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Original Research Article

Cyfluthrin-induced toxicity on testes of Swiss albino mice

N.K.Rajawat[1], I.Soni[1], P.Mathur[2] and D.Gupta[2]

[1]Centre for Advanced Studies, Department of Zoology, University of Rajasthan, Jaipur, India
[2]Department of Zoology, IIS University, Jaipur, Rajasthan, India
Corresponding author

A B S T R A C T

Widespread use of insecticides for many years leads to contamination in food chain and environment. Cyfluthrin, a synthetic pyrethroid insecticide is used widely against chewing and sucking insects. Toxic effects of cyfluthrin were evaluated on testes of male Swiss albino mice. The test animals were divided into two groups- Control and Experimental. Three doses of cyfluthrin: 2.65 mg/kg b.wt. (low dose), 5.3 mg/kg b.wt. (medium dose) and 10.6 mg/kg b.wt. (high dose), were administered to the animals of Experimental group. They were given the pesticide dissolved in distilled water once orally and were autopsied on Day 2, 7 and 30 after pesticide administration. Quantitative aspects viz. body weight, testes weight and organ/somatic Index were assessed. Histopathological examination of testes was performed and biochemical parameters viz. Glycogen, Fructose, Cholesterol and Sialic acid were evaluated. The medium and high dose significantly decreased the body weight and organ/somatic index. The germinal epithelium showed shrinkage, vacuolization occurred in the interstitial spaces, seminiferous tubules were displaced and lumen diameter decreased. A highly significant decrease in Glycogen, Fructose, Cholesterol and Sialic acid occurred. It may therefore be inferred that cyfluthrin exerts hazardous effects on the testes of Swiss albino mice.

Keywords

Cyfluthrin, insecticide, testes; biochemical parameters; histopathology

Introduction

Pyrethroids are used effectively against a wide variety of economically important pests. They have a low oral toxicity to mammals; their insect to mammal toxicity ratio is much higher than that of the other major classes of insecticides. However, pyrethroids are lipophilic in nature, hence bio accumulate and affect humans indirectly (Kumar *et al*., 2006; Suman *et al*., 2006). They are reported to elicit reproductive toxicity, neuro-developmental toxicity, endocrine disruption and adverse immune system effects (Wang *et al*., 2009). Cyfluthrin is a relatively new insecticide that is classified as a synthetic type II pyrethroid because its chemical structure is a synthetic analogue of naturally occurring pyrethrins (Adams *et al*., 2002). Its ISO approved common name is cyano (4-fluoro-3-

phenoxy phenyl) methyl 3-(2,2 – dichloroethenyl)-2, 2- dimethyl – cyclopropane carboxylate. It is the active ingredient of many insecticide formulations including Baythroid, Baygon, Attatox, Contur, Loser, Responsar, Solfac and Tempo (Meister, 1992).

Cyfluthrin is a neurotoxicant. It induces alterations in nerve membrane, causing abnormal sodium and potassium ion flow. This results in repetitive discharge from the neurons, causing convulsions and also blockage of further nerve impulses (Narahashi, 1996). The most common symptom of acute exposure to cyfluthrin is paresthesia progressing to numbness (Singh *et al*., 1999). Six synthetic pyrethroids (Eil and Nisula, 1990) are reported to bind with androgen (a male sex hormone) receptors, and disrupt normal androgen function. Permethrin is known to reduce male reproductive hormone levels, sperm number and motility (Zhang *et al*., 2007). Some animal and in vitro studies suggest that some pyrethroid insecticides or their metabolites may possess endocrine disrupting properties (Sun *et al*., 2007) and adversely affect the semen quality (Tyler *et al*., 2007). In humans, few recent occupational studies have reported significant or suggestive associations of urinary pyrethroid insecticide metabolite concentrations with reduced sperm concentration, motility and morphology and increased DNA damage (Meeker *et al*., 2007). This study is designed to assess the toxicity of cyfluthrin on testes of Swiss albino mice.

Materials and Methods

Test material

A commercial formulation (product type 18) Solfac® EW 050 produced by Bayer CropScience, Mumbai, India, containing 5% w\w cyfluthrin purchased from Sigma Aldriech Laborchemikalien GmbH D-30918 Seelze was used.

Animals

Swiss albino mice were obtained from the Indian Veterinary Research Institute, Bareilly (UP, India), and were housed in air cooled room. The animals were kept in polypropylene cages covered by steel wire grid and having bedding of wood savings. The colony was maintained on standard mice feed obtained from Hindustan Lever Ltd., New Delhi and tap water was provided *ad libitum.* An inbred colony was maintained. In the animal room temperature was maintained at 25±2ºC, relative humidity at approximately 30-40% and a 12 hrs light-12 hrs dark photoperiod. Healthy young male mice (6-8 weeks old) with an average body weight 22±2g were selected for the study purpose. The test animals were divided into two groups- Control and Experimental. Cyfluthrin dissolved in distilled water was administered to the Experimental animals once daily by oral intubation. The animals of Experimental group were divided into three sub groups: (Group L) receiving low dose - 2.65 mg/kg b.wt., (Group M) receiving medium dose – 5.3 mg/kg b.wt. and (Group H) receiving high dose - 10.6 mg/kg b.wt. The animals of each sub group were autopsied on Day 2 (Groups L2, M2 and H2), Day 7 (Groups L7, M7 and H7) and Day 30 (Groups L30, M30 and H30) after pesticide administration. The control animals received the vehicle alone i.e. distilled water.

On the day of autopsy, the animals were weighed and sacrificed. Testes were removed and weighed and organ/somatic index were calculated. Fifty percent tissue was fixed in Bouin's fluid for histopathological examination and the remaining used for biochemical estimations viz. Glycogen (Montgomery, 1957), Fructose (Foreman *et al*., 1973) Cholesterol (Zlatkis *et al*., 1953) and Sialic acid (Svennerholm *et al*., 1957).

The experiments were performed according to the guidelines for care and use of animals in scientific research of the Indian National Science Academy (2000), New Delhi and approved by Institutional Ethical committee (1678/GO/a/12/CPCSEA).

Body and organ weight

The body weights of mice were recorded before treatment and on the day of autopsy. The animals were sacrificed and testes were dissected out, trimmed off the attached tissues and weighed and organ/somatic index was calculated.

Histopathology

After removal, the testes were fixed in Bouin's fluid and embedded in paraffin and bees wax (3:1). After 24 hrs. of fixation 5 µm thick sections were taken and stained with hematoxylin and eosin. Each slide was examined for histopathological changes under light microscope.

Statistical analysis

The results obtained were expressed as Mean±SD, and the obtained data statistically analyzed using Student's 't' test. The significance level was set at $P<0.01$ and $P<0.001$.

Results and Discussion

The biochemical observations obtained after oral administration of cyfluthrin at the three dose levels are shown in the Graph I-IV. Testis is a complex organ containing three important cell types viz. germ cells, sertoli cells and leydig cells in close proximity (Figure.1). Histopathological examination of testes indicates that cyfluthrin caused various structural abnormalities. The germinal epithelium was shrunken and broken at some places. Vacuolization occurred in the interstitial spaces. The seminiferous tubules were displaced and lumen diameter decreased, thereby increasing the interstitial spaces (Figure B-E). There was a highly significant decrease ($P<0.001$) in body weight at all dose levels (Table I). Testes weight decreased in sub group M2, M7 and M30. Organ/somatic Index was affected by cyfluthrin treatment (Table I). Glycogen content in the testes was found to decrease highly significantly ($P<0.001$) as shown in (Graph I) at all dose levels. Fructose content in testes significantly decreased in a day dependent manner (Graph II). The cholesterol content decreased highly significant ($P<0.001$) on day 7 and day 30 at all dose levels (Graph III). Sialic acid decreased ($P<0.001$) in all sub groups (Graph IV).

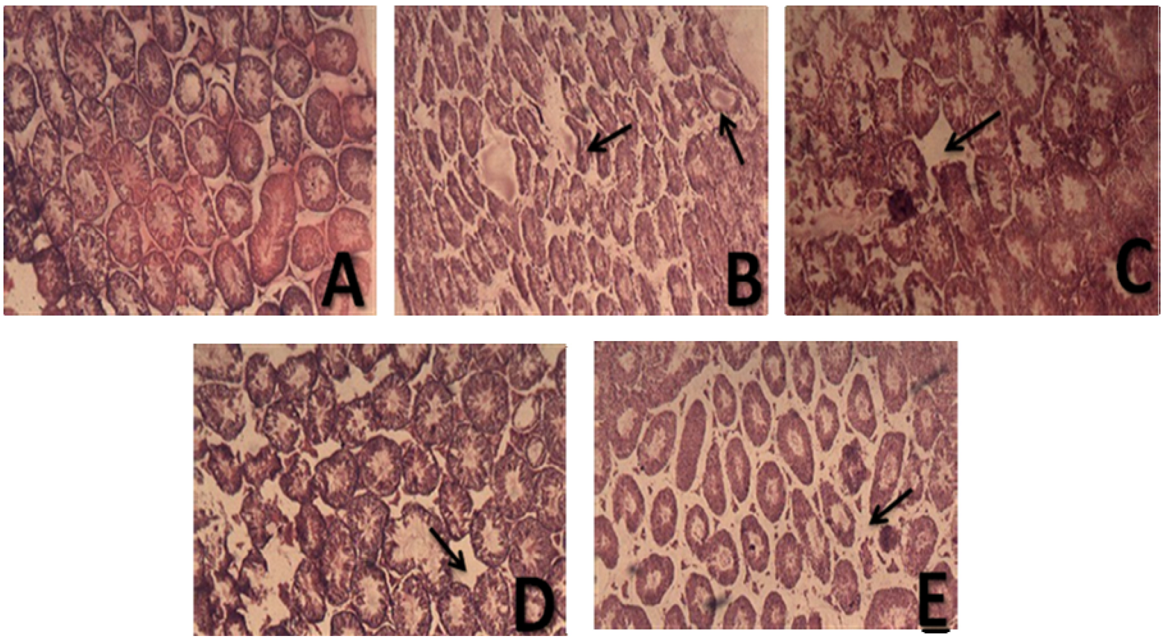
A significant decrease in body weight of animals was observed. This may be due to direct cytotoxic effect of the pesticide on somatic cells or indirectly through the central nervous system which controls the feed and water intake and regulates the endocrine function (Yousef *et al*., 1995). Testis is both an endocrine gland and a reproductive organ, responsible for the production of hormones and male gametes, and an important target for endocrine disruption. The testis consists of two types of tissues: seminiferous tubules, supported by sertoli cells, and the interstitial compartment, comprised of leydig cells (Fisher, 2004; Akingbemi and Estrogen, 2005). Testicular functions (spermatogenesis, steroidogenesis) are regulated by the hypothalamic-pituitary-testicular (HPT) axis which involves the pituitary gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Dettin *et al*., 2003; Jana *et al*., 2006). Testicular functions are proposed to be regulated by a number of hormones and growth factors in addition to FSH, LH, and androgens, including insulin-like growth factor, oxytocin, and transforming growth factor- and estrogens (Pryor *et al*., 200). As indicated by histopathogical examinations, cyfluthrin caused various structural abnormalities in testes. Seminiferous tubules were shrunken and appeared to be displaced, lumen diameter was decreased and there occurred vacuolization in the interstitial spaces. Similar results were also reported with chlorpyrifos (Joshi *et al*., 2007). This may probably explain the decrease in testes weight.

Carbohydrates are stored in the animal tissue in the form of glycogen, which acts as an energy producing source. Glucose plays a major role in energy metabolism and is stored as a readily available energy source in the form of glycogen in cells during various developmental and physiological stages (Thong and Graham, 2004; Sinclair *et al*., 2003; Gruetter and Glycogen, 2003; Ferrer *et al*., 2003). Glucose has also been shown to be an essential substrate for maintaining tissue integrity, ATP production and protein synthesis in the rat testis (Bajpai *et al*., 2008). Klip *et al.*, (1994) observed that the testicular interstitial cells are a good source of glycogen. In early pubertal period, spermatogenesis takes place in which glycogen is degraded to release glucose, which is used for metabolism of actively growing tissue. In the present work a highly significant decrease ($P<0.001$) in glycogen occurred, which could affect energy requirements of cells. This is in accordance with the view of Zuping *et al.*, (2009), who speculated that protein synthesis in spermatogenic cells is dependent upon glucose. Hence a decrease in the glycogen content could affect protein synthesis and thus subsequently inhibit spermatogenesis.

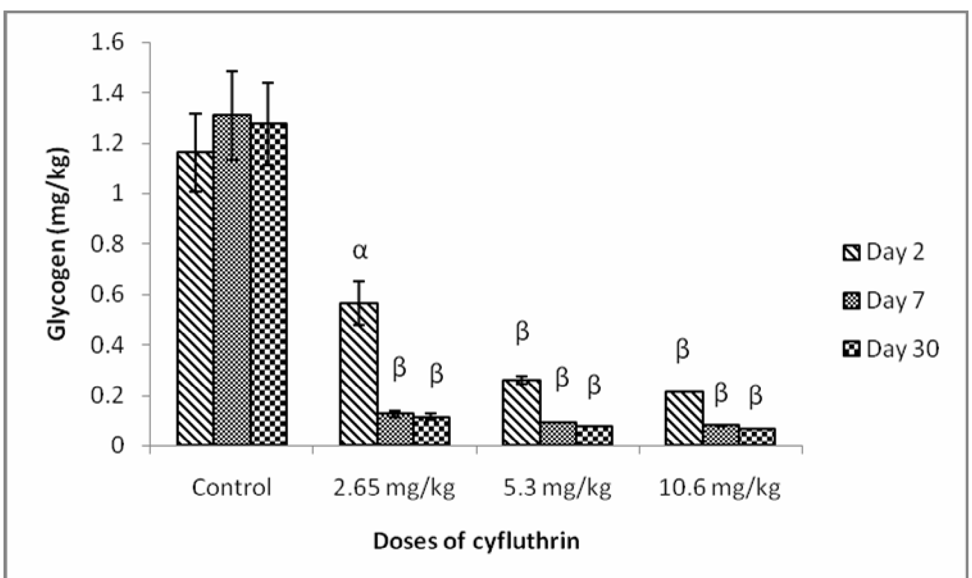
A decrease in fructose level in the testis of treated animals was also observed. Since the function of fructose is to induce the glycolytic metabolism of spermatozoa, it can be suggested that the decrease in fructose content due to cyfluthrin treatment hampers the glycolytic metabolism of spermatozoa. This in turn may lead to abnormal sperm function which ultimately may cause complete male sterility (Sarkar *et al*., 2000).

Cholesterol, containing a mono atomic alcohol and one double bond is considered to be most important precursor of all the steroid hormones including androgens (Chang *et al*., 2004). Testes and other tissues actively synthesize cholesterol. Sharpe *et al.*, (1993) speculated that most of the tissues of the body are dependent on dietary cholesterol as their source, while testis relies heavily on its endogenous synthesis. Since cholesterol is known to be a precursor in androgen synthesis in the testis, changes in the testicular cholesterol levels are considered to be important, as it is implicated in the inhibition\stimulation of spermatogenesis (Meroni *et al*., 2002). Androgens are very essential for normal functioning of accessory reproductive organs. Zhang (2007) found that pesticides pose anti – gonadal action or deprived levels of androgens. In the present study, cyfluthrin reduced the cholesterol content significantly. This is supported by the

Figure.1 T.S. of Testes showing (A) normal structure of testes (B) reduced diameter of seminiferous tubules and broken germinal epithelium (C) vacuolization in the interstitial spaces at low dose and (D) vacuolization in the interstitial spaces at high dose (E) increased interstitial spaces, decreased lumen diameter and distantly placed tubules at high dose. (400X)

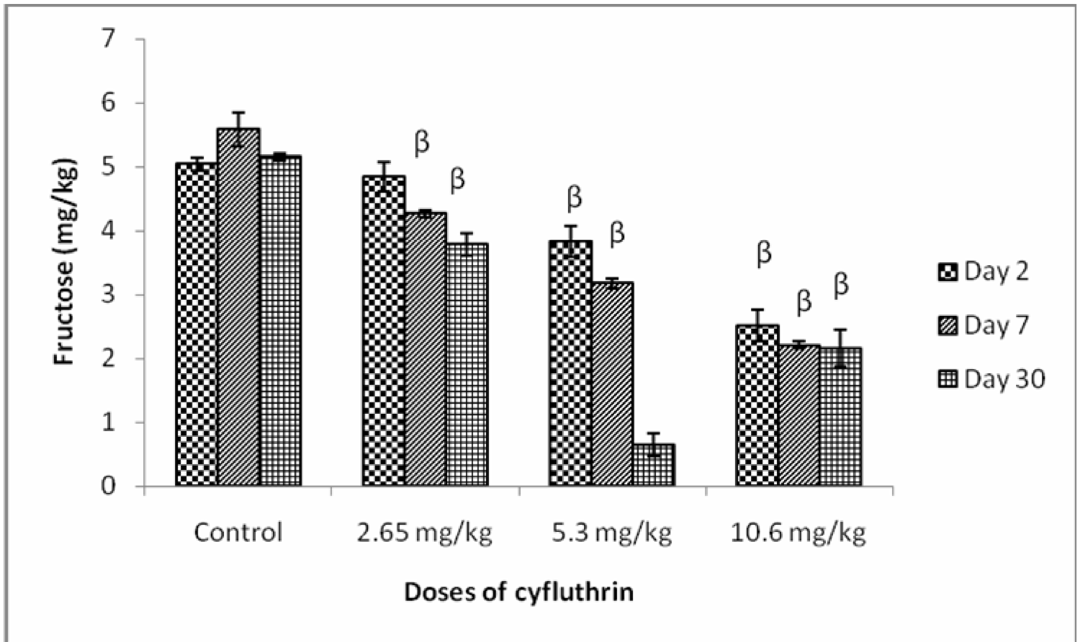


Graph.I Effect of different doses of Cyfluthrin on Glycogen content in testis



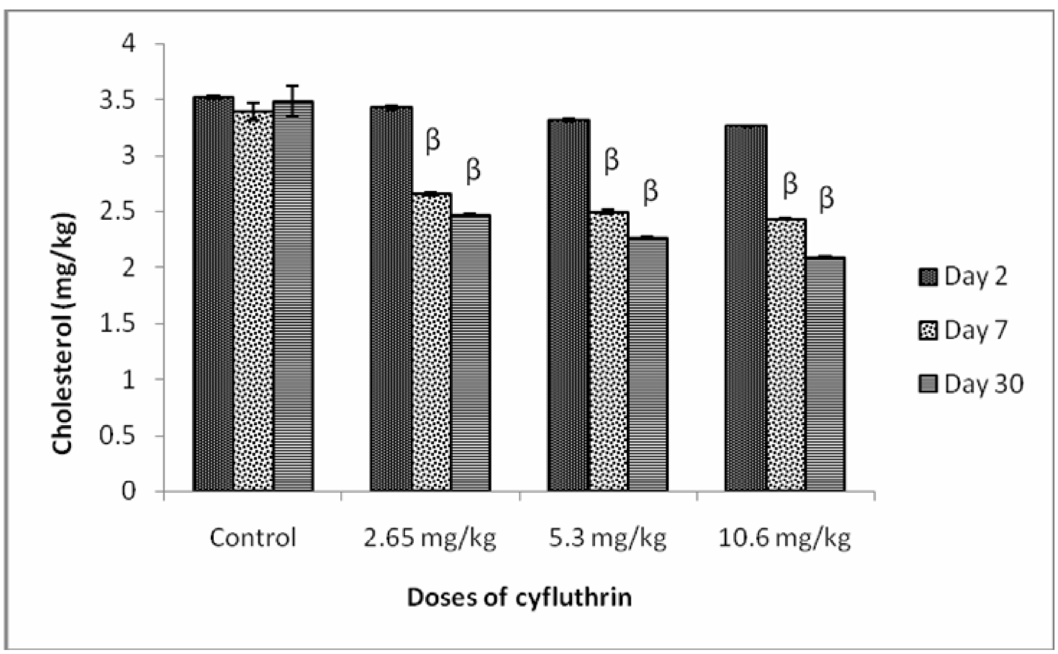
$^{\alpha}$ Significant difference (P<0.01), $^{\beta}$ Highly Significant difference (P<0.001)

Graph.II Effect of different doses of Cyfluthrin on Fructose content in testis



$^{\alpha}$ Significant difference (P<0.01), $^{\beta}$ Highly Significant difference (P<0.001)

Graph.III Effect of different doses of Cyfluthrin on Cholesterol content in testis



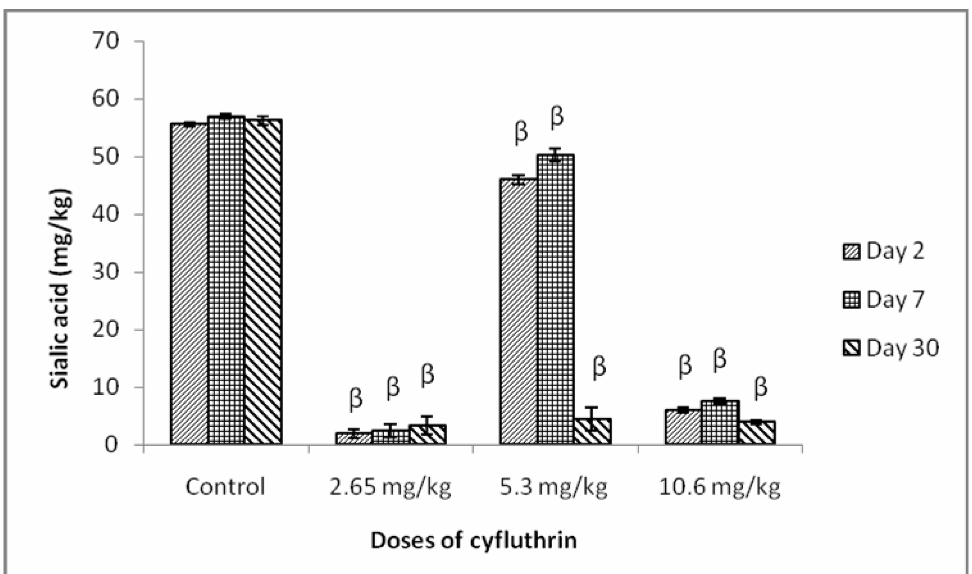
$^{\alpha}$ Significant difference (P<0.01), $^{\beta}$ Highly Significant difference (P<0.001)

Table.1 Effect of Cyfluthrin on testis/somatic index

Days / Animals	Day 2			Day 7			Day 30		
	Body Weight	**Testis weight**	**Testis /body Weight ratio**	**Body Weight**	**Testis weight**	**Testis /body Weight ratio**	**Body Weight**	**Testis weight**	**Testis /body Weight ratio**
Control	27.79 ±0.16	0.26128 ±0.0057	0.0098±0.0002	27.79±0.16	0.26128±0.0057	0.0098±0.0002	27.79±0.16	0.26128±0.0057	0.0098±0.0002
Low Dose (2.65 mg/kg)	25.91 ±0.18 **	0.26046 ±0.0055	0.0095±0.0001	25.9±0.17**	0.25902±0.0046	0.0098±7.48	25.53±0.34*	0.25148±0.0047	0.0096±0.0001
Medium Dose (5.3 mg/kg)	25.18 ±0.26 **	0.25148 ±0.0047 **	0.0057±0.0002**	24.59±0.17**	0.28158±0.005	0.011±0.0003*	22.64±0.25**	0.17402±0.0068**	0.0076±0.00037**
High Dose (10.6 mg/kg)	24.59 ±0.19 **	0.26128 ±0.0057 *	0.0093±0.0002	24.84±0.22**	0.38132±0.0058**	0.04±0.024	21.47±0.38**	0.24896±0.0095	0.011±0.00031*

Student's t test: * Significant difference (P<0.01), **Highly Significant difference (P<0.001)

Graph.IV Effect of different doses of Cyfluthrin on Sialic acid content in testis



$^{\alpha}$ Significant difference (P<0.01), $^{\beta}$ Highly Significant difference (P<0.001)

study in which permethrin reduced the testosterone levels and sperm count in adult male mice. This leads to decreased testosterone level in the testes and blood, increased blood levels of the signalling luteinizing hormone (LH), altered mitochondrial membranes in leydig cells, changed gene expression which controls important proteins and reduced sperm health and numbers (Zhang *et al*., 2007).

Sialic acid is a carbohydrate component attached with protein to form glycoprotein. It is found at the end of the oligosaccharide chains of many soluble glyco-proteins which determine whether the protein will continue to circulate in the blood stream or be removed by the liver. Sialic acid is also concerned with the stabilization of the plasma membrane, maintenance of sperms in a decapitated state, ionic balance in the epididymal plasma and antigen interaction between sperm and epididymal epithelium (Thomas *et al*., 2008). The synthesis and secretion of sialic acid is under androgen control. Gupta *et al*., (2002) had earlier indicated that possible role of androgen dependent sialic acid is to inhibit the stabilization of the acrosome of the maturing spermatozoa by contributing to surface negative charge. Epididymal epithelium is involved in the synthesis and secretion of compounds containing sialic acid. Alteration in sialic acid level in reproductive tissues indicates changes in the level of glycoprotein\ FSH and LH which is needed for normal functioning of gonads and accessory reproductive organs (Gupta at al., 2002). In the present study, sialic acid content of testis significantly decreased in all the dose groups. Depletion in the testicular sialic acid content in the mouse possibly reflects the androgen and gonadotrophic deficiency resulting in the inhibition of spermatogenesis, loss of spermatozoal motility and fertilizing ability (Gheri *et al*., 2009).

Cyfluthrin treatment also caused a decrease in cholesterol content. Since it is a precursor of androgens, it may be correlated with decrease in the sialic acid. All the biochemical parameters studied play important physiological role by coordinating with one another and they were affected by cyfluthrin treatment. It thus indicates adverse effects on the male reproductive organs. Therefore it may be inferred that cyfluthrin elicits toxicity at the tested dose levels in mice. Results from this study can be extrapolated to humans and this may help to create awareness among the general public to take precautions while using pesticides.

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