Effect of Synthetic Pyrethroid Fenvalerate Induced Carbohydrate metabolism in Liver and Kidney tissues of *Rana Tigrina* (Indian Bull Frog).

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ABSTRACT

Fenvalerate is a pyrethroid insecticide extensively used in agricultural crops such as rice, wheat, sorghum, pulses, groundnut, vegetables and on cotton to kill the stem borers, leaf folders, fruit borers and head borers. They are affecting several non-target organisms like fishes and other aquatic organisms. Healthy frogs, *Rana tigrina* weighing 50 ± 3 gms were collected from the pond, acclimated to the laboratory conditions in large glass aquaria with water. The frogs were divided into groups of ten animals. They were exposed to different pesticide concentrations of fenvalerate, both commercial and technical grade, according to biomass ratio as suggested by *Doundroff et al.*, (1951). The total carbohydrate content in the present study Control liver has shown higher amount of carbohydrates than the other tissues. Under fenvalerate intoxication, the frog tissues showed consistent decrement in all the periods and it may be due rapid utilization to withstand pesticide of stress. Total protein content in control frog tissues the liver showed higher concentration of proteins than kidney. The protein content in experimental frog tissues showed depleted levels when compared to controls. The significant decrease in protein and it may be due rapid utilization to withstand pesticide of stress. Total free amino acid (FAA) levels in fenvalerate exposed frog tissues were increased significantly when compared to control. This elevated levels may be due to enhanced protease activity or enhanced transamination. From the above observations fenvalerate seems to be hazards to the aquatic life, causing drastic biochemical changes and irrepairable architectural changes investigated in the present investigation.

Key words: Fenvalerate, Ttotal Ccarbohydrate content, Total Protein content, Total free amino acid (FAA) levels, *Rana Tigrina* (Indian Bull Frog).

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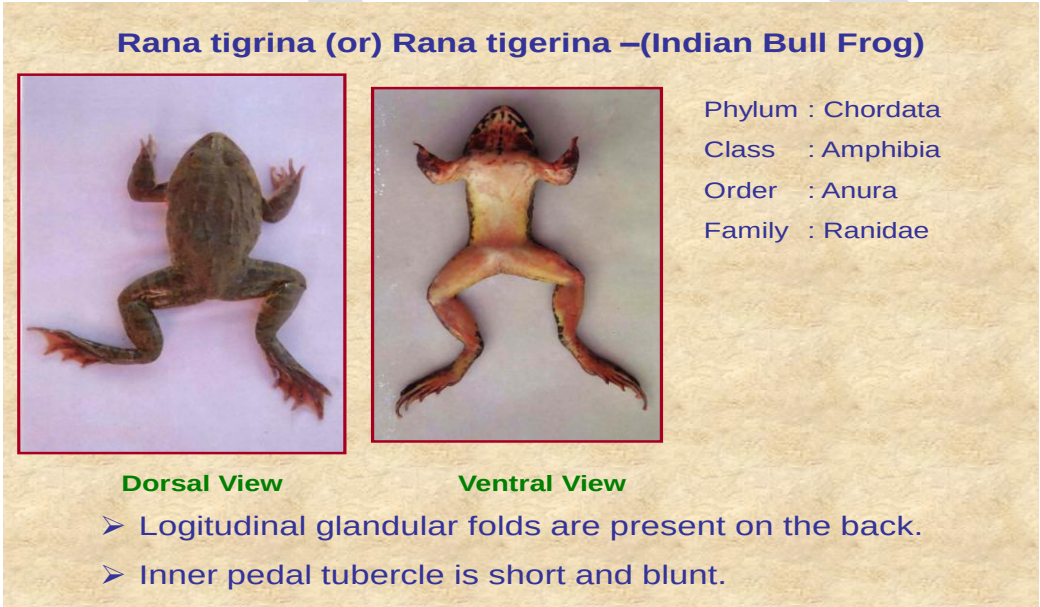
INTRODUCTION

The Amphibians originated about 300 million years ago in the Devonian Period of Palaeozoic era. They were flourished in the Carboniferous period which was the age of Amphibians. They occupy an intermediate position in vertebrata phylogeny.The amphibian fauna of India consists of 205 species. These include one Salamander (Caudata), about 20 Caecilians (Gymnophiana) and a little over 180 species of frogs and toads (Anura). General consensus is that there are more species in Peninsular India – many being endemic to the Western Ghats i.e. nearly 129 (63%) and 76 (37%) are non-endemics. American, Indian Magicians used parts of frogs and toads in magic. Frogs are used as bait in fish hunting. Frogs are also kept in decorative aquaria. A study of frog habitat brought out interesting reports. Frogs inhabit swamps and low lying areas especially irrigated fields **(**Abdulaile, 1985**).** As frog breed during rainy season and release their eggs in the ponds, puddles etc., the developing tadpoles face ill effects of the accumulated pesticides (Pandian & Marian, 1986***).***



Convention on international trade in endangered species in West Germany has placed Indian bull frog, *Rana tigrina* in the protected list. Indian Government had banned the export of frog legs and Indian Board for wild life had recommended that *Rana* species should be included in Schedule - II, Part-II : of wild life (protection) Act 1972, thus providing frog special status of protection. Conservation action and recommendations made by some education working group

(Deuti *et al*., 1998) have given by some suggestions. This species is classified as *Least Concern* in the IUCN red list. (Anand Padhye *et al*., 2010).

Synthetic pyrethroids have emerged as a new class of agricultural pesticides and are widely used over organochlorine and organophosphate pesticides. Synthetic pyrethroids account for more than 30%of insecticide use worldwide in household, agricultural, and veterinary applications (Williamson, E.G *et al*., 1989).

S.NO.	NAME OF THE PESTICIDE	TRADE NAMES
1	Permethrin	Permaset – 25.0 EC
2	Decamethrin	Decis – 2.8 EC
3	Fenvalerate	Sumicidin – 20.0 EC, Agroten – 20.0 EC, Fenval – 20.0 EC.
4	Cypermethrin	Ripcord – 10.0 EC, Cymbush – 25.0 EC, Cyperkil – 25.0 EC

Source: The following synthetic pyrethroids are available in India. **(**S.V.Subbareddy, 2007**)**

Saleh *et al* **.,** (1986) reported the persistence and distribution of Cypermethrin, Deltamethrin and Fenvalerate in various tissues viz., fat, skin, blood, heart, brain, liver, kidney, ovary and eggs of White Leghorn hens as long as for 14 days after treatment. Fenvalerate is about 1.5 to 2.0 times more persistent in the tissues than Cypermethrin and Deltamethrin. The results also indicated that the residues of the parent compound were more persistent in the brain than in the other tissues. Fenvalerate, a synthetic pyrethroid of third generation insecticide has been proved to be remarkably toxic to pests with a relative low toxicity to mammals and other vertebrates. When present in the water at low concentration, it appears to be highly toxic to fishes (Radhaiah, 1988), Crustaceans (Ayanna, 1991) and to various organisms (Tagatz and Ivey, 1981). Fenvalerate is the most widely used compound of the cyanophenoxy-benzyl group of the synthetic pyrethroid pesticides and it is used in agriculture to protect a wide variety of crops including cotton, soybeans, corn, vegetables, apples, peaches, pears and nuts from insect pests .

High doses of Fenvalerate has been reported to be associated with reduction of body mass, increase in liver mass, and proliferation of the smooth endoplasmic reticulum in hepatic cells, and induction of the activity of microsomal enzymes (El-Sewedy SM *et al*.,1982; WHO,1991). Fenvalerate is one of the most persistent synthetic pyrethroids in soils. Fenvalerate is highly toxic for fish (Madhuban Datta Bhattacharya and Anilava kaviraj, 2006) and bees, while for birds and mammals its toxicity is low. Hence, the present study was made to understand the effect of synthetic pyrethroid compound, fenvalerate on some metabolic aspects of different tissues of an Indian Bull Frog, *Rana tigirna*.

Material and Methods:

Procurement of the experimental animal:

Rana tigrina is commonly known as Indian Bull Frog. They are occurring near the tanks and ponds in and around Tirupati (A.P.). Besides experimental frogs other species of frogs were also collected and their morphological features were studied. For the present study, the locally available frog, *Rana tigrina* was selected.

Selection of the test chemical:

Fenvalerate (Sumcidin (R) (5-5602 OMS – 2000) a synthetic pyrthroid compound both commercial (Fenvalerate EC 20) and Technical grade, 93.7% (wt/vol) supplied as gratis by Rallis India Limited, Bangalore (India) was used. The following are the physico-chemical properties of fenvalerate used in the present study.

Preparation of Stock Solution

The active ingredient of commercial grade 93.7% of fenvalerate was used for present investigation. A stock solution of fenvalerate was prepared by dissolving the fenvalerate in Acetone. Available literature indicates that low levels of acetone are harmless to the biological system *(*Pickering *et al*., 1962). The quantity of acetone used was found to be non-toxic to non-target animals and it was biologically safe in the preparation of stock solution of pesticides (Jagannatha Rao**,** 1981). One gram of technical grade of fenvalerate (93.7%) is dissolved in minimal quantity of acetone and this was made upto 937 ml with water to make 1000 ppm of stock solution. Fresh stock solution was prepared for experimental use.

Experimental Design:

Healthy frogs, *Rana tigrina* weighing 50 ± 3 gms were collected from the pond, acclimated to the laboratory conditions in large glass aquaria with water (Temperature 27 ± 2°C; P^H 7.0 ± 0.2, light period – 12 hours) for 7 days. They were fed with cockroaches and earthworms *ad libitum*, with change of water daily. They were exposed for 1 week, 2 week, and 4 week in sublethal concentration (9.4 mg/lt) of fenvalerate i.e $1/5^{th}$ of LC_{50} of 48 h. After stipulated period, the liver and kidney tissue was isolated from Control and fenvalerate exposed frogs, The tissues stored at -80 ^oC for further biochemical analysis.

Histology

Histological examination of the tissues was followed as per Humason (1972). The tissues like liver, Kidney were isolated both from control and experimental frogs. They were gently rinsed with physiological saline solution (0.09% NaCl) to remove blood and debris adhering to the tissues. They were fixed in 5% formalin for 24 hours. The fixative was removed by washing through running tap water for overnight. After dehydration through a graded series of alcohols the tissues were cleared in methyl benzoate, embedded in paraffin wax. Sections were cut at 6 µ (microns) thickness and stained with Harris haematoxylin (Harris**,** 1900) and counter stained with eosin (dissolved in 95% alcohol). After dehydration and clearing, sections were mounted in DPX and photographed.

Biochemical Analysis:

The total carbohydrate content was estimated in the control and experimental tissues by the method of Carrol *et al*., (1956). The tissues were isolated and 2% homogenates in 10% trichloroacetic acid were prepared. The homogenates were centrifuged at 2500 rpm for 15 minutes. 0.5 ml of the clear supernatant was taken, followed by 5 ml of anthrone reagent. The contents were boiled for 15 minutes. The tubes were cooled and the color developed was read at 620 nm in a spectrophotometer using blank, containing trichloro acetic acid and anthrone reagents in the same proportion. The OD of the sample was compared with that of the standard

and the total carbohydrates with that of the standard and the total carbohydrates content was expressed as mg/g wet weight of the tissues.

Total protein content was estimated by the method of Lowery *et al.***,** (1951). Different tissues were isolated and 2% homogenates were prepared in 10% trichloroacetic acid. 1 ml of the crude homogenate was taken and centrifuged at 2500 rpm for 10 minutes. The sediment was dissolved in 5 ml of 1N sodium hydroxide by thorough shaking. From this 0.1 ml of solutions were taken and 4 ml of alkaline copper reagent was added followed by 4 ml of folin phenol reagent (1:1, Folin:H_2O). The color (Light blue) was read at 600 nm against the blank in spectrophotometer. The standard graph was prepared with bovine serum albumin. The protein content was expressed in mg/g wet weight of the tissue.

Total free amino acid content in control and experimental animal tissues was estimated by the method of Moore and Stein (1954) as described by Colowick and Kaplan (1951). 2% homogenate of tissues were prepared in 10% trichloro acetic acid. The contents were centrifuged at 2500 rpm for 15 minutes. To 0.05 ml of the supernatant 2 ml of ninhydrin reagent was added and kept in boiling waterbath for exactly 12 ½ minutes and cooled immediately to room temperature. The solution was then made upto 10 ml with distilled water and the bluish pink color developed was read at 570 nm in spectrophotometer against blank. The free amino acid content was expressed as "μ" moles of tyrosine equivalent/g wet weight of the tissue.

Statistical Analysis:

Statistical analysis has been carried out using INSTAT software. The data was analyzed for the significance and the results were presented with the P-value.

RESULTS:

The total carbohydrate:

The total carbohydrate levels in control and fenvalerate exposed frogs of different tissue are presented in Table:1 Control liver has shown the higher amount of carbohydrates than kidney tissues, The highest part of carbohydrates in liver may be in the form of glycogen which forms a source of reserve energy. Exposure to fenvalerate resulted in the decrease of the carbohydrate in

all the tissues (Table:1). The decrease was consistently progressive and significant in all the three exposures of I, II and IV weeks. Kidney carbohydrate levels decreased to 39.70% after four weeks which is the lowest percentage and it is two and half fold more compared to first week. The decrease was statistically significant (P> 0.001) over control. The decreased trend in the fourth week exposed tissues of frog is as follows: (**Liver > Kidney).**

Total protein content:

Total protein content levels in control and fenvaleate exposed frogs of different tissues are presented in the Table:2 In control frog tissues the liver showed higher concentration of proteins than kidney, being the seat of metabolic regulation has more proteins. However, fenvalerate has brought about negative changes in the protein levels in liver and kidney tissue studied Table 2. Exposure to sub lethal concentrations of fenvalerate for I, II and IV weeks reduced the protein levels in the tissues. The decrease in liver and kidney tissues of exposure periods was statistically significant (P< 0.001). The decreased trend in the fourth week exposed tissues is as follows: (**Kidney > Liver)**

Free amino acid content:

Free amino acid content estimated in liver and kidney tissues of control and pesticide exposed of frogs are presented in the Table: 3 Among control tissues liver has recorded more free amino acid content than kidney tissues. Thus the kidney showed the least amino acid content. The fenvelarate intoxication resulted in elevted levels of FAA in all the tissues studied in experimental animals. The maximum increase observed was in liver (40.75%) after four weeks of fenvalerate exposure and it is three fold when compared to first week and two fold increase was observed in second week. However the increased levels in fourth week tissues were as follows: (**Liver > Kidney).**

Table 1: Total Carbodydrate levels in the tissues of control and fenvalerate exposed frogs mg/gm wet wt.)

	I week		II week		IV week	
	Control	Experiment	Control	Experiment	Control	Experiment
Liver	79.61 ±0.553	68.43 ±1.255 -14.04	85.29 ±0.589	60.23 ±2.720 -29.38	83.92 ±0.870	45.57 ±2.310 -45.69
Kidney	64.12 ±1.720	53.33 ±0.877 -16.82	65.71 ±1.323	44.18 ±1.818 -32.77	64.71 ±1.79	39.02 ±0.809 -39.70

Values represent mean of six individual observations, ± S.D., Figures in parenthesis indicate per cent change over control. P=`t' test. All values are significant at P<0.001.

Table 2: Protein levels in the tissues of control and fenvalerate exposed frogs (mg/gm wet wt.)

	I week		II week		IV week	
	Control	Experiment	Control	Experiment	Control	Experiment
Liver	163.58 ±3.45	147.16 ±3.88 -10.04	168.98 ±3.53	130.28 ±3.38 -22.90	177.47 ±3.44	99.54 ±2.32 -43.91
Kidney	86.42 ±3.45	73.29 ±3.18 -15.19	91.05 ±3.45	54.01 ±3.45 -40.68	85.65 ±2.32	43.21 ±2.38 -49.55

Values represent mean of six individual observations, ± S.D., Figures in parenthesis indicate percent change over control. P=`t' test. All values are significant at P<0.001.

Table 3: Free Amino Acid levels in the tissues of control and fenvalerate exposed frogs (mg/gm wet wt.)

	I week		II week		IV week	
	Control	Experiment	Control	Experiment	Control	Experiment
Liver	17.62 ±0.435	19.94 ±0.298 +13.17	17.16 ±0.633	21.53 ±0.409 +25.47	18.18 ±0.578	25.59 ±0.704 +40.75
Kidney	10.52 ±0.742	12.19 ±0.746 +15.87	10.72 ±0.486	13.19 ±0.412 +23.04	12.01 ±0.408	15.87 ±1.222 +32.14

Values represent mean of six individual observations, ± S.D., Figures in parenthesis indicate per cent change over control. P=`t' test. All values are significant at P<0.001.

PLATE-1: LIVER TISSUE

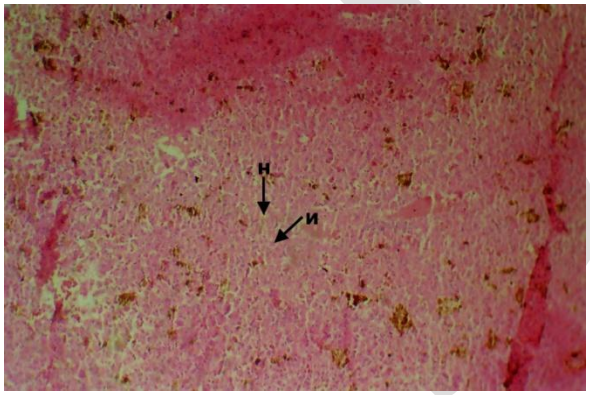


Fig.A

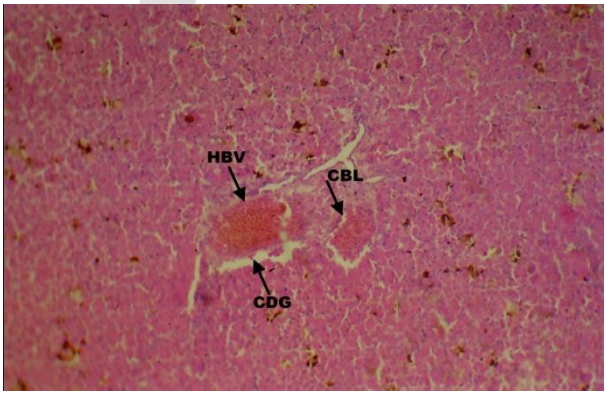


Fig.B

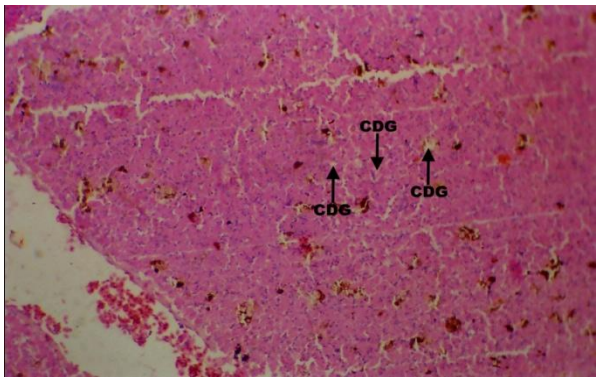


Fig.C

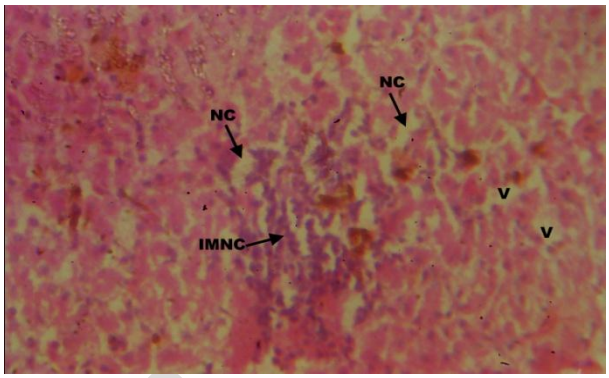


Fig.D

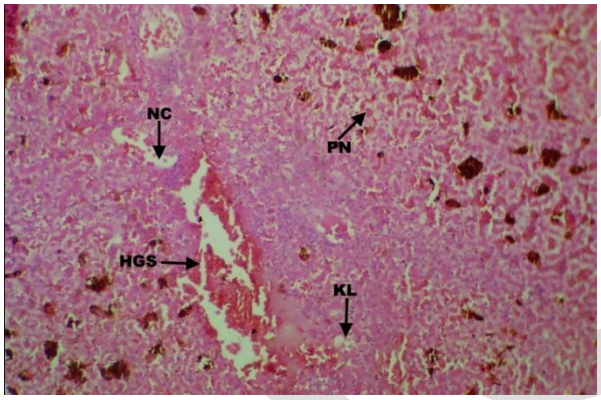


Fig.E

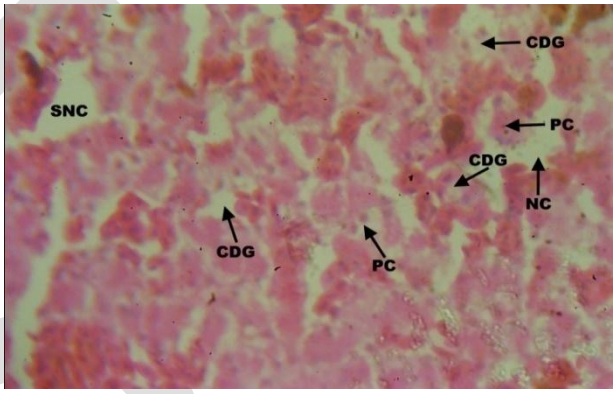


Fig.F

Fig A: Control liver of frog showing hepatocytes (H) with centrally placed nucleus (N) and sinusoids (H & E). X50

Fig B: Frog liver under one week fenvalerate exposure – showing congestion of blood vessel (CBV) with moderate cellular degenerative change (MCDG) around blood vessel (H & E).X50

Fig C: Frog liver under two weeks of fenvalerate exposure showing initiation of cytoplasmic degeneration (CDG) and appearance of vacuoles (V) (H&E).X50

Fig D: Frog liver under two weeks of fenvalerate exposure showing focal mononuclear infiltration (FMI) and degenerative changes (DG) in hepatocytes(H&E). X280

Fig. E: Frog liver under four weeks of fenvalerate exposure showing haemorrhage in sinusoids (HGS), Fibrosis of blood vessels (FBS) and thickened blood vessels (TBV). (H&E). X50.

Fig . F: Same as above higher magnification showing severe necrosis, vacuoles (V), karyolysis (KL), cytoplasmic degeneration (CDG) and pycnotic nuclei (PN) (H& E).X 280

PLATE: 2 KIDNEY TISSUE

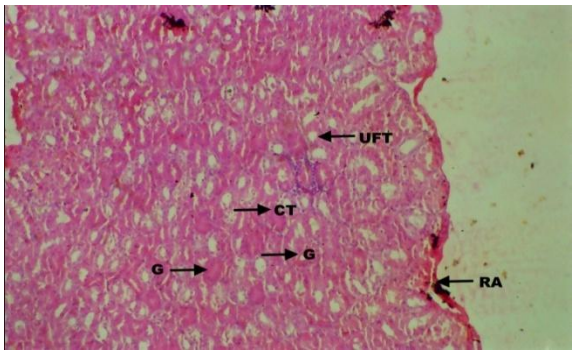


Fig.G

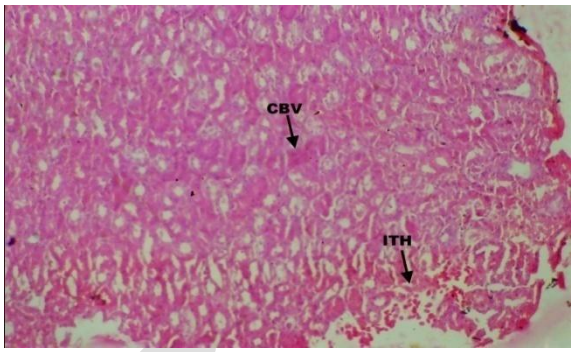


Fig.H

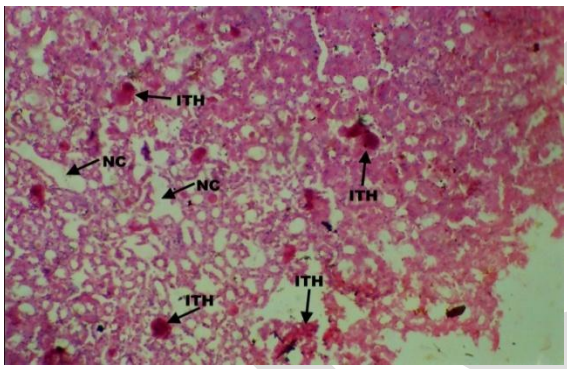


Fig.I

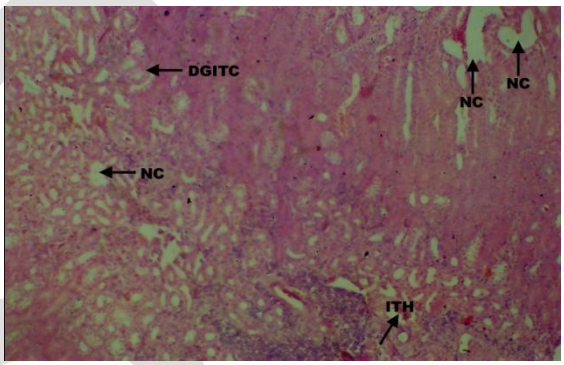


Fig.J

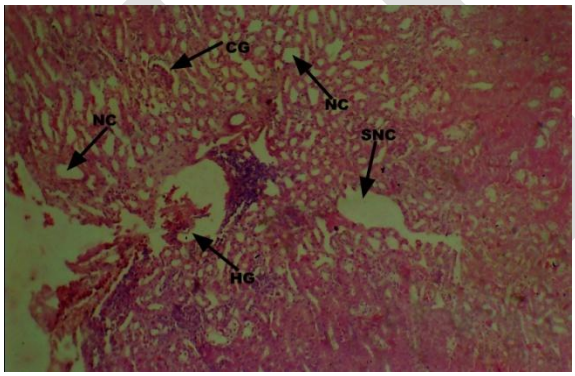


Fig.K

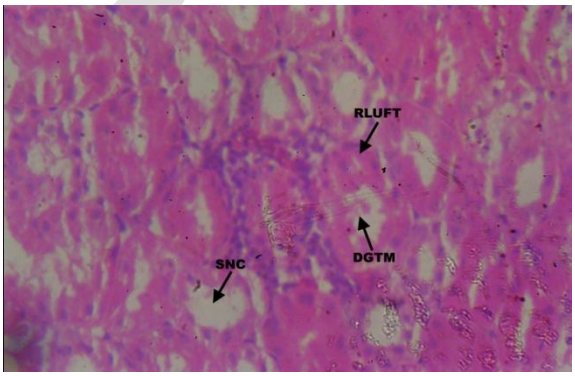


Fig.L

Figure G: Control kidney of frog, Rana tigrina-showing uriniferous tubules (UFT), connective tissue (CT), glomerulus (G) and renal artery (RA) (H & E). X50

Figure H: Frog kidney under seven days of fenvelarate exposure showing proximal tubules (PT) and glomuruli (G), intertubular haemorrhage (ITH) and congestion of blood vessel (CBV) (H & E). X50

Figure I: Frog kidney under two weeks of fenvalerate exposure showing intertubular haemorrhage (ITH) and necrosis in inter tubular regions (NCT) (H & E). X50

Figure J: Frog kidney under four weeks of fenvalerate exposure showing intertubular necrosis (ITNC), appearance of vacuoles (V) and intertubular haemorrhage (ITH) (H&E). X50

Figure K: Frog kidney under four weeks of fenvalerate showing severe necrosis (SNC), severe infiltration of tubular mononuclear (SIFMNC) in between renal tubules and haemorrhages (H&E). X50

Figure L: Frog Kidney under four weeks of fenvalerate exposure showing reduced lumen (RL) of uriniferous tubules and degenerative changes in tubular membrane (DGTM) (H&E). X280.

DISCUSSION:

Total carbohydrates:

In the present investigations the total carbohydrates are estimated. Control liver has shown higher amount of carbohydrates than the Kidney tissue. Under fenvalerate intoxication, the frog tissues showed consistant decrement in all the periods and it may be due to rapid utilization to withstand pesticidal stress.

Carbohydrates serve as a fuel to provide energy for the metabolic activities in an organism. They are stored as such in the body of an animal as glycogen (only to a limited amount) and glycogen is considered as one of the major sources of energy and maintenance of its reserves is an important feature of cellular metabolism (Turner and Manchester, 1972). The major function of carbohydrates in metabolism is as a fuel to be oxidized and provide energy for other metabolic processes (Martin *et al*., 1983). Carbohydrate metabolism takes place both in aerobic and anaerobic conditions. In anaerobic condition glycogen is broken down to release energy after going on a series of reactions. Aerobic condition consists of pyruvate oxidation to acetyl Co A to be utilized through another cycle viz., citric acid cycle. Utilization of reduced co-enzymes leads to ATP synthesis through oxidative phosphorylation (Lehninger**,** 1983). There is an alternative respiratory parthway, which does not require glycolysis. This pathway and

operation of glycogenesis and gluconeogenesis from amino acids impart a great importance to the carbohydrate metabolism, especially under stress condition which include pesticidal stress also. Disturbances in carbohydrate metabolism are among the most understanding biochemical lesions arising by the action of toxic compounds and the compensatory shift form aerobic towards anaerobic metabolism in the presence of toxic substances seems to be inevitable in tissue cells for survivability (Bhatia *et al*., 1973). This may prove to be of negative survival value for the affected organisms.

Proteins:

The present study reveals significant variation in protein metabolism and associated enzymes systems after exposure of fenvalerate in Liver and Kidney tissues of *Rana tigrina*. The physiological and biochemical activities in the frog were disturbed after sublethal concentration of fenvalerate change indicates stress. The reason for decrement of protein is that tissue protein might be metabolized to produce glucose by the process of gluconeogenesis and glucose is utilized for energy production during stress condition. The significant decrease in protein of experimental animals could be due to increased proteolysis under fenvalerate toxicity.

Proteins are the important biomolecules in a wide spectrum of cellular and metabolic functions. They serve indispensible functions in cellular architecture, catalysis, metabolic regulation and contractile processes and are weapons in the defense arsenal of many higher organisms. They are highly complex macromolecular compounds of a large number of different aminoacids. They also serve as precursors for several other important biomolecules such as hormones, purines, pyrimidines, porphyrins and some vitamins. Moreover, they also serve as a source of energy, particularly when they are ingested in excess, and their consumption was accomplished either through gluconeogensis or oxidation to CO_2 via tricarboxylic acid cycle. Protein profiles of the cell are indicative of the physiological status of the animal (Harper, 1985) and there exists a dynamic equilibrium between the synthetic and degenerative pathways associated with these molecules. The protein metabolism constitutes as one of the physiological events involved in the compensatory mechanism interms of homeostasis during any stress condition (Krishnamurthy**,** 1981). In view of this, an attempt has been made in the present investigation to study the effects of fenvalerate on total protein levels in different tissues of *Rana tigrina*.

Free Amino Acids:

The free amino acid (FAA) levels in fenvalerate exposed frog tissues were increased significantly when compared to control. This elevated levels may be due to enhanced protease activity or enhanced transamination. The physiological charactersitcs and enzyme functions of the animals were known to alter as a consequence to pesticide toxicity (Knox and Green gard, 1965; Mayers *et al.*, 1985). The free aminoacids play a vital role in maintaining the intracellular osmotic balance, during physiological stress conditions. The rate of synthesis of proteins also depends on the levels of elimination of nitrogen from various amino acids is the result of such catalysis. These keto acids are the source for the TCA cycle and gluconegenesis, thus regulating the protein ad carbohydrate metabolism (Knox and Green gard, 1965). The physiological state of the cell can be understood by means of quality and quantity of FAA pool which can be considered as the best diagnostic tool (Adibi, 1980). The amino acids released during protein degradation due to activation of proteolysis will once again return to the amino acid pool and thus the FAAs are the currency through which the protein metabolism operates (Munro, 1970) showing the interdependence of both amino acids and proteins (Mahle and cordes, 1970). An abnormality in the protein or amino acid metabolism will have its own consequences in the tissues due to increased flow of the protein catabolic products. In view of this, the levels of FAA in liver and kidney tissues of control and fenvalerate exposure of frogs were studied to gain an insight into the pattern of amino acid metabolism.

CONCLUSION:

This investigation draw a conclusion stating that, In the present investigation hisotpathological studies were also carried out by light microscope to elucidatet the toxic potential of the pesticide in frog exposed to different periods of fenvalerate. Descrite histopathological changes were observed in liver, kidney, of fenvalerate exposed frogs. The severity of damage was more in four weeks exposed animals when compared to first and second weeks. We believe that is it is necessary to assess amphibian fauna of this area before their natural habitat are altered or damaged beyond a true reflection of their species diversity and population abundance.

Reference:

1. Abdulali, H (1985): On the export of frog legs from India, *J. Bombay Nat. Hist. Soc.*, 82: 347- 375.
2. Pandian, T.J and M.P. Marian (1986): Production and utilization of frogs: an ecological view. *Proc. Ind. Acad. Sci.* 95 (3): 289-301.
3. Deuti, K. Deshpande, S.C. Gupta, R.C and M.R. Yadav (1998): Amphibians of India – Report summary. Zoos print. Volume XIII, No. 12: P: 28-29.
4. Anand Padhye, Kelum Manamendra-Arachchi, Anslem de Silva, Sushil Dutta, Tej Kumar Shrestha, Sabitry Bordoloi, (2010). Theodore Papenfuss, Steven Anderson, Sergius Kuzmin, Muhammad Sharif Khan & Ronald Nussbaum . "*Hoplobatrachus tigerinus*". *IUCN Red List of Threatened pecies.* . International Union for Conservation of Nature. *Version 2009.2*
5. Williamson, E.G., S. F. Long, M. J. Kallman, and M. C. Wilson. (1989). A comparative nalysis of the cute toxicity of technical-gradepyrethroid insecticides and their commercial formulations. *Ecotoxicology and Environ mental Safety* 18:27-34.
6. Subbareddy S.V. (2007). Survey of Amphibians and impact of Synthetic pyrethroid Fenvalerate on *RANA TIGRINA* (Indian Bull frog).). Ph.D. Thesis ,Sri Venkateswara University.Tirupathi.
7. Saleh, M.A., Ibrahim, N.A., Saliman, N.Z and M.K. Sheimy (1986): Persitence and distribution of cypermethrin, deltamethrin and fenvalerate in laying chickens. *J. Agri. Food. Chem.*, 34: 895-898.
8. Radhaiah, V. (1988): Studies on the toxic impact of a pyrethroid insecticide, fenvalerate on some metabolic and histopathology of a freshwater Teleost, *Tilapia mossambica (peter*s).Ph.D. Thesis, S.V.University, Tirupati, India.
9. Ayanna, K (1991): Impact of a synthetic pyrethroid on the Crab *Oziotelophusa senex senex*, M.Phil. thesis, S.V.University, Tirupati.

10. Tagatz, M.E and J.M. Ivey (1981): Effect of fenvalerate on field and laboratory developed estuarine benthic communities. *Bull. Environ. Contam. Toxicol.* 27: 256-267.

11. El-Sewedy SM, Mostafa MH, El-Bassiouni EA, Abdel-Rafee A, El-Sebae AH: (1982) Effect of fenvalerate on kynurenine metabolizing enzymes and acid ribonuclease of mouse liver. *J Environ Sci Health B,* , 17, 571-579.

12. WHO (Ed): (1991) Occupational exposure in insecticide application, and some pesticides. International Agency for Research on Cancer. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, 53, 309- 328.

13. Madhuban Datta Bhattacharya, Anilava kaviraj; (2006). Dietary ascorbic acid supplementation to ameliorate chronic toxicity of fenvalerate to*clarias gariepinus*. Australasian Journal of Ecotoxicology, vol. 12, pp. 129-134,

14. Pickering, O.H., Henderson, C and A.E. Lemke (1962): Toxicity of organophosphorous insecticides to different species of warm water fishes. *Trans. Am. Fish. Soc.*, 91(2): 175-184.

15. Jagannatha Rao, K.S (1981): Solvent validity test models, Action Committee report FRAME, Royal society buildings, London. U.K. 120.

16. Humason, G.L. (1972): Animal tissue techniques. III Edn; W.H. Freeman and Co., San fransisco.

17. Harris, H.F (1900): On the rapid conversion of haematoxylin into haematin in staining reaction. *J. appl. Micro. Lab. Meth.,* 3: 777.

18. Carrol, N.V., Longley, R.W and J.M. Row (1956): Glycogen determination in liver and muscle By use of anthronereagent.*J.Biol.Chem.*22: 583-593.

19. Lowry, O.H., Rosenbrough, N.J., Farr, A.L and R.J. Randall (1951): Protein measurement with Folin phenol reagent: *J. Biol. Chem.* 193: 265 – 275.

20. Moore, S and W.H. Stein (1954): A modified ninhydrin reagent for the photometric Determination of Aminoacids and related compounds. *J. Boil. Chem.,* 221: 907.

21. Colowick, S.P and N.C. Kaplan (1951): In methods in enzymology. Acad. Press, New York, 3: 501

22. Turner, L.V. and K.L. Manchester (1972) : Effects of denervation on the glycogen content and on the activities of enzymes of glucose and glycogen metabolism in rat diaphragm muscle. *Biochem.J.*,128: 789 – 801.

23. Martin, D.W., Mayes, A and W.V. Rodwell (1983). In: Harper's review of Biochemistry, Lange medical publications, Maruzan, Asia.

24. Lehninger, A.L (1983): In: Biochemistry, Kalyani publishers. Ludhiana, New Delhi.

25. Bhatia, S.C., Sharma, S.C and T.A. Venkitasubramanian (1973): Effects of dieldrin on hepatic carbohydrate metabolism and protein biosynthesis in vivo. *Toxicol. Appl. Pharmocol.*, 24: 216- 221.

26. Harper, A (1985): In 'Harper's Review of Biochemistry'. (ed: D.W.Martin, P.A. Mayes and V.W. Rodwell), 2oth edition, Lange Medical Publications, Maruzen. Asia (Singapore).

27. Krishna Murthy, C.R (1981): Biological effects of environmental pollution. *Indian J. biophys. Biochem.*, 8: 245.

28. Knox, W.E and O. Greengard (1965): In: An introduction to enzyme physiology. Advan. Enzyme Regul. Ed weber, G. Pergman Press, New York, London, Vol III, 247 – 248.

29. Mayers, A (1985): The citric acid cycle In. Harper's Review of Biochemistry. (eds. Martin, D. W.,Mayars, P.A., Rodwe;;, V.W. and Granner, D.K). Lamge Medical Publications,California. USA. 20: 158-166.

30. Adibi, S.A (1980): Role of branched – Chain amino acids in metabolic regulation *J. Lan. Clin. Med.*, 95: 475.

31. Munro, H.N (1970). In: Mammalian Protein metabolism (ed: H.N. Munro). Academic Press, New York, London, 4:291.

32. Mahle, H.R and Cordes, E.H. (1970): In: "Biological Chemistry". (eds: Harper and Row). Academic Press, New York.