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HISTOPATHOLOGICAL CHANGES IN LIVER OF *HETEROPNEUSTES FOSSILIS* EXPOSED TO PENTACHLOROROPHENOL (PCP)

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ABSTRACT

Endocrine disrupting chemicals (EDCs) are the substances which change the course of endocrine systems in a way that adversely affects the organism itself or its progeny. EDCs call for greater attention because of their increasing utility in daily products and possible correlation with compromised health.It has been reported that PCP is the most important degradation products of phenolic compounds due of its enhanced resistance towards biodegradation.

Effect of Pentachlorophenol (PCP) on the histology of Liver of a fresh water Catfish Heteropneustes fossilis was studied by exposing the fish to 32µg/l/day (1/10 of LC_{50}) of sub lethal concentration of PCP for a period of 14 and 28 days.

Histopathological changes observed in liver was vacuolization, necrosis, rupturing of hepatocytes during different time of exposure i.e., 14^{th} and 28^{th} days.

In our present study PCP (32µg/l/day) showed an adverse impact on liver of fresh water Catfish H. fossilis.

Key words: *Liver, Pentachlorophenol, Histopathological, Heteropneustes fossilis.*

INTRODUCTION

The use of pesticides rises exponentially with the industrial development and agricultural growth. Side by side these pesticides create serious threat to the non-target organisms both in terrestrial as well aquatic ecosystems. Hazardous chemicals from industrial waste water and agricultural runoff are the main cause of water pollution. An aquatic organism mainly fishes accumulate many contaminants and toxicants directly through their gills and skin and indirectly via their food chain, which may causes diverse alternations in their vital organs.

Histopathology showed to be a suitable biomarker in the evaluation of the health of organism exposed to pollutants and can be used as biomonitoring tools for toxicity studies (Meyers and Hendricks, 1985). One of the great advantages of using histopathological biomarkers in environmental monitoring is that this study allows examining specific target organs, including gills, kidney and liver, that are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish and other animals (Dubey et. al., 2017; Dubey and Shah 2017; Gernhofer et al., 2001). Pentachlorophenol (PCP) is used globally in the production of plastics, pesticides, wood preservatives, herbicides and is present in sewage effluents around the world (Bennie, 1999, Talmage, 1994). It has been reported that PCP is the most important degradation products of phenolic compounds because of its enhanced resistance towards biodegradation, toxicity, ability to bio accumulate in aquatic organisms, and estrogenicity (Ahelet al.,1994).The frequently occurring persistent environmental pollutant pentachlorophenol (PCP) has been prposed to be carcinogenic (WHO 2003). However, literature on toxic mechanisms of action of PCP at the cellular level is scarce. Additionally, it has been shown that several

environmental chemicals, such as lindane, pentachloronitrobenzene, and pentachlorobenzene, can be metabolized to PCP in animals and plants (Koss and Koransky 1978; Van ommen et al. 1985; Renner and Mücke 1986; WHO 2003).

The objective of the present study was to investigate toxicity effects of PCP on the liver of the catfish (*Heteropneustes fossilis*). Histological changes were monitored through the short and long term exposure of 14 and 28 days respectively.

MATERIALS AND METHODS

Pentachlorophenol (Crystalline, 99% pure,Acros organics Geel, Belgium), was dissolved in ethanol and then diluted with water. To estimate $LC_{50}$for PCP, the protocol of Secretria Estatual Do Meio Ambient (SEMA;1988) was followed. Acclimatized cat fish (30-40g) were divided in to 14 groups of 15 fish each and kept in 10 L aquaria. each group was exposed to the following nominal PCP concentration 0,5,10,20,30,40,50,60,70,80,100,200,400 and 800µg/L ,PCP was added to the water. The fish were maintained for 96 hrs. Mortality and abnormal behavioral responses were recorded every 12 hrs during 96 hr. Mortality of fish was recorded for each of the concentration during the 96 hrs exposure.

Mature male catfish *Heteropneustes fossilis* (35-45g) were purchased from local fish market in the spawning phase (June last week) of the annual reproductive cycle (Senthilkumaran and joy, 1994). They were maintained in the laboratory under normal photo period (13.0L:11.0D) and temperature (25 ± 2^{0}c) until use for experiments. The fish were fed egg white daily ad libitum. The adapted adult fish classified into two groups (12 fish each group), first group control, second group PCP treated (for 14 days and 28 days with 32µg/l day). The experiments were performed in accordance with guidelines for experimentation in animals and all care was taken to prevent cruelty of any kind.

Small pieces of the liver was taken and fixed in Bouin's fluid to be embedded in paraffin wax and sectioned at 7µ in thickness. They were stained with Ehrlisch hematoxylin and eosin stain (H & E) according to Bancroft and Steven, (1982) and mounted in DPX, to visualize the section using Lac Zene microscope model HL-23(Lac Zene Biosciences, India).

RESULTS AND DISCUSSION

The liver shows continuous mass of hepatic cells and cord like pattern of hepatocytes interrupted by blood vessels and sinusoids. The hepatocytes are large in size, polygonal in shape with spherical centrally located nuclei and have homogenous eosinophilic cytoplasm. The sinusoids are seen as communicating channels occupied by blood cells.

After 14 days of exposure the hepatocytes became irregular and after 28 days some cells exhibited expanded contour and loosen their polygonal shape. There were many regions in the liver of experimental fish, where cells were highly vacuolated. Many cells have exhibited necrosis. Liver also showed degeneration and disintegration in most cytoplasmic content.

Endocrine disrupting chemicals (EDCs) are the substances which change the course of endocrine systems in a way that adversely affects the organism itself or its progeny (Marcoccia et al 2017). These chemicals can be found in a variety of everyday products and goods, such as in foods, water, plastics, shampoos, clothes, toothpastes, soaps, fertilizers, paper, textiles, carpets, utensils, bedding, toy, cosmetics, deodorant, etc. (Vilela et al 2014, Zhu et al 2016). EDCs call for greater attention because of their increasing utility in daily products and possible correlation with compromised health. The endocrine system maintains homeostasis of the bodily systems through hormones that can travel long distances in the body and often have amplified effects. Differences have also been reported in urban versus rural areas showing a statistical correlation between poor semen quality and higher levels of EDCs found in pesticides,

such as alachlor, diazinon, atrazine, metolachlor, and 2,4-dichlorophenoxyacetic acid (Fisher JS 2004). Such evidence linking the increasing prevalence of EDCs to declining semen quality and male reproductive health calls attention to the detrimental effects of EDCs.It has been reported that PCP is the most important degradation products of phenolic compounds because of its enhanced resistance towards biodegradation (Ahel et al.1994). The frequently occurring persistent environmental pollutant pentachlorophenol (PCP) has been proposed to be carcinogenic (WHO 2003). However, literature on toxic mechanisms of action of PCP at the cellular level is scarce. In addition to the health risks caused by PCP itself, polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), which are the impurities of commercial Na-PCP, released into the environment (Bao et al., 1995; Zheng et al., 2008). The problems associated with Pentachlorophenol (PCP) exposure indicate the adverse effects of PCP include immunotoxicity, carcinogenicity, oxidative stress and metabolic disorders (Yin et al.,2006; Fang et al., 2010; Pietsch et al., 2014; Chen et al., 2015).Other chlorophenol studies revealed damaging effect on the liver and gonads of fish tissues (Christtiansen et al.1998; Jobling et al.1996; Lech et al. 1996) and the corresponding metabolism.Pentachlorophenol was found to accumulate in the liver, gill, gut, fat, and kidney tissue (Ahel et al. 1993). In our present study PCP 32µg/l/ day showed adverse impact on liver of Indian catfish *H.fossilis*, however, effect on liver enzymes will be studied further.

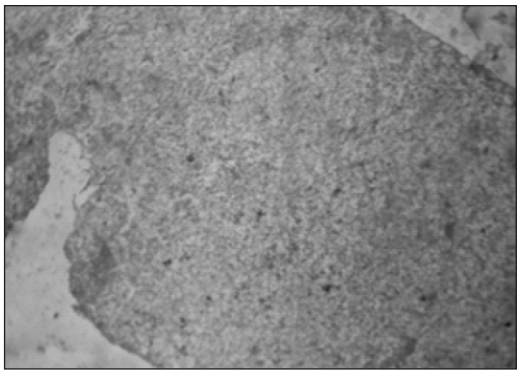

Fig A: Liver showing normal hepatocytes with visible nuclei in control groups (10X)

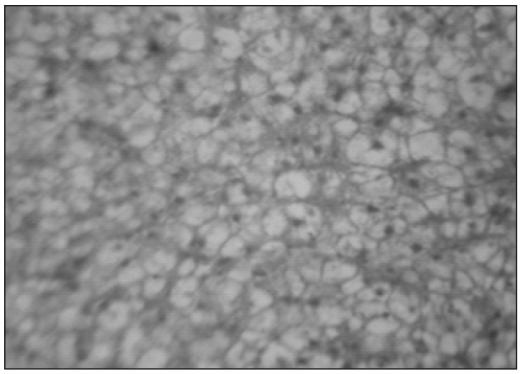

Fig B: Liver showing clearly visible Polygonal hepatocytes with central spherical nucleus. (40X)

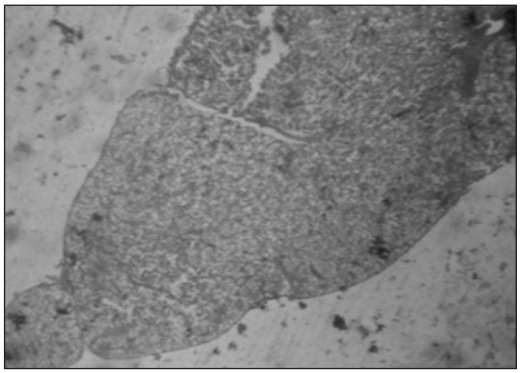

Fig C: Liver showing rupturing of hepatocytes and vacuolation of cytoplasm in 14 days PCP treated groups. (10X)

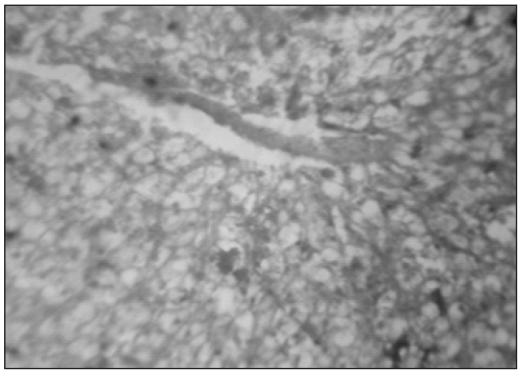

Fig D: Liver showing irregular hepatocytes with vacuolation in 14 days PCP treated groups (40X).

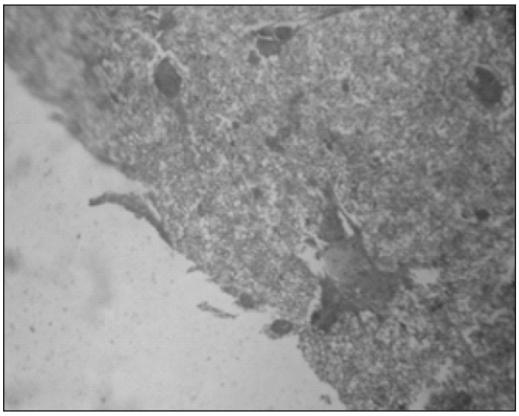

Fig E: Liver showing intensive vacuolation of cytoplasm and disintegration of hepatocytes in 28 days PCP treated groups. (10X) groups (40X).

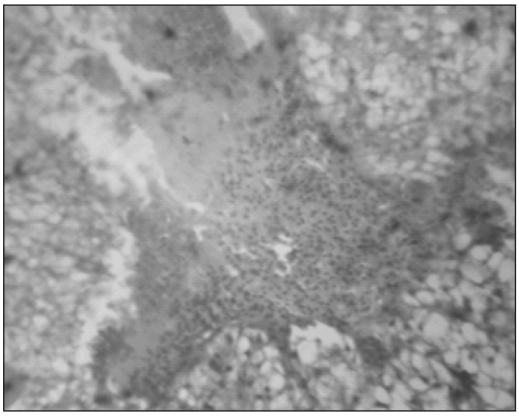

Fig F: Liver showing more irregular hepatocytes, loosing their polygonal shape, more vacuolation of hepatocytes and necrosis in 28 days PCP treated

REFERENCES

Ahel, M., Giger, W., Koch, M. 1994: Behaviour of alkylphenolpolyethoxylate surfactants in the aquatic environment - I. Occurrence and transformation in sewage treatment. *Water Res.* 28, 1131–1142.

Ahel, M., McEvoy, J. and Giger, W. 1993: Bioaccumulation of the lipophilic metabolites of nonionic surfactants in freshwater organisms. *Environ Pollut* 79: 243-248.

Bancroft, J. & Stevens, A. 1982. Theory and Practice of Histological Techniques, 2nd Ed. Churchill-Livingston, NY, pp 131-135.

Bao, Z.C., Wang, K.O., Kang, J.X. and Zhao, L.W. 1995: Analysis of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans in pentachlorophenol and sodiumpentachlorophenol. *Environ Chem;* 14:317–21 (in Chinese).

Bennie, D. T. 1999: Review of the environmental occurrence of alkylphenols and alkylphenolethoxylates. Water Qual. Res. *J. Canada* 34, 79–122.

Chen, H.M., Lee Y.H. and Wang, Y.J. 2015: Ros-triggered signaling pathways involved in the cytotoxicity and tumor promotion effects of pentachlorophenol and tetrachlorohydroquinone. *Chem. Res. Toxicol.* 28 (3), 339e350.

Christiansen, T., Korsgaard, B. and Jespersen, Å. 1998: Effects of nonylphenol and 17β-oestradiol on vitellogenin synthesis, testicular structure and cytology in male eelpout Zoarcesviviparus. *J. Exp. Biol.* 201, 179-192

Fang, Y., Gao, X., Zha, J., Ning, B., Li, X., Gao, Z. and Chao, F. 2010: Identification of differential hepatic proteins in rare minnow (Gobiocypris rarus) exposed to pentachlorophenol (PCP) by proteomic analysis. *Toxicol. Lett.* 199, 69e79.

Fisher, J.S. 2004. Environmental anti-androgens and male reproductive health: focus on phthalates and testicular dysgenesis syndrome. *Reproduction* ;127:305-15. 10.1530/rep.1.00025

Gernhofer, M., Pawet, M., Schramm, M., Müller, E. and Triebskorn, R. 2001. Ultrastructural biomarkers

astools to characterize the health status of fish incontaminated streams. *Journal of AquaticEcosystem, Stress and Recovery,* Vol. 8, pp.241-260.

Jobling, S., Sheahan, D., Osborne, J. A., Matthiessen, P. and Sumpter, J. P. 1996: Inhibition of testicular growth in rainbow trout (Oncorhynchusmykiss) exposed to estrogenic alkylphenolic compounds. *Environ. Toxicol. Chem.* 15, 194-202.

Koss G. and Koransky, W. 1978: Pentachlorophenol in different species of vertebrates after administration of hexachlorobenzene and pentachlorobenzene. In: Rao KR, editor. Pentachlorophenol: chemistry, pharmacology, and environmental toxicology. New York: Plenum Press;. p. 131–7.

Lech, J. J., Lewis, S. K. and Ren, L. 1996: In vivo estrogenic activity of nonylphenol in rainbow trout. *Fundam. Appl. Toxicol.* 30, 229-232.

Marcoccia, D., Pellegrini, M., Fiocchetti, M., Lorenzetti, S. and Marino, M. 2017: Food components and contaminants as (anti)androgenic molecules. *Genes Nutr* ;12:6. 10.1186/s12263- 017-0555-5

Meyers, T. R. and Hendricks, J. D. 1985.*Histopathology*. In: Loux, D.B., Dorfman, M.,(Eds.), Fundamentals of Aquatic Toxicology: Methods and Applications, Hemisphere USA, pp. 283–330.

Pietsch, C., Hollender, J., Dorusch, F.and Burkhardt-Holm, P., 2014: Cytotoxic effects of pentachlorophenol (PCP) and its metabolite tetrachlorohydroquinone (TCHQ) on liver cells are modulated by antioxidants. *Cell Biol. Toxicol.* 30, 233e252.

Renner, G. and Mücke, W. 1986: Transformation of pentachlorophenol. *Toxicol Environ Chem.;*11:9–29.

Dubey, S. and Shah, S. 2017: Histological Alteration Inflicted By Tulsi in Thyroid and Parathyroid Gland of Rat*. Proceedings of Zoological Society of India.* 16(2):57- 62:ISSN NO: 0972-6683.

Dubey, S., Mehrotra, M. and Yadav, S. N. P. 2017: Effect of para-chlorophenyl Alanine and Melatonin on Immune and Reproductive Status of Indian Passeriformes Finch, *Lonchura punctulata. Proceedings of Zoological Society of India*. pg 47-52 ISSN no: 0972-6683.

SEMA., 1988. Avaliacao da toxicidadeparapeixes (D.3). In Manual de Testes Para Avaliacao da Ecotoxicidade de AgentesQuimicos. *Brasilia,* pp: 10.

Senthikumaran B. and Joy K.P. 1994: Effects of ovariectomy and estradiol replacement in hypothalamic serotonergic and monoamine oxidase activity in the catfish, *Heteropneustesfossilis* : a study correlating plasma oestradiol and gonadotropin levels. *J. Endocrinol.* 142:193-203.IF 2.636.

Talmage S. S. 1994: Environmental and human safety of major surfactants: Alcohol ethoxylates and alkylphenolethoxylates. 374.

van Ommen B., van Bladeren P.J., Temmink J.H. and Muller F. 1985: Formation of pentachlorophenol as the major product of microsomal oxidation of hexachlorobenzene. *Biochem Biophys Res Commun.;*126:25–32.

Vilela J., Hartmann A., Silva E.F., Cardoso, T., Corcini, C.D., Varela-Junior, A.S., Martinez, P.E., and Colares E.P. 2014. Sperm impairments in adult vesper mice (Calomyslaucha) caused by in utero exposure to bisphenol *A. Andrologia ;* 46:971-8. 10.1111/and.12182.

WHO 2003. Pentachlorophenol in drinking-water. Background document for preparation of WHO Guidelines for drinking-water quality. Geneva: World Health Organization;. WHO/ SDE/WSH/03.04/62).

Yin, D., Gu, Y., Li, Y., Wang, X. and Zhao, Q. 2006. Pentachlorophenol treatment in vivo elevates point mutation rate in zebrafish p53 gene. Mutat. Rese-Gen. Tox. *Environ. Mutag* 609, 92e101.

Zheng, G.J., Leung, A.O.W., Jiao, L.P., Wong, M.H. 2008. Polychlorinated dibenzo-p-dioxins and dibenzofurans pollution in China: sources, environmental levels and potential human health impacts. Envion Int;34:1050–61.

Zhu, W., Zhang H., Tong C., Xie, C., Fan, G., Zhao, S., Yu, X., Tian, Y. and Zhang J. 2016. Environmental Exposure to Triclosan and Semen Quality. *Int J Environ Res Public Health* ;13:224. 10.3390/ijerph13020224.