ISSN 0975-6299 Vol.1/Issue-3/Jul-Sep.2010

International Journal of Pharma and Bio Sciences

CYPERMETHRIN AND SODIUM FLUORIDE SYNERGISTIC EFFECT ON OXIDATIVE ENZYMES IN MUSCLE AND KIDNEY OF ALBINO MICE

P. RAVI SEKHAR, Y. SAVITHRI, Y. PRAKASH RAO, S. KISHORE AND K. JAYANTHA RAO*

Division of Toxicology, Department of Zoology, Sri Venkateswara University, Tirupati -517 502. AP. INDIA.

***Corresponding Author** kjrao_1954@rediffmail.com , pesala1980@gmail.com

ABSTRACT

Present study was aimed to investigate the synergistic effect of cypermethrin and sodium fluoride on oxidative enzymes. Albino mice were treated orally with cypermethrin and sodium fluoride individually and combination, for individual administration 1/10th LD_{50} of cypermethrin and sodium fluoride selected (i.e. 8.5 mg/kg body weight and 5.6 mg/kg body weight) and for combined administration 1/20th LD_{50} was selected (i.e 4.25mg/kg body weight and 2.8 mg/kg body weight) for 15 days and 30 days with 48 hours intervals. It reveals significant variation in glucose-6-phosphate dehydrogenase (G6PDH), lactate dehydrogense (LDH), succinate dehydrogenase (SDH) and glutamate dehydrogenase (GDH) activities. Glucose-6-phosphate dehydrogenase, lactae dehydrogenase and glutamate dehydrogenase activities were enhanced where as succinate dehydrogenase activity was decreased in muscle and kidney tissues of experimental animals. The Changes were more pronounced in combination treated tissues than individual treated tissues and it may be due to synergistic effect of cypermethrin and sodium fluoride.

KEY WORDS

Cypermethrin, Sodium fluoride, Synergism, oxidative enzymes, albino mice

INTRODUCTION

Water is the most precious natural resource that exists on our planet. Although water covers more than 70% of the Earth, only 1% of the Earth's water is available as a source of drinking. Yet, our society continues to contaminate this precious resource. Water is known as a natural solvent. Before it reaches the consumer's tap, it comes into contact with many different substances, including organic and inorganic matter, chemicals, and other contaminants. Water dissolves numerous substances in large amounts, pure water rarely occurs in nature. Precipitation absorbs carbon dioxide and other gases, as well as traces of organic and inorganic material from

the atmosphere. Because water reacts with minerals in the soil and rocks, surface and groundwater may contain many different dissolved substances. Surface waters may also contain domestic sewage and industrial wastes.

Pesticides are one of the most common causes of water pollution. Continuous use of these pesticides results in their accumulation in various components of the environment. Presence of these pesticides above safe level in the environment will pose serious threat to non-target organisms. The pesticides contribute much to the chemical pollution.[1] However, most of the chemicals that are used as pesticides are not highly selective but are generally toxic to many non-target species, including man, and other desirable forms of life that co-inhabit the environment. Extended occupational exposure of many environmental chemicals caused adverse effects in the biological system.[2]

Synthetic pyrethroids such as cypermethrin, permethrin, and deltamethrin are increasingly used for indoor pest control because of their high insecticidal activity and considerably lower mammalian toxicity compared with other pesticides.[3] Inspite of the low toxicity of pyrethroids, persistence of these compounds in mammalian tissues may be dangerous.[4] Liberal use of pyrethroids has increased the risk of intoxication for non-target species, such as birds, animals and organisms present in soil and water. Several studies have indicated that pyrethroids induce oxidative stress.[5] Cypermethrin is synthetic pyrethroid insecticide widely used against pests all over the world and there is increased risk of food being contaminated with the insecticide[6]. Thus contaminated food may harm humans and the domesticated animals. It produces reverse effects on the non-target organisms including both invertebrates and vertebrates.[7]

Among minerals, fluoride is one of the contaminants of water. Fluoride is an essential trace element for human beings and animals. In small amounts fluoride is beneficial as it is believed to impart stability to bone and enamel, thereby preventing dental carries and osteoporosis to some extent but its higher concentration is highly toxic to humans and animals alike. The permissible limits of fluoride in drinking water as suggested by Bureau of Indian Standards varies between 0.6 to 1.2 ppm BIS[8], and World Health organization WHO[9] permits a maximum of 1.5 ppm of it. Chronic exposure to fluoride above the permissible limits, causes a disease called “Fluorosis”. Fluorosis is an important clinical and public health problem in several parts of the world. As fluoride is found in small quantities in almost all foods, it enters the human body mainly through the oral route along with food and water. It can be rapidly absorbed by passive diffusion through stomach, small intestine, mouth, lungs and skin.[10]

Drinking water containing fluoride is the major source of fluorosis due to geological crust contamination. An estimated 103 million people around the world live in areas where optimal fluoride concentrations occur naturally.[11] High fluoride in several regions of the world. Excess fluoride problem prevails in 27 countries of the world. In India, 20 states are endemic for fluorosis with an estimated 60 million people at risk and 6 million people disabled; about 600,000 might develop a neurological disorder as a consequence.[12] Fluoride when ingested beyond the limit of tolerance (i.e 1.5 ppm) of the body may cause a dreaded diseae called “fluorosis”.

The effect of fluoride on human health has long been of interest to medical researchers. Fluorosis is an important clinical and public health problem in several part of the world.[13] In high concentrations fluoride compounds are toxic. It enters the human body mainly through the oral route along with food and water. It can be rapidly absorbed by passive diffusion through

stomach, small intestine, mouth, lungs and skin.[10].

Several independent studies on pesticide toxicity and fluoride toxicity carried out in different parts of the world. However, not many attempts have been made to understand the combined toxic effect of pesticides and fluoride. The present study is designed to investigate the combined toxic effect of cypermethrin and sodium fluoride. Combined poisoning of cypermethrin and fluoride through drinking water is an exceptional condition and cause more severe toxicity. In view of this, the present study is carried out in the albino mice to understand the toxic potentials of cypermethrin and sodium fluoride.

MATERIAL AND METHODS

Test Chemicals selected: Cypermethrin technical (92% purity; *cis:trans* isomers ratio 40:60) was obtained from Tagros Chemicals India Limited, Chennai; and Sodium fluoride (99%) supplied by BDH Chemical Division, Bombay.

Animal model : Albino mice

Healthy adult male albino mice of the same age group 75±5 days and weight (35 g) were taken from parental stock obtained from Veterinary College, Bangalore and maintained a colony. They were kept in well cleaned and sterilized cages. Mice were maintained at laboratory conditions (26±2℃; 12 hrs light and 12 hrs darkness) throughout the course of the present study. The animals were fed on rat feed supplied by Hindustan Lever Limited, Bombay and water was supplied *ad libitium*.

Experimental Design

Healthy adult male albino mice were divided into seven groups having ten animals each. Toxicity of cypermethrin and sodium fluoride was evaluated by static bioassay method of Finney [14] and the LD_{50} of cypermethrin and sodium fluoride to albino mice were found to be as 85 mg/kg bw and 56 mg/kg bw respectively. 1/10 of LD_{50} value of cypermethrin and sodium fluoride (i.e 8.5mg/kg bw and 5.6 mg/kg bw) for individual administration and $1/20^{th}$ LD_{50} for combined administration were selected. The first group animals treated as control animals. Second and third group animals were treated for 15 days and 30 days with cypermethrin respectively with 48 hours intervals. Fourth and fifth group animals were treated with sodium fluoride for 15 days and 30 days with 48 hours interval. Sixth and seventh group animals were treated with combined dose of cypermethrin and Sodium fluoride for15 and 30 days through oral gavage with 48 hours interval.

Experimental Protocol

Group	Treatment	Duration (days)	Day of Sacrifice
I	Controls	-	-
II	Treated with cypermethrin (8.5 mg/kg bw)	15	16th
III	Treated with cypermethrin (8.5 mg/kg bw)	30	31st
IV	Treated with sodium fluoride (5.6 mg/kg bw)	15	16th
V	Treated with sodium fluoride (5.6 mg/kg bw)	30	31st
VI	Treated with cypermethrin + sodium fluoride (4.25 mg/kg bw + 2.8 mg/kg bw)	15	16th
VII	Treated with cypermethrin + sodium fluoride (4.25 mg/kg bw + 2.8 mg/kg bw)	30	31st

Enzyme assays

5% homogenate of tissues were prepared in 0.25 ice-cold sucrose solution for estimation of Glucose-6-Phosphate Dehydrogenase (Lohr and Waller[15]) as modifiec by Mastanaiah *et al*[16], Succinate Dehydrogenase (Nachalas *et al*[17)], Lactate dehydrogenase (Srikanthan and Krishnamoothy[18]) and the GDH activity was assayed by the method of Lee and Lardy[19], and these were centrifuged 10,000 rpm for 15 min in a refrigerated centrifuge at 4 ˚C to remove cell debris, and clear extracts were used as enzyme source. The reaction mixture containing 100 µ moles of sodium potassium phosphate buffer, 40 µ moles of INT and 0.3 µ moles of NADP for G-6-PDH, 0.1 µ moles of NAD for LDH and 10 µ moles INT for GDH, 40 µ moles of different substrates and is initiated by adding 1 ml of required enzyme. Then incubate the contents for 30 min at 37 ˚C, the reaction stopped by adding 5 ml of glacial acetic acid and 5 ml of toluene, kept overnight and the colour extract was read at 495 nm by using Hitachi U-2800 model spectrophotometer.

RESULTS

The results of Glucose-6-Phosphate dehydrogenase, Succinate dehydrogenase, Lactate dehydrogenase and Glutamate dehydrogenase enzyme activities of control and experimental mice under cypermethrin and sodium fluoride toxicity in muscle and kidney were shown in table-1, 2. The enzyme activities in experimental mice exposed to cypermethrin and sodium fluoride individual and combination showed statistically significant ($p<0.05$) increase of G-6-PDH, LDH and GDH activities, where as SDH activity was significantly ($p<0.05$) decreased. Alterations in oxidative enzyme activities were in the form of a dose and time dependent manner in experimental animals.

Table 1.

Changes in the activity levels of oxidative enzymes in muscle tissues of cypermethrin and sodium fluoride treated albino mice (μ moles of formazan formed/ mg protein/hr)

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
G-6-PDH							
Mean	0.657	0.821	0.967	0.785	1.084	0.942	1.136
SD±	0.016	0.024	0.016	0.026	0.030	0.012	0.014
PC		(24.961)	(47.184)	(19.482)	(64.992)	(43.378)	(72.907)
SDH							
Mean	0.680	0.577	0.475	0.602	0.400	0.466	0.338
SD±	0.011	0.017	0.012	0.013	0.016	0.015	0.017
PC		(-15.147)	(-30.147)	(-11.470)	(-41.176)	(-31.470)	(-50.294)
LDH	3.321	3.750	4.019	3.615	4.183	3.983	4.310
Mean	0.025	0.022	0.030	0.018	0.012	0.021	0.015
SD±		(12.917)	(21.017)	(8.852)	(25.956)	(19.933)	(29.780)
PC							
GDH							
Mean	0.167	0.190	0.225	0.183	0.238	0.217	0.245
SD±	0.013	0.017	.014	0.011	0.015	0.011	0.022
PC		(13.772)	(34.730)	(9.580)	(42.514)	(29.940)	(46.706)

All the values are mean of six individual observations.
PC – percent change over control, SD ± Standard deviation
All the values are significant at p<0.05

Table 2.

Changes in the activity levels of oxidative enzymes in kidney tissues of cypermethrin and sodium fluoride treated albino mice (μ moles of formazan formed/ mg protein/hr)

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
G-6-PDH							
Mean	0.737	0.848	0.951	0.811	1.037	0.968	1.104
SD±	0.022	0.016	0.009	0.028	0.020	0.010	0.012
PC		(15.061)	(29.036)	(10.040)	(40.705)	(31.343)	(49.796)
SDH							
Mean	0.745	0.663	0.582	0.685	0.512	0.575	0.425
SD±	0.020	0.010	0.012	0.015	0.014	0.012	0.014
PC		(-11.006)	(-21.879)	(-8.053)	(-31.275)	(-22.818)	(-42.953)
LDH							
Mean	2.019	2.273	2.388	2.227	2.467	2.322	2.515
SD±	0.020	0.015	0.016	0.018	0.011	0.044	0.048
PC		(12.580)	(18.276)	(10.302)	(22.189)	(15.007)	(24.566)
GDH							
Mean	0.196	0.215	0.237	0.202	0.243	0.226	0.255
SD±	0.013	0.021	0.009	0.015	0.021	0.008	0.031
PC		(9.693)	(20.918)	(3.061)	(23.979)	(15.306)	(30.102)

All the values are mean of six individual observations.
PC – percent change over control, SD ± Standard deviation
All the values are significant at p<0.05

DISCUSSION

Glucose-6-phosphate dehydrogenase (G-6-PDH) is an important enzyme, which pioneers the hexose monophosphate (HMP) shunt by catalyzing reversible oxidation of glucose-6-phosphate to 6- phospho gluconolactone. This pathway has great importance in the carbohydrate metabolism because it is not only an alternate pathway for glucose oxidation but also produces pentose sugars and reduced NADP which are much needed for the synthesis of nucleic acids, fatty acids and amino acid.[20]

An increased glucose-6-Phosphate dehydrogenase activity was observed in the present investigation (Tables 1 & 2). G-6-PDH is the key enzyme of hexose monophosphate path way and is used to generate NADPH and ribose -5-Phosphate. If energy needs are high, this pathway serves to generate glycolytic intermediates for the production of energy.[21] The increased oxidation of glucose through switched over HMP shunt by G-6-PDH is due to the prevalence of anaerobiosis.[22]

The common feature of the tissue under stress conditions is a general elevation in the activities of NADP dependent dehydrogenases and reduction in the activities of NAD dependent dehydrogenases. The elevation in G-6-PDH activity was also supported by many investigators. An increased G-6-PDH activity in selected tissues of fresh water mussel, *Lamellidens marginalis* under copper sulphate toxicity.[23] Increased G-6-PDH activity observec in albino rat treated with cypermethrin[24] , increased G-6-PDG activity with individual and combined treatments of 2,4-D and azinphospomethyl.[25] OruC and Uner[26] demonstrated increased glucose-6-phosphate dehydrogenase activity in response to pesticide toxicity.

Lactate dehydrogenase is hydrogen transferring enzyme that catalyzes the oxidation of L- lactate to pyruvate with mediation of NAD^+ as hydrogen acceptor. Any alterations in LDH activity indicates change in the production of pyruvate to lactate under anaerobic conditions favoring the reoxidation of NADH. This allows glycolysis to proceed in the absence of oxygen by generating sufficient NAD. Elevation in the specific activity of LDH in experimental animals when compared to respective controls (Tables 1 & 2) observed in the present investigation may be due to forward reaction of LDH, namely pyruvate to lactate which may be operative during cypermethrin and fluoride toxicity. It is also to meet energy demands when aerobic conditions are lowered due to diminished TCA cycle enzyme activities.[27]

Increased activity of LDH is a characteristic feature of a shift from aerobic to anaerobic metabolism leading to an elevated rate of pyruvate conversion into lactate, resulting in lactic acidosis.[28] The LDH activity increases during conditions favoring anaerobic respiration to meet energy demands, when aerobic respiration is lowered.[29]

Increased LDH activity was supported by several authors.Albino rat treated with sodium selenete[30] , with arsenite treatment in fresh water fishes[31], in albino mice with sodium fluoride[32] and in kidney of young pigs[33] . John Sushma *et al.*[34] also observed Similar increase in LDH activity in albino mice treated with aluminium acetate.

SDH is a vital enzyme of citric acid cycle catalyses the reversible oxidation of succinate to fumarate. This decrease would affect the conversion of succinate to fumarate and might cause a block in the Krebs cycle. The activity of succinate dehydrogenase, an oxidative enzyme involved in the Krebs cycle, was significantly decreased in muscle and kidney tissues after treatment of cypermethrin and sodium fluoride, corroborating earlier findings with sodium fluoride exposure. Chinoy and Memon[35] reported decreased SDH activity in gastrocnemius muscle and liver of mice with combined exposure of calcium fluoride and Aluminum.

The SDH activity was decreased treated with sodium fluoride, in pectorials and gastrocnemius muscles of mice[36] and in gastocnemius muscle, liver and brain of mice.[37] Decreased SDH activity in rat by zinc sulphate treatment.[38] This decrease would affect the conversion of succinate to fumarate and might cause a block in the Krebs cycle. Decreased SDH activity of all tissues in the present study clearly indicates depletion in the oxidative metabolism at the level of mitochondria leading to depression of TCA cycle under the cypermethrin and sodium fluoride exposure.

Glutamate dehydrogenase (GDH) is an enzyme, present in cytoplasm and mitochondria of eukaryotes and is an important enzyme between carbon and nitrogen metabolism, as some of the other enzymes required for urea synthesis, that catalyzes the reversible oxidative deamination of glutamate to α-ketoglutarate and ammonia.[39] GDH plays a crucial role in the nitrogen metabolism by functioning both in amino acid catabolism and their biosynthesis. GDH allows the incorporation of ammonia into α-ketoglutarate before being transferred by transamination to other α-keto acids7).[40]

GDH activity increased in the tissues of mice exposed to sublethal doses of cypermethrin and sodium fluoride individually and combined (Tables 1 & 2). The increased levels of GDH activity in cypermethrin and sodium fluoride treated animals may be due to increased mitochondrial permeability in the present investigation. Increased GDH activity cypermethrin toxicity in fish.[41] Increased, GDH activities are reported by several authors in mice treated with sublethal dose of aluminium acetate,[34] in albino rats exposed to hexachlorophene.[42] Birkner *et al*.[43] reported increased GDH activity in liver tissues of rat after acute poisoning with sodium fluoride. Thus the activity levels of Glutamate dehydrogenase has been recorded under cypermethrin, sodium fluoride individual and combination treatment in the present investigation.

The results of the present study clearly shows significant alterations in oxidative enzymes due to intoxification of cypermethrin and sodium fluoride oxidative stress in albino mice. Finally it can be stated that long term exposure to sublethal doses of pyretroid pesticides and fluorides can result in cell metabolism toxicosis. Cypermethrin and sodium fluoride might have synergistic effects on muscle and kidney functions leading to physiological impairment. It is concluded that combination of cypermethrin and sodium fluoride has pronounced more toxic than individual treatment.

ACKNOWLEDGMENT

One of the authors, P. Ravi Sekhar is highly thankful to UGC for the financial assistance through Rajiv Gandhi National Fellowship (RGNF).

REFERENCES

1. Brown, A.W.A: Ecology of pesticides, A Wiley Interscience Publications, Chickester, Brisbance, Toronto, p-5, New York (1978).
2. Abdollahi., Akaram Ranjbar., Shahin Shadnia., Shekoufeh Nikfar., and Ali Rezone, Pesticides and oxidative stress: a review, *(C) Med. Sci. Monit*., 10(6) a: 141-147, (2004).
3. Leng, G., K. Kuch and H. Idel ,. Biological monitoring of pyrethroid metabolites in urine of pest control operators. Toxicol. Letter., 88: 215-220, (1996).
4. Crawford,M.J., A . Croucher and D.H Huston, Metabolism of cis and trans-cypermethrin in rats. Balance and tissue retention study. J. Agric. Food Chem., 29: 130-135, (1981).
5. Gabbianelli R, Falcioni G, Nasuti C and Canthalamessa F. Cypermethrin induced plasma membrane perturbation in

erythrocytes from rats: reduction of fluidity in the hydrophobic core and in glutathione peroxidase activity. *Toxicology*; 21: 1003-1009, (2002).

6. Usmani, K.A and C. O. Knowles: Toxicity of pyrethroid and effect of synergists to larval and adult *Helicoverpa zea*, *Spodoptera frufiperda* and *Agrotis ipsilon* (Lepidoptera: Noctuidae*). J. Econ. Entomal*., 94:868-873 (2001).
7. Gowlen, B.T., Moffat, C.F., Stagg, R.M., Houlihan, D.F and I.M. Davies: Cypermethrin induces Glutathione S-Transferase activity in the shore crab, Carcinus maenas. Mar. *Environ. Res*., 54: 169-77 (2002).
8. BIS (Bureau of Indian Standards), ISI specification for drinking water, IS 10500; 1983, New Delhiv, (1984).
9. WHO (World Health Organisation: Guideline for drinking water quality recommendations, *Geneva,* 1 : 130 (1984).
10. Khandare, A.L., Kumar P.U and N. Lakshmaiah, Fluoride toxicity–Dietary intervention. National Institute of Nutrition, Hyderabad, 22 (1) (2001).
11. Murray JJ. Appropriate use of fluoride for human health, WHO Geneva.(1986)
12. Reddy DR. "Neurology of endemic skeletal fluorosis". Neurol India, 57 (1): 7–12, (2009).
13. Singh, A and SS. Jolly, Chronic toxic effects on the skeletal system. In: Fluorides and human health. WHO Monograph Series No. 59. Geneva: WHO; p. 238-49, (1970).
14. Finney, D.J: Probit analysis, III Edition, Cambridge Univ. press, London, p.20 (1971).
15. Lohr, G.D and H.D. Waller, In : Methods of Enzymatic Analysis (Ed) H.V. Bergmeyer Academic Press, New York, London (1965).
16. Mastanaiah, S. Chengalraju, D and K.S. Swamy, Circadian rhythmic activity of lipase in the scorpion, Heterometrus fulvipes (C. Koch). *Curr. Sci.,* 20 (47): 130-131,(1978).
17. Nachlas, M.M., Margulism, S.D and A.M. Seligman, A colorimetric method for estimation of Succinic dehydrogenase activity. *J. Biol. Chem.,* 235 : 499-504, (1968).
18. Srikanthan, T.N and C. Krishnamoorthy, Tetrazolium test for dehydrogenases. *J. Sci. Indust. Res.,* 14: 206, (1955).
19. Lee, Y.L and A.A. Lardy, Influence of thyroid hormones on L-glycerophosphate dehydrogenases in various organs of the rat. *Journal of Biological Chemistry,* 240: 1427-1430, (1965).
20. Wenger, E., Wenger, B.and P. Kisto, Pentose phosphate pathway of the chick embryo. *J. Exp. Zool.,* 166 : 263-270 (1966).
21. Voet, D and J.G. Veot, Biochemistry, John wiley and Sns, New York, (1995).
22. Bhatia, S.C., Sharma, S.C and T.A. Venkatasubramanian,. *Arch. Environ. Health.*, 20: 993, (1972).
23. Venkata Ramana, P and K. Radhakrishnaiah, Copper influenced changes in lactate dehydrogenase and G-6-PDH activities of the fresh water teleost, *Labeo rohita*. *Bull. Environ. Contam. Toxicol.,* 67: 257-263 (2001).
24. Ravi Sekhar, P., Pushpa Raj, CJ., Savithri, Y., Madhava Rao, S and K. Jayantha Rao, Alterations in oxidative enzymes in liver and kidney tissues of albino rats under cypermethrin toxicity. *Bull. Boil. Sci.* 7 (1): 25-31 (2009).
25. Elif Ozcan Oruç and Nevin Uner, Combined effects of 2,4-D and azinphosmethyl on antioxidant enzymes and lipid peroxidation in liver of *Oreochromis niloticus .Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology,* 127 (3): 291-296, (2000).
26. OruC E.O. and N Uner. Combined effects of 2,4 D and azinphosmethyl on antioxidant enzymes and lipid peroxidation in liver of

*Oreochromis niloticus,*Comp.Biochem.Physiol.,Part C,127,291-296 page 669, (2000).

27. Martin, D.W., Mayers, P.A. and V.W. Rodwell, Harper's Review of Biochemistry, Lange Medical Publications, Maruzan, Asia (1983).
28. Chinoy, N.J., Sequier, A.E and M. Michael, Effects of zinc sulphate on the reproductive functions of male mouse. *Indian J. Environ. Toxicol.,* 6 (1) : 14-18, (1996).
29. Murray, R.K., Granner, D.K., Mayes, P.A and V.W. Road well, Harper's Biochemistry. Lange Medical Publications. Appleton and Lange, California, (1995).
30. Vasantha Sena, J, Selenium effect on mice with special reference to teratological, histological and selected biochemical parameters. Ph.D. Thesis, Sri Venkateswara University, Tirupati, Andhra Pradesh, India (2002).
31. Shobha Rani, A. Reddy, R.S and T.N. Raju, Alterations in the levels of dehydrogenases in a fresh water fish *Tilapia mossambica* exposed to arsenite toxicity. *Ind. J. Environ. Health.,* 42 : 130-133 (2000).
32. Manna, S., Bhattacharyya, D., Basak, D. K and T. K. Mandal, Single oral dose toxicity study of α-cypermethrin in rats. *Indian J Pharmacol;* 36 (1): 25-28, (2004).
33. Xiu-An Zhan, Min Wang, Zi-Rong Xu, and Jian-Xin Li, Toxic effects of fluoride on kidney function and histological structure in young pigs. *Fluoride,* 39(1)22–26, (2006).
34. John Sushma, N., Sivaiah, U., John Suraj, N. and K. Jayantha Rao, Aluminium acetate : Role in oxidative metabolism of Albino mice. *International J. Zoological Research,* 3 (1): 48-52, (2007).
35. Chinoy, NJ and MR. Memon, Beneficial effects of some vitamins and Calcium on fluoride and aluminium toxicity on gastrocnemius muscle and liver of male mice. *Fluoride*, 34 (1) 21-33, (2001).
36. Chinoy, NJ., Patel, KG and C. Sunita, Antifertility investigations of alcoholic papaya seed extract in female rats. Journal of Medicinal and Aromatic Plant Sciences, 19(2): 422-426, (1997).
37. Lakshmi Vani, M and K. Pratap Reddy, Effects of Fluoride accumulation on some enzymes of brain and gastrocnemius muscle of mice. *Fluoride;* 33 (1): 17-26 (2000).
38. Chinoy, N.J., Sequier, A.E and M. Michael, Effects of zinc sulphate on the reproductive functions of male mouse. *Indian J. Environ. Toxicol.,* 6 (1) : 14-18 (1996).
39. Stillman, TJ., Baker, PJ., Britton, KL. and DW. Rice, Conformational Flexibility in Glutamate Dehydrogenase Role of Water in Substrate Recognition and Catalysis. *J. Mol. Biol,* 234(4): 1131-1139 (1993).
40. Murray Robert, K., Daryl Granner, K., Peter Mayes, A and W. Victor Rodwell, In: Harper's Illustrated Biochemistry. International 26th Edition, The McGraw-Hill Companies, Inc. pp 46, 47 (2007).
41. Begum, G, Cypermethrin – induced biochemical perturbations in freshwater fish *Clarias batrachus* at sublethal exposure and after released into freshwater. *Drug and Chemical Toxicology,* 30: 55-65 (2007).
42. Suhasini, N., Lokanatha, V., Sahitya, C.P and W. Rajendra, Alterations in the protein catabolism and transamination pattern in the rat liver on repeated hexachlorophene treatment. *Toxicology International,* 13(1):33-38 (2006).
43. Birkner, E., Grucka-Mamczar, E., Machoy, Z., Tarnawski, R. and R. Polaniak, Disturbance of protein metabolism in rats after acute poisoning with sodium fluoride. *Fluoride*, 33 (4) 182-186, (2000).