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Research Article
Adverse Effects of Sixty Days Sub-chronic Exposure to β-cyfluthrin on Male Rats

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Abstract

Background and Objective: Beta-cyfluthrin, one of the most widely used pyrethroid insecticides against broad-spectrum insects in agriculture and in public health sectors. In domestic and agriculture uses, humans in all life stages e.g., adult (men and woman), pregnant women, newborns and children may have suffered potential exposure to β-cyfluthrin. Therefore, the present study was conducted to evaluate the adverse effects of 60 days sub-chronic exposure to β-cyfluthrin on male rats. **Methodology:** Twenty four male rats were assigned to four groups, control group and β-cyfluthrin-treated groups. Rats of β-cyfluthrin-treated groups were orally administrated β-cyfluthrin at doses 15.2 mg kg^{-1} b.wt. (1/25 LD_{50}), 7.6 mg kg^{-1} b.wt. (1/50 LD_{50}) and 3.8 mg kg^{-1} b.wt. (1/100 LD_{50}) in corn oil, daily for 60 days, respectively. Blood hematological parameters, (Red Blood Cells (RBC), White Blood Cells (WBCs), hemoglobin (Hb), hematocrit (PCV) and platelets (PLT)) serum liver and kidney biomarkers (aspartate aminotransferases (AST), alanine aminotransferases (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), protein, albumin, uric acid and creatinine) and testosterone hormone were determined, in addition to histopathological investigations. **Results:** Results showed that β-cyfluthrin caused significant reduction in body weights (b.wt.) and increase in relative liver weights at high and medium doses. It caused significant decrease in RBC's and PLT count, Hb level, HCT volume and PCV% and significant increase in WBC's count. Significant increase in AST, ALT, ALP, LDH, globulin, protein uric acid and creatinine, while decrease in albumin and testosterone hormone were recorded in β-cyfluthrin-treated rats in dose dependent manner. It induced histopathological alteration in liver, kidney and testes tissues. **Conclusion:** In light of the results in the present study, it can deduce that β-cyfluthrin induced liver, kidney and testes damage in male rats. Therefore, some precautions must take mainly among agriculture workers especially in poor rural where workers and their families (woman, pregnant women, newborns and children) are working and housing close to area of pesticides application and storage.

Key words: Pyrethroid, β-cyfluthrin, biochemical, hormone, sub-chronic, histopathology, rats

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Synthetic pesticides are widely use in agriculture and public health sectors to control pests in addition to reduce vector borne diseases[1]. Globally, about five billion pounds of pesticides formulation are used every year[2]. Therefore, it has caused severe and new hazards to human, domestic animal and eco-system. According to the World Health Organization, pesticides induced poisonings to 3 million cases and more than 220,000 deaths every year, especially in third world[3]. Although only 25% of the pesticides production worldwide are used in developing countries, the deaths due to pesticides toxicity[3] account 99%. The high percent of poisonings and mortality occurs in these countries, where poor knowledge of adverse effects and risks of pesticides on health, no or low protection against exposure especially between workers in agriculture sector, safe use is limited and pesticides are easily handy. Moreover, chronic exposure to pesticides is very important especially in rural populations of workers in agriculture sector. It has been reported that pesticides can cause biochemical and histopathological alteration in human and experimental animals. It cause organs dysfunction and injure after acute and chronic exposure[4-8]. Exposure to pesticides may cause cancer in human such as leukemia, brain and testes cancer[9]. It cause endocrine disruptor and reduced reproductive functions in animals and humans[9,10]. In addition, some pesticides can alter level of reproductive hormone as a result to their effect on hormone synthesis, storage, release and recognition[1].

Pyrethroid insecticides are the most active insecticides used in agriculture sector for insect-pests control and in public health for control mosquito, housefly and other domestic insects. This class of insecticides comprise about 25% of insecticide market worldwide[11]. In agriculture and domestic uses, human in all life stage e.g., adult (men and woman), pregnant women, newborns and children are exposure to pyrethroid insecticides[12-14] in addition to occupational exposure[15,16]. Previous studies reported biochemical and histopathological alteration in experimental animals after exposure to pyrethroid insecticides including, cypermethrin[17-21], fenvalerate[22], prallethrin[23] and cyfluthrin[24].

Beta-cyfluthrin, 3-(2, 2-dichloro-vinyl)-2, 2-dimethyl-cyclopropane-carboxylic acid cyano-(4-fluoro-3-phenoxy-phenyl)-methyl ester is a type II synthetic pyrethroid and wide use in agriculture and in public health insects[25,1]. One formulation of β-cyfluthrin is register under tread name Bulldock® (12.5% SC) as insecticide for agriculture using in Egypt. In addition, other formulation of β-cyfluthrin insecticides is widely used for control domestic insects e.g., mosquitoes, flies and cockroaches[26,1]. Therefore, humans could be exposure to β-cyfluthrin and others pyrethroid insecticides as direct exposure during manufacturing, agriculture and household application and indirect exposure via insecticides residues in air, water and food.

At this time, there is a shortage of information concerning the adverse effects of sub-chronic exposure to β-cyfluthrin on hemato-biochemicals and reproductive effects on male rats. Therefore, the present study was designed to evaluate the adverse effects of 60 days sub-chronic exposure to β-cyfluthrin on hematological, biochemical and reproductive parameters on male albino rats.

MATERIALS AND METHODS

Insecticide: Technical grade β-cyfluthrin (95.3% purity), obtained from Jiangsu Yangnong Chemical Co., Ltd., China was used in this study.

Kits and reagents: Kits of aspartate aminotransferases (AST; EC 2.6.1.1.), alanine aminotransferases (ALT; EC 2.6.1.2), creatinine and uric acid were obtained from Biodiagnostic Co., 29 Tahrir Street, Dokki, Giza, Egypt. Kit of alkaline phosphatase (ALP; EC 3.1.3.1) from Spectrum, Egyptian Company for Biotechnology, Obour City industrial area, block 20008 piece 19 A, Cairo, Egypt. Kits of total protein and albumin from Stanbio Laboratory, Texas, USA. Kit of LDH from BioSystems S.A. Costa-Brava 30, Barcelona, Spain. The ELIZA kit of total testosterone was obtained from Immunospec Corporation 7018 Owensmouth Ave. Suite 103 Canoga Park, CA, 91303.

All other chemicals and reagent used in this study were analytical reagent and purchased from Sigma-Aldrich.

Experimental animals: Male albino rats (*Rattus norvegicus*) weighing 170±10 g were obtained from the Animal Breeding House (ABH) of the National Research Centre (NRC), Dokki, Giza, Egypt. Rats housing in clean plastic cages in animal house on standard pellet diet, 12 h dark/light cycle, 40% humidity and 22±3°C temperature. Before treatment, rats were acclimated 1 week under the laboratory conditions. The experimental work on rats done with the approval of the Animal Care and Experimental Committee, National Research Centre, Giza, Egypt and the protocol conforms to the guidelines of the National Institutes of Health[27].

Experimental protocol: Three doses of β-cyfluthrin were selected based on the acute oral LD_{50}, equal 380 mg b.wt.$^{-1}$, of rats according to the e-Pesticide Manual[25]. The doses equal to 1/25 LD_{50} (15.2 mg kg^{-1} b.wt.), 1/50 LD_{50}

(7.6 mg kg^{-1} b.wt.) and 1/100 LD_{50} (3.8 mg kg^{-1} b.wt.) of β-cyfluthrin. Rats were orally administrated β-cyfluthrin in corn oil at a fixed volume of 0.5 mL rat^{-1} daily for 60 days. The dosages were adjusted weekly according to body weights changes. In the present study, 24 rats were divided into 4 different groups (6 rats per group), control group (G1) and β-cyfluthrin-treated groups (G2, G3 and G4). Control group was received (0.5 mL rat^{-1}) corn oil. Beta-cyfluthrin-treated groups (G2, G3 and G4) were received β-cyfluthrin orally at doses 15.2, 7.6 and 3.8 mg kg^{-1} b.wt., in corn oil for 60 days, respectively.

Organs, body weights and blood samples: At the end of experimental period, rats were fasted overnight; blood samples either were collected from the retero-orbital venous plexus in EDTA-tubes or in normal glass tubes for hematological and biochemical studies, respectively. Then, rats were sacrificed by cervical dislocation. Serum were collected after blood centrifugation at 3000 rpm for 10 min (4°C) using Heraeus Labofuge 400R (Kendro Laboratory Products GmbH, Germany). All serum samples were stored at -20°C for biomarker measurements by using Shimadzu UV-VIS Recording 2401 PC (Japan). Liver, kidney and testes were removed, cleaned, weighted and kept in 10% natural formalin for histopathological studies.

Hematological studies: Hematology Analyzer (URIT-3300) was used for Complete Blood Count (CBC) such as Red Blood Cell (RBCs), haematocrit (PCV), haemoglobin concentration (Hg), White Blood Cell (WBCs) and total platelet (PLT).

Liver and kidney dysfunction biomarkers: All liver and kidney biomarkers measurements (AST, ALT, ALP, LDH, protein, uric acid and creatinine) were determined in serum according to the methods given in the kit's instructions using a spectrophotometer (Shimadzu UV-VIS Recording 2401, PC, Japan). The AST and ALT[28], ALP[29], LDH[30], uric acid[31], total protein[32], albumin[33] and creatinine[34] were determined.

Globulins concentration: Globulins (G) concentration in serum was calculated based on total protein in serum comprise of globulins (alpha, beta and gamma) and albumin (A). So, globulins concentration can calculated as follows: Globulins = total protein-albumin. In addition, albumin/globulin ratio (A/G) could be estimated.

Determination of testosterone hormone in serum: Total testosterone hormone was determined by ELIZA kits in serum according to the immunoassay method[35].

Histopathological studies: After scarification, rat's organs (liver, kidney and testes) were removed and washed. Then, organs sections were cut (5 μm think), dehydrated in alcohol, embedded in paraffin wax and stained by haematoxylin and eosin (H and E)[36]. All organs sections (two slides of each section) were investigated using a light microscope (Olympus CX41) with a digital camera (Olympus DP12). The histopathological alteration in each tissues were scored to severe (+++), moderate (++), mild (+) and normal appearance (-)[37].

Statistical analysis: All data were analyzed using SPSS version 18.0 for windows and the statistical analysis was done by using one-way ANOVA analysis followed by Duncan's test. The differences were statistically significant at $p<0.05$. All data were expressed as Mean±Standard Error (SE).

RESULTS

Body and relative organs weights: A significant decrease in body weights and increase in relative liver weights were recorded in rats exposed to β-cyfluthrin at high and medium doses (Fig. 1a, b). In addition, there was no significant changes in relative kidney and testes weights either in high or low doses of β-cyfluthrin, compared to untreated rats (Fig. 1c, d). At the end of experimental periods (60 days), the decreased in body weight in rat exposed to β-cyfluthrin at high and medium doses accounted to -25.65 and -15.69%, respectively. The change in body and liver weights appeared to be occurred in a dose-dependent manner.

Hematological studies: Hematological parameters of rats exposed to β-cyfluthrin for 60 days are shown in Table 1. A significant decreased in red blood cell count (RBCs), haemoglobin concentration (Hg), haematocrit (PCV) and total platelet (PLT) were recorded and white blood cell (WBCs) was significant increased. The decrease or increase in hematological parameters of rats exposed to tested doses of β-cyfluthrin occurred in a dose-dependent manner. For example, the decrease in RBCs of rat exposed to β-cyfluthrin accounted to -6.46, -10.51 and -12.53% at doses 3.8, 7.6 and 15.2 mg kg^{-1} b.wt., of β-cyfluthrin compared to control, respectively. In contrast, the increase in WBCs accounted

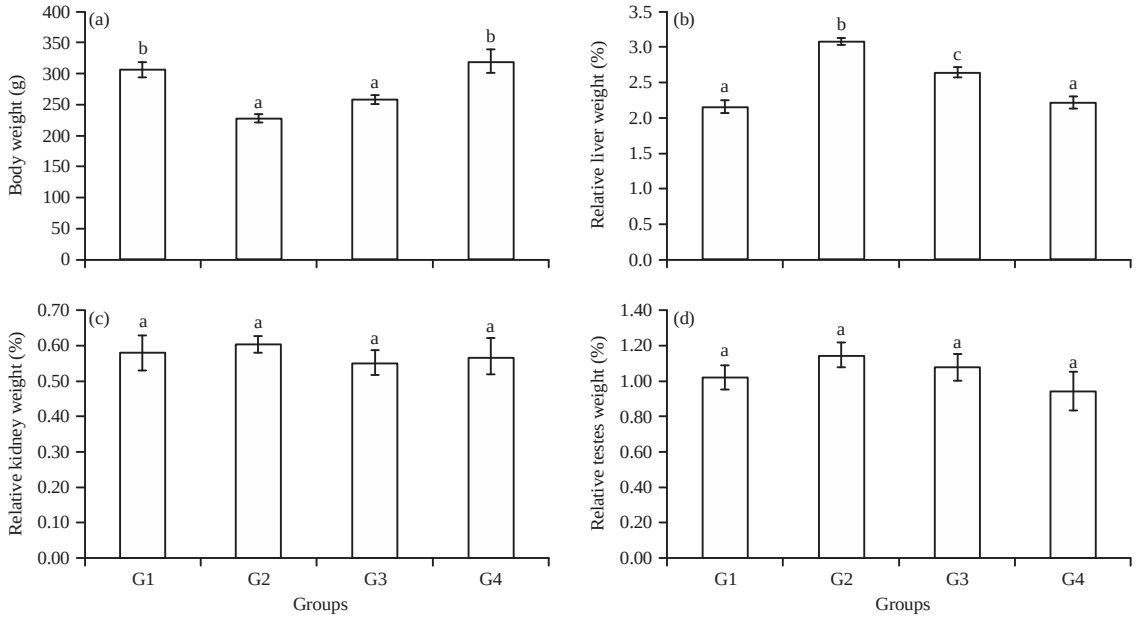

Fig. 1(a-d): (a) Body weight, (b) Relative liver, (c) Kidney and (d) Testes weights of male rats exposed to β-cyfluthrin for 60 days. G1: Control, G2: 1/25 LD_{50}, G3: 1/50 LD_{50} and G4: 1/100 LD_{50} of β-cyfluthrin. Bars represented mean of 6 rats±SE

Table 1: Effect of exposure to β-cyfluthrin for 60 days on some hematological parameters of rats

Treatments	RBCs (10^6 mm^{-3})	Hemoglobin (g dL^{-1})	Hematocrit (PCV%)	WBCs (10^3 mm^{-3})	Platelets (10^3 mm^{-3})
G1	4.95±0.03^{d}	15.35±0.03^{d}	47.65±0.09^{d}	6.95±0.44^{a}	365.50±8.97^{cd}
G4	4.63±0.06^{c}	14.75±0.27^{c}	45.73±0.89^{c}	7.75±0.31^{a}	354.75±4.57^{c}
G3	4.43±0.08^{b}	13.80±0.13^{b}	43.08±0.31^{b}	12.50±0.31^{b}	336.25±4.01^{b}
G2	4.33±0.06^{a}	13.35±0.12^{a}	42.25±0.35^{a}	20.10±0.12^{c}	308.00±1.16^{a}

RBCs: Red blood cells, WBCs: White blood cells, G1: Control, G2: 1/25 LD_{50}, G3: 1/50 LD_{50}, G4: 1/100 LD_{50} of β-cyfluthrin. Each value is a mean of six rat's±SE

Table 2: Liver enzymes in serum of rats exposed to β-cyfluthrin for 60 days

Treatments	AST (U L^{-1})	ALT (U L^{-1})	AST/ALT	ALP (U L^{-1})	LDH (U L^{-1})
G1	32.74±1.31^{a}	21.95±0.33^{a}	1.49±0.05	61.36±1.62^{a}	169.99±2.55^{a}
G4	42.95±1.47^{b}	28.50±1.05^{b}	1.52±0.14	93.17±2.36^{b}	221.96±3.01^{b}
G3	76.48±3.49^{c}	50.38±1.41^{c}	1.53±0.09	220.89±3.44^{c}	285.59±3.52^{c}
G2	83.81±4.28^{c}	69.75±1.39^{d}	1.21±0.08	290.88±4.75^{d}	416.89±3.63^{d}

AST: Aspartate aminotransferases, ALT: Alanine aminotransferases, ALP: Alkaline phosphatase, LDH: Lactate dehydrogenase, G1: Control, G2: 1/25 LD_{50}, G3: 1/50 LD_{50}, G4: 1/100 LD_{50} of β-cyfluthrin. Each value is a mean of six rat's±SE

Table 3: Protein, albumin and globulin in serum of rats exposed to β-cyfluthrin for 60 days

Treatments	Protein (g dL^{-1})	Albumin (g dL^{-1})	Globulin (g dL^{-1})	Albumin/globulin (A/G) ratio
G1	6.63±0.49^{a}	4.28±0.21^{d}	2.35±0.43^{a}	1.99±0.31^{d}
G4	8.55±0.40^{b}	3.99±0.21^{c}	4.57±0.46^{b}	0.91±0.11^{bc}
G3	9.88±0.48^{c}	3.35±0.11^{b}	6.54±0.43^{c}	0.52±0.03^{bc}
G2	11.18±0.32^{d}	2.82±0.16^{a}	8.36±0.16^{d}	0.34±0.01^{a}

G1: Control, G2: 1/25 LD_{50}, G3: 1/50 LD_{50}, G4: 1/100 LD_{50} of β-cyfluthrin. Each value is a mean of six rat's±SE

11.51, 79.86 and 189.21% of control at doses 3.8, 7.6 and 15.2 mg kg^{-1} b.wt., of β-cyfluthrin, respectively.

Liver and kidney biomarkers: The data of the liver biomarkers in serum of male rats exposed to β-cyfluthrin are shown in Table 2 and 3. A significant increase in the liver enzymes e.g., AST, ALT, ALP and LDH were recorded in β-cyfluthrin-treated rats (Table 1), while protein and globulin showed a significant increase and albumin and albumin/globulin (A/G) ratio showed a significant decrease (Table 2) in a dose dependent-manner. The increase in AST activity, if calculated, for β-cyfluthrin-treated groups accounted to 31.18, 133.60

and 155.99% at doses 3.8, 7.6 and 15.2 mg kg^{-1} b.wt., compared to control group, respectively. The change in ALT also, accounted to 29.84, 129.52 and 217.78% at doses 3.8, 7.6 and 15.2 mg kg^{-1} b.wt., of β-cyfluthrin compared to control group, respectively. However, the change in ALP and LDH activities in rats exposed to β-cyfluthrin at high dose (15.2 mg kg^{-1} b.wt.) accounted to 374.06 and 145.24%, while at low dose (3.8 mg kg^{-1} b.wt.), the activity accounted to 51.84 and 30.57% of control values, respectively. The total protein concentration accounted to 6.63 g dL^{-1} for untreated rats and accounted to 8.55, 9.88 and 11.18 g dL^{-1} for β-cyfluthrin-treated rats at doses 3.8, 7.6 and 15.2 mg kg^{-1} b.wt., respectively. In contrast, albumin concentration accounted to 4.28 g dL^{-1} of control and decreased to 2.82 g dL^{-1} in rats exposed to β-cyfluthrin at high dose (15.2 mg kg^{-1} b.wt.).

Results of kidney biomarkers in serum of male rats exposed to β-cyfluthrin are shown in Fig. 2. A significant increase in uric acid and creatinine concentrations were observed in β-cyfluthrin-treated rats in a dose dependent-manner. The concentration of uric acid accounted 4.79 mg dL^{-1} of control and increased to 6.86, 8.16 and 14.46 mg dL^{-1} of β-cyfluthrin-treated rats at doses 3.8, 7.6 and 15.2 mg kg^{-1} b.wt., respectively. In contrast, creatinine concentration accounted 0.87 mg dL^{-1} of control and increased to 1.28, 1.55 and 1.61 mg dL^{-1} of β-cyfluthrin-treated rats at doses at doses 3.8, 7.6 and 15.2 mg kg^{-1} b.wt., respectively.

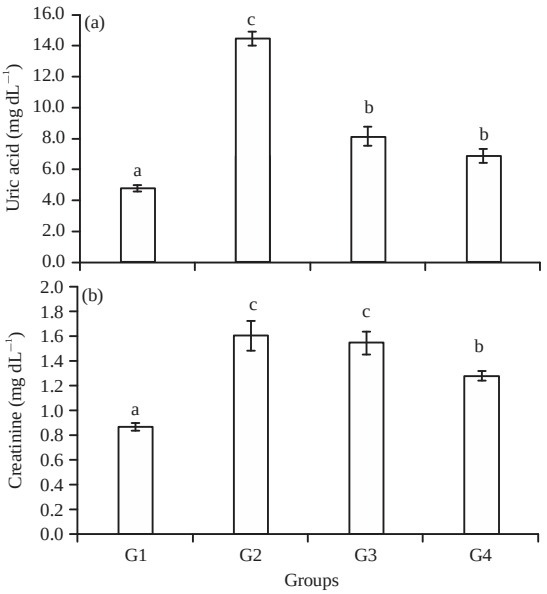

Fig. 2(a-b): Uric acid and creatinine concentration (mg dL^{-1}) in serum of male rats exposed to β-cyfluthrin for 60 days. G1: Control, G2: 1/25 LD_{50}, G3: 1/50 LD_{50} and G4: 1/100 LD_{50} of β-cyfluthrin. Bars represented mean of 6 rats±SE

Testosterone hormone: Results of testosterone hormone concentration are show in Fig. 3. Significant decrease in testosterone hormone level was noted in β-cyfluthrin-treated rats compared to control group. The testosterone hormone values accounted 4.72 ng mL^{-1} of control and decreased to 2.38, 3.16 and 351 ng mL^{-1} of β-cyfluthrin-treated rats at dose 15.2, 7.6 and 3.8 mg kg^{-1} b.wt., respectively. The decrease in testosterone hormone after exposure to β-cyfluthrin were found -49.58, -33.05 and -25.64% of β-cyfluthrin at dose 15.2, 7.6 and 3.8 mg kg^{-1} b.wt., respectively.

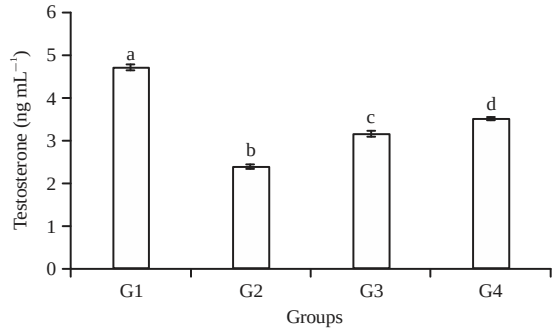

Fig. 3: Testosterone hormone concentration in serum of male rats exposed to β-cyfluthrin for 60 days. G1: Control, G2: 1/25 LD_{50}, G3: 1/50 LD_{50} and G4: 1/100 LD_{50} of β-cyfluthrin. Bars represented mean of 6 rats±SE

Histopathological studies: The histopathological alterations in liver, kidney and testis tissues were shown in Fig. 4-6. Liver tissue sections of control rats showed normal hepatocytes, while β-cyfluthrin at high dose (15.2 mg kg^{-1} b.wt.) showed severe degeneration of hepatic cells, infiltration of hemorrhage in sinusoids, severe portal tract fibrosis and infiltration by inflammatory cells. β-cyfluthrin at medium dose (7.6 mg kg^{-1} b.wt.) showed moderate-mild pyknotic nuclei, dilatation, vacuolization and hemorrhage in bile duct surround by inflammatory cells and degeneration, while β-cyfluthrin at low dose (3.8 mg kg^{-1} b.wt.) showed dilated central vein with moderate infiltration by inflammatory cells (Fig. 4). Kidney tissue sections of control rats showed normal renal tissue with many glomeruli, while β-cyfluthrin at high dose (15.2 mg kg^{-1} b.wt.) showed severe changes and multiple atrophic glomeruli, vacuolation, hemorrhage, atrophy in some of glomerular tuft and cystic dilatation. β-cyfluthrin at medium dose (7.6 mg kg^{-1} b.wt.) showed moderate vacuoles in epithelial lining tubules, mild infiltration

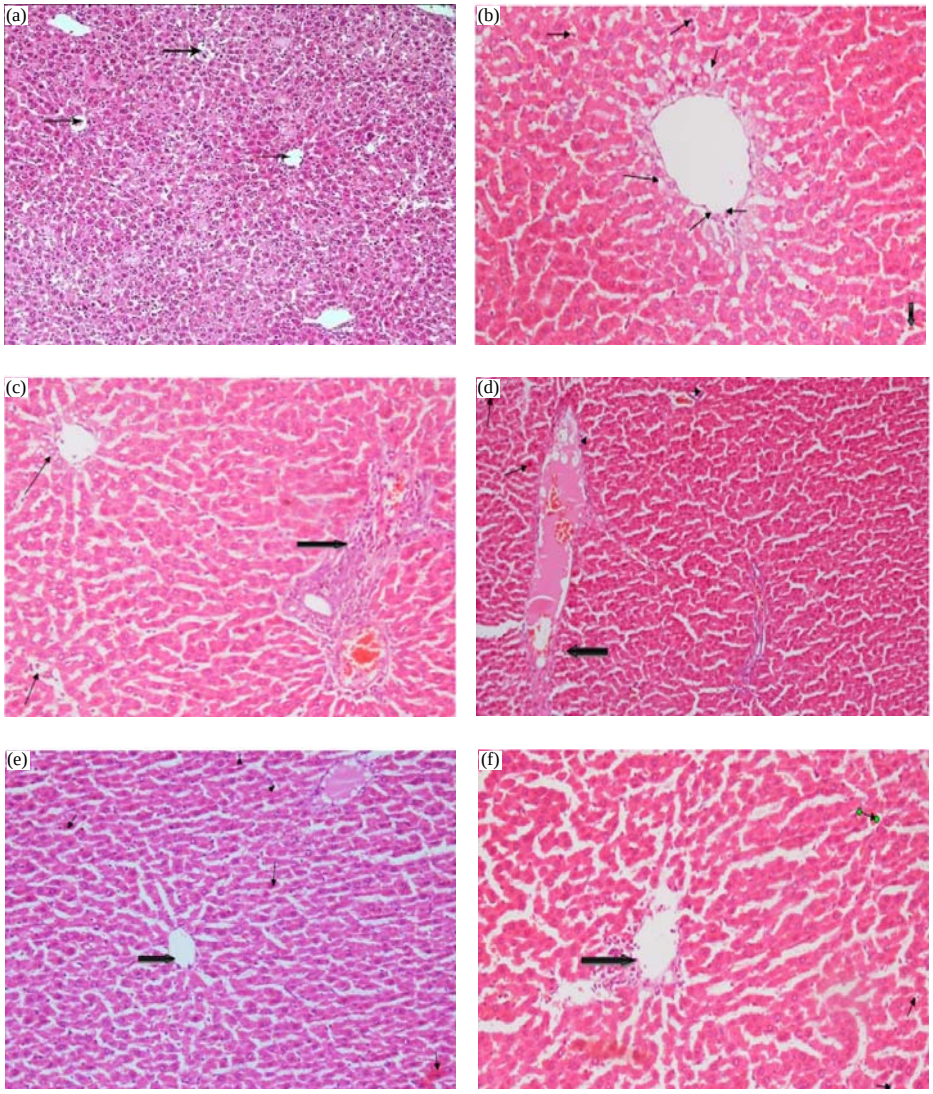

Fig. 4(a-f): Photomicrograph of liver tissue stained by haematoxylin and eosin (H and E) of (a) Control group (G1) showing normal liver tissue, the central vein (thin arrow) (x100), (b-c) β-cyfluthrin-treated groups, G2 (1/25 LD_{50}) showing A central veins that surrounded by severe degeneration of hepatic cells (thin arrow), also scattered in other areas and infiltration of hemorrhage in sinusoids, B showing central veins that surrounded by severe degeneration of hepatic cells (thin arrow) and severe portal tract fibrosis and infiltration by inflammatory cells (thick arrow) (x200), (d-e) G3 (1/50 LD_{50}) showing A preserved architecture, mild appearance of pyknotic nuclei (thin arrow) and moderate dilatation, vacuolization and hemorrhage in bile duct (thick arrow), surround by moderate inflammatory cells (arrow head) (x100), B showing preserved architecture, mild appearance of pyknotic nuclei (thin arrow), normal dilated central vein (thick arrow) and mild degeneration of few cells (arrow head) (x200) and (f) G4 (1/100 LD_{50}) showing dilated central vein with moderate infiltration by inflammatory cells (thick arrow) (x200)

by inflammatory cells and hemorrhage in scattered areas, while β-cyfluthrin at low dose (3.8 mg kg^{-1} b.wt.) showed mild vacuolation of epithelial lining of few of tubules and normal appereance of glomeruli (Fig. 5). Testis tissue sections of control rats showed normal testicular tissue with many seminiferous tubules, complete spermatogenesis process and interstitial tissue with leydig cells, while β-cyfluthrin at high dose (15.2 mg kg^{-1} b.wt.) showed dilated tubules, severe degeneration, severe-moderate deposition of hyaline material, loss of leydig cells and inflammatory cells. The β-cyfluthrin

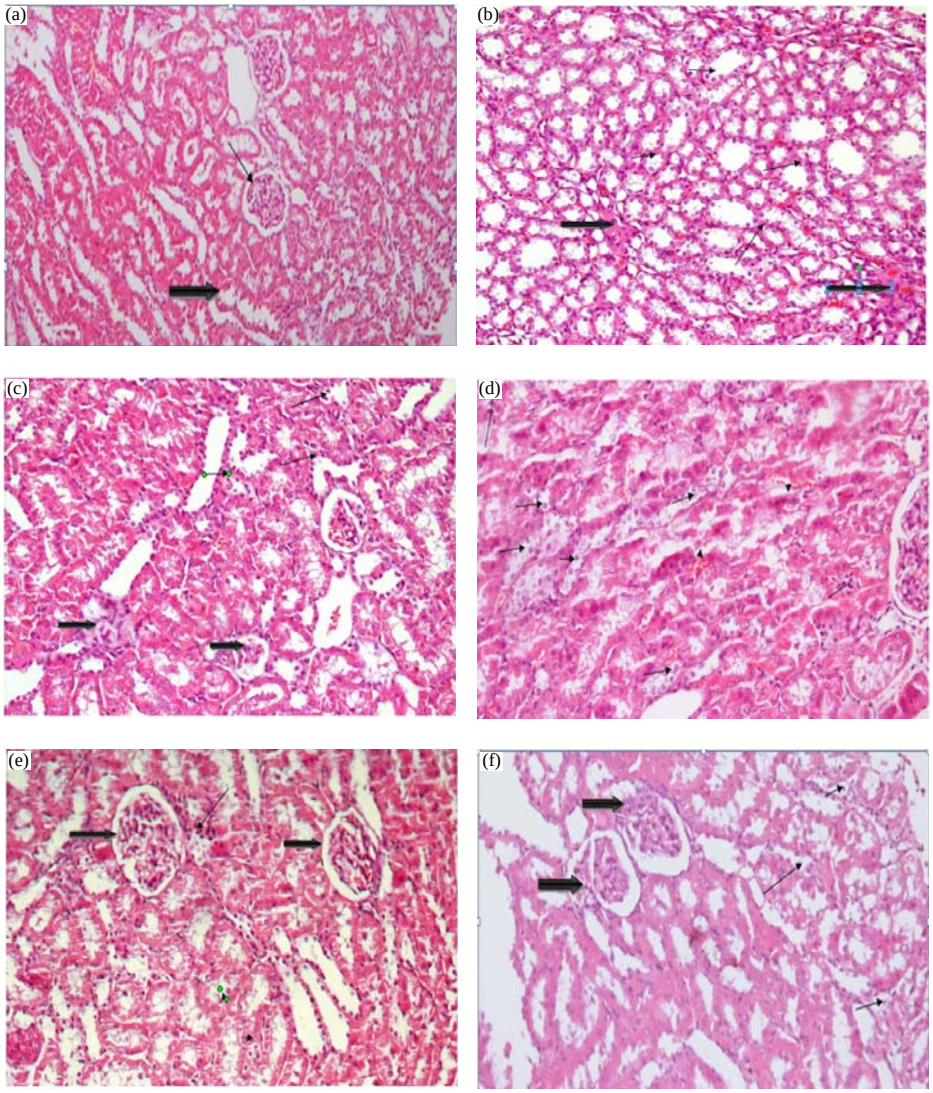

Fig. 5(a-f): Photomicrograph of kidney tissue stained by haematoxylin and eosin (H and E) of (a) Control group (G1) showing normal renal tissue with many glomeruli, (thin arrow) and normal appearing tubule (thick arrow) (x100), (b-c) β-cyfluthrin-treated groups, G2 (1/25 LD_{50}) showing A severe changes and multiple atrophic glomeruli (thick arrow), most tubules showed vacuolation in its epithelial lining and scattered hemorrhage, B atrophy in some of glomerular tuft (thick arrow), vacuolation of epithelial lining of most renal tubules (thin arrow) and cystic dilatation in most of them (x200), (d-e) G3 (1/50 LD_{50}) showing A moderate appereance of vacuoles in epithelial lining of most of tubules (thin arrow), B mild infiltration by inflammatory cells (line), appereance of hemorrhage in scattered areas (arrowhead) (x200) and (f) G4 (1/100 LD_{50}) showing normal appereance of glomeruli (thick arrow), mild vacuolation of epithelial lining of few of tubules (thin arrow) (x200)

at medium dose (7.6 mg kg^{-1} b.wt.) showed marked depression of the spermatogensis, decrease in the spermatid count/tubule with arrest of spermatogensis at the level of 2ry spermatocytes, hemorrhage, degeneration and vacuolation. While, β-cyfluthrin at low dose (3.8 mg kg^{-1} b.wt.) showed mild degeneration, deposition of hyaline material, hyaline deposition in the interstitial tissue and thickened blood vessels (Fig. 6). The histopathological alteration in β-cyfluthrin-treated rats occurred in a dose-dependent manner.

DISCUSSION

Physiological changes in experimental animals such as body weights and relative organs weights are important criteria for toxicological studies of xenobiotic. In the present

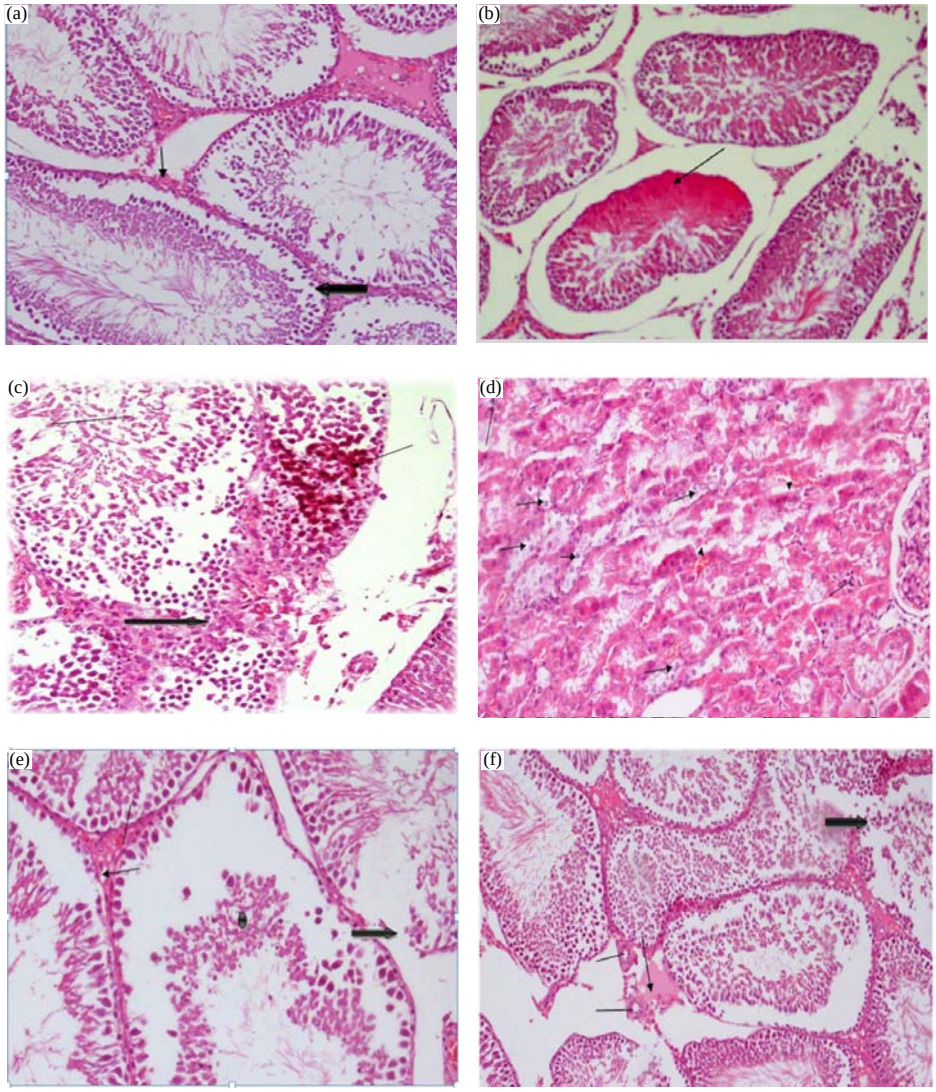

Fig. 6(a-f): Photomicrograph of testis tissue stained by haematoxylin and eosin (H and E) of (a) Control group (G1) showing normal testicular tissue with many seminiferous tubules, complete spermatogenesis process (thick arrow) and interstitial tissue with leydig cells (thin arrow) (x200), (b-c) β-cyfluthrin-treated groups: G2 (1/25 LD_{50}) showing A dilated tubules, severe changes in the testicular tissue, severe degeneration of its cellular component (thin arrow) with moderate to severe deposition of hyaline material and loss of leydig cells between tubules (x100), B showing dilated tubules, severe changes in the testicular tissue, severe infiltration by inflammatory cells (thin arrow) with moderate to severe deposition of hyaline material (line) and destruction at one side of the basement membrane (thick arrow) (x200), (d-e) G3 (1/50 LD_{50}) showing A marked depression of the spermatogensis and marked decrease in the spermatid count/tubule with arrest of spermatogensis at the level of 2ry spermatocytes in most of tubules (thick arrow), loss of boundaries between most of tubules (fusion) (thin arrow) and hemorrhage in most areas (x100), B showing dilated tubules with moderate changes in the testicular tissue, moderate cellular degeneration in the lumen (dot) with mild deposition of hyaline material, mild hemorrhage (line), vacuolation (thin arrow) and partial destruction of the basement membrane of normal thickness (thick arrow) (x400) and (f) G4 (1/100 LD_{50}) showing dilated tubules, moderate changes in the testicular tissue, mild degeneration of its cellular component with mild deposition of hyaline material, the basement membrane of normal thickness and lost in most tubules show fusion with each other (thick arrow), hyaline deposition in the interstitial tissue (thin arrow) and thickened blood vessels (line) (x100)

study, significant decrease in body weighs of rats exposed to β-cyfluthrin at high and medium doses was observed. The decrease in body weight can be due to the toxic action of β-cyfluthrin on rats that cause decrease in hungriness and absorption of nutrients from gut of intoxicated-rats[38]. This effect could be due to the toxic action of β-cyfluthrin on gastrointestinal tract, which cause decrease in food consumption of treated animals[39]. Previous studies reported decrease in body weight of mice exposed to β-cyfluthrin[39] at 1/20 and 1/10 LD_{50}. In addition, other pyrethroid insecticides caused loss in body weight of male rats such as deltamethrin[40], cypermethrin[7] and prallethrin[23] and on weanling female rats and male mice such as cypermethrin[20,21], respectively. Moreover, organs and relative organs weights were executively use as marker for organ toxicity. In the present study, β-cyfluthrin caused increase in relative liver weight in treated-rats. This increase could be due to increase cell division as adaptive mechanism of liver to improve liver function[41-43,6,24]. However, liver weight was increased after exposure to pyrethroid insecticides such as β-cyfluthrin[37,24], cypermethrin[41,20,21], deltamethrin[42] and other insecticides such as diazinon[43] and chlorpyrifos[6]. In contrast, there is no significant changes in kidney and testis weights were observed in this study.

Hematological parameters are an important biomarkers for studying the adverse effects of xenobiotic e.g., insecticides on blood constituents of vertebrates. Previous studies reported direct correlation between concentration of haemoglobin (Hg) and values of haematocrite (PCV%) with RBCs count[4]. In this correlation, synergistic linking between the account of RBCs and other hematological parameters such as concentration of Hg and PCV% were recorded in all vertebrates including human[44]. In the present study, β-cyfluthrin induced decrease in RBCs count, Hb concentration and PCV% in a dose dependent manner. The decreased in RBCs in β-cyfluthrin-treated rats could be due to increase rat of RBCs breakdown and or due to the toxic effect of β-cyfluthrin on bone marrow[4,45,46]. Therefore, the decrease in Hb concentration could be due to the reducing of RBCs numbers or the increase of the breakdown of RBCs[4,46]. In addition, other studies reported toxic effect of β-cyfluthrin on bone marrow cells and induced cytotoxicity[47,39]. It has been reported that pyrethroid insecticide bifenthrin decreased RBCs in rabbits[48]. The researchers explained this reduces in RBCs count to excessive injury, inhibition of formation and breakdown of erythrocytes (RBCs). In addition, insecticides such as deltamethrin[46], cypermethrin[49,46], dimethoate[4], bifenthrin[48], tribenuron-methyl[5] and spinosad[50] reduced RBCs count, Hb concentration and PCV% and increase WBCs as a direct toxic effect on animals.

Liver and kidney biomarkers in serum such as AST, ALP, ALT, LDH, protein, albumin, uric acid and creatinine are extensively use in evaluation the hepatic and renal damage. In fact, the liver is the main organ for metabolism and detoxification process of xenobiotics, while kidney is play a central role for exertions. Therefore, both liver and kidney are extreme exposure to xenobiotics such as pesticides and their metabolic compounds[51]. In the present study, β-cyfluthrin caused significant elevation in the activity of serum liver enzymes (AST, ALT, ALP and LDH) and decrease albumin concentration in a dose dependent-manner. It caused increase in total protein and globulin concentration in serum of male rats. The increase of serum liver enzymes (AST, ALT and ALP) in the present study could be due to the toxic action of β-cyfluthrin on hepatic cells lead to hepatic dysfunction and biosynthesis disruption with change in membrane permeability[52,43,21]. It has been reported that increase serum liver enzymes is a good correlation between liver enzymes leakage and hepatic cell damage[53]. In addition, increase activity of LDH in serum of β-cyfluthrin-intoxicated rats could be due to hepatocellular necrosis, change in hepatic cells membrane permeability and outflow of LDH into blood stream[22]. The increase of protein concentration in serum of rat exposed to β-cyfluthrin can be due to increase leakage of liver enzymes and disturbance in protein catabolism, while decrease albumin concentration may be due to liver dysfunction and disturbance in the biosynthesis of Ab in hepatocytes[50,46]. In contrast, increase of globulin concentration may be due to the toxic stress of β-cyfluthrin. Other studies reported increase of globulin concentration under stress of some insecticides such as cypermethrin[17,18] and endosulfan[54]. It has been reported that the toxicity of pyrethroid insecticides such as cypermethrin can be due to the stress conditions induced by these insecticides that cause release of adrenocorticotropic hormone, activating consequent secretion of cortisol by the adrenal cortex[55]. The increase in this hormone lead to reduces cellular protein stores. Therefore, proteins synthesis by hepatocytes come to be out into blood, increasing the protein concentration[19]. Previous studies reported hepatotoxicity in experimental animals and increase serum liver enzymes after exposure to some insecticides. For example β-cyfluthrin[24], deltamethrin[46,40], cypermethrin[38,49,46,20], dimethoate[4], bifenthrin[48], chlorpyrifos[6] and diazinon[43]. The alteration in liver dysfunction biomarkers

in rats exposed to β-cyfluthrin in the present study, suggests probable liver injury. This damage in the liver can observed in the histopathological alterations in liver tissue of β-cyfluthrin-treated rats. The histopathological lesions including degeneration, infiltration, hemorrhage, fibrosis, inflammations, dilatation and vacuolization. Some insecticides such as β-cyfluthrin[24], cypermethrin[18,46], deltamethrin[46], diazinon[43], chlorpyrifos[6] and fipronil[56] were reported early pathological alterations.

Our results revealed the clear dose dependent increase in uric acid and creatinine levels in serum with sub-chronic β-cyfluthrin exposure. The increase in uric acid and creatinine may attributed to the decrease in glomerular filtration, tubules dysfunction and kidney damage[57,8]. The increase in uric acid and creatinine in β-cyfluthrin-intoxicated rats was associated with the histopathological lesions in the kidney. These lesions including multiple atrophic glomeruli, vacuolation, hemorrhage, cystic dilatation and infiltration by inflammatory cells and hemorrhage in scattered areas. Changes in uric acid and creatinine concentration along with histopathological alteration in kidney were reported in the animals exposed to various pesticides such as β-cyfluthrin[24], abamectin[8], cypermethrin[46], endosulfan[58] and chlorpyrifos[46,6].

In fact, testosterone hormone is necessary for normal testicle function and reproduction. In addition, many complex cellular mechanism is involves in testosterone production in testis[59]. It play as essential role for citric acid and fructose production from androgen dependent organs (the epididymis and accessory sex glands), that are the important for vehicle and energy for sperm in the ejaculate[60]. In the present study, β-cyfluthrin caused significant decrease in testosterone hormone concentration in male rats. This effect may be due to the effect of β-cyfluthrin and their toxic metabolites on testis leading to testicular failure. It has been reported that occupational exposure to pyrethroid insecticides can reduce testosterone concentration by 10% as a results to effect of their toxic metabolites, which paralleled to decrease sperm concentration and motility and increase abnormal sperm morphology[61,62]. In rats exposed to fenvalerate, low testosterone and sperm concentration and motility were recorded. These decreases could be due to the reduced of 17 β-hydroxysteroid dehydrogenase and glucose-6-phosphate dehydrogenase in fenvalerate-treated rats[63]. However, the decrease in testosterone concentration in rats exposed to β-cyfluthrin in the present study, suggests probable testis injury, which observed in the histopathological alterations in testis tissue. The histopathological lesions including dilated tubules severe degeneration, severe-moderate deposition of hyaline material, loss of leydig cells, inflammatory cells, hemorrhage, degeneration and vacuolation. Other studies reported reproductive effect of pyrethroid and other pesticides on human and animals[38,63,61,16].

CONCLUSION

Based on the results of the present study, it can conclude that β-cyfluthrin caused significant alteration in liver, kidney and testes function biomarkers in male rats. It induced histopathological changes and damage of the liver, kidney and testes tissues. Therefore, some precautions must take mainly among agriculture workers especially in poor rural where workers and their families (woman, pregnant women, newborns and children) are working and housing close to area of pesticides application and storage.

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