Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 4 [2] January 2015: 128-134

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804

ORIGINAL ARTICLE

Hepatotoxicity Of Cyfluthrin After Acute Exposure In Swiss Albino Mice

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ABSTRACT
Widespread use of insecticides for many years can lead to contamination in food chain and environment. Cyfluthrin is a type II synthetic pyrethroid insecticide. It is used against chewing and sucking insects on crops. For the present study a formulation of cyfluthrin"SOLFAC 050 EW" was used and its toxic effects were evaluated in the liver of male Swissalbino mice. The test animals were divided into two groups- control and treated. Three doses of cyfluthrin were administered to the animals of treated group: low dose 0.16 µl in 40 µl of distilled water, medium dose 0.32 µl in 40 µl of distilled water and high dose 0.64 µl in 40 µlof distilled water. Doses were calculated according to cyfluthrin recommended for field usei.e. 8 ml in 1 liter. The animals of control group received distilled water only. Animals of treated group were given the pesticide orally once and they were autopsied after 2, 7 and 30days. Liver was removed and weighed. Liver enzymes viz. AST or SGOT, ALT or SGPT and ALP in the serum and glycogen and cholesterol in the tissue were evaluated. Weight of liver and enzymes viz. ALP, SGPT and SGOT increased significantly and glycogen, cholesterolcontent of liver was also affected. It may therefore be inferred that day today use of pesticidesmay cause hepatotoxicity in Swiss albino mice.
Keywords: *Cyfluthrin, SGPT, SGOT, glycogen, cholesterol, hepatotoxicity.*

Received 12.10.2014 **Revised 10.11.2014** **Accepted 29.12.2014**

INTRODUCTION

Pesticides are widely used to protect crops against insects, weeds, fungi, and rodents butthese compounds are also toxic to non - target species including humans. The ruralpopulation is exposed to a higher concentration of pesticides due to their application in fields.Most pesticides cause harm to the environment and animals by entering the food chain. There are reports that, our vegetables, dairy products, meat products and even mother's milk have pesticide residues [1].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 pyrethroid [2].Its ISO approved common name is cyano (4-fluoro-3-phenoxy phenil) methyl 3-(2,2 –dichloroethenyl)-2, 2- dimethyl – cyclopropanecarboxylate [3]. It is the active ingredient of many insecticide formulations including Baythroid, Baygon, Attatox, Contur, Loser,Responsar, Solfac, Tempo and Tempo H [4]. Cyfluthrin is a neurotoxicant. It induces alterations in nerve membrane, causing abnormal sodium and potassium ion flow. Thisresults in repetitive discharge from the neurons, causing convulsions and also blockage offurther nerve impulses [5-6].The most common symptom of acute exposure to cyfluthrin is paresthesia progressing to numbness [7]. It is estimated by EPA that each year, 3 million workers in agriculture in the developing world experience severe poisoning from pesticides about 18,000 of whom die [8].The liver being the primary site for biotransformation of foreign compounds is particularly vulnerable to chemical assaults. Various pesticides cause pathological and biochemical alterations in the liver. Determination of the activity of the hepatic enzymes released into the blood by the damaged liver has become one of the most useful tools in the study of hepatotoxicity. Serum enzymes are useful indicators for the assessment of liver function in the toxicity studies of chemicals and environmental pollutants. Luty *et al* (2000) reported degenerative changes and inflammatory infiltration in liver and kidney of albino rats aftertreatment with high dose of alpha cypermethrin [9]. Watanable *et al* (1982) reported liver necrosis and increased chromatin in the nuclei of hepatic cells after the administration of cyfluthrin [10].

The present study aims to study the hepatotoxic effect of cyflthrin by detecting the biochemical milieu and serum enzymes of male Swiss albino mice.

MATERIALS AND METHODS

Swiss albino mice obtained from CDRI, Lucknow, were housed in an air cooled room and acolony was maintained. They were supplied with standard mice feed, obtained from Hindustan Lever Ltd., New Delhi and tap water was given *ad libitum.* Solfac 050 EW, a formulated product containing 5% w/w cyfluthrin was used for the study. Doses werecalculated according to the recommended dose of cyfluthrin for field use i.e. 8 ml in 1 liter.Three doses of cyfluthrin were used: low dose 0.16 µl in 40 µl of distilled water, mediumdose 0.32 µl in 40 µl of distilled water and high dose 0.64 µl in 40 µl of distilled water. Adult male mice were mainly divided into two groups according to the dose administration. Group Ii.e. control group; that received vehicle i.e. distilled water orally, Group II i.e. Experimental group that received pesticide orally once. Group II again divided into three sub groups according to the concentration of doses as mentioned above, low dose 0.16 µl in 40 µl of distilled water, medium dose 0.32 µl in 40 µl of distilled water and high dose 0.64 µl in 40 µl of distilled water. There were 45 male mice in experimental group, which were divided intolow, medium and high dose group of 15 animals each. Then each dose group of 15 animalswas further divided into three groups consisting of 5 animals each according to autopsy interval as after 2days, 7 days and 30 days. Animals were sacrificed. Liver was removed and weighed and hepatosomatic index was calculated. Fifty percent tissue was fixed in Bouin' s fluid for histopathological examination and the remaining used for biochemical estimations viz. Glycogen [11] and Cholesterol [12]. During the time of autopsy, blood was taken in dry sterilized vials. It was allowed to clot at room temperature for 60 minutes. The collected blood was then centrifuged at 3500 to 3700 rpm for 15 minutes and the serum thus obtained was used for estimation of enzymes. Serum alkaline phosphatase activity was measured by using commercially accessible kits (sigma Pvt. Ltd.) and optical density was measured at 405nm. The SGPT and SGOT activity was measured by International federation of Clinical Chemistry (IFCC) method.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).

Statistical analysis

The obtained data was statistically analyzed using students'' test and significance level was set at different levels as $P<0.05$, $P<0.01$ and $P<0.001$.

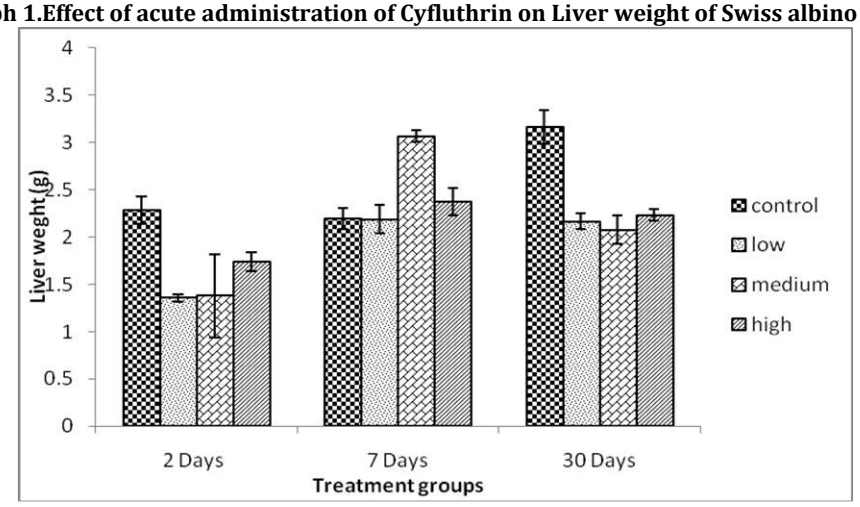
RESULTS AND DISCUSSION

The exponential increase in the production, use and disposition of pesticides has a profound impact on the environment and creates unforeseen hazards to man's wellbeing [13-14]. Liver,the primary target of biotransformation and excretion of xenobiotics. The observations obtained after oral administration of cyfluthrin at various dose levels are shown in the graph 1-6 and table 1-4. In the present study a highly significant decrease was observed in the weight of liver on 2nd day of the acute administration of cyfluthrin (Graph1, Table 1).Histopathological examination of liver revealed mild to marked cellular changes upon administration of the pesticide like widening of sinusoidal spaces, cytoplasmic degeneration,foamy appearance in hepatocytes, damaged cell walls, various aberrations and triadmultinucleated cells (Figure 1). Cyfluthrin caused hypertrophy and hydropic degeneration.The glycogen content showed significant decrease after administration of cyfluthrin. In the present study a highly significant decrease ($P<0.001$) in glycogen occurred at all the thre edose levels (graph 2) at all the autopsy intervals. The acute administration of cyfluthrin caused a significant increase ($P<0.01$) after two days and highly significant increase ($P<0.001$) in cholesterol content of liver at 30 days autopsy intervals (graph 3). In acutetoxicity studies, increased activity of SGPT and SGOT, were recorded which wassignificantly high ($P<0.001$) at all autopsy intervals except after 2 days of medium dose($P<0.05$) and high dose ($P<0.01$) treatment at which significant decrease was observed inSGPT values (Graph 4) and after 2 days a highly significant ($P<0.001$) decrease was observedin SGOT on medium as well as on high dose of cyfluthrin (Grapg 5).On acute administration of cyfluthrin a highly significant increase ($P<0.001$) was observed in the Alkaline phosphatase (ALP) activity on all the dose levels (Graph 6, Table 2-4).

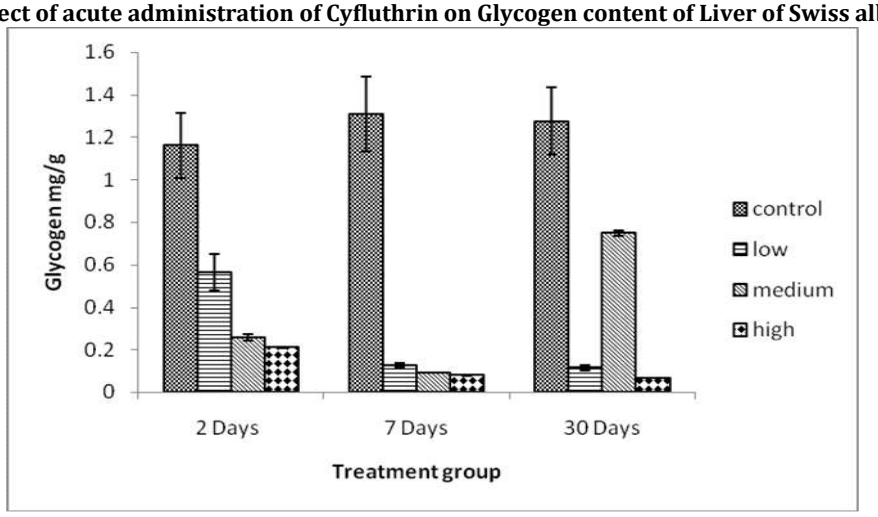
In the present study decreased liver weight by cyfluthrin is supported by the studies of other workers with different pesticides on mammals as well as on birds [15-16]. The changes in liver weight after cyfluthrin intoxication may be associated with hydropic changes of hepatocytes andhypertrophy of hepatocytes. The decrease in liver weight may be due to tissue necrosis [17].As indicated by histopathological examination of liver revealed mild to marked cellular changes by cyfluthrin treatment. The central vein appeared intact and sinusoids and Kuffer cells were normal and lobular structure

remained unaltered in control rats whereas cellular degeneration and hypertrophic changes were found in liver which is the direct effect of pesticide on cellular structure of liver.

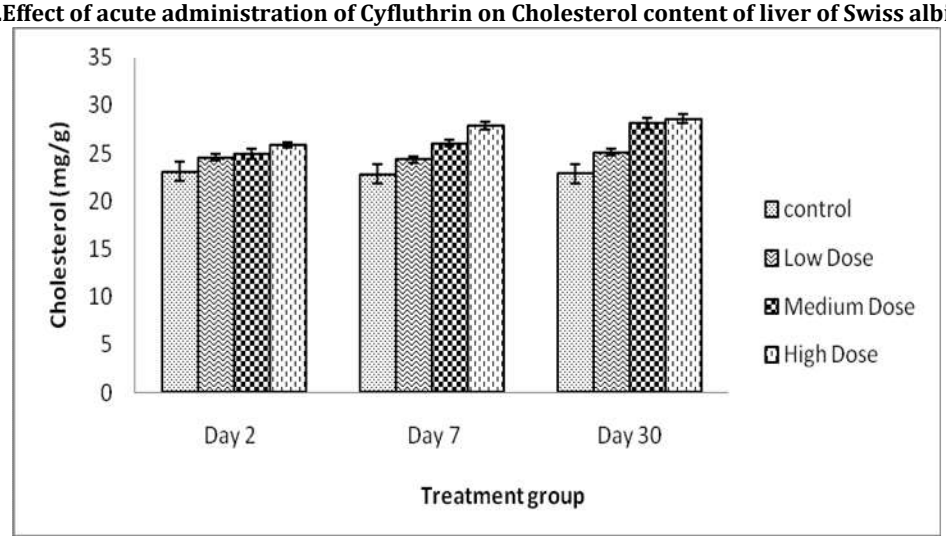
Graph 1.Effect of acute administration of Cyfluthrin on Liver weight of Swiss albino mice.



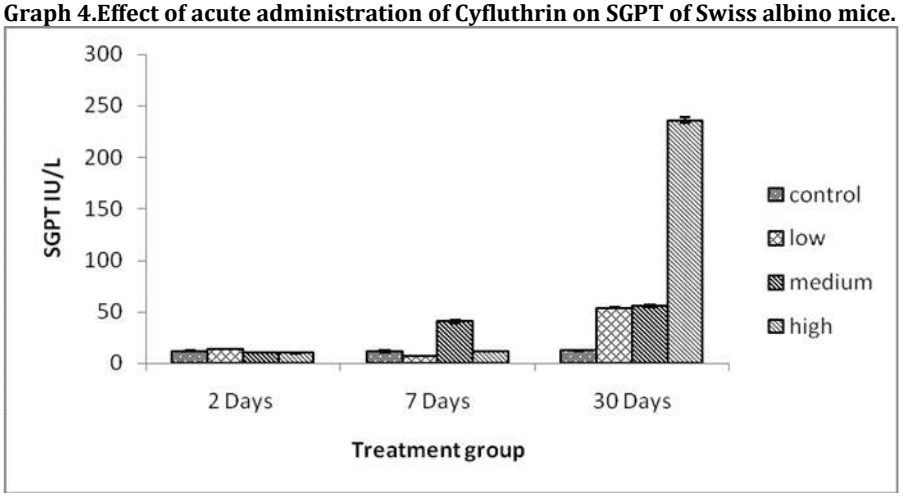
Graph 2.Effect of acute administration of Cyfluthrin on Glycogen content of Liver of Swiss albino mice.



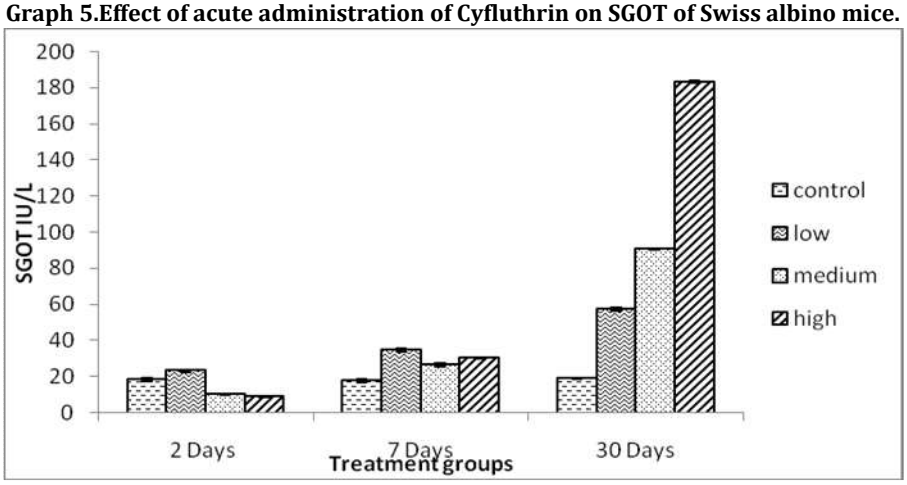
Graph 3.Effect of acute administration of Cyfluthrin on Cholesterol content of liver of Swiss albino mice.



Graph 4.Effect of acute administration of Cyfluthrin on SGPT of Swiss albino mice.



Graph 5.Effect of acute administration of Cyfluthrin on SGOT of Swiss albino mice.



Graph 6.Effect of acute administration of Cyfluthrin on ALP of Swiss albino mice.

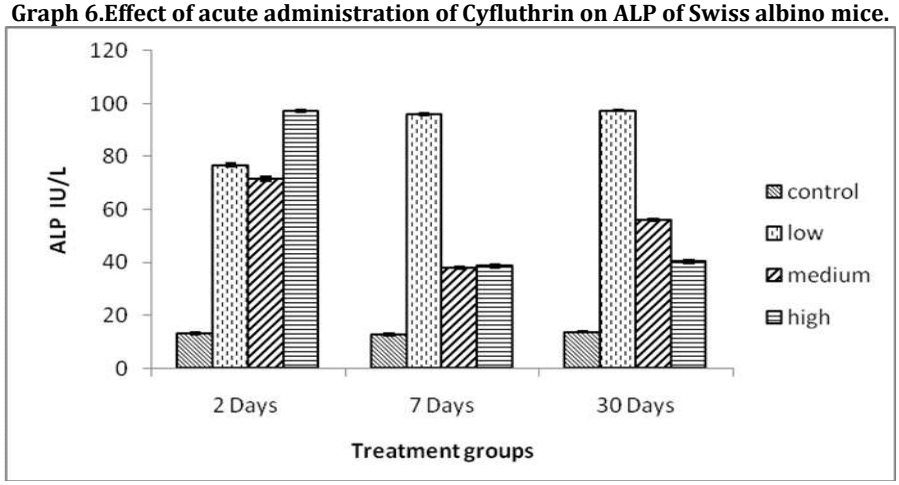


Table 1: Effect of Acute treatment of Cyfluthrin on liver weight (gm) of Swiss albino mice.

Days / Dose	2 Days	7 Days	30 Days
Control	2.284±0.142	2.19628±0.1077	3.1648±0.147
Low Dose	1.357±0.035***	2.190±0.147	2.168±0.084
Medium Dose	1.38±0.044***	3.07±0.057***	2.074±0.152
High Dose	1.74±0.098***	2.374±0.14**	2.234±0.066

Significance difference: P*<0.05, P**<0.01, P***<0.001

Table 2: Effect of acute (0.16$\mu l/day$) low dose of Cyfluthrin on Glycogen, Cholesterol, SGPT, SGOT and ALP in male Swiss Albino mice.

Autopsy Interval	Glycogen mg/g	Cholesterol mg/g	SGPT IU/L	SGOT IU/L	ALP IU/L
Control	1.1622±0.15421	23.09±0.35	11.678±0.468	18.056±1.03	13.108±0.429
2 Days	0.5642±0.08485**	24.58±0.37	13.744±0.833	23.008±0.99**	76.888±0.646***
7 Days	0.126±0.01101***	24.37±0.32*	6.654±0.215***	34.41±0.86***	96.064±0.536***
30 Days	0.114±0.01305***	25.09±0.32*	53.648±1.429***	57.14±0.95***	97.498±0.782***

Significance difference: P*<0.05, P**<0.01, P***<0.001

Table 3: Effect of acute (0.32$\mu l/day$) medium dose of Cyfluthrin on Glycogen, Cholesterol, SGPT, SGOT and ALP in male Swiss Albino mice.

Autopsy Interval	Glycogen mg/g	Cholesterol mg/g	SGPT IU/L	SGOT IU/L	ALP IU/L
Control	1.3084±0.1767	22.82±0.22	11.281±0.3252	17.7744±0.4767	12.7828±0.2605
2 Days	0.2596±0.01551***	24.88±0.54*	10.268±0.351*	10.07±0.327***	71.652±0.836***
7 Days	0.092±0.001612***	26.03±0.34**	40.242±0.466***	26.518±0.919***	38.02±0.451***
30 Days	0.75±0.01305***	28.09±0.56**	55.636±0.873***	90.344±0.34***	56.05±0.434***

Significance difference: P*<0.05, P**<0.01, P***<0.001

Table 4: Effect of acute (0.64$\mu l/day$) high dose of Cyfluthrin on Glycogen, Cholesterol, SGPT, SGOT and ALP in male Swiss Albino mice.

Autopsy Interval	Glycogen mg/g	Cholesterol mg/g	SGPT IU/L	SGOT IU/L	ALP IU/L
Control	1.2756±0.1601	22.84±0.46	12.154±0.4504	18.8592±0.2514	13.7964±0.3616
2 Days	0.211±0.0016***	25.91±0.26**	9.868±0.27**	8.872±0.162***	97.294±0.283***
7 Days	0.08±0.0015***	27.88±0.36**	11.408±0.326	30.154±0.43***	38.808±0.602***
30 Days	0.067±0.001***	28.6±0.5**	235.842±2.885**	183.09±0.601***	40.454±0.581***

Significance difference: P*<0.05, P**<0.01, P***<0.001

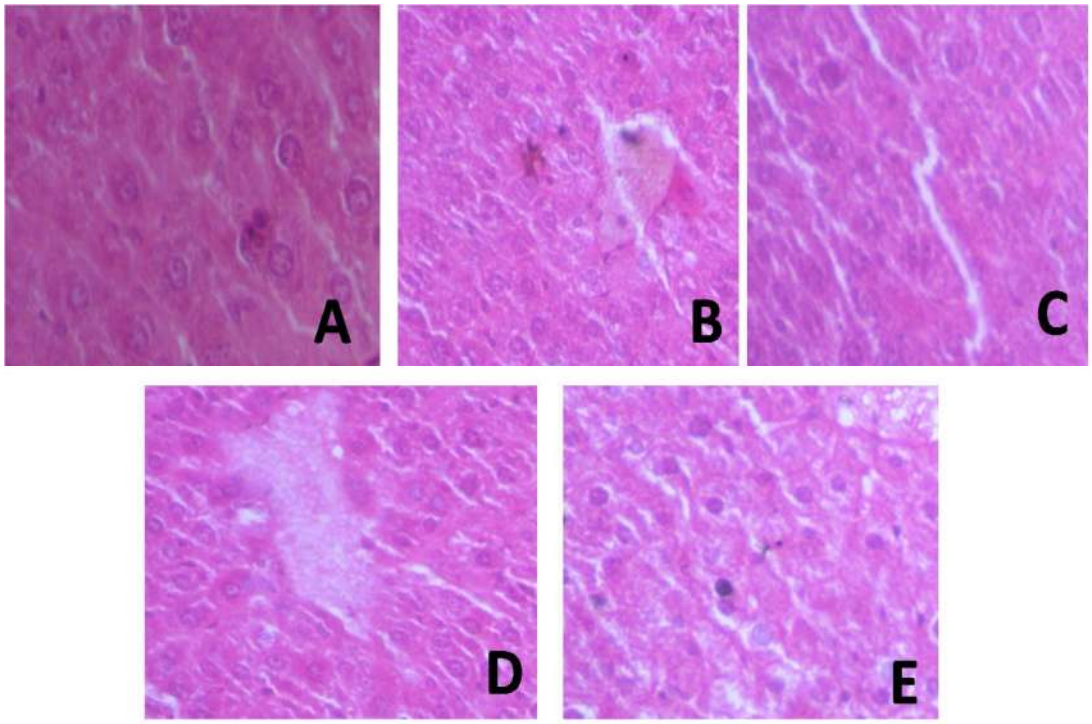


Figure 1. Histopathological examination of liver in control group (A)widening of sinusoidal spaces, cytoplasmic degeneration in animals treated with low dose at autopsy interval 30 day (B) Mild cytoplasmic degeneration with few nuclear aberration, foamy appearance in hepatocytes medium dose treatment group after 30 days (C) Damaged cell walls, various aberrations and triad multinucleated cells in high dose treatment group after 7 days (D) nuclear hypertrophy and hydropic degeneration high dose treatment after 30 days (E) (400x).

The liver is the largest organ in the body that performs both endocrine and exocrine functions. An initial step in detecting liver damage is a simple test to determine the presence of certain liver enzymes (proteins) in the blood. Under normal circumstances, these enzymes are side within the cells of the liver. But when the liver is injured for any reason, these enzymes are spilled into the blood stream. Among the most sensitive and widely used liver enzymes are the aminotransferases. Theyinclude aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The enzymeaspartate aminotransferase (AST) is also known as serum glutamic oxaloacetic transaminase (SGOT); and alanine aminotransferase (ALT) is also known as serum glutamic pyruvictransaminase (SGPT). These enzymes are normally contained within liver cells. If the liver is injured or damaged, the liver cells spill these enzymes into the blood, raising the enzyme levels in the blood and signaling the liver disease. The aminotransferases catalyze chemical reactions in which an amino group is transferred from a donor molecule to a recipientmolecule.AST (SGOT) is normally found in many tissues including liver, heart, muscle, kidney, andbrain. It is released into serum when any one of these tissues is damaged. ALT (SGPT) is, bycontrast, normally found largely in the liver. This is not to say that it is exclusively located inliver, but that is where it is most concentrated. It is released into the blood stream as a resultof liver injury. It therefore serves as a fairly specific indicator of liver status. Increased activity of SGPT and SGOT, were recorded. The enzyme (GOT) and (GPT) gain entrance into the serum and consequently their concentration rises markedly in hepatocellular diseases. These findings can also be correlated with the view of Kamal *et al*., [18]) that long term effect of exposure to organophosphorus pesticides caused significant increase in SGPT and ALP.Increase in SGPT activity in present investigation can be correlated with the studies of Oikawa and Luatomy [17] who reported that males at 1000 mg/kg of cyfluthrin feed had significant increased SGPT activities. These parameters suggest that metabolism in liver is affected by cyfluthrin administration.Alkaline Phosphatase (ALP) is an important enzyme which helps in body metabolism invarious ways. Though it is present in all tissues, maximum amount of alkaline phosphatase is found in liver and in bones. Alkaline phosphatase is also present in bile duct, intestinal lining, placenta, kidneys, etc. It is required by the body for 'dephosphorylation', the removal of phosphate groups from different molecules. The liver makes more alkaline phosphatase than other organs, hence an increment in its level could be an indication that all is not well with the liver. Increase in the activity of ALP probably indicates that the pesticide has a stimulatory effecton cell growth and proliferation. Other reason may be due to improper functioning of the liver, bile ducts or gall bladder system [19].Congestion or obstruction of the biliary tract, raises the levels of alkaline phosphatase in the blood. Liver conditions such as hepatitis, cholecystitis, cholangitis, and cirrhosis, fatty liver and liver tumor can cause elevated liver enzymes, and eventually alkaline phosphatase. Elevated alkaline phosphatase levels thus mean that it could probably be the liver cell damage.

Carbohydrates are stored in the animal tissue in the form of glycogen, which acts as an energy producing source. Glucose has also been shown to be an essential substrate for maintaining tissue integrity, ATP production and protein synthesis [20]. Hepatic glycogen gets converted into glucose which passes into systemic circulation [21], [22]. Tissue carbohydrates are affected by pesticide toxicity. Sharma and Mahajan (1983) showed that aldrin affected biochemical factors like blood glucose and liver and muscle glycogen [23].In the present study a highly significant decrease ($P<0.001$) in glycogen occurred at all the three dose levels at all the autopsy intervals. This is in accordance with the view of Tripathi and Verma [24] that treated the fresh water fish, *Clarias batrachus*L with endosulfan and studied the changes in protein, glycogen and lipid in the liver and muscle tissues [24].

Cholesterol, containing a mono atomic alcohol and one double bond is considered to be mostimportant precursor of all the steroid hormones including androgens [25]. Cyfluthrin administration caused increase in cholesterol content of liver. Tissues actively synthesize cholesterol. Pesticides can cause changes in blood cholesterol levels by altering the permeability of hepatic cells and disrupting lipid metabolism [21]. Widely used synthetic insecticide permethrin dramatically reduced testosterone levels [26].

CONCLUSION

Since all biochemical parameters studied play important physiological role by coordinating with one another. In the present work all the values significantly disturbed as compared to control group animals which can cause adverse effects on hepatic system. Hence it is concluded from the present findings that carelessly use of cyfluthrin as an insecticide is hazardous to non-target species as well.

ACKNOWLEDGEMENT

This work was funded by University Grants Commission, New Delhi, India and other facilities were provided by Center for Advanced Studies in Zoology, University of Rajasthan, Jaipur.

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CITATION OF THIS ARTICLE

Neelu K R, Inderpal S and Priyanka M. Hepatotoxicity Of Cyfluthrin After Acute Exposure In Swiss Albino Mice. Bull. Env. Pharmacol. Life Sci., Vol 4 [2] January 2015: 128-134