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HISTOPATHOLOGICAL CHANGES OF LIVER UNDER ACUTE TOXIC EFFECT OF CHLORPYRIFOS IN ALBINO RATS

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ABSTRACT

Histology, is the study of micro anatomy of specific tissues, has been successfully employed as a diagnostic tool in medical and veterinary science. The present study was aimed to examine the histopathological changes in the liver of albino rats under Transmission electron microscopy (TEM) with oral sub-lethal (20 mg/kg) administration of organo phosphate compound chlorpyrifos as single, double and multiple doses with 48 hr intervals. Structural changes were observed in liver tissues of albino rats. Chlorpyrifos-induced histopathological changes suggest that the structural integrity of certain organ systems can be disrupted to a great extent from chlorpyrifos exposure.

KEY WORDS: Chlorpyrifos, Liver, Histopathology, Albino rat.

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INTRODUCTION

Pesticides enter in to the body via an internal digestive system after oral administration. They are not subjected initially either to the detoxifying reactions of the liver or to excrete via the biliary system. Compounds transported by oral feeding in effect can be distributed to all parts of the body in their unmetabolised form [1]. The examination and study of normal cells and tissues by microscopy is called histology or microscopic anatomy. The study of abnormal cells and tissues is histopathology[2]. Toxicological histopathology gives useful data concerning the changes induced by chemicals at the tissue and cellular level. All the tissues and organs in the body of an animal may be potential targets for the toxic effects of any chemical or metal. The architectural dynamics of a tissue are very essential for maintaining the tissue integrity and for effective physiological, biochemical and metabolic functions. The cellular and sub-cellular constituents of tissue in terms of size, shape, number and position play an important role in the physiological and metabolic functions. Therefore, the histological structure of tissue in an animal has a profound influence on its function. Histology, the study of the microanatomy of specific tissues, has been successfully employed as a diagnostic tool in medical and veterinary sciences since the first cellular investigations carried out in the nineteenth century[3]. The knowledge of the histology is useful to distinguish normal cells from abnormal or diseased ones, which helps in diagnosis of many diseases[4]. Physiological studies do not satisfy in complete understanding the impact of any deleterious chemical. The toxicity of any chemical necessarily impairs the metabolic strategy of animal physiology. To have a clear understanding, as to how these chemicals cause injury to the tissues, it is essential to have an insight into histopathological analysis of the tissues, wherein, one can envisage a better understanding of the pathological conditions of tissues under toxic stress of pestilent. Thus, histopathology helps in diagnosing the damages of the tissues of an animal subjected to toxic stress of pestilence[5]. Several workers reported that the pesticides cause damage at cell organelles[6,7]. Electron microscopy provides a static morphological assessment of cells, its ability to characterize changes in sub cellular organelles can provide valuable information about any functional deficits. Electron microscope is scientific instrument that use a beam of highly energetic electrons to examine objects on a very fine scale. Inspite of the apparent similarities there are great differences between the Light and the Electron Microscopy. In case of EM, molecules or supramolecular structures are now possible to obtain more detailed information. The electron microscope (EM) permits a direct study of biological ultra structure. Its resolving power is much greater than that of the light microscope [8, 9]. In view of this the present investigation has been made an attempt to study the possible changes at cell organelles under chlorpyrifos intoxication in albino rats under transmission electron microscopy (TEM).

MATERIALS AND METHODS

Test Chemical

Chlorpyrifos Technical (95.30%) was obtained from Nagarjuna Agri Chem Limited, Ravulapalem Mandal, East Godavari District, A.P., India.

Animals

Healthy adult albino rats of the same age group (100±10 days) and weight (200±10g) were obtained from the Indian Institute of Science (IISc) Bangalore, India, and maintained at 25±2ºC, with 12 hr light, 12 hr dark cycles, food and water *ad libitum*.

Experimental Design

Toxicity of chlorpyrifos was evaluated by Probit method of Finney[10] and the LD_{50} for albino rats was found to be 200 mg/kg bw. One-tenth of the LD_{50} value (20 mg/kg bw) was selected as sublethal dose. The animals were divided into four groups having ten animals each, first group

of animals was considered as control.The second, third and fourth groups of animals were termed as experimental animals. To the animals of second group single dose of pesticide (i.e. on 1st day) was administered orally by gavage method. To the third group of animals' double doses was given i.e. on 1st and 3rd day. Similarly multiple doses i.e., 1st, 3rd, 5th and 7th day was given to the fourth group of animals.

Transmission Electron Microscopy

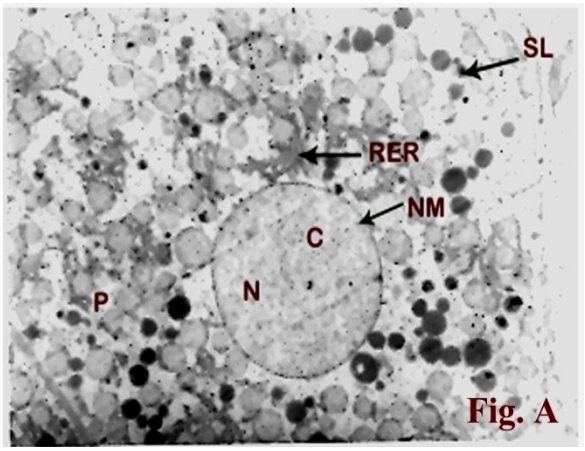
Tissue samples were isolated from freshly sacrificed control and experimental rats. The tissues were gently rinsed in physiological solution (0.9% NaCl) to remove blood and debris adhering to the tissue. Tissues were transferred to vials and fixed in 2.5% gluteraldehyde in 0.05M phosphate buffer (pH 7.2) for 24 hr at 4°C and post fixed 0.5% aqueous osmium tetroxide in the same buffer for 2 hr. After the post fixation samples were dehydrated in a series of graded alcohol, infiltrated and embedded in Araldite 6005 resin. Both Semi thin and Ultra thin sections were cut with a glass knife on a Leica Ultra cut UCT-GA-D/E-1/00 ultra microtome, Semi thin of 200-300nm thick were stained with toludine blue and ultra thin sections (50-70 nm thickness) were mounted on grids. Then the Ultra thin sections were stained with saturated aqueous uranyl acetate and counter stained with 4% lead citrate. Observed at various magnifications[8] and electron micrographic photographs taken under Hitachi H-7500 model (from JAPAN) transmission electron microscope at Ruska Lab, College of Veterinary Sciences, Rajendra Nagar, Hyderabad, A.P.

RESULTS

The cells of the liver are called hepatocytes. They are large sized and polygonal cells. Hepatocytes have prominent nuclei uniformly distributed chromatin and centrally placed, uniform distribution of cell organelles, rough endoplasmic reticulum, smooth endoplasmic reticulum, many mitochondria, lysosomes, rich in peroxisomes, rich in secretary vesicles and secondary lysosomes were seen in control liver tissues of albino rat (Figs. A & B).

Ultra structural changes of rat liver hepatocytes under chlorpyrifos intoxication

Ultra structural changes in hepatocytes of double and multiple dose administered liver tissues of the chlorpyrifos showed paler nucleus with dense condensation of chromatin centrally very little condensed chromatin is attached to periphery of nucleus, nucleus at the periphery site of the cell, condensation of cell organelles towards the nucleus, loss of cytoplasmic organelles, cell organelles absent at the periphery site of cell, lucent areas of cytoplasm containing the residues of cell organelles and lipid vacuoles, total cell size decrease, ruptured mitochondria, disappearance of secretory vesicles, decrease in the number of mitochondria, increase in the number of lipid vacuoles, increase in the number and rupture of peroxisomes, proliferation of endoplasmic reticulum and nick of plasma membrane. Mitochondria were usually swollen, showed a clearance of the matrix and destruction of cristae (Figs. C & D).



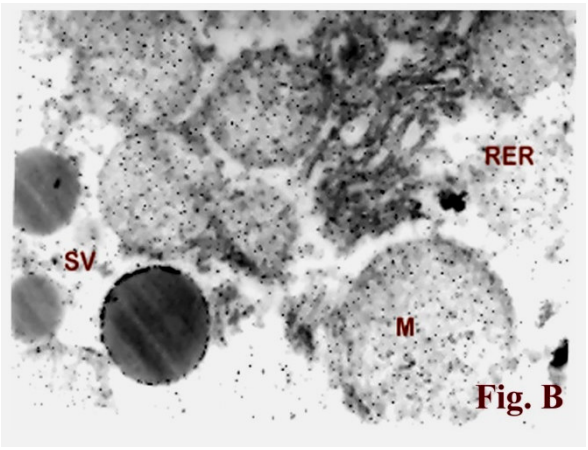
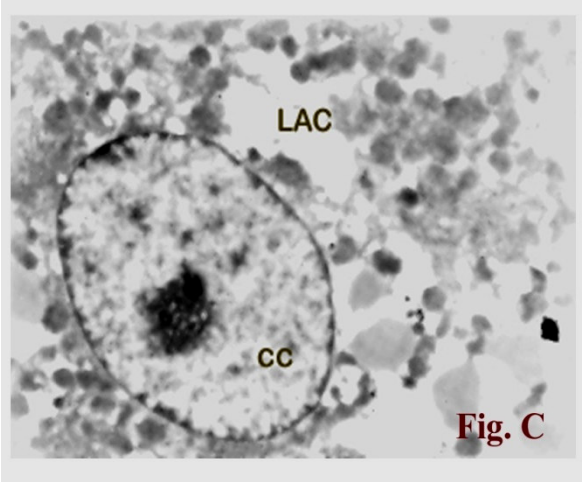


Figure A

Electron micrograph of control rat liver showing prominent nucleus (N), clear chromatin (C), nuclear membrane (NM), rough endoplasmic reticulum (RER), secondary lysosomes (SL), peroxysomes (P) and secretory vesicles (SV) (Stain: Uranyl acetate and lead citrate). 2Kx

Figure B

Electron micrograph of control rat liver cell showing rough endoplasmic reticulum (RER), more number of mitochondria (M) and secretory vesicle (SV) (Stain: Uranyl acetate and lead citrate). 10Kx



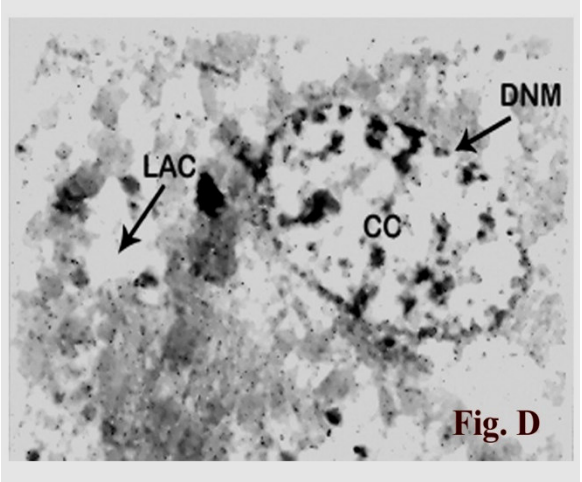


Figure C

Electron micrograph of double dose chlorpyrifos exposed rat liver cell showing dense condensation of chromatin (CC), ruptured peroxisomes (RP), lucent area of cytoplasm (LAC) consist of residual organelles and lipid vacuoles (LV) (Stain: Uranyl acetate and lead citrate). 4Kx

Figure D

Electron micrograph of multiple dose chlorpyrifos exposed rat liver cell showing lucent area of cytoplasm (LAC) consists of residual organelles and vacuoles (V), degenerative changes in nuclear membrane (Stain: Uranyl acetate and lead citrate). 6Kx

DISCUSSION

The electron microscopic observation of liver under double and multiple doses of chlorpyrifos administration showed pronounced pathological changes in cell organelles. Dense chromatin condensation and fragments of rough endoplasmic reticulumn increase in the number of autophagous vacuoles observed in the present investigation. This may suggest an intensification of the processes of intracellular digestion. Changes in mitochondria were connected with disturbances in oxido-reduction processes taking place in the organelle. Increase in the number of peroxisomes may be associated with the cellular response to the toxic effect of free radicals induced by chlorpyrifos, as peroxisomes contain enzymes which inactive these radicals. Focal degeneration of the cytoplasm was also observed in the present investigation in tubular cells, manifested by the presence of autophagous vacuoles. Changes of this type are irreversible and are undoubtedly associated with the destruction of the protein-lipid structure of intracellular membranes and lysis of cytoplasm. According to the authors, this is a manifestation of the toxic effect on cells, because the integrity of protein-lipid membranes ensures the normal functioning of the cells[11]. In studies *in vitro* concerning the effect of organophosphorus pesticides, on protein-lipid membranes in mammals it was shown that these compounds change physical and chemical properties of the membranes. Due to their hydrophobic nature and small molecular size, chlorpyrifos passes through the cell membrane and reaches the nucleus. It is suggested that within the nucleus chlorpyrifos binds to DNA through the reactive groups of its acid moiety, leading to destabilization as well as unwinding of the DNA, which could be possible for its genotoxicity. The similar results are reported for acute toxicity of fenthion on liver and kidney functions, which indicated that exposed these pesticide lead to induce physiological and biochemical disturbances in experimental animals[12]. Animals administered with the combination of endosulfan and cypermethrin (Ratio 1:1) cause hepatic and medullary congestion, leading to mild pathological change in liver and kidney tissues[13]. Different other studies, supporting the results of this present study, showed that malathion and other pesticides induced histopathological alteration in liver and kidney of the experimental animals[14, 15]. The most consistent changes were seen in the liver of animals, of all treatment groups, were varying degrees of degenerative changes and vascular changes were observed in cypermethrin toxicity in rats[16]. Diazinon induced histopathological alterations in the liver of rabbit showed congestion of veins, leucocytic infiltrations, cytoplasmic vacuolation of the hepatocytes and fatty degeneration [17]. Tos-Luty *et al.*[18] reported decrease in amounts of the rough endoplasmic reticulum, as well as an increase in the amounts of the smooth endoplasmic reticulum in the cytoplasm of hepatocytes in the liver of rats exposed to carbaryl. Tos-Luty *et al.*[19] observed widening of ergastopasma tubules and slightly swollen mitochondria in hepatocytes of rat liver dermal exposed to malathion.

CONCLUSION

The pathological changes observed in the present investigation clearly indicate that chlorpyrifos not only caused damaged at cellular level of these organs but also caused damage at sub cellular level. All these changes were more pronounced in multiple dose chlorpyrifos administered rats clearly indicates that the frequent exposure of non-target organisms including human beings to pesticides may result vulnerability and eventual death. In nature's conservationist point of view, pesticides should not affect the non-target life adversely but should degrade in the ecosystem at a faster rate, otherwise problems like biomagnifications and cumulative effects will arise.

REFERENCES

1. TurnerJ.C and V. Shanks, Absorption of some organochlorine compounds by rat small intestine in vivo, *Bulletin of Environmental Contamination and Toxicology,* 24: 625 (1980).
2. Aughey E and Frye F.L, Comparative veterinary histology with clinical correlates. Manson Publishing Ltd., London, New York, UK (2001).
3. Virchow R, Die cellular pathologic in Hrer Begriundung and Physiologische gewelehre, Berlin: A. Hireshwald (1858).
4. Majumdar N.N, In: *A* Textbook of Histology*,* I edition, Vikas Pub. House Pvt. Ltd., U.P., India. pp. 252-253, (1980).
5. Jayantha Rao K, Histopathology as a diagnostic tool in evaluation of toxicity, Manual of Environ. Toxicol. Selected Lectures and Methods, pp. 35-40, (1984).
6. Tos-Luty S, Przebirowska D, Latuszynska J, Tokarska-Rodak M and Haratym-Maj A, Dermal and oral toxicity of malathion in rats. Ann Agric Environ Medicine*,* 10:101-106, (2003).
7. Ravi Sekhar P, Savithri Y and Jayantha Rao K, Synergistic Effect of Cypermethrin and Sodium Fluoride on Liver tissues of Albino Mice. Drug Invention Today, 4(7): 401-405, (2012).
8. Bozzola John J and Russell D Lonnie, In: Electron Microscopy principles and Techniques for Biologists 2nd Edition, Jones and Bartlett publishers, Sudbury, Massachusetts, pp. 19-45 and 72-144, (1999).
9. Aughey E and Frye F.L, Comparative veterinary histology with clinical correlates. Manson Publishing Ltd., London, New York, UK (2001).
10. Finney D.J, Probit analysis, III Edition, Cambridge Univ. press, London, p.20, (1971).
11. Videira RA., Antunes-Madeira MC, Lopes VI and Madeira VM, Changes induced by malathion, methyl parathion and parathion on membrane lipid physicochemical properties correlate with their toxicity. Biochim Biophys Acta*.,* 1511:360-368, (2001).
12. Kerem M, Bedirli N, Gurbuz N, Ekinci O, Bedirli A, Effects of acute fenthion toxicity on liver and kidney function and histology in rats, Turk J Med Sci. 37:281–8 (2007).
13. Jaya Raj, Mohineesh, Ruma Ray, Dogra T. D and Anupuma Raina, Acute Oral Toxicity and Histopathological Study of Combination of Endosulfan and Cypermethrin in Wistar Rats. Toxicol Int. Jan-Apr; 20(1): 61–67, (2013).
14. Saadi L, Lebaili N, Benyoussi M. Exploration of cytotoxic effect of malathion on some rat organs structure. Commun Agric Applied Biol Sci. 73:875–81, (2008).
15. Afshar S, Farshid AA, Heidari R, Ilkhanipour M. Histopathological changes in the liver and kidney tissues of Wistar albino rat exposed to fenitrothion. Toxicol Ind Health, 24:581–586, (2008).
16. Grewal KK, Sandhu GS, Kaur RR, Brar RS, Sandhu HS, Toxic impact of cypermethrin on behavior and histology of certain tissues of albino rats. Toxicol Int. 17:94–98 (2010).
17. O.M.M.Sarhan and Z.Y. Al-Sahhaf, Histological and Biochemical Effects of Diazinon on Liver and Kidney of Rabbits: Life Science Journal, 8 (4)-1183-1189, (2011).
18. Tos-Luty S, Przebirowska D, Latuszynska J and Tokarska-Rodak M, Histological and ultra structural studies of rats exposed to carbaryl. Ann Agric Environ Medicine*,* 8:137-144, (2001).
19. Tos-Luty S, Przebirowska D, Latuszynska J and Tokarska-Rodak M and Haratym-Maj A, Dermal and oral toxicity of malathion in rats. Ann Agric Environ Medicine*,* 10:101-106, (2003).