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Full Length Article

Cypermethrin Induced Biochemical and Hepato-renal Pathological Changes in Rabbits

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ABSTRACT

The effects of intra-peritoneal administration of cypermethrin (CY) on biochemistry and histology of liver and kidneys in rabbits were studied. Male rabbits (n = 10 x 4 = 40) in groups B, C and D received low (50 mg.kg^{-1} body weight), medium (100 mg.kg^{-1} body weight) and high (150 mg.kg^{-1} body weight) CY doses, respectively in mustard oil at weekly interval up to day 71. Group A served as control and each animal in this group received equivalent volume of mustard oil. Blood samples without anticoagulant from all animals were collected prior to start of experiment (day 0) and after every treatment, which were used for extraction of serum. The serum was used for analysis of proteins (serum total proteins/STP, serum albumin, serum globulins), aminotransferases (AST & ALT), alkaline phosphatase (ALP), urea and creatinine. Two animals from each group were euthanized fortnightly for histopathological studies. Increases in AST level in sera of CY-treated rabbits were accompanied by histological lesions in liver (different stages of degeneration & bile duct hyperplasia). Increased urea and creatinine concentrations and decreased STP, albumin and globulins in sera of CY-treated rabbits could be due to renal damage. The renal damage appeared histologically in the form of different lesions (pyknotic nuclei, necrosis, sloughed tubular epithelium, cast deposition & increased urinary space) in CY-treated rabbits. It was concluded that cypermethrin at various doses administered during the study produced moderate histological lesions in liver and kidneys along-with biochemical alterations in serum samples.

Key Words: Cypermethrin; Liver enzymes; Proteins; Kidneys; Creatinine; Histopathpology

INTRODUCTION

A wide range of pests including those challenging the public health are being killed/inactivated with cypermethrin (CY), which is very forceful synthetic pyrethroid pesticide. It is extensively used as insecticidal sprays on cotton, vegetables and other crops. Likewise, some veterinary products are based on CY (e.g., Ecofleece), which are popularly used for dipping or showering of food animals (Shah *et al*., 2007). Pyrethroids have less mammalian toxicity among the pesticides but have been reported to affect physiological activities and produce pathological entities in animals (Khan *et al*., 2009). Among pyrethroids, CY is rapidly absorbed in the body and exhibit clinical signs of neurotoxicity including loss of coordination, muscular twitching and death due to respiratory failure (Sharaf *et al*., 2010). Due to being lipid soluble and of smaller size (V = 536.40 Å^3), CY easily passes through cell membrane and may damage DNA by causing destabilization and unwinding of the DNA helix and chromosomal injuries (Saxena *et al*., 2005). The DNA damage has led to different consequences in different body systems (Sharaf *et al*., 2010).

Although hepato-renal consequences have been addressed in a few studies in animals, yet present study is peculiar in regard to long term repeated treatments and follow up. Changes produced in the pyrethroid exposed animals can be regarded as immediate, since pyrethroids are rapidly metabolized in the body (Sayim *et al*., 2005). However, it was realized that frequent stress, debility and residue accumulation etc. with repeated CY exposure could reveal more pronounced lesions than single exposure. The half-life of orally administered *cis*-isomer of CY in fat, liver and kidneys has been reported to be about 12 days, although residues in liver and kidneys were much lower as compared to fat at a given time (Crawford *et al*., 1981). On the other hand, the *in vivo* half-life of trans-isomer of CY has been reported to be 3.4 days (Sayim *et al*., 2005). So, *in vivo* half-life was one of the factors considered, while establishing weekly interval between intraperitoneal injections in the present study. Furthermore, almost weekly agricultural spraying practices and anti-parasite application in animals also required the weekly interval to make contrast of the study to animal exposures under field conditions.

When exogenous and endogenous toxic products enter the body, their detoxification is carried out with active participation of liver. So, there is a greater risk of hepatocyte

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injury (Wight, 1982). Serum enzyme levels are considered indicators of overall health status of an individual especially hepatocyte injury and related stress (Khan *et al*., 2009). By processing blood plasma and excreting urine, the kidneys play a vital role in the clearance and excretion of xenobiotics including drugs and drug-products from the body. Valuable facts about the nephrotic health can be known from the estimation of several waste products of metabolism (such as urea & creatinine), which are excreted completely through kidneys (Garba *et al*., 2007). Urea and creatinine are waste products of protein metabolism and are withheld in blood in cellular damage (Aslam *et al*., 2010). Therefore, these are the most sensitive biochemical markers for the diagnosis of renal damage (Garba *et al*., 2007). The major reasons for decreased proteins in serum include: renal and intestinal protein loss, hemorrhages, malabsorption and liver failure (Khan, 2008). So determination of proteins in CY-treated animals might yield interesting information in connection with hepato-renal toxicity.

Numerous pesticides are in use for the control of agricultural pests and animal disease causing vectors in Pakistan. Despite the fact that the pyrethroids (including CY) have potential adverse health effects, they are still being widely used in agriculture and animal husbandry (Shah *et al*., 2007). During the previous decade, pyrethroid application has increased tremendously (Ahmad *et al*., 2011). Although, many studies are available addressing hepato-renal pathology in pyretroid exposed animals, yet present study is peculiar with regard to duration and more clearly correlates the biochemical alterations with renal and hepatic histopathology.

MATERIALS AND METHODS

Synopsis of this experiment was tailored keeping in view all the national legislations and research ethics laid down by the Ethics Committee about the animal welfare and following the strategies and guidelines of the Advanced Studies and Research Board (ASRB) of the University. Before implementation, the experimental proposal was approved by the ASRB.

Experimental animals and protocol: Apparently healthy New Zealand white adult male rabbits (*Oryctolagus cuniculus*) (n = 40) almost of the same age (about 6 months) and weight (990±50 g), were procured from the local market and were kept under similar management conditions. The animal room temperature was maintained at 25-27°C with 45-70% humidity and 12-h light–dark cycle throughout the study. Drinking water was available *ad libitum*. The green fodder Berseem (*Trifolium alexandrinum*) was offered in the morning and evening. After 5 days acclimatization, the rabbits were randomly divided into four equal groups i.e., A-D. Cypermethrin (92%) used in the study was gifted by M/S Pak-China Chemicals, Lahore. Oral LD_{50} of CY in rabbits is 2400 $mg.kg^{-1}$ body weight (Hartley & Kidd, 1990), $1/48^{th}$, $1/24^{th}$ and $1/16^{th}$ of it were administered intra-peritonealy. Since CY is not soluble in water, it was dissolved in mustard oil for intra-peritoneal (IP) injection. Dose to be injected was adjusted according to body weight. Groups B, C and D received low, medium and high (50, 100 & 150 $mg.kg^{-1}$ body weight) CY doses, respectively at weekly interval up to day 71. Group A served as control and each animal in this group received equivalent volume of mustard oil.

Sample collection and analysis: Blood samples from all animals were collected prior to first treatment (d 0) and then after every treatment and subjected to extraction of serum. The serum samples were stored in aliquots at -20°C for biochemical studies. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities were measured with commercially available colorimetric kits (AMP Medizintechnic GmbH, Austria Cat # BR0415, BR061 & BR0202, respectively). The principles for the leakage enzymes (AST & ALT) measurements were to monitor the concentration of L-lactate or L-Malate with α-ketoglutarate at wavelength of 340 nm, while that for ALP was to monitor the concentration of p-nitrophenol formed with water at wavelength of 405 nm with a spectrophotometer. Urea and creatinine levels in serum were measured with commercially available colorimetric kits (AMP Medizintechnic GmbH, Austria Cat # BR04006 & BR2810, respectively) using spectro-photometer. The principle for enzymatic determination of urea was measurement of L-glutamate with water and α-ketoglutarate, while that for creatinine determination was to measure the speed of production of colored complex between creatinine and alkaline picrate using spectrophotometer at wavelengths of 340 and 500 nm, respectively.

The serum total proteins (STP) were estimated by Biuret method and serum albumin by bromocresol green (BCG) dye binding method (Khan *et al*., 2011) using spectrophotometer. The serum globulin was calculated by subtracting albumin from STP (Javed *et al.*, 2010).

Two animals randomly selected from each group were euthanized fortnightly (on experimental days 15, 29, 43, 57 & 71). The liver and kidneys from each animal were carefully dissected and processed for histopathology (Awaad *et al*., 2010; Khan *et al*., 2010). Briefly, about 5 mm thick pieces of the morbid organs were fixed in 10% buffered formalin and later processed for histopathological studies using routine methods of dehydration, paraffin embedding, sectioning (4-5 µm) and staining (H & E). During the microscopic examination of slides of each group at a particular period, histological lesions were scored on a scale of ---- to ++++. From this a cumulative lesion score was derived for the overall severity of pathology in a particular group.

Data analysis: Randomized Complete Block Design was used and serum parameters' data collected were subjected to analysis of variance using Minitab statistical software package on personal computer. Mean values of various

treatments were compared by Duncan's Multiple Range Test (DMR) at P<0.05.

RESULTS

Biochemical parameters: Significantly ($P \leq 0.05$) higher AST was recorded in all treated groups at day 43 and in group D (150 $mg.kg^{-1}$ body weight) at days 36, 57 and 71 (Table I). Significantly ($P \leq 0.05$) lower ALT was recorded in all treated groups at day 71, while significantly higher ALP at day 15 only in group B (Table I). A dose and time dependent increasing trend was observed in the concentration of urea (Fig. 1) and creatinine concentration (Table II) in all treatment groups Significantly ($P \leq 0.05$) lower serum urea was recorded in all treated groups at day 1. Contrarily, significantly ($P \leq 0.05$) higher serum urea was recorded in all treatment groups at day 64 and 71, in groups C and D at days 43 and 57 and in group D at days 29 (Table II). Significantly ($P \leq 0.05$) higher serum creatinine was recorded in all treated groups at day 64, in groups C and D at days 1, 29 and 71 and in group D at day 43.

A dose and time dependent decreasing trend was observed in the concentration of STP in all treatment groups (Fig. 1). Significantly ($P \leq 0.05$) lower STP were recorded in all treated groups at days 8 and 57, in groups B at day 15, in group C at day 36, in group D at day 15 and in groups C and D at days 50, 64 and 71 (Fig. 1). Significantly ($P \leq 0.05$) lower serum albumin was recorded in all treated groups at day 8 and in group B at day 15 (Table II). Significantly ($P \leq 0.05$) lower serum globulins were recorded in all treated groups at day 57, in groups C and D at days 64 and 71 and in group B at day 8 (Table II).

Gross and histopathology: In group A (control) throughout the course of experiment, the liver and kidneys did not exhibit any gross morphological alteration. All treated groups were having dose and time related frequency

Table I: Serum aspartate transaminase, alanine transaminase and alkaline phosphatase concentrations (IU) in cypermethrin treated rabbits

Parameters/Experimental Days	Group (CY dose: $mg.kg^{-1}$ body weight)				P-Value
	A (0)	B (50)	C (100)	D (150)	
Aspartate transaminase					
0	95.2 ± 13.4	91.3 ± 17.5	90.3 ± 14.4	94.7 ± 20.7	0.945
1	88.0 ± 17.9	84.8 ± 38.2	116.3 ± 70.9	95.7 ± 28.0	0.602
8	93.2 ± 20.2	171.7 ± 56.0	119.0 ± 36.8	115.3 ± 71.2	0.098
15	112.6 ± 48.8	155.2 ± 50.3	127.7 ± 24.0	126.7 ± 32.9	0.367
22	97.6 ± 36.6	160.2 ± 35.0	135.8 ± 45.8	146.2 ± 56.9	0.184
29	95.8 ± 61.7	129.6 ± 46.5	177.0 ± 86.0	144.8 ± 99.2	0.465
36	75.8 ± 15.7	118.0 ± 46.3	149.5 ± 13.2	178.0 ± 72.2*	0.036
43	80.0 ± 8.7	149.5 ± 7.4*	172.5 ± 31.3*	157.3 ± 47.5*	0.003
50	104.3 ± 16.0	95.0 ± 21.9	139.0 ± 35.7	162.7 ± 65.4	0.213
57	90.3 ± 18.5	52.0 ± 12.1	118.0 ± 45.0	277.0 ± 114.0*	0.010
64	94.0 ± 49.5	91.0 ± 32.5	114.5 ± 46.0	154.0 ± 59.2	0.400
71	91.0 ± 11.3	138.0 ± 28.3	149.5 ± 20.5	207.5 ± 21.9*	0.025
Alanine transaminase					
0	114.3 ± 6.0	131.2 ± 6.5	113.17 ± 26.06	128.2 ± 60.7	0.709
1	144.0 ± 34.3	135.7 ± 70.5	106.00 ± 69.13	154.5 ± 72.8	0.597
8	140.6 ± 85.1	121.2 ± 54.4	172.17 ± 56.92	99.3 ± 31.9	0.207
15	156.4 ± 41.9	140.8 ± 30.6	112.67 ± 20.92	158.2 ± 34.6	0.093
22	163.2 ± 47.8	125.7 ± 31.1	191.17 ± 90.66	201.2 ± 94.5	0.302
29	148.6 ± 25.4	105.0 ± 47.5	157.60 ± 91.75	205.4 ± 57.0	0.113
36	152.0 ± 66.5	125.6 ± 52.9	114.75 ± 10.87	115.4 ± 27.8	0.577
43	164.7 ± 53.4	116.0 ± 33.1	104.25 ± 16.78	123.8 ± 36.8	0.170
50	162.7 ± 144.0	88.3 ± 16.7	99.00 ± 42.29	86.3 ± 48.8	0.501
57	116.0 ± 12.5	116.0 ± 12.0	145.00 ± 12.00	87.7 ± 48.7	0.150
64	159.0 ± 14.0	97.0 ± 21.0	153.67 ± 62.50	79.0 ± 49.0	0.109
71	155.0 ± 25.9	80.0 ± 19.4*	69.00 ± 17.57*	45.0 ± 14.2*	0.000
Alkaline phosphatase					
0	273.0 ± 165.5	284.0 ± 104.5	326.2 ± 164.7	239.3 ± 99.9	0.749
1	220.2 ± 74.8	386.7 ± 227.0	311.3 ± 114.0	290.7 ± 46.0	0.278
8	239.2 ± 39.6	291.2 ± 106.0	309.3 ± 87.8	254.2 ± 72.9	0.471
15	232.2 ± 31.1	364.3 ± 79.4*	275.2 ± 35.7	307.7 ± 59.3	0.007
22	267.2 ± 29.3	262.8 ± 29.7	245.8 ± 47.0	272.8 ± 53.3	0.755
29	226.8 ± 43.6	182.2 ± 64.9	174.0 ± 44.2	243.6 ± 112.0	0.421
36	252.7 ± 32.9	285.0 ± 98.9	247.3 ± 66.9	244.8 ± 69.1	0.840
43	231.8 ± 81.4	343.0 ± 84.1	317.8 ± 133.1	208.0 ± 150.2	0.330
50	200.7 ± 22.5	333.7 ± 44.5	281.0 ± 140.0	284.0 ± 82.9	0.353
57	235.0 ± 12.0	324.0 ± 16.0	222.0 ± 19.0	261.7 ± 110.4	0.201
64	239.5 ± 67.2	274.0 ± 32.5	331.0 ± 79.2	255.0 ± 12.7	0.457
71	227.5 ± 61.5	256.5 ± 26.2	362.0 ± 168.3	243.5 ± 34.7	0.525

The values (mean ± SD) bearing asterisks in a row differ significantly ($P \leq 0.05$)

Fig. 1: Serum urea (upper) and total proteins (lower) concentrations (mean ± SD) in cypermethrin treated rabbits.

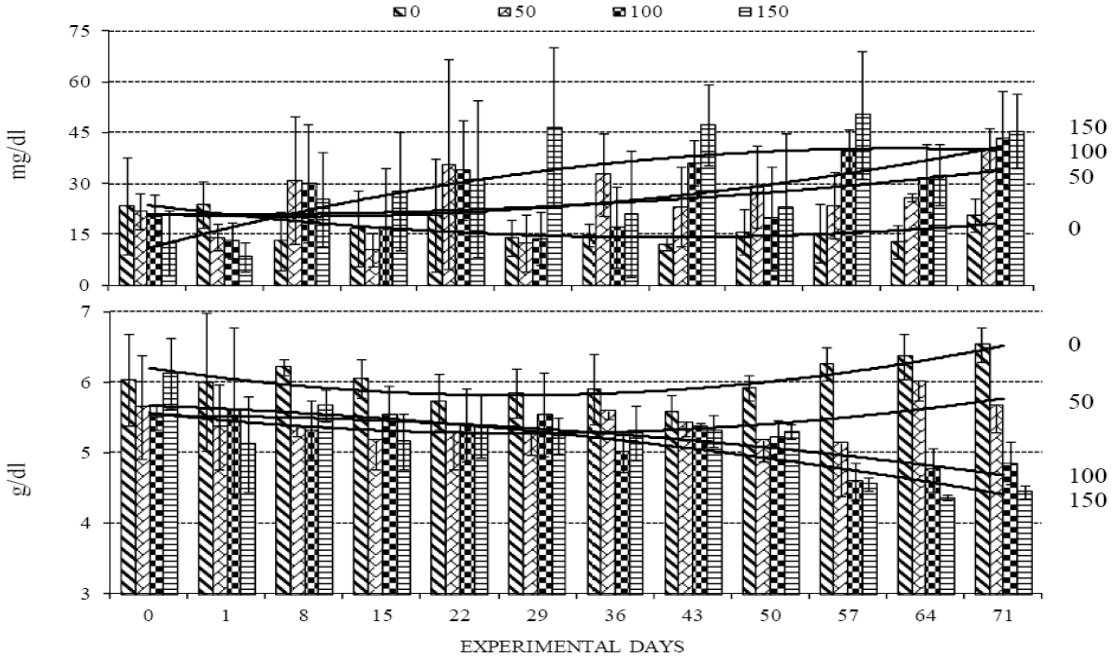


Table II: Serum creatinine, albumin and globulin concentrations in cypermethrin treated rabbits

Parameters/Experimental days	Group (CY dose: mg.kg^{-1} body weight)				P-Value
	A (0)	B (50)	C (100)	D (150)	
Creatinine (mg.dl^{-1})					
0	1.45 ± 0.51	1.35 ± 0.77	1.84 ± 0.34	1.57 ± 1.15	0.711
1	1.62 ± 0.38	1.91 ± 0.42	2.88 ± 0.55*****	2.77 ± 0.60*****	0.000
8	2.32 ± 0.90	2.45 ± 0.36	1.04 ± 0.22	0.95 ± 0.71	0.533
15	1.37 ± 0.31	1.53 ± 0.22	2.02 ± 0.49	2.76 ± 0.33	0.528
22	1.21 ± 0.72	1.34 ± 0.57	4.95 ± 0.47	4.03 ± 0.23	0.072
29	1.04 ± 0.27	1.04 ± 0.54	4.21 ± 0.42*****	5.39 ± 0.12*****	0.011
36	1.04 ± 0.36	4.11 ± 3.12	3.30 ± 2.19	3.99 ± 2.24	0.150
43	1.00 ± 0.148	3.27 ± 1.76	3.08 ± 1.69	5.52 ± 1.66*****	0.008
50	1.53 ± 0.66	2.49 ± 2.17	1.56 ± 1.53	2.75 ± 2.07	0.851
57	1.27 ± 0.26	2.75 ± 2.69	1.62 ± 0.08	2.87 ± 0.20	0.419
64	1.88 ± 0.77	4.90 ± 0.85*****	5.15 ± 0.50*****	6.47 ± 0.26*****	0.008
71	2.28 ± 1.01	4.65 ± 0.93	5.93 ± 1.96*****	6.95 ± 1.58*****	0.004
Albumin (g.dl^{-1})					
0	2.61 ± 0.38	2.88 ±0.46	2.75 ± 0.20	3.00 ± 0.24	0.242
1	2.63 ± 0.45	2.55 ±0.59	1.94 ± 0.55	2.07 ± 0.69	0.140
8	3.60 ± 0.09	3.30 ± 0.07*	2.92 ± 0.20*	2.98 ± 0.34*	0.000
15	3.25 ± 0.19	2.56 ± 0.40*	2.76 ± 0.38	2.76 ± 0.22	0.015
22	2.75 ± 0.76	2.70 ± 0.48	2.60 ± 0.41	3.00 ± 0.43	0.599
29	3.04 ± 0.26	2.71 ± 0.35	2.97 ± 0.40	2.92 ± 0.58	0.622
36	3.21 ± 0.38	3.01 ± 0.20	2.81 ± 0.14	3.37 ± 0.48	0.113
43	3.10 ± 0.17	3.28 ± 0.43	2.95 ± 0.25	3.18 ± 0.19	0.409
50	3.12 ± 0.11	3.48 ± 0.78	3.07 ± 0.43	2.96 ± 0.23	0.445
57	3.20 ± 0.12	3.41 ± 0.50	3.46 ± 0.23	3.40 ± 0.11	0.695
64	3.22 ± 0.02	3.20 ± 0.08	3.48 ± 0.43	3.63 ± 0.12	0.307
71	3.23 ± 0.16	3.23 ± 0.21	3.45 ± 0.20	3.63 ± 0.12	0.201
Globulin (g.dl^{-1})					
0	3.18 ± 0.71	3.11 ± 0.51	2.75 ± 0.30	2.90 ± 0.61	0.530
1	2.71 ± 0.68	1.82 ± 0.42	2.66 ± 1.62	2.06 ± 0.46	0.280
8	2.61 ± 0.14	2.07 ± 0.11*	2.40 ± 0.36	2.69 ± 0.24	0.001
15	2.30 ± 0.33	2.13 ± 0.31	2.27 ± 0.17	2.10 ± 0.21	0.459
22	2.97 ± 0.62	2.59 ± 0.41	2.79 ± 0.25	2.37 ± 0.60	0.259
29	2.81 ± 0.35	2.57 ± 0.33	2.57 ± 0.31	1.32 ± 0.60	0.429
36	2.69 ± 0.25	2.59 ± 0.09	2.20 ± 0.25	1.91 ± 0.76	0.090
43	2.47 ± 0.24	2.14 ± 0.36	2.42 ± 0.30	2.13 ± 0.37	0.373
50	2.30 ± 0.22	1.70 ± 0.70	2.55 ± 0.44	2.34 ± 0.21	0.089
57	3.06 ± 0.35	1.73 ± 0.76*	1.13 ± 0.47*	1.16 ± 0.18*	0.004
64	3.15 ± 0.34	2.83 ± 0.35	1.29 ± 0.74*	0.74 ± 0.08*	0.014
71	3.30 ± 0.09	2.45 ± 0.59	1.40 ± 0.50*	0.81 ± 0.03*	0.011

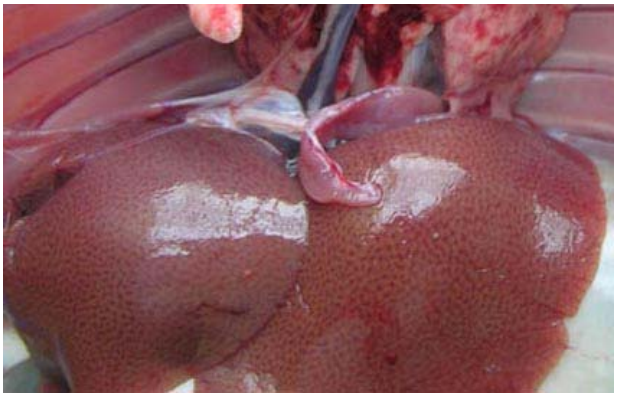
The values (mean ± SD) bearing asterisks in a row differ significantly (P≤0.05)

Table III: Frequency and incidence of gross and histological lesions in liver and kidneys of cypermethrin treated rabbits

Organ/Lesion	Groups (Doses of CY: mg.kg^{-1} body weight)					
	B (50)		C (100)		D (150)	
	F*	I **	F	I	F	I
Liver						
Paleness (grossly)	+---	30	+---	50	++--	70
Fat deposits (grossly)	----	0	+---	50	++--	70
Hyperplasia of bile ducts	+---	20	++--	40	++++	80
Degeneration	+---	30	++--	60	+++-	90
Cytoplasmic vacuolation	++--	60	++--	80	+++-	100
Kidneys						
Swelling (grossly)	+---	40	++--	60	+++-	80
Paleness (grossly)	+---	40	+---	60	+++-	80
Hemorrhage	+---	60	++--	80	++++	90
Degeneration	++--	40	++--	70	+++-	100
Increased urinary space	+---	30	++--	60	++++	90
Sloughed epithelium	++--	40	++--	80	+++-	90
Cast deposition	+---	30	++--	60	++++	90

*F= Frequency; **I = Incidence (%). Various concentrations of cypermethrin mixed in mustard oil were injected IP to male rabbits (n = 10 x 4 = 40) at weekly interval.

Fig. 2: Liver showing enhanced lobular pattern at day 71 in the rabbit treated with high (150 mg.kg^{-1} body weight) cypermethrin dose



of paleness of liver (Table III). The liver in group D (150 mg.kg^{-1} body weight) showed enhanced lobular pattern (Fig. 2) during later stages of the study (d 57-71). Swollen and pale kidneys of variable intensity were observed in all treated groups at various experimental days (Table III).

Rabbits of control group (A) did not show any histological alteration in liver and kidneys. Condensation of hepatic nuclei and cytoplasmic vacuolation (Fig. 3) were observed initially in 50% animals and later in all treated animals. In the later stages, these changes became more severe and extensive. Bile duct hyperplasia/newly formed bile ducts along with various degrees of cellular degeneration in the parenchyma were observed in dose dependent manner (Fig. 4). Regenerating hepatocytes were found in later stages of exposure. Hemorrhages in renal

Fig. 3: Photomicrograph of liver of rabbit treated with cypermethrin (100 mg.kg^{-1} body weight) at experimental day 57 showing condensed nuclei (arrow) and necrosed hepatocytes. H and E, Lens 40X

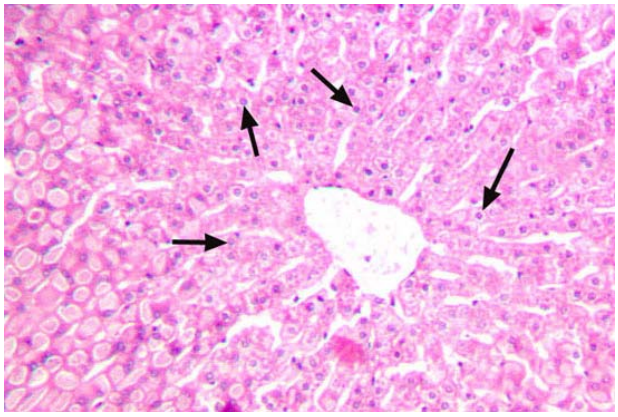
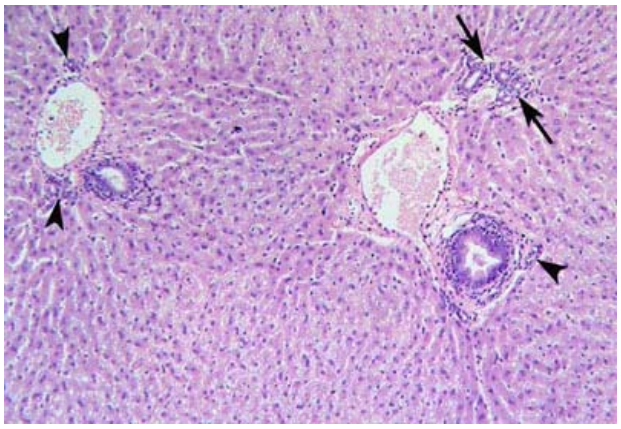


Fig. 4: Photomicrograph of liver of rabbit treated with cypermethrin (150 mg.kg^{-1} body weight) on experimental day 57 showing bile duct hyperplasia (arrow) and newly formed bile ducts (arrow head) along with necrosis of hepatocytes. H and E, Lens 10X



tubules, pyknosis and increased urinary space (Fig. 5) in treated animals were observed. Tubular epithelial detachment from the basement membrane and cast deposition in the renal tubules along with different stages of degeneration (Fig. 6) were noted. The kidney lesions appeared earlier in group D, followed by groups C and B. Variable frequency and incidence of different lesions were observed in dose related pattern (Table III).

DISCUSSION

In the present study, liver was slightly enlarged with pale color and friable consistency at initial stage, but darker/hemorrhagic at later stage with the high dose group showing enhanced lobular pattern; the latter denoting decreased partial pressure of oxygen in blood.

Fig. 5: Photomicrograph of kidney from cypermethrin (100 mg.kg^{-1} body weight) treated rabbit on day 29 showing increased urinary space (blank arrows) and condensation of nuclei (arrow head) along with degeneration of lining epithelium in renal tubules (filled arrow). H and E, Lens 40X

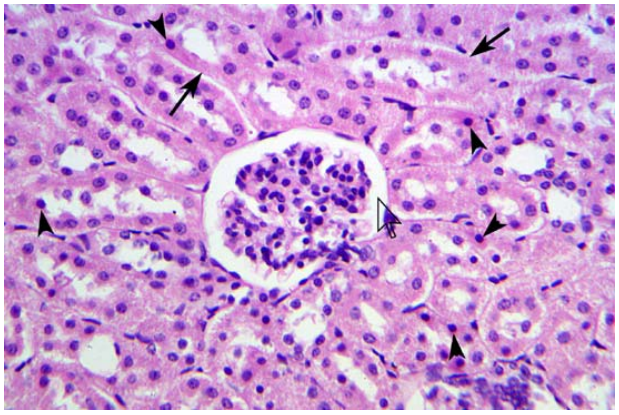
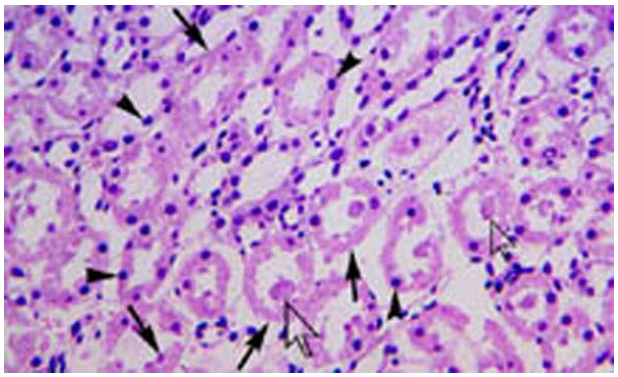


Fig. 6: Photomicrograph of kidney from cypermethrin (150 mg.kg^{-1} body weight) treated rabbit on day 71 showing degenerated tubules (filled arrows), condensed nuclei (arrow heads) and cast deposition in the lumen of renal tubules (blank arrow). H and E, Lens 40X



The greatly branched portal venous arrangement retorts quickly to minute alterations in metabolic activity. This striking vascular reaction to cellular damage and the intricacy of hepatic acinar blood streaming model may elucidate the enhanced lobular pattern in a variety of liver injuries (Zimmon, 1977). Histologically, liver parenchyma in treated animals exhibited vacuolar degeneration and moderate proliferation of bile ducts in the present study. The latter is mostly obvious in cholestatic injuries (Burt & MacSween, 1993). Pathologic changes in hepatocytes due to CY can be related to its inhibitory effect on total adenine tri-phosphate activity in the liver, which may disturb active transport of Na^+, K^+ and Ca^{2+} ions, thus injuring hepatocytes (Khan *et al*., 2009).

The present study revealed increased serum AST with CY treatment, whereas serum ALT activity at the end of the study was decreased significantly. Serum enzyme levels are considered indicators of overall health status of an individual especially hepatocyte injury and related stress (Khan *et al*., 2009). Increases of blood aminotransferases are associated with liver injury and change in hepatic functions (Manna *et al*., 2003). Increase of these enzymes has been credited to their seepage into the blood stream (Yousef *et al*., 2006). Free radicals produced by pyrethroids lead to hepatocyte alterations (Manna *et al*., 2004). Chemical-induced cellular alterations vary from simple increase of metabolism to cell death. Since liver stores AST and synthesizes ALT (Nduka, 1999), focal, multifocal and diffused hepatic necrosis lead to variable magnitude and frequency of alteration in these enzymes (Onyesom & Anosike, 2007). Due to mitochondrial as well as cytoplasmic origin, AST activity is increased in large proportion in various types of liver damage (Theal & Scott, 1996). The increase or decrease of enzyme activity depends on the intensity of cellular damage. Rarely serum AST and ALT levels decrease in toxicology studies. Among the reasons of decreased aminotransferases could include low synthesis/release from hepatocytes, inhibited/reduced enzyme activity, assay interference and decreased vitamin B (pyridoxal 5-phosphate). However, decreased amino-trasferases have been documented to not have any toxicologically significant effects on liver, regardless of the mechanism involved (Hayes, 2007).

ALP is membrane bound enzyme, it is found on all cell membranes, where active transport occurs and is hydrolase and transphosphorylase in function. The highest concentrations of ALP are found in the liver, biliary tract epithelium, bone and intestinal mucosa (Ravel, 1995). Serum ALP activity increases in case of damage to hepatic cells and obstruction of bile duct through proliferation of hepatic cells (El-Demerdash *et al*., 2003). Its decreased activity is taken as an index of parenchymal damage (Anwar, 2003). Differences in concentrations of ALP were in-consistent, which might rule out the cholestatsis in CY pathogenesis (Wulkan & Leijnse, 1986), although moderate bile duct hyperplasia was observed histologically. The latter might have been produced due to non-cholestatic conditions (Hulzebos *et al*., 2005).

Microscopically, hemorrhages in renal tubules, different stages of degeneration, cast deposition and increased urinary spaces were observed with dose dependent frequency and incidence in CY treated rabbits in the present study. In kidneys, filtration barrier is made up of endothelium and podocytes, which are arranged on basement membrane inside and outside, respectively and all these structures are negatively charged whereas protein is also negatively charged (Khan, 2008). Pyrethroids produce oxidative stress by generating free radicals which could damage the filtration barrier and negative charge on the structures (endothelium, podocytes & basement membrane) become positive or charge is removed, thereby proteins go out freely. Due to various degenerative changes in the kidneys caused by CY, epithelial cells are detached from the

tubular basement membranes. When detached epithelial cells are mixed with leaked proteins, the resultant mixture appears in the form of epithelial casts (Khan *et al*., 2009).

The significant increase ($P \leq 0.05$) in urea and creatinine levels noticed in this study is a classical sign that the kidney was unfavorably affected by CY exposure. Creatinine is more specific to kidneys since renal damage is the only significant parameter that increases serum creatinine in mammals (Garba *et al*., 2007). Like many other waste products of metabolism, most (> ¾th) of the creatinine is removed from the body through glomerular filtration and the rest (<25%) through tubular secretion (Ravel, 1995). The increased serum concentrations of creatinine thus might be a result of alteration in these two mechanisms. Urea is also excreted by kidneys, so impaired kidney function causes diminished ability to excrete urea from the blood into urine (Aslam *et al*., 2010). Other than renal tissue damage, causes of increased serum urea include: (1) Rapid urea production from ammonia and proteins, (2) Hampered excretion of urea (Garba *et al*., 2007), (3) Dehydration, (4) Increased activities of urea enzymes (ornithine carbomoyl transferase, arginase) (Guven *et al*., 2006), (5) Decreased serum proteins and (6) Low blood volume (Garba *et al*., 2007). Possibly dehydration with CY-treatment (Sharaf *et al*., 2010) led to increased serum proteins first, which were used for rapid urea production leading to high serum urea. Excessive urea production from proteins might have then led to hypoproteinemia and more urea production might continue after development of hypoproteinemia. The erythropoeitin production is inhibited in renal malfunctioning leading to high urea (Garba *et al*., 2007). Definitely blood volume decreases along with decreased erythrocytes, which might be a reason for anaemia reported in CY-treated animals (Ahmad *et al*., 2009) but the types of cells involved in inflammation and white blood cells are increased, making the body more vulnerable to infections (Garba *et al*., 2007). Alterations in cellular renal structure diminish the ability of the kidneys to filter the waste products from the blood and excrete them. As a result clearance values for creatinine and urea in CY-treated animals might be lowered and blood levels of creatinine and urea were increased (Yousef *et al*., 2006).

Present study revealed a dose and time dependent decreasing trend in the concentration of STP, serum albumin and serum globulins with CY treatment. Decreased STP results either from reduced synthesis or increased breakdown/degradation. Both mechanisms have been proposed for pyrethroid induced hypoproteinemia. Firstly the pyrethroids have been reported to prevent adenine triphosphate production by inhibiting mitochondria complex I (Gassner *et al*., 1997) and oxygen consumption (Reddy & Philip, 1992) in the cell. In this way, Na^+/K^+ pumps are disturbed (Khan *et al*., 2009), with sodium and water being transported into the cell cytosol, resulting in cellular water overload and deranged protein synthesis (Guyton & Hall, 2000) leading to hypoproteinemia. Secondly, degradation of proteins by pyrethroids might occur due to oxidative stress as discussed above in the mechanism of epithelial cast formation or it may occur due to increased activities of urea enzymes. These enzymes are mostly associated with liver damage, since the urea cycle is confined to liver in which proteins are broken down into urea (Woodman, 1980). Decreased STP could be credited due in part to the damaging outcome of pyrethroids on hepatocytes as confirmed by the increase in the activities of liver enzymes (Yousef *et al*., 2006). Decreased proteins may be encountered in renal and intestinal protein loss, hemorrhages, malabsorption and liver failure (Khan, 2008). Decreased serum proteins in the present study implied that increase in serum urea levels could be due to increased protein breakdown, in which case the hypoproteinemia could be due to increased breakdown of proteins and not lesser synthesis.

Decreased albumin in animals treated with pesticides might be related with altered metabolic activities of proteins and free amino acids in liver (Rivarola & Balegno, 1991). When amino acids in the liver are altered, albumin synthesis is hampered and decreased serum albumin results. Decreased serum globulins were recorded in the present study with CY-treatment. The endoplasmic reticulum (ER) of plasma cells is principally involved with globulin synthesis, which is also reported to be adversely affected during pesticide toxicity (Reyes & More, 1979). It has been proposed that ER might accumulate calcium by Ca^{2+} pump, then either inositol 1, 4, 5-trisphosphate or cyclic adenosine di-phosphate ribose cause release of Ca^{2+}. When ER is disturbed, there is also lesser globulin synthesis (He *et al*., 2006). Therefore, it is apparent that decrease in serum globulin was due to reduction in its synthesis by the plasma cells.

From the results of the present work, it was concluded that CY at various doses administered produced moderate histological lesions in liver and kidneys along-with increased levels of various enzymes, urea and creatinine and decreased levels of proteins in serum samples. All of these changes were mostly dose and time dependent.

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REFERENCES

Ahmad, L., A. Khan, M.Z. Khan and I. Hussain, 2009. Cypermethrin induced anaemia in male rabbits. *Pakistan Vet. J.*, 29: 191–195

Ahmad, L., A. Khan and M.Z. Khan, 2011. Pyrethroid-induced reproductive toxico-pathology in non-target species. *Pakistan Vet. J.*, 31: In Press

Aslam, F., A. Khan, M.Z. Khan, S. Sharaf, S.T. Gul and M.K. Saleemi, 2010. Toxico-pathological changes induced by cypermethrin in broiler chicks: Their attenuation with Vitamin E and selenium. *Exp. Toxicol. Pathol.*, 62: 441–450

Anwar, K., 2003. Cypermethrin, a pyrethroid insecticide induces teratological and biochemical changes in young chick embryos. *Pakistan J. Biol. Sci.,* 6: 1698–1705

Awaad, M.H.H., G.A. Abdel-Alim, K.S.S. Sayed, Kawkab, A. Ahmed, A.A. Nada, A.S.Z. Metwalli and A.N. Alkhalaf, 2010. Immunostimulant effects of essential oils of peppermint and eucalyptus in chickens. *Pakistan Vet. J.,* 30: 61–66

Burt, A.D. and R.N.M. MacSween, 1993. Bile duct proliferation: Its true significance? *Histopathology*, 23: 599–602

Crawford, M.J., A. Croucher and D.H. Hutson, 1981. Metabolism of cis- and trans-cypermethrin in rats: Balance and tissue retention study. *J. Agric. Food Chem.,* 29: 130–135

El-Demerdash, F.M., M.I. Yousef and K.S. Al-Salhen, 2003. Protective effects of isoflavone on some biochemical parameters affected by cypermethrin in male rabbits. *J. Environ. Sci. Health B.*, 38: 365–378

Garba, S.H., A.B. Adelaiye and L.Y. Mshelia, 2007. Histopathological and biochemical changes in the rats' kidney following exposure to a pyrethroid based mosquito coil. *J Appl. Sci. Res.,* 3: 1788–1793

Gassner, B., A. Wüthrich, G. Scholtysik and M. Solioz, 1997. The pyrethroids permethrin and cyhalothrin are potent inhibitors of the mitochondrial complex I. *J. Pharmacol. Exp. Therapeut.,* 281: 855–860

Guven, A., M. Erisir, N.N. Kamiloglu, I. Kaya, A. Devecl and A.K. Deverim, 2006. *Kafkas Univ Vet Fakultesi Dergisi*, 12: 53–56

Guyton, A.C. and J.E. Hall, 2000. *Text Book of Medical Physiology*, 10th edition, pp: 14–20. WB Saunders, Philadelphia

Hartley, D. and H. Kidd, 1990. *The Agrochemicals Handbook*, 2nd edition. Royal Society of Chemistry, Cambridge, UK

Hayes, A.W., 2007. *Principles and Methods of Toxicology*, 5th edition, pp: 1343–1345. Informa Healthcare, London, UK

He, J., J.F. Chen, R. Liu, L. Song, H.C. Chang and X.R. Wang, 2006. Fenvalerate-induced alterations in calcium homeostasis in rat ovary. *Biomed. Environ. Sci.,* 19: 15–20

Hulzebos, C.V., P.J. Voshol, H. Wolters, J.K. Kruit, R. Ottenhof, A.K. Groen, F. Stellaard, H.J. Verkade and F. Kuipers, 2005. Bile duct proliferation associated with bile salt-induced hypercholeresis in Mdr2 P-glycoprotein-deficient mice. *Liver Int.,* 25: 604–612

Javed, M.T., L. Ahmad, M. Irfan, I. Ali, A. Khan, M. Wasiq, F.A. Farooqi, M.S. Latif and M. Cagiola, 2010. Haematological and serum protein values in tuberculin reactor and non-reactor water buffaloes, cattle, sheep and goats. *Pakistan Vet. J.,* 30: 100–104

Khan, A., 2008. *Veterinary Clinical Pathology,* University of Agriculture Press, Fasialabad, Pakistan

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

Khan, I.A., A. Khan, A. Hussain, A. Riaz and A. Aziz, 2011. Hemato-biochemical alterations in cross bred cattle affected with bovine theileriosis in semi arid zone. *Pakistan Vet. J.,* 31: 137–140

Khan, W.A., M.Z. Khan, A. Khan and I. Hussain, 2010. Pathological effects of aflatoxin and their amelioration by vitamin E in White Leghorn layers. *Pakistan Vet. J.,* 30: 155–162

Manna, S., D. Bhattacharyya, T.K. Mandal and S. Das, 2004. Repeated dose toxicity of alfa-cypermethrin in rats. *J. Vet. Sci.,* 5: 241–245

Manna, P.R., D.W. Eubank, E. Lalli, P. Sassone-Corsi and D.M. Stocco, 2003. Transcriptional regulation of the mouse steroidogenic acute regulatory protein gene by the cAMP response-element binding protein and steroidogenic factor 1. *J. Mol. Endocrinol.,* 30: 381–397

Nduka, N., 1999. *Clinical Biochemistry for Students of Pathology*. Longman, Nigeria

Onyesom, I. and E.O. Anosike, 2007. Changes in rabbit liver function markers after chronic exposure to ethanol. *Asian J. Biochem.,* 2: 334–342

Ravel, R., 1995. *Clinical Application of Laboratory Data, In: Clinical Laboratory Medicine*, 6th edition, pp: 309–330. Mosby-Year Book Inc., St Louis

Reddy, P.M. and H. Philip, 1992. Changes in the levels of respiration and ions in the tissue of fresh water fish, *Labeo rohita* under fenvalerate stress. *Chemosphere*, 25: 843–852

Reyes, M.D. and E.C. More, 1979. Tissue residues and ultrastructural changes induced by DDT in chicken. *Poult. Sci.,* 58: 1183–1191

Rivarola, V. and H. Balegno, 1991. 2,4-dichlorophenoxyacetic acid effects on polyamine synthesis. *Toxicology,* 68: 109–119

Saxena, P.N., L.K.S. Chauhan and S.K. Gupta, 2005. Cytogenetic effects of commercial formulation of cypermethrin in root meristem cells of *Allium sativum*: Spectroscopic basis of chromosome damage. *Toxicology*, 216: 244–252

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

Shah, M.K., A. Khan, F. Rizvi, M. Siddique and S.U. Rehman, 2007. Effect of cypermethrin on clinico-haematological parameters in rabbits. *Pakistan Vet. J.,* 27: 171–175

Sharaf, S., A. Khan, M.Z. Khan, F. Aslam, M.K. Saleemi and F. Mahmood, 2010. Clinico-hematological and micronuclear changes induced by cypermethrin in broiler chicks: Their attenuation with vitamin E and selenium. *Exp. Toxicol. Pathol.,* 62: 333–341

Theal, R.M. and K. Scott, 1996. Evaluating asymptomatic patients with abnormal liver function test results. *American Family Phys.,* 53: 2111–2119

Wight, D.G.D., 1982. *Atlas of Liver Pathology*, pp: 176–181. MTP Press Limited, Lancaster, UK

Woodman, D.D., 1980. Assessment of hepatic function and damage in animal species: A review of the current approach of the academic, governmental and industrial institutions represented by the Animal Clinical Chemistry Association. *J. Appl. Toxicol.,* 8: 249–254

Wulkan, R.W. and B. Leijnse, 1986. Alkaline phosphatase and cholestasis. *Ann. Clin. Biochem.,* 4: 405–412

Yousef, M.I., T.I. Awad and E.H. Mohamed, 2006. Deltamethrin-induced oxidative damage and biochemical alterations in rat and its attenuation by Vitamin E. *Toxicology*, 227: 240–247

Zimmon, D.S., 1977. The hepatic vasculature and its response to hepatic injury: A working hypothesis. *Yale J. Biol. Med.,* 50: 497–506

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