Beta-Cyfluthrin Induced Histochemical Alterations in the Liver of the Albino Rat

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Summary

Pesticide poisoning and related diseases have gained considerable attention in the recent past. Beta-cyfluthrin is a type II pyrethroid pesticide and is widely used against crop and house hold pests. The present study has been designed to assess histochemical alterations following beta-cyfluthrin intoxication in the liver of the female albino rat (*Rattus norvegicus*). Beta-cyfluthrin was orally administrated, 35.48 mg/kg b.wt. for acute (1 day) and 5.06, 2.53 and 1.68 mg/kg b.wt. for sub-acute (7, 14 and 21 days) treatments respectively. Beta-cyfluthrin caused marked hepatotoxicity in the form of altered hepatosomatic index viz. liver weight, body weight and liver weight-body weight ratio and histochemical localization of DNA and proteins in the liver of albino rat. On the basis of histochemical study, it could be revealed that beta-cyfluthrin causes a reduction in hepatic proteins and an enhancement in the hepatic DNA. The centrilobular zone was the zone of maximum alteration followed by the midzone and periportal zone of the hepatic lobule. Decreased protein content is a consequence of lysis of structural proteins and the need to support increased cell proliferation under stress of beta-cyfluthrin, whereas increased DNA is a consequence of increased mitogenic activity resulting in excessive cell proliferation.. It is proposed that xenobiotic metabolising enzymes, which are most concentrated in the centrilobular zone, cause the formation of metabolites of beta-cyfluthrin, which are toxic to the liver. Lower oxygen levels in the centrilobular zone, compared to other parts of the liver lobule, may have also contributed to the observed histochemical changes being greatest in the centrilobular zone.

Introduction

Pesticides are presently a cynosure both among farmers as well as the toxicological community. The mighty role of pesticides to farmers surely merits appreciation but their widespread use is associated with serious health hazards to various non-target organisms (*Bian et al., 2004; Bhalli et al., 2006; Farrag & Shalby, 2007*).

Regardless of the ill effects, unsafe and indiscriminate pesticidal use is widespread in developing and underdeveloped nations. Owing to poor economic conditions in these countries, agriculture is still the mainstay of the economy, which in turn plays a key role in continuous pesticidal use (*Patil et al., 2003; Jamil et al., 2007; Narendra et al., 2008*). Presently, pyrethroids account for about 25% of the total pesticidal market (*Anand et al., 2006; McCarthy et al. 2006*). Attributes favouring use of pyrethroids include limited soil persistence, photostability and high insect/mammalian ratio *(Atessahin et al.; 2005; Singh et al., 2005)*. Pyrethroids are broadly divided into type I and type II, based on absence and presence of cyano group respectively and behavioural profiles *(Sayim et al., 2005)*. Beta-cyfluthrin is a type II pyrethroid pesticide with a fluorine atom attached to position 4 in its structure and having broad potential against target as well as non-target species (*Cox, 1994; Khambay, 2002; Omotuyi et al., 2006*).

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The present study has been undertaken to assess histochemically qualitative alterations in hepatocytic DNA and proteins under stress of beta-cyfluthrin.

Materials and Methods

Experimentation was conducted on 40 adult female albino rats, *Rattus norvegicus* (Berkenhout). These animals were kept in standard polypropylene cages at a temperature of 25±5^{0}C and a photoperiod of 10 hours/day. Rats were fed on Goldmohar brand rat feed procured from Hindustan Uni Lever Ltd., Mumbai and provided water *ad libitum*. After 10 days of acclimatization these animals were divided into 8 groups, 4 serving as treated and other 4 as control corresponding to each treated set. Experimental compound, beta-cyfluthrin was obtained from Bayer India Ltd., Mumbai and its calculated LD_{50} is 354.8 mg/kg/b.wt (*Singh, 2003*). Albino rats weighing 100±5 gm were administered an acute dose (35.48 mg/kg b.wt.) and sub-acute dose (5.06, 2.53 and 1.68 mg/kg b.wt.) of beta-cyfluthrin by gavage tube for acute (1 day) and sub-acute (7, 14 & 21 days) treatment respectively. Animals were autopsied at predetermined time intervals, quickly dissected and liver tissue was excised out. Liver was soon weighed, sliced and fixed in Carnoy's fixative (*Gatenby and Beams, 1950*). Fixation was followed by dehydration, embedding in wax and sectioning (*Pearse, 1980; Humason, 1979*). Sections of 5 micrometer thickness were taken on clean slides, stained with mercuric bromophenol blue (*Chapman, 1975*) for localization of hepatocytic proteins and Feulgen reaction (*Feulgen & Rossenback, 1924*) was conducted for histochemical localization of DNA in liver. Quantitative aspects viz. body weight, liver weight and liver weight-body weight ratio (hepatosomatic index) were also considered and student's 't' test (*Fisher & Yates, 1950*) was conducted to assess statistical significance.

Results

The investigations have demonstrated histochemically a decrease in hepatic protein following acute (1 day) as well as sub-acute (7, 14 & 21 days) beta-cyfluthrin intoxication, on the contrary a simultaneous increase of hepatic DNA has been observed. The lobule is the functional unit of liver and contains three zones viz. centrilobular, midzonal and periportal zone. Our observations revealed the centrilobular zone to be the most sensitive, followed by midzone & periportal zone, with regard to the effect of beta-cyfluthrin on proteins and DNA (Plate-Ia-Ie; Plate-IIa-IIe).

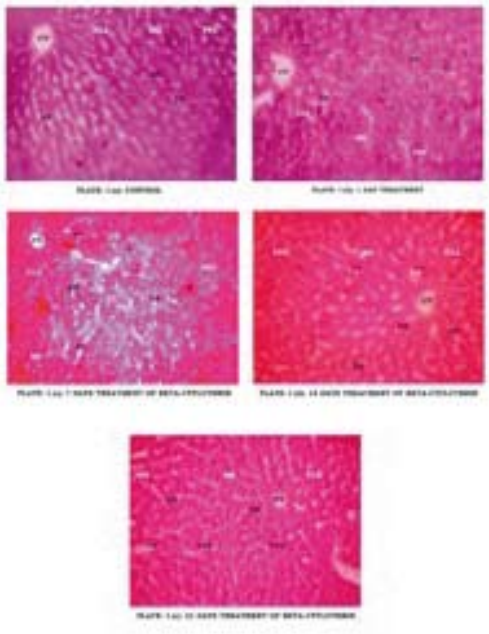

Plate-I(a): Histochemical localization of hepatic DNA in control albino rat (400X)
Plate-I(b): Histochemical localization of hepatic DNA in albino rat after acute (1day) beta-cyfluthrin intoxication (400X)
Plate-I(c): Histochemical localization of hepatic DNA in albino rat after sub-acute (7days) beta-cyfluthrin intoxication (400X)
Plate-I(d): Histochemical localization of hepatic DNA in albino rat after sub-acute (14days) beta-cyfluthrin intoxication (400X)
Plate-I(e): Histochemical localization of hepatic DNA in albino rat after sub-acute (21days) beta-cyfluthrin intoxication (400X)

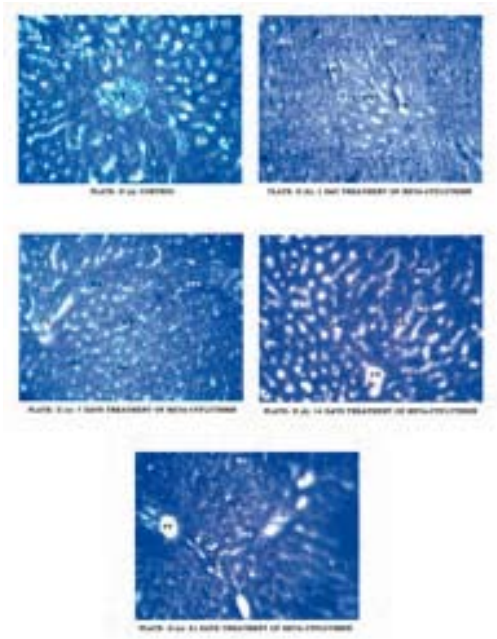

Plate-II(a): Histochemical localization of hepatic proteins in control albino rat (400X)
Plate-II(b): Histochemical localization of hepatic proteins in albino rat after acute (1day) beta-cyfluthrin intoxication (400X)
Plate-II(c): Histochemical localization of hepatic proteins in albino rat after sub-acute (7days) beta-cyfluthrin intoxication (400X)
Plate-II(d): Histochemical localization of hepatic proteins in albino rat after sub-acute (14days) beta-cyfluthrin intoxication (400X)
Plate-II(e): Histochemical localization of hepatic proteins in albino rat after sub-acute (21days) beta-cyfluthrin intoxication (400X)
CV- Central vein, SN- Sinusoids
CLZ- Centrilobular zone, MZ- Mid zone
PPZ- Periportal zone, – : Mild decrease
– – : Moderate decrease, – – – : Severe decrease
+ : Mild increase, ++: Moderate increase
+++: Severe increase

Quantitative aspects viz. body weight, liver weight & liver weight-body weight ratio (hepatosomatic index) were found to increase significantly in treatment groups (Table-1).

Discussion

DNA has a central role due to its unique functions and is present mainly on chromosomes in association with nucleoproteins in the nucleus of mamma-

Table 1. Body weight, liver weight and liver weight-body weight ratio of *Rattus norvegicus* after acute (1 day) and sub-acute (7, 14 and 21 days) beta-cyfluthrin intoxication

Treatment time (in days)	Treatment	Dose (mg/kg b.wt.)	No. of rats	Body weight (gm) Mean±S.E.	Liver weight (gm) Mean±S.E.	Liver wt.-Body wt. ratio Mean±S.E.
1 day	Control	-	5	109.7±2.90	3.17±0.11	0.028±0.0002
	Acute	35.48	5	113.3±3.28*	3.25±0.09*	0.029±0.0003*
7 days	Control	-	5	112.0±1.73	3.28±0.12	0.029±0.0006
	Sub-acute	5.06	5	120.7±2.40**	3.40±0.01*	0.028±0.0004*
14 days	Control	-	5	116.3±1.20	3.38±0.03	0.029±0.0004
	Sub-acute	2.53	5	124.0±2.31**	3.76±0.09***	0.030±0.0007*
21 days	Control	-	5	120.0±2.31	3.46±0.01	0.030±0.0004
	Sub-acute	1.68	5	127.3±0.03***	3.79±0.09****	0.031±0.005**

* P>0.05, ** P<0.05, *** P<0.01, ****P<0.001

lian cells. In addition to being part of chromosomes, proteins play a significant role in structure and metabolism of the cell. The bulk of the proteins within the cell is present in cellular as well as subcellular membranes. Membrane proteins primarily aid to maintain integrity of the cell & cellular organelles, with a secondary function being selective passage of materials through channels & pumps (*Lodish et al., 2001*). The liver is a hub for protein synthesis and also a target organ for xenobiotics due to its detoxifying function and unique relationship to the gastrointestinal tract. Beta-cyfluthrin probably produces its toxicity in a two step manner, initially due to affinity towards certain components of cell membranes causing membrane damage and later by forming more toxic intermediates (*Vandenberghe, 1996*).

Decrease in proteins as observed histochemically in this investigation[Plate II(a)-II(e)] may be a result of damage through both these steps. Initial damage to proteins includes lysis of structural proteins whereas secondary damage may be at genetic level by altering cell cycle leading to proliferation of hepatic cells. (*Sakr et al. 2004; Manna et al., 2004: Ksheerasagar & Kaliwal, 2006; Omotuyi et al. 2006*).

Qualitative enhancement of hepatic DNA has been found histochemically following acute as well as sub acute beta-cyfluthrin intoxication. DNA is the genetic material of the cell and although enhanced DNA is suggestive of increased mitogenic activity, it is noteworthy that beta-cyfluthrin has been reported to have genotoxic effects (*Rana et al.,2008*). Qualitative decrease in hepatic proteins is an indicator of damage to membrane integrity, which in turn gives a clue to the entry of toxicant into the cell.

Within the cell, both toxicant as well as its metabolic intermediates can cause a variety of damage including the release of various hydrolytic enzymes (e.g. nucleases and proteases). Cells with damage to these enzymes are very prone to chromosomal damage and altered cell cycle (Singh & Saxena, 2002). Beta-cyfluthrin might have caused excessive DNA synthesis in the present study due to altered mitogenic activity (*Kolaja et al. 1996; Kostka et al. 1996*). Significant increase in liver weight and liver weight-body weight ratio is also an indicator of hepatocellular proliferation under stress of beta-cyfluthrin and may have increased due to excessive cell division (*Shakoori et al. 1988; Gupta & Kumar, 1991; Singh et al., 2005*). Body weight has been found to increase which may be due to altered CNS functions as evident in the form of excessive food intake in treated sets (*Ahmed et al., 1989; Sharma, 1997; Kumar, 2001; Singh, 2003*).

Within the three zones of a hepatic lobule, the presence of both DNA & proteins has been found to be altered greatly in the centilobular zone but less so in the midzone and periportal zone. This may be due to the fact that when blood flows from portal triad towards the central vein, oxygen content goes on decreasing towards the successive zones and also most of the xenobiotic metabolising enzymes are concentrated in the centrilobular and midzone. There must be thus the formation of toxic intermediates during processing in these zones, which is responsible for pronouncing the effect in these regions (*Anadon et al., 1995; Hinton, 2000*).

The above facts strongly suggest that beta-cyfluthrin has the potential to disrupt normal hepatic functions in mammals and may lead to more deleterious consequences. These facts thus advocate the necessity of proper precautions to be taken during use of this pesticide.

References

Ahmed, N., P.K Gupta. and K.C. George. Sub acute toxicity of cypermethrin in rats. J. Environ. Biol. 1989, *10*(3), 309-317.

Anadon, A., M.R. Martinez – Larranaga and M.L. Fernandez – Cruz. Effect of flumethrin on hepatic drug metabolizing enzymes and antipyrine disposition in rats. Toxicol. Appl. Pharmacol. 1995, *141*, 8-16.

Anand S.S., K. Kyu-Bon., S.Padilla, S. Muralidhara, H.J. Kim, J.W. Fisher and J.V. Bruckner. Ontogeny of hepatic and plasma metabolism of deltamethrin in vitro: role in age dependent acute neurotoxicity. Drug Metab. Dispos. 2006,

34(3), 389-397.

Atessahin A., S. Yilma., I. Karahan, I. Pirincc and B. Tasdemir. The effects of vitamin E and selenium on cypermethrin induced oxidative stress in rats. Turk. J. Vet. Anim. Sci., 2005, *29*, 385-391.

Bhalli J.A., Q.M. Khan, M.A. Haq, A.M. Khalid and A. Nasim. Cytogenic analysis of Pakistani individuals occupationally exposed to pesticides in a pesticide production industry. Mutagenesis. 2006, *21*(4), 143-148.

Bian Q., L.C. Xu, S.L.Wang, Y.K. Xia, L.F.Tan, J.F.Chen, L.Song, H.C. Chang and X.R. Wang. Study on the relation between occupational fenvalerate exposure and spermatozoa DNA damage of pesticide factory workers. Occup. Environ. Med., 2004, *61*, 999-1005.

Chapman, D.M. Dichromatism of bromophenol blue, with an improvement in the mercuric bromophenol blue technique for pòntein. Stain Technology. 1975, *50*, 25-30.

Cox, C. Insecticide fact sheet cyfluthrin. Pestic. Reform. 1994, *14* (2), 28-34.

Farrag A.R.H., S.E.M. Shalby. Comparative histopathological and Hisônchemical studies of IGR, lufenuron and pònfenofos insecticide in albino rats. J. Appl. Sci. Res. 2007, *3*(5), 377-386.

Feulgen, R. and H. Rossenback. Mikroskoisch. Chemischer Nachweis einer Nukle in same von Typus Thymusnucleinsame and die darant peruhende elective forbung von zellkernen in mikroskopischen paraparaten. Zeitschsift fur. Physiologische chemic. 1924, *135*, 203-248.

Fisher, R.A. and F. Yates. Statistical tables for biological agriculture and medical research, (VIth ed.) Hing Yip Printing Co. Hong Kong, 1950, *pp 146*.

Gatenby J.B., Beams H.M. The microtomist's vademecum, London. J. and A. Churchill. 1950

Gupta, P.K. and S.Kumar. Cumulative toxicity of deltamethrin in mice. J. Environ. Biol. 1991, *12*(1), 45-50.

Hinton R.H. and P. Grasoo. Hepatotoxicity. In General and Applied Toxicology (eds. Ballantyne B., Marrs T., Syversen T.) Grove's Dictionaries Inc., New York, USA. 2000, *2*. pp 853-892

Humason, G.L. Animal tissue techniques IVth ed. W.H. Freeman and company, San Francisco U.S.A. 1979

Jamil K., G.P. Das, A.P. Shaik, S.S .Dharmi and S. Murthy. Epidemiological studies of pesticide exposed individuals and their clinical implications. Current Science, 2007, *92*(3), 340-344.

Khambay B.P.S. Pyrethroid insecticides. Pesticide Outlook. 2002, 49-54.

Kolaja K.L., D.E. Stevenson, J.T. Johnson, E.F. Walborg and J.E. Klaunig. Subchronic effects of dieldrin and Phenobarbital on hepatic DNA synthesis in mice and rats. Fundam. Appl. Toxicol., 1996, *29*, 219-228.

Kostka G., J. Kopec–Szlezak and D. Palut. Early hepatic changes induced in rats by two hepatocarcinogenic organohalogen pesticides: bromopropylate and DDT. Carcinogenesis. 1996, *17*(3), 407-412.

Ksheerasagar R.L. and B.B. Kaliwal. Histological and biochemical changes in the liver of albino mice on exposure to insecticide, carbosulfan. Caspian. J. Env. Sci. 2006, *4*(1), 67-70.

Kumar, R. Biochemical alteration in rat brain following type II pyrethroid intoxication. M. Phil. Dissertation. Dr. B.R. Ambedkar University, Agra. 2001.

Lodish H., A. Berk, S.L. Zipursky, P. Matsudaira, D. Baltimore and J. Darnell. Nucleic acids, the genetic code and the synthesis of macromolecules. In Molecular Cell Biology (IVth Ed.) W.H. Freeman and Company, New York. 2001, pp 101-139.

Manna, S., D. Bhattacharyya, T.K. Mandal and S. Das. Repeated dose toxicity of alfa-cypermethrin in rats. J. Vet. Sci. 2004, *5* (3), 241-245.

Mc Carthy A.R., B.M. Thomson, I.C. Shaw and A.D. Abdel.l Estrogenecity of pyrethroid insecticide metabolites. J. Environ. Monit. 2006, *197*(8), 197-202.

Narendra M. G. Kavitha, P. Padmavathi, A.H. Kiranmai and N.C. Varadacharyulu. Allethrin

induced biochemical changes and properties of human erythrocyte membrane. Afr. J. Biochem. Res. 2008, *2*(1), 24-29.

Omotuyi I.O., K.A. Oluyemi, C.O. Omofoma, S.J. Josiah, O.A. Adesany and L.C. Saalu. Cyfluthrin induced hepatotoxicity in rats. Afr. J. Biotechnol., 2006, *5*(20), 1909-1912.

Patil J.A., A.J. Patil and S.P. Govindwar. Biochemical effects of various pesticides on sprayers of grape gardens. Ind. J. Clin. Biochem. 2003, *18*(2), 16-22.

Pearse, A.G.E. Histochemistry: Theoretical and applied. Vol I, IVth ed. Chuschill livingstone, London. 1980.

Rana,N., *N. Saxena, H.N. Sharma and P.N. Saxena*. Comparative genotoxicity of alphacyano pyrethroids in *Drosophila melanogaster.* Entamon. 2008, *33*(2), 135-138.

Sakr S.A, H.A. Abdel Samei and M.E. Soliman. Exploring hepatotoxicity of Benomyl. Histological and histochemical study of albino rats. J. Med. Sci. 2004, *4*(1), 77-83.

Sayim F., N.U.K. Yavasoglu, Y. Uyanikgil, H. Aktug, A. Yavasoglu and M. Turgut. Neurotoxic effects of cypermethrin in wistar rats: a haematological, biochemical and histopathological study. J. Hlth. Sci. 2005, *51*(3), 300-307.

Shakoori, A., S.S. Al and M.A. Salee. Effects of cypermethrin on the blood and liver of albino rats. J. Biochem. Toxicol. 1988, *3*, 59-71.

Sharma, D.C. Brain biochemical changes of albino rat after hafen –20 EC intoxication. M. Phil. Dissertation. Dr. B.R. Ambedkar University, Agra. 1997.

Singh V.K., P. Dixit and P.N. Saxena. Cybil induced hepatobiochemical changes in wistar rats. J. Environ Biol. 2005, *26*(4), 725-727.

Singh V.K. and P.N. Saxena. Genotoxic potential of cypermethrin in mammalian haemopoietic system. Him J. Env. Zool. 2002, *16*(2), 195-202.

Singh, A.K. Stress of Beta Cyfluthrin on Brain lipids of Rattus norvegicus. M. Phil. Dissertation Dr. B. R. Ambedkar University, Agra. 2003.

Vandenberghe J. Hepatotoxicity. In Toxicology Principles and Applications (eds. Niesink R.J.M., Vries J., Hollinga M.A.), CRC New York. 1996, pp 668-723