ASIAN JOURNAL
ISSN: 0976-3376

Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

Asian Journal of Science and Technology
Vol. 8, pp.159-165, December, 2010

RESEARCH ARTICLE

A STUDY OF EFFECT OF LUFENURON ON BIOCHEMICAL PARAMETERS IN SERUM OF MICE *Mus musculus species*

Deivanayagam, C[1]*., Rajasekar, S[1]., Asokan, S[2] and Suganthy, K[3]

[1]Advanced Zoology and Biotechnology, Dr. S.R.K. Government College, Yanam, Pondicherry- 533 464
[2]PG & Research Department of Advanced Zoology & Biotechinology, AVC College (Autonomous), Mannampandal - 609 305, Mayiladuthurai, Tamil Nadu
[3]Vinayka missions Medical College & Hospital, Karikal 609 609

Received 7th September, 2010; Received in revised form; 25th October, 2010; Accepted 27thNovember, 2010; Published online 15th December, 2010

The present study examined the impact of Lufenuron on biochemical parameters of albino mice. The biochemical parameters such as Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) and Total protein in serum were observed. The mice exposed with Lufenuron (0.1520 mg/kg) for 30 days. The present study suggests that the level of AST, ALT and ALP were increased and Total protein content was decreased in Lufenuron treated mice. The present study concludes that the Lufenuron damages the liver tissue. Due to the damage of liver tissue these enzyme increased in the serum.

Key words: *Lufenuron, AST, ALT, ALP, Total protein, Mice*

INTRODUCTION

Pesticides and polychlorinated biphenyls (PCBs) have been widely used throughout the world since the middle of the last century; however, most of them have been banned since the 1970s (many of the organochlorine compounds). Pesticides were mainly used in agriculture and animal production and PCBs as industrial fluids, both including substances with high toxic effects and persistence in the environment, posing considerable hazards. The problem becomes more serious when bioaccumulation of these lipophilic compounds is taken into consideration (Biziuk, 2001; Doyle, 2004). Organo Phosphorus Compounds (OPC) is among the most widely used pesticides, mainly as insecticides in agriculture, health campaigns and urban pest control. In last decade, adverse effects of OPC exposure on the male reproductive stem, along with their mutagenic and carcinogenic activities have attracted attention (IARC, 1991). Pesticides are widely used throughout the world in agriculture to protect crops and in public health to control diseases transmitted by vectors or intermediate hosts. Insect Growth Regulators (IGRs) are third-generation insecticides less toxic and compatible with insect pest management that were developed to reduce the pollution of food and environment. These compounds have a specific mode of action on insects and a lower toxicity against vertebrates than conventional insecticides. IGRs include compounds that affect moulting and metamorphosis by mimicking juvenile hormone (JH agonists) or usually antagonizing JH activity (ecdysteroid agonists) or by interfering with cuticle formation (chitin synthesis inhibitors) (Smet *et al*., Oberlander *et al.,* 1991*;* Oberlander *et al.,*1997).

***Corresponding author:** *15deivayagam69@gmail.com*

Organophosphorus pesticides, in addition to their intended effects like the control of insects or other pests, are sometimes found to affect non target organisms including humans (Chaudhuri *et al., 1999*). Exposure to organophosphorus pesticides is also a potential cause of longer-term damage to the nervous system, with reports of poor mental health and deficits in memory and concentration *(*Davis, *1991;* Nigg *et al., 2000*) Because of the serious environmental problems resulting from the use of pesticides in the agricultural sector, several governments are seeking to employ biological and other nonpolluting methods for combating pests. Several biocides and their metabolites are suggested to be prior mutagenic and/or teratogenic compounds *(*Ridgway *et al.,* 1978*)*.

Lufenuron may be co-formulated with profenofos or fenoxycarb. Lufenuron is an insecticide which has not been evaluated by the FAO/WHO JMPR or WHO/IPCS. An EU review (according to EU directive 91/414/EEC) is expected to be completed by the end of 2008). Lufenuron is a racemic mixture of two enantiomers. It is a solid of low vapour pressure, having low solubility in water and a high octanol/water partition coefficient. Hydrolysis occurs slowly at pH 9 and very slowly at pH5 and 7. Photolysis occurs slowly. Lufenuron is a selective insecticide, which provides control of the larvae of insect some Diptera (leaf miners & fruit flies), some Homoptera (Psyllids & flocculent whitefly), and rust mites of the family riophiidae. It is used on a wide range of crops, including cotton, maize, sugar beet, potatoes, other vegetables, grapes, citrus, other fruit, and ornamentals.

MATERIALS AND METHODS

Chemical

Lufenuron 5.4% (w/w) (Cigna) Chemical composition of Lufenuron 540% w/w Emulsifying agents caster of polyglcal, ether 36.40.6.00 w/w. Emulsifying agents linear alkylbenzone sulfonic acid. Calcium 4.00% w/w Solvent cycotoexanaon 20.00 solvent. (Solvent) 64.60% w/w. Manfactured by. Syngenta India limited. 14.1. Tata Road Mumbai.

Animals

Male albino mice, 7-8 weeks old, weighing 130-140g were used for the study. The animals were obtained from National Institute of Nutrition, Hyderabad and maintained in Central animal house, Rajah Muthiah Institute of Health Science, Annamalai University, Annamalainagar, India. The rats were housed in polypropylene cages at room temperatures (27 ± 2°C) with relative humidity 55±5%, in an experimental room. In Annamalainagar, the LD (light: dark) cycle is almost 12:12h. The local institutional animal ethics committee (Registration Number 160/1999/CPCSEA), Annamalai University, Annamalainagar, India, approved the experimental design (Proposal No. 527, dated 25.05.2007). The animals were maintained as per the principles and guidelines of the ethical committee for animal care of Annamalai University in accordance with the Indian National Law on animal care and use. The animals were provided with standard pellet diet (Amrut Laboratory Animal Feed, Mysore Feeds Limited, Bangalore, India) and water *ad libitum*. The mice were divided into two groups. Each group having 6 mice. The group I was control and Group II was treated with Lufenuron (0.1520 mg/kg). After the treatment, the blood samples were collected from venipuncture of mice. The centrifuged blood samples were stored and serum were separated and used for various biochemical estimations.

Estimation of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT)

The conversion of NADH to NAD^+ is proportional to the concentration of AST in serum, and is measured at 340nm as rate of decrease in absorbance. To 100µl of serum, 1ml of given reagent mix is added. The Mixture is mixed thoroughly and contents are transferred into cuvette. The first reading is recorded at 60^{th} second, and subsequently three more readings are taken with 30 seconds interval at 340 nm. The conversion of NADH to NAD^+ is proportional to the concentration of ALT in serum, and is measured at 340nm as rate of decrease in absorbance. To 100µl of serum, 1ml of given reagent mix is added. The Mixture is mixed

thoroughly and contents are transferred into cuvette. The first reading is recorded at 60th second, and subsequently three more readings are taken with 30 seconds interval at 340 nm.

Assay of alkaline phosphatase (ALP) in serum

Alkaline phosphatase in a sample hydrolyses para – nitrophenyl phosphate into para – nitrophenol and phosphate, in the presence of magnesium ions. The rate of increase in absorbance of the reaction mixture at 405nm due to liberation of paranitrophenol is proportional to the alkaline phosphatase activity. Twenty microlitres of serum is mixed with 1 ml of given buffered substrate, mixed well and absorbance is read at 30,60,90 and 120 seconds at 405nm.The mean change in absorbance per minute is determined and test results are calculated.

Estimation of total protein in serum

The peptide bonds of protein react with copper ions in alkaline solution to form blue – violet coloured complex (biuret reaction). Each copper ion complexes with 5 or 6 peptide bonds. The colour formed is proportional to the protein concentration and is measured at 546nm. To 20µl of serum and 20µl of standard, 1 ml of reagent is added in two different test tubes, mixed well and incubated for 10 minutes at 37° C. The absorbance of test and standard are measured at 546nm against reagent blank.

Estimation of serum Albumin

Albumin binds with the dye Bromocresol Green in a buffered medium to form a green coloured complex. The intensity of the colour formed is directly proportional to the amount of albumin present in the sample. To 10µl of serum and 10µl of standard, 1 ml of reagent is added in two different test tubes, mixed well and incubated for 5 minutes at room temperature. The absorbance of test and standard are measured at 630nm against reagent blank.

Estimation of urea

To 0.1 ml of serum 1.0 ml of enzyme solution was added, mixed well and incubated for three minutes at 37 ^{0}C (Bumas et *al.,* 1971). Then 1 ml of chromogen solution was added. This was mixed well and incubated for five minutes at 37 ^{0} C. The standard and blank were treated similarly. The absorbances of the test and standard were measured against blank at 578 nm.

Estimation of serum creatinine

Fifty microlitres of serum was mixed with 1.0 ml of working solution **(**Henry *et al.,* 1974**)**. The absorbance of assay mixture was read at exactly 30 seconds after the addition of serum and then again at 90 seconds. The standard was treated in a similar manner.

Estimation of serum glucose

To 10 µl of serum, 1 ml of enzyme reagent was added. Mixed well and incubated for ten minutes at 37^{0}C (Barham *et al.,* 1972). Similarly 10 µl of standard and 10 µl of deionized water as blank were treated. The absorbance of standard and test was measured against reagent blank at 505 nm.

RESULTS

The level of AST was 82±1.3 U/L. in the serum of control mice. During the Lufenuron exposure the activity of AST was significantly increased in the serum (133±1.9) U/L. In the control mice, the level of ALT was 67±1.3 U/L. At sublethal dose of Lufenuron the ALT increased upto114±1.7 U/L. in serum of Lufenuron treated mice. Serum Glucose, Urea, Creatinnine, was observed to be in normal limit in both the groups.

The level of ALP was 92±1.3 U/L in control mice and 286±1.1 in treated serum of mice. The level of total protein was 5 ± 1.1 g/dl in normal mice. During the Lufenuron treatment the level of total protein was decreased (4.4 ± 1.5) in serum of mice.

DISCUSSION

The determination of the patho-physiological enzymes like AST and ALT is a common mean of

Table 1. The level of liver enzymes, total protein, Albumin of treated and control mice

Parameters	Control Group I	Treated Group II
AST(U/L)	82±1.3	133±1.9*
ALT(U/L)	67±1.3	114±1.7*
ALP(U/L)	92±1.3	286±1.1*
Total protein (g/dl)		
Albino (g/dl	5 ± 1.1	4.4 ± 1.5*

Values are expressed as mean ± SD (n = 06). Values that are not sharing a common superscript letter in the same column differ significantly differ at $p < 0.05$ (DMRT).

Table 2. The level of General Biochemical Parameters in serum of Lufenuron treated & control mice

Parameters	Control Group I	Treated Group II
Glucose (mg/dl)	161±7.2	106±6.7*
Urea(mg/dl)	45±3.5	24±2.5*
Creatinine(mg/dl)	0.58±24	0.38±0.18*

Values are expressed as Mean±SD(n=06) (Significan P Value <0.0.5)

detecting the liver status. Alterations in AST and ALT values are reported in hepatic disease or damage. AST, ALT and ALP are considered the bio-markers for liver functions (Martin *et al*., 1981; Mazumder, 1999). AST is responsible for transferring amino group from aspartate to α- β glutaric acid forming glutamate and oxaloacetate. The rise in AST level is virtually responsible for all types of hepatic disease. Its peak concentration and ratio to other enzymes reflect the type of hepatic damage (Tiwari and Srivastava, 2001).

ALT is responsible for transferring an amino group from alanine to α- ketaglutaric acid forming glutamate and pyruvate. It is well known that AST is very specific enzyme for hepatic tissue. It is more sensitive to hepatic damage and its level rises faster and higher in most types of hepato cellular damage (Tiwari and Srivastava, 2001). The present study shows significant increase in the level of AST and ALT in the serum of mice treated with sub lethal dose Lufenuron. This result indicates that the increase in AST and ALT in serum may be due to hepato cellular necrosis, which causes increase in the permeability of the cell membrane resulting in the release of transaminase in the blood stream. Similar results were found by Shalan *et al.* (2005) and Hassainn, (1994). They reported that the serum AST and ALT are elevated due to heavy metals in chronic hepatic disease indicating toxic liver damage (Beuer, 1982) and correlating with the development of fibrosis (Latner, 1975). Similarly, Tandon *et al.* (1997), Sankar Samipillai *et al.,* 2010;Sankar Samipillai and Jagadeesan,2009) and Khan *et al*, (1993) reported disturbances in the liver functions after exposure reported the increase in the level of AST and ALT activities in the serum of mice when treated with. Hwang *et al*., (2000) have also observed similar results in mice serum when treated with lufenuron. The increased AST and ALT activity in rats exposed to may reveal possible leakage of enzymes across damaged plasma membranes and or the increased synthesis of enzyme in the liver. Meanwhile, the elevation of ALP also correlates with exposure level and time of animals. The Increased serum ALP has been explained by pathological processes such as liver impairment and kidney dysfunction (Atroshi *et al*., 2000; Bogin *et al*., 1994).

Alkaline phosphatase is a brush border enzymes, splits various phosphate esters at an alkaline pH and mediates membrane transport (Smith *et al.*, 1983). Alkaline phosphatase is a membrane bound enzyme and its inactivation leads to membrane damage of hepatic cells (Flora *et al.*, 1994). Increased Alkaline phosphatase is responsible for intra-and extra-hepatic disease. These enzymes are indicative of various aspects of metabolism and they have been used to evaluate the physiological, biochemical and metabolic defects in the brain, liver and kidney tissues. Alkaline phosphatase is involved in the synthesis of nuclear proteins, nucleic acids and phospholipids as well as in the cleavage of phosphate esters. These enzymes are associated with transmembrane transport mechanism, ion transport, maintenance of ionic strength and cell growth in the organ (Moog, 1946). The significant increase in the activities of acid and alkaline phosphatases may be attributed to the destruction of all membranes and lysosomes which in turn might cause tissue damage (Saxena and Sarin, 1980, Ramalingam *et al.*, 1999). Similar observations were made by various authors (Farley *et al*, 1983; Blood *et al*, 1983; Teotia and Teotia, 1991).

Proteins are important organic constituents of the animal cells. It plays a vital role in the process of interactions between intra and extra-cellular media being a part of cell membrane and an enzyme. It participates the intricately balanced subceullar fraction (Amudha *et al*., 2002; Ramalingam *et al*., 2002). Proteins are important organic substances required by an organism in the tissue building, the cellular organelles repair and also cellular metabolism (Yeragi *et al*., 2000). Its synthesis is considered as premier biochemical parameters since it is the most sensitive and earlier indicator of stress. It can be reflected by a large number of exogenous substances, mainly through reduction of the endoplasmic reticulum in the cells (Syverson, 1977).

The present study showed that the level of total protein content significantly decreased in serum of mice when treated with sub-lethal dose of Lufenuron. This result suggests that the decreased level of total protein might be due to their catabolism to liberate energy during the stress of Lufenuron toxicity similar type of results was observed by Furkas (1975); Dhar and Banerjee (1983) in rat and mice when they treated with lead and cadmium respectively. The liver enzymes in the present work (AST, ALT and ALP) was increased and total protein in serum were significantly increased were observed in Lufenuron intoxicated animals (Table 1). These results suggested that the Lufenuron has induced treated mice hepatotoxicity Serum glucose, urea, Creatinine to be is normal limit in both the groups.

These results may be due to hepato cellular necrosis which causes increase in the permeability of cell membrane resulting in the release of these enzymes in the blood stream (Sharma *et al.,* 2002; Rana *et al.,* 1996; Sankar Samipillai *et al.,* 2010; Sankar Samipillai and Jagadeesan, 2009). As the liver is the centre for detioxifying many foreign compounds entering the body. Lufenuron which is admimsiered to mice is neither not properly dextoxified in body or have caused its direct toxic effect on hepatic tissue. So in the present study, It is concluded that treated albino mice with Lufenuron showed significant changes in the level of ALT,AST,& ALP without returning to normal levels even at the end of 30 days of recovery period. So further toxic effects of Lufenuron on hepatic tissue is to be considered.

Acknowledgement

The authors are thankful to Professor and Head, PG & Research Department of Advanced Zoology & Biotechnology, AVC, College, Mayiladuthurai for providing necessary lab facilities to carry out the work successfully.

REFERENCES

Amutha, P. Sangeetha, G. and Mabalingam, S. 2002. Dairy effluent induced alterations in the protein, carbohydrate and lipid metabolism of freshwater teleost fish *Oreochromis mossambicus*. *Poll. Res*., 21: 51-53.

Atroshi, F., Rizzo, A., Sankari, S., Bieso, I., WesterMark, T. and Veijalaiman. T. 2000. Liver enzyme activities of rats exposed to ochratoxin A and T-2 toxin with antioxidants. *Bull. Environ. Contam. Toxicol.,* 64: 586-592.

Bauer, J.D. 1982. Enzymology. In : Clinical laboratory methods 9th ed. The CV Mosby Co., St Louis MO USA.

Blood, D.C., Radostits, O.M., Henderson, J.A. Arundel, J.H. Gay, C.C. 1983. In: Veterinary Medicine, sixth ed. Bailliere Tindal and Cassel Ltd. London.

Bogin, E., Marom, M. and Levi, M. 1994. Changes in serum, liver and kidneys of cisplatin-treated rats: Effects of antioxidant. *Eur. J. Clin. Chem. Clin. Biochem.,* 32: 843-851.

Chaudhuri, K Selvaraj, S. and A. K. Pal, 1999."Studies on the genotoxicity of endosulfan in bacterial systems," Mutation Research, vol. 439 (1): 63–67.

Davis. J.E. 1991. "Neurotoxic concerns of human pesticide exposures," *American Journal of Industrial Medicine,* 18(3): 327–331.

Dhar, A. and Banerjee, P.K. 1983. Impact of lead on nucleic acids and incorporation of labeled amino acid into protein. *I. J. Vit. Nut. Res*., 53: 349-354.

Dudley R.E., Gammel L.M. and Klasson C.D. 1985. *Toxicl. Appl. Pharmacol.,* 74: 299.

Farley, J.R., Wergedal, J.E., Baylink, D.J. 1983. Fluoride directly stimulates proliferation and alkaline phosphatase activity of bone farming cells. *Science,* 222: 330-332.

Flora, G.J.S., Mathur, R., Sandhu, N. and Dua, K.K. 1994. Time dependent preventive effects of simultaneous administration of selenium or zinc during mercury exposure in rats. *Ind. J. Pharmacol*., 26: 209-212.

Furkas, W.R. 1975. Effect of plumbous ion on messenger RNA. *Chem. Biol. Interact.,* 11: 253-263.

Hassanin, L.A.M. 1994. The effects of lead ollution on the susceptibility of rats to anticoagulants rodenticides M.Sc. Thesis, Zoology Department. Faculty of science. Cairo University. GIZA, Egypt. Henry R.J et al: 1974

Hu, C.C., Yem, C.J., Jang, M.L., Liu, C.B., Chen, W.K.,Chung, C., 1991. Cadmium induced serum biochemicalschanges in subchronically exposed rats. *Chung Shan Med. J.,* 2, 97.

Hwang D.F., Hour J.L. and Cheng H.M. 2000. Effect of taurine on toxicity of oxidized fish oil in rats. *Food. Chem. Toxicol.,* 38: 585.

Hwang, D.F., Hour, J.L. and Cheng, H.M. 2000. Effect of taurine on toxicity of oxidized fish oil in rats. *Food. Chem.. Toxicol*., 38: 585.

Khan, M.Z., Szarek, J., Krasondebska-Depta, A., Koncicki, A. Effect of concurrent administration of lead and selenium on some haematological and biochemical parameters of boiler chickens *Acta. Vet. Hang.,* 41: 123-137.

King, J. 1965. In: Practical clinical enzymology var Nortant, D. Company. London. 106-107.

Latner, A.L. 1975. In Contarow, Tramper (Eds) Clinical biochemistry. Sunders *Philadelphia.* Lowry, O.H. Rosenbrough, N.J., Farr, A and Randall, R.J. 1951. Protein measurement with folinphenol reagent. *J. Biol. Chem*., 193: 265- 273.

Marie, P.J. and Hott, M. 1986. Short-term effects of fluoride and strontium on bone formation and resorption in the mouse. *Metabolism*, 35: 547-551.

Martin, D.W.Jr., Mayes, P.A and Rodwell, V.W. 1981. Herper's review of biochemistry. 18th edn. (Lange medical publication Califonia) 61. Mazumder, U.K., Gupta, N., Chakrabarti, S. and Pal, D. 1999. Evaluation of hematological and hepato renal functions of methanolic extract of Moringa oleifera (Lam) root treated mice *Ind. J Expl. Biol*., 37: 612-614.

Moog, F. 1946. Physiological significance of phosphomonoesterases. *Boil. Rev*., 21: 24-28. Nigg,H.N. and Knaak, J.B. 2000."Blood cholinesterases as human biomarkers of organophosphorus pesticide exposure," *Reviews of Environmental Contamination & Toxicology*, 163: 29–111.

Oberlander, H., D.L. Silhacek, E. Leach, I. Ishaaya and E. Shaaya. 1991. Benzoylphenyl urea inhibits chitin synthesis without interfering with amino sugar uptake in imaginal wing discs of Plodia interpuntella, Arch. *Insect Biochem. Physiol.,* 18: 219-227.

Oberlander, H., D.L. Silhacek, E. Shaaya and I. Ishaaya, 1997. Current status and future perspectives of the use of insect growth regulators for the control of stored product insects. *J. Stored Prod. Res.,* 33:1-6.

Ramalingam, V., Prabhakaran, P., Vimaladevi, V. and Narmadharaj, R. 2002. Effect of mercuric chloride in the brain of male rats. Impact of adenosine triphosphate. *Poll. Res.,* 21: 7-11.

Ramalingam, V., Suganthy, O.M.A., Arunadevy, R., and Jaya, A. 1999. Mercuric chloride induced biochemical changes in the liver of mature male albino rats. *Ind. J. Environ. Toxicol*., 9: 56-58.

Rana S.V.S., Singh R. and Verma S. 1996. Protective effects of few antioxidants on liver function in rats treated with cadmium and mercury. *I. J. Exp. Biol.,* 34: 177.

Reddy, J., Kolarani, B, Tharankandha, B., Reddy, D.C. and Ramamurthi, R. 1998. Changes in energy metabolism of the fish *Labeo rohita* in relation to prolonged lead exposure and recovery. *J. Ecotoxicol. Enviorn. Monit.,* 8 : 45- 53.

Ridgway, R.L., J. C. Tinney, J. T. MacGregor, and N. J. Starler, 1978."Pesticide use in agriculture," Environmental Health Perspectives, 27: 103–112.

Sankar samipillai, S,G.Jagadeesan, S.Ramesh and

P.Arumugam. 2010. Role of Taurine and Glutathine treatement on lipid peroxidation and antioxidant defense in mercury induced toxicity in rats. *Int. J .Hum. Sci. Tech.,* 1:72-81.

Sankar Samipillai, S and G. Jagadeesan. 2009. Protective role of Taurine against mercuric chloride intoxicated rats. *Rec. Res. Sci. Tec.,* 1: 81-87

Saxena, A.K. and Sarin, K. 1980. Pathological and biochemical changes in the liver and testes of the desert gerbil, marines hurrine (Jerdon): Effect of a single intraperitomial injection of phorate. *Ind. J. exp. Biol.,* 18: 1001-1004.

Shalan, M.G., Mostafa, M.S., hassouna, M.M., Hassab, E. Nabi, S.E. and E1-Refaie, A. 2005. Amelioration of lead toxicity on rat liver with vitamin C and silymarin supplements. *Toxicology*., 206: 1-15.

Sharma M.K., Kumar M. and Kumar A. 2002. Ocimum sanctum aqueous leaf extract provides protection against mercury induced toxicity in swiss albino mice. *I. J. Expl. Biol*., 40: 1079- 1082.

Shalan, M.G, M.S. Mostafa, M.M. Hassouna, S.E. Hassab El-Nabi, A. El-Refaie. 2005. Amelioration of lead toxicity on rat liver with Vitamin C and silymarin supplements. Toxicology. 206. 1–15.

Smet, H., M.Rans and A. De loof, 1990. Compartive effectiveness of insect growth regulators with juvenile hormone, antijuvenile hormone and chitin synthesis inhibiting activity against several stored food insect pests, In: Fleualbino mice-Lessard, F., Ducon, P.(Eds.) Proceedings 5th International Working Conference on Sotred Product Protection, France, pp: 649-657.

Smith, E.L., Hill, R.L., Lehman, J.R., Lefkowitz, R.J., Handler, P. and White, A. 1983. Principles of biochemistry. In: Mammalian biochemistry. McGraw. Hill Book. Company. Japan. 317-321.

Syverson, TLM. 1977. Effects of methyl mercury in vivo protein synthesis in isolated cerebellar neurons. *Peuropathol. Appl. Neurobiol*. 3: 225- 236.

Tandon, S.K., Snigh, S., Prasad, S., and Mathur, 1997. Influence of L-lysine and zinc administration during exposure to lead or lead and ethonal in rats. *Biol. Trace elements Res*., 576: 51-58.

Teotia, S.P.S. and Teotia, M. 1991. Endemic fluoride bone and teeth update. *Ind. J. Environ. Toxicol*., 1:1.

Tiwari, A. and Srivastava, V.K. 2001. Acetaminophen poisoning induced enzymological and biochemical changes in liver and kidney of male mice *Mus musculus. Biochem. Cell. Arch*., 1: 71-76.

Yeragi, S.G., Koli, A. and Yeragi,S. 2000. Effect of pesticide malathion on protein metabolism of the marine crab,*Uca marionis. J. Ecotoxicol. Environ Monot.,* 10: 59-62.

****