	$P < 0.001$
11	Recovery control	28 (60)	–	0.4 – 0.5	0.4 ± 0.02		
12	Recovery treated	28 (60)	10.7	0.4 – 0.6	0.5 ± 0.03	+25.0	$P > 0.05$

impedes its excretion. Cholestasis leads to regurgitation of enzymes due to obstruction of its passage through the bile duct. The back pressure results in leaching of the enzyme into blood, raising its concentration. Similar explanation can be suggested for biliary impairment. Increased activity of hepatic AST, ALT and LDH along with a rise in serum bilirubin level, indicate cholestasis leading to enhanced levels of ALP in blood (Pande 2001). Hepatic tissue destruction (necrosis), in addition might have accounted for increase in serum levels of ALP. Hepatic parenchymal cells are degenerated and necrosed due to pesticide treatment, thereby releasing enzymes into the circulating blood stream, resulting in increased levels (Singh, Saxena 2001; Manna et al. 2004; Yavasoglu et al. 2006; Attia and Nasr 2009; Ahmed et al. 2011; Bhushan et al. 2013).

Elevation in serum level of LDH is an outcome of tissue necrosis, particularly of liver (Pande 2001). Further, cypermethrin is also known to cause haemolytic anaemia resulting in lysis of the red blood cell membrane, which also causes enhanced LDH levels in serum of cypermethrin-intoxicated rats (Manna et al. 2004; Fetoui et al. 2008; Attia, Nasr 2009; Nair et al. 2010).

Type II pyrethroid pesticides have strong genotoxic potential and can cause hypoxic conditions in liver of intoxicated rats, which additionally contributes to over expression of these enzymes (AST, ALT, ALP and LDH) at the gene level in respective cells (El-Tawil, Abdel-Rehman 2001; Singh, Saxena 2002; Rana et al. 2008; Bhushan et al. 2010).

Bilirubin is formed from haemoglobin in the reticulo-

Table 6. Effect of cypermethrin on serum total protein level (mg dL^{-1}) in *Rattus norvegicus*. Control were given groundnut oil only. Recovery group were given oil (11) and cypermethrin (12) treatment for 28 days only and effects were assessed after 60 days. Number of rats analyzed for every treatment were six

No.	Treatment	Treatment duration (days)	Dose (mg kg^{-1} b.w.)	Range	Mean ± SE	Change (%)	Significance level
1	Control	1	–	6.4 – 7.1	6.8 ± 0.1		
2	Acute	1	300	7.1 – 8.3	7.3 ± 0.8	+6.9	$P < 0.05$
3	Control	7	–	6.8 – 7.9	7.2 ± 0.2		
4	Subchronic	7	10.7	6.5 – 8.2	7.2 ± 1.0	+0.7	$P < 0.05$
5	Control	14	–	6.4 – 7.0	6.7 ± 0.1		
6	Subchronic	14	10.7	7.0 – 7.3	7.1 ± 0.7	+6.9	$P < 0.05$
7	Control	21	–	6.4 – 6.9	6.7 ± 0.1		
8	Subchronic	21	10.7	8.2 – 9.7	8.8 ± 1.0	+31.1	$P < 0.05$
9	Control	28	–	6.8 – 7.4	7.1 ± 0.1		
10	Subchronic	28	10.7	8.4 – 12.9	10.7 ± 0.8	+52.1	$P < 0.01$
11	Control	28 (60)	–	6.4 – 6.8	6.7 ± 0.1		
12	Treated	28 (60)	10.7	6.4 – 7.4	6.8 ± 0.1	+2.4	$P < 0.05$

Table 7. Effect of cypermethrin on serum aspartate aminotransferase activity (U L^{-1}) in *Rattus norvegicus*. Control were given groundnut oil only. Recovery group were given oil (11) and cypermethrin (12) treatment for 28 days only and effects were assessed after 60 days. Number of rats analyzed for every treatment were six

No.	Treatment	Treatment duration (days)	Dose (mg kg^{-1} b.w.)	Range	Mean ± SE	Change (%)	Significance level
1	Control	1	–	108.0 – 131.5	119.6 ± 0.6		
2	Acute	1	300	109.5-– 147.2	124.3 ± 1.4	+3.9	$P < 0.05$
3	Control	7	–	100.1 – 152.2	121.4 ± 0.4		
4	Subchronic	7	10.7	111.5 – 141.3	126.2 ± 1.7	+4.0	$P < 0.05$
5	Control	14	–	119.5 – 135.3	123.7 ± 1.3		
6	Subchronic	14	10.7	123.6 – 132.7	127.6 ± 1.8	+3.2	$P > 0.05$
7	Control	21	–	108.8 – 133.5	120.8 ± 0.9		
8	Subchronic	21	10.7	122.5 – 159.6	136.0 ± 1.1	+12.6	$P < 0.001$
9	Control	28	–	116.7 – 121.7	120.1 ± 0.9		
10	Subchronic	28	10.7	125.2 – 158.0	141.1 ± 2.2	+17.5	$P < 0.001$
11	Recovery control	28 (60)	–	108.0 – 133.4	121.2 ± 0.9		
12	Recovery treated	28 (60)	10.7	119.2 – 127.0	123.5 ± 1.5	+1.9	$P > 0.05$

endothelial system, and then circulates attached to plasma albumen. Approximately 80% of circulating bilirubin is derived from red blood cells; the remaining 20% bilirubin is formed from ineffective erythropoiesis resulting from destruction of erythroid cells in bone marrow (Tortora, Grobawiski 2003).

Increased destruction of red blood cells releases increased amounts of haemoglobin, and the subsequent rise in bilirubin content of blood causes hyperbilirubinemia. Water insoluble bilirubin attached to plasma albumen that is later taken up by hepatocytes requires dissociation of bilirubin-albumen complex for transport across the hepatocytes and binding to ligand in the hepatocytes. Pesticide-induced disruption of this phase of bilirubin along-with disturbed membrane integrity of hepatocytes following cypermethrin toxicity might also affect transport and binding of bilirubin. Further, bilirubin within the hepatocytes is conjugated with glucuronic acid, which is excreted in the bile in water soluble form. The conjugation requires hepatic glucoronosyl transferase activity. Cypermethrin-induced inhibition of this enzyme might also have contributed towards hyperbilirubinemia in cypermethrin intoxicated rats (Pande 2001).

Since liver plays a key role in bilirubin metabolism, any damage to liver cells, which probably was inflammation in the present study resulting in disturbed bile excretion, might be responsible for hyperbiliribinemia in cypermethrin intoxicated rats (Vaittnen et al. 1995; Rani, Dua 1999; Singh et al. 2005).

Cypermethrin has a profound influence on proteins

Table 8. Effect of cypermethrin on serum alanine aminotransferase activity (U L^{-1}) in *Rattus norvegicus*. Control were given groundnut oil only. Recovery group were given oil (11) and cypermethrin (12) treatment for 28 days only and effects were assessed after 60 days. Number of rats analyzed for every treatment were six

No.	Treatment	Treatment duration (days)	Dose (mg kg^{-1} b.w.)	Range	Mean ± SE	Change (%)	Significance level
1	Control	1	–	22.5 – 46.2	34.6 ± 0.8		
2	Acute	1	300	30.1 – 38.7	35.4 ± 1.5	+2.31	$P > 0.05$
3	Control	7	–	20.3 – 45.5	32.5 ± 1.2		
4	Subchronic	7	10.7	30.5 – 48.5	38.0 ± 1.8	+16.92	$P < 0.05$
5	Control	14	–	31.2 – 47.0	34.4 ± 1.0		
6	Subchronic	14	10.7	25.3 – 58.2	42.6 ± 2.3	+23.83	$P < 0.05$
7	Control	21	–	28.2 – 47.7	35.3 ± 0.8		
8	Subchronic	21	10.7	30.7 – 52.3	46.8 ± 2.2	+32.58	$P < 0.01$
9	Control	28	–	30.5 – 48.1	34.4 ± 1.2		
10	Subchronic	28	10.7	32.5 – 69.7	52.4 ± 2.6	+52.32	$P < 0.001$
11	Recovery control	28 (60)	–	26.2 – 48.4	35.6 ± 0.9		
12	Recovery treated	28 (60)	10.7	23.4 – 47.5	34.2 ± 1.0	+3.93	$P > 0.05$

Table 9. Effect of cypermethrin on serum alkaline phosphatase activity (U L^{-1}) in *Rattus norvegicus*. Control were given groundnut oil only. Recovery group were given oil (11) and cypermethrin (12) treatment for 28 days only and effects were assessed after 60 days. Number of rats analyzed for every treatment were six

No.	Treatment	Treatment duration (days)	Dose (mg kg^{-1} b.w.)	Range	Mean ± SE	Change (%)	Significance level
1	Control	1	–	1.85 – 3.57	2.51 ± 0.03		
2	Acute	1	300	2.41 – 2.78	2.61 ± 0.02	+3.98	$P < 0.05$
3	Control	7	–	2.08 – 3.12	2.47 ± 0.07		
4	Subchronic	7	10.7	2.29 – 3.60	2.55 ± 0.02	+3.24	$P < 0.05$
5	Control	14	–	2.39 – 2.93	2.55 ± 0.05		
6	Subchronic	14	10.7	2.25 – 2.88	2.62 ± 0.04	+2.74	$P < 0.05$
7	Control	21	–	2.13 – 3.08	2.50 ± 0.02		
8	Subchronic	21	10.7	2.50 – 2.80	2.64 ± 0.02	+6.4	$P < 0.001$
9	Control	28	–	1.27 – 3.59	2.45 ± 0.05		
10	Subchronic	28	10.7	2.81 – 3.02	2.87 ± 0.01	+17.14	$P < 0.001$
11	Recovery control	28 (60)	–	2.16 – 2.94	2.50 ± 0.01		
12	Recovery treated	28 (60)	10.7	2.12 – 2.87	2.49 ± 0.03	–0.40	$P > 0.05$

that play an important role in fluid homeostasis necessary for cellular activity. Globulin concentration increases under stress caused by xenobiotic substances (Khan et al. 2009; Abdel-Rahim et al. 2010; Nawaz et al. 2010; Saxena, Saxena 2010).

The toxicity of cypermethrin possibly is due to its stress-causing effect (Singh et al. 2009; Sadeghi-Hashjin et al. 2011). Stress conditions cause release of adrenocorticotropic hormone, triggering consequent secretion of cortisol by the adrenal cortex (Hayes, Laws 1991), which reduces cellular protein stores, excepting in liver. Further, plasma proteins produced by liver become released into blood, raising the protein level.

Cypermethrin also has effect on serum lipids. Serum lipids include triglycerides, cholesterol, phospholipids and free fatty acids (Tortora, Grabowski 2003). Any increase in the level of these forms will lead to an increase of total lipid concentration in serum. One of the causes of increased total lipid concentration appears to be disturbance of carbohydrate metabolism, due to probable cytotoxic effect of cypermethrin on cells of the pancreas leading to relative deficiency of insulin (Kalender et al. 2005). In such conditions, carbohydrates are not available to body tissues as insulin is not available to facilitate glucose transport in cells. In insulin deficiency, carbohydrates are not used to meet energy demands of body and most of the energy is derived from fats. The fat stored in adipose tissue is then hydrolysed and thus the amount of free fatty acids in blood is increased resulting in increased serum total lipid concentration (Guyton, Hall 2001; Rezg et al. 2004).

Table 10. Effect of cypermethrin on serum lactate dehydrogenase activity (U L^{-1}) in *Rattus norvegicus*. Control were given groundnut oil only. Recovery group were given oil (11) and cypermethrin (12) treatment for 28 days only and effects were assessed after 60 days. Number of rats analyzed for every treatment were six

No.	Treatment	Treatment duration (days)	Dose (mg kg^{-1} b.w.)	Range	Mean ± SE	Change (%)	Significance level
1	Control	1	–	52.0 – 58.4	55.5 ± 1.1		
2	Acute	1	300	56.0 – 60.2	57.9 ± 0.7	+4.3	$P > 0.05$
3	Control	7	–	50.1 – 62.6	56.3 ± 2.1		
4	Subchronic	7	10.7	54.3 – 64.7	59.4 ± 1.7	+5.5	$P > 0.05$
5	Control	14	–	53.5 – 60.2	57.3 ± 1.3		
6	Subchronic	14	10.7	58.2-– 65.9	62.3 ± 1.1	+8.7	$P > 0.05$
7	Control*	21	–	52.3-– 54.8	53.4 ± 0.4		
8	Subchronic	21	10.7	62.1 – 70.4	66.7 ± 1.1	+24.9	$P < 0.001$
9	Control	28	–	53.2 – 57.4	55.1 ± 1.8		
10	Subchronic	28	10.7	61.5 – 72.0	67.5 ± 1.9	+22.5	$P < 0.001$
11	Recovery control	28 (60)	–	50.5 – 60.2	55.6 ± 1.7		
12	Recovery treated	28 (60)	10.7	53.1 – 62.5	57.3 ± 1.7	+3.1	$P > 0.05$

Xenobiotic substances activate the sympathetic nervous system, resulting in release of epinephrine and norepinephrine by adrenal medulla (Harrison 1994; Sadeghi-Hashjin et al. 2011), which activates hormone-sensitive triglyceride lipase in tissue, resulting in hydrolysis of stored triglycerides from fat stores and mobilization of free fatty acids in the blood stream causing raised serum total lipid concentration (Rani, Dua 1995; Guyton, Hall 2001).

A similar explanation can be applied for cypermethrin intoxication leading to alteration in the serum lipid profile.

Triglycerides are esters of free fatty acids with glycerol, an important cause of excess carbohydrates in the blood. Probably, the altered carbohydrate levels along with that of serum protein observed in the present study might have been due to conversion to fats including triglycerides via intermediatory metabolism (Bhushan 2011; Guyton, Hall 2001).

Phospholipids have both metabolic and structural function in mammals and are the main precursors of lipoproteins, the carriers for triglyceride transport (Zubay et al. 1995). Cypermethrin has been found to decrease serum cholinesterase activity (Kale et al. 1999), also responsible for enhanced serum phospholipid concentration.

Increased levels of cholesterol in serum observed in the present investigation under cypermethrin stress might be an outcome of cholestasis, along with endogenous synthesis of cholesterol (Saxena, Sharma 1999).

These results suggest that cypermethrin has strong toxic potential to alter normal body physiology. However, the effects can be normalized over time, as observed in the recovery sets.

In conclusion, cypermethrin has strong potential to disturb normal blood biochemistry of mammals. In the present study, all the considered biochemical components: AST, ALT, ALP and LDH, total lipids, phospholipids, glycerides, total proteins, cholesterol and total bilirubin in serum of albino rats were found to increase significantly following cypermethrin intoxication. This strongly indicates that proper precautions need to be taken in use of this pesticide.

References

Abdel-Rahim E.A., Abdel-Rahim G.A., Fayed S.A., Mahmoud G.I. 2009. Antioxidant diet as protective agents against biochemical perturbation effects induced by cypermethrin on lipids and protein fractions as well as kidneys function of blood rat. *Austr. J. Basic Appl. Sci.* 3: 267–276.

Abdou H.S., Salah S.H., Abdel Rahim E.A. 2009. The ability of vitamins A, C and E on antioxidants against the genotoxic potential of tefluthrin. *Austr. J. Basic Appl. Sci.* 3: 4190–4198.

Addy-Orduna L.M., Zaccagnini M.-E., Canavelli S.B., Mineau P. 2011. Formulated beta-cyfluthrin shows wide divergence in toxicity among bird species. *J. Toxicol.* doi:10.1155/2011/803451.

Adjrah Y., Karou S.D., Agbonon A., Ameyapoh Y., de Souza C., Gbeassor M. 2013. Effect of cypermethrin-treated lettuce (*Lactuca sativa*) on wistar rat liver. *J. Appl. Pharmacol. Sci.* 3: 128–132.

Ahmad L., Khan A., Khan M.Z. 2011. Cypermethrin induced biochemical and hepato-renal pathological changes in rabbits. *Int. J. Agric. Biol.* 13: 865–872.

Aldana L., Tsutsumi V., Craigmill A., Silveira M.I., de Mejia E.G. 2001. Alpha-tocopherol modulates liver toxicity of the pyrethroid cypermethrin. *Toxicol. Lett.* 135: 107–116.

Anadon A., Martinz-Larranaga M.R., Ferandez-Cruz M.L. 1995. Effect of flumethrin on hepatic drug metabolizing enzymes and antipyrine disposition in rats. *Toxicol. Appl. Pharmacol.* 141: 8–16.

Anwar K. 2004. Toxic effects of cypermethrin on the development of muscle in chick embryo of *Gallus domesticus*. *Int. J. Agricult. Biol.* 6: 400–406.

Assayed M.E., Khalaf A.A., Salem H.A. 2010. Protective effects of garlic extract and vitamin C against *in vivo* cypermethrin-induced teratogenic effect in rat offspring. *Food Chem. Toxicol.* 48: 3153–3158.

Attia A.M., Nasr H.M. 2009. Evaluation of protective effect of omega-3 fatty acids and selenium on paraquat intoxicated rats. *Slovak J. Anim. Sci.* 42: 180–187.

Bhushan B. 2011. Comparative hepatotoxicity under stress of cypermethrin and beta-cyfluthrin in albino rats. Ph.D. Thesis. Dr. B.R. Ambedkar University, Agra.

Bhushan B., Saxena N., Saxena P.N. 2010. Beta-cyfluthrin induced histochemical alteration in the liver of the albino rat. *Scand. J. Lab. Anim. Sci.* 37: 61–66.

Bhushan B., Saxena P.N., Saxena N. 2013. Biochemical and histological changes in rat liver caused by cypermethrin and beta-cyfluthrin. *Arh. Hig. Rada Toksikol.* 64: 57–67.

Bian Q., Xu L.C., Wang S.L., Xia Y.K., Tan L.F., Chen J.F., Song L., Chang H.C., Wang X.R. 2004. Study on the relation between occupational fenvalerate exposure and spermatozoa DNA damage of pesticide factory workers. *Occup. Environ. Med.* 61: 999–1005.

Dumas B.T. 1971. Determination of total protein and albumin in serum. *Clin. Chem. Acta* 31: 87–96.

El-Magd S.A.A., Sabik L.M.E., Shoukry A. 2011. Pyrethroid toxic effects on some hormonal profile and biochemical markers among workers in pyrethroid insecticide company. *Life Sci. J.* 8: 311–322.

El-Tawil O.S., Abdel-Rehman M.S. 2001. The role of enzyme induction and inhibition on cypermethrin hepatotoxicity. *Pharmacol. Res.* 44: 33–40.

Fetoui H., Garoui E.M., Zeghal N. 2009. Lambda-cyhalothrin-included biochemical and histopathological changes in the liver of rats: Ameliorative effect of ascorbic acid. *Exp. Toxicol. Pathol.* 61: 189–196.

Finey D.J. 1971. *Probit Analysis.* Cambridge University Press. 303 p.

Fisher R.A., Yates F. 1963. Statistical tables for biological agriculture and medical research. VIth ed. Hing Yip printing Co. Hong Kong. 146 p.

Folch J., Lees M., Stanley G.H.S. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226: 497–509.

Gupta PK and Kumar S. 1991. Cumulative toxicity of deltamethrin in mice. *J. Env. Biol.* 12: 45–50.

Guyton A.C., Hall J.E. 2001. *Textbook of Medical Physiology.* 10th ed. Prism Books Limited, Bangalore, India. 1148 p.

Harisson T.R. 1994. *Principles of Internal Medicine.* Mc Graw Hill, New York, 154 p.

Hayes W.J.Jr., Laws E.R. 1991. *Handbook of Pesticide Toxicology.* Vol. 3. Academic Press Inc., San Diego, New York. 1190 p.

Hinton R.H., Grasoo P. 2000. Hepatotoxicity. In Ballantyne B., Marrs T., Syversen T. (eds) *General and Applied Toxicology.* Vol. 2, Grove's Dictionaries Inc., New York, pp. 853-892.

Kale M., Rathore N., John S., Bhatnagar D. 1999. Lipid peroxidative damage on pyrethroid exposure and alterations in antioxidant status in rat erythrocytes: a possible involvement of reactive oxygen species. *Toxicol. Lett.* 105: 197–205.

Kalender S., Ogutcu A., Uzunhisaracikli M., Acikzoz F., Durak D., Ulusoy Y., Kalender Y. 2005. Diazonin induced hepatotoxicity and protective effect of vitamins E on some biochemical indices and ultra-structural changes. *Toxicology* 21: 197–206.

Keele C.A., Neil E. 1971. *Samson Wright's Applied Physiology.* 12th Ed. The English Language Book Society and Oxford University Press. 576 p.

Khan A., Faridi H.A.M., Ali M., Khan M.Z., Siddique M., Hussain I., Ahmad M. 2009. Effects of cypermethrin on some clinico-hemato-biochemical and pathological parameters in male dwarf goats (*Capra hircus*). *Exp. Toxicol. Pathol.* 61: 151–160.

King J. 1965. The dehydrogenase of oxidoreductase-lactate dehydrogenase. In King J.C., Van D. *Practical Clinical Enzymology*. Nostrand Co, London, pp. 83–93.

Kind P.R.N., King E.J. 1954. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. *J. Clin. Pathol.* 7: 332–326.

Malloy H.T., Evelyn K.A. 1937. Oxidation method for bilirubin determinations in bile and meconium with the photoelectric colorimeter. *J. Biol. Chem.* 122: 597–603.

Manna S., Bhattacharya D., Mandal T.K., Das S. 2005. Repeated dose toxicity of deltamethrin in rats. *Indian J. Phrmacol.* 37: 160–164.

McGowan M.W., Artiss J.D., Strandbergh D.R., Zak B. 1983. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clin. Chem.* 29 538–542.

Muthuviveganandavel V., Muthuraman P., Muthu S., Srikumar KS. 2011. Individual and combined biochemical and histological effect of cypermethrin and carbendazim in male albino rats. *J. Appl. Pharmaceut. Sci.* 1: 121–129.

Nair R.R., Abraham M.J., Nair N.D., Lalithakunjamma C.R., Aravindakshan C.M. 2010. Hematological and biochemical profile in sublethal toxicity of cypermethrin in rats. *Int. J. Biol. Med. Res.* 1: 211–214.

Nair R.R., Abraham M.J., Lalithakunjamma C.R., Nair N.D., Aravindakshan C.M. 2011. A pathomorphological study of the sublethal toxicity of cypermethrin in Sprague Dawley rats. *Int. J. Nutr. Pharmacol. Neurol. Dis.* 1: 179–183.

Nawaz S.K., Batool R., Arshad M., Arshad N. 2010. Alpha tocopherol may reduce endosulfan induced toxicity in mice. *Pakistan J. Zool.* 42: 205–210.

Pande S. 2001. Effect of synthetic pyrethroids on certain haematobiochemical parameters of *Rattus norvegicus*. Ph.D. Thesis, Dr. B.R. Ambedkar University, Agra.

Rani S., Dua K.K. 1999. Cypermethrin toxicity induced histological and biochemical changes in the liver of albino rats (*Rattus norvegicus*). *J. Ecotoxicol. Environ. Monitor.* 9: 41–46.

Rana N., Saxena N., Sharma H.N., Saxena P.N. 2008. Comparative genotoxicity of alpha-cyano pyrethroids on *Drosophila melanogaster. Entamon* 33: 135–138.

Reitman S., Frankel S. 1957. A colorimetric method for aspartate and alanine aminotransferase in serum. *J. Clin. Pathol.* 28: 56–58.

Rezg R., Mornagui B., Kamoun A., El-Fazza S., Gharbi N. 2007. Effect of subchronic exposure to matathion on metabolic parameters in rats. *C.R. Biology* 330: 143–147.

Sadeghi-Hashjin G., Koohi M.K., Fallah F. 2011. Influence of Permethrin and Cypermethrin on behavior in the mouse. *Int. J. Veterin. Res.* 5: 119–124.

Saka W.A., Akhigbe R.E., Azeez O.M., Babatunde T.R. 2011. Effect of pyrethroid exposure on haematological and haemostatic profiles in rats. *Pakistan J. Biol. Sci.* 14: 1024–1027.

Sangha G.K., Kaur K., Khera K.S. 2013. Cypermethrin induced pathological and biochemical changes in reproductive organs of female rats. *J. Environ. Biol.* 34: 99–105.

Salama A.K., Osman K.A., Saber N.A., Soliman S.A. 2005. Oxidative stress induced by different pesticides in the Land

Snails, *Helix aspersa*. *Pakistan J. Biol. Sci.* 8: 92–96.

Saxena P., Saxena A.K. 2010. Cypermethrin induced biochemical alterations in the blood of albino rats. *Jordan J. Biol. Sci.* 3: 111–114.

Saxena PN and Sharma DC. 1999. Effect of hafen 20 EC on brain cholesterol, glycogen and total lipids in albino rat. *Indian J. Environ. Toxicol.* 9: 72–73.

Sayim F., Yavasoglu N.U.K., Uyanikgil Y., Aktug H., Yavasoglu A., Turgut M. 2005. Neurotoxic effects of cypermethrin in Wistar rats: a haematological, biochemical and histopathological study. *J. Health Sci.* 51: 300–307.

Singh A.K., Saxena P.N., Sharma H.N. 2009. Stress induced by beta-cyfluthrin, a type-2 pyrethroid, on brain biochemistry of albino rat (*Rattus norvegicus*). *Biol. Med.* 1: 74–86.

Singh V.K., Dixit P., Saxena P.N. 2005. Cybil induced hepatobiochemical changes in wistar rats. *J. Environ. Biol.* 26: 725–727.

Singh V.K., Saxena P.N. 2001. Effect of Cybil (cypermethrin 25EC) and Cybil-sevin (carbaryl 50EC) combination on liver and serum phosphates in wistar albino rats. *J. Ecophysiol. Occup. Health* 1: 229–234.

Singh V.K., Saxena P.N. 2002. Genotoxic potential of cypermethrin in mammalian haemopoietic system. *Him. J. Eniron. Zool.* 16: 195–202.

Spencer C.I., Sham J.S.K. 2005. Mechanisms underlying the effects of the pyrethroid tefluthrin on action potential duration in isolated rat ventricular myocytes. *J. Pharmacol. Exp. Therapeut.* 315: 16–23.

Stonard M.D., Evans G.O. 1995. Clinical Chemistry. In: Ballantyne B, Marrs T, Turner P (eds). *General and Applied Toxicology.* Macmillan Press, London. 247 p.

Tortora G.J., Grabowski S.R. 2003. The digestive system. In: *Principles of Anatomy and Physiology*. Xth ed. John Wiley & Sons. Inc., USA, pp. 851–903.

Vaittinen S.L., Komulainen H., Kosma V.M., Julkunen A., Mäki-Paakkanen J., Jansson K., Vartiainen T., Tuomisto J. 1995. Subchronic toxicity of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) in Wistar rats. *Food Chem. Toxicol.* 33: 1027–1037.

Varley H., Gowenlock A.H., Bill M. 1980. *Practical Clinical Biochemistry.* Vol. I, 5th ed. William Heinmenn Medical Books Ltd., London. 1235 p.

Wolansky M.J., Gennings C., Crofton K.M. 2006. Relative protencies for acute effects of pyrethroids on motor functions in rats. *Toxicol. Sci.* 8: 271–277.

Wybenga D.R., Pileggi V.J., Dirstine H., Giorgio J.D. 1970. Direct manual determination of serum total cholesterol with a single stable reagent. *Clin. Chem.* 16: 980–984.

Yavasoglu A., Sayim F., Uyanikgil Y., Turgut M., Yavasoglu N.U.K. 2006. The pyrethroid cypermethrin induced biochemical and histological alterations in rat liver. *J. Health Sci.* 52: 774–780.

Zilversmith D.B., Davis A.K., Memphis B.S. 1950. Microdetermination of plasma phospholipids by trichloracetic acid precipitation. *J. Lab. Clin. Med.* 35: 155–160.

Zubay G.L. Parson W.W., Vance D.E. 1995. Biosynthesis of membrane lipids. In: *Principles of Biochemistry*. William C. Brown, New York, pp. 438–441.

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