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Research Paper

Histopathology of the liver of Indin Murrel *Channa punctatus* (Bloch) exposed to Phenolic Effluents

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Abstract: *The aim of this study was to examine the histological changes in the liver of fish Channa punctatus (Bloch) for a short term (8day) and long term (30 days) exposure in response to the Phenolic Industrial effluents of Bhilai Steel plant. Fishes were exposed in different concentration (low .01%, .015%, .02, .025%) and (high concentration 10%, 20%, 30%) of Phenolic effluent. Tissu sections of the liver were stained with haematoxyline-eosin and changes in the tissues were examined using light microscope. No histopathological changes were found in control, although no lesions were observed in response to short term exposure to both low and high concentration of toxicants, however remarkable changes in liver structure were observed in response to long term exposure .These included nuclear and cytoplasmic degeneration, leucocytic infiltration, vascular congestion hemorrhage. The degree of damage depended on the concentration of effluents and period of exposure.*

Keywords: Histopathology, Liver, Phenol, Effluents, *Channa punctatus*.

Introduction

Indiscriminate discharge of such compounds that contain mixtures of heavy metals, such as herbicide, pesticides, detergents, phenols etc. in water bodies, have harmful effects on the fish population and other forms of aquatic life found in that area[1,2,3]. The result was the generation of large amounts of waste going straight in to the environment seriously damaging its natural processes. The consequences were deleterious, giving birth to three major types of contaminations of our precious natural resources- air, water and soil.

Water is a universal solvent is an essential for life on earth. Hence, discharge of various toxic chemicals and substances into water makes life difficult, If discharged directly in to the environment are responsible for various acute and chronic diseases, such as skin burns and rashes, bone abnormalities, lung and digestive system disorders in aquatic and surrounding land fauna[4].

The Bhilai Steel Plant is the largest integrated steel plant, a unit of Steel Authority of India Ltd. and a public sector undertaking was conceived under aegis of Indo-USSR Treaty in the 2nd Five year plan in 1959. The plant is located at the central position of India, Besides the major marketable product which is good quality steel, it also produces important by products, such as, Coal tar, Naphthalene and Benzol. Left this it also produce large amount of waste water streams, generated from the cooling of the coke oven gas and the processing of ammonia, tar, naphthalene, phenol, and light oil. If they are directly dumped into the river it carries harmful effects on the water bodies.

Phenols are hydroxy-derivatives of aromatic hydrocarbons and are formed during the decomposition of organic materials under natural conditions. They are an important constituent of coal tar. The major portion of the phenol Present in the environment is of anthropogenic origin. Production and use of phenol and its products (wood, iron and steel industry),

exhaust gases, residential wood burning, cigarette smoke and smoked food are potential sources of phenols. Phenol is toxic with a probable oral lethal dose to humans of 50-500 mg/kg. Some individuals may be hypersensitive with lethality or serious effects at very low exposures. Rapid absorption and severe systemic toxicity can occur after any route of exposure including skin through the food. Death and severe toxicity are usually due to effects on the CNS, heart, blood vessels, lung, and kidneys. Acute exposure may lead to shock, delirium, coma, pulmonary distress, phenolic breath, scanty/dark urine, and death. Protracted or chronic exposure usually results in major damage to the liver, kidneys and eyes. Pigmentary changes of the skin have also been noted. Consumption of water contaminated with phenol resulted in diarrhea, mouth sores, burning of the mouth, and dark urine. Phenol is highly caustic to tissues. Skin exposure results in pain, then numbness, blanching, severe burns and scar formations. Ingestion leads to burning of throat and severe gastrointestinal inflammation. Inhalation can result in pulmonary irritation and edema
Fishes are aquatic and poikilothermic animals. Hence, their existence and performance is dominated by the quality of their environment. All species of fish perform best under certain optimal conditions, but the amplitude of such conditions is quite narrow. Thus, in the aquatic environment, life goes on under dynamic and unstable circumstances, forcing fishes to acclimatize to various factors, such as-changes in population density, pressure, temperature, dissolved gases, light, pH, etc, which impose a considerable amount of stress on their lives and predispose them to diseases.

The liver is the largest gland of the body which plays a primary role in the metabolism and excretion of xenobiotic compounds with morphological alterations occurring in some toxic conditions[7]. It also acts as storage center for many substances[8]. Fish liver is a very interesting organ for study of the interactions between environmental factors and hepatic structures and functions[9-12]. It is the site of glycogen synthesis, storage and deamination of amino acid. Several proteins, including those required for blood clotting, are also synthesized in the liver cells. Abdel (1984) observed that the liver of fish subjected to phenol showed high score of histopathological symptoms such as inflammation, central necrosis and cell degeneration[2].

Material and Methods

The effluents were collected from the origin point at Purena Nala for analysis of its physic-chemical characteristics by APHA-AWWA method (1975) and also calculated the LC -50 value of model fish (*Channa punctatus*) by the method of Probit analysis. A preliminary experiment was conducted involving a range of percentages of the effluent to find the percentage that resulted in 50% mortality in a given time. Laboratory acclimatized fishes, 20 in each concentration, were exposed to these dilution percentages of the Effluent with one set maintained along as control. In all six concentrations of the effluent (10%, 30%, 50%, 70%, 90% and 100%) were taken for experiment after dilution by tap water and percent mortality calculated by the formula:Number of fishes dead X 100 / Total number of fishes exposed. Similarly, 20 fishes each, were also exposed to different concentrations (10,12,14,16,18 mg/l) of commercially available phenol solution for 24 hours and the LC_{50} determined. The corresponding Probit values for each concentration were used further for calculating LC_{50}. Accordingly, the LC_{50} for effluent was calculated to be 70% (Table 3.1) and Phenol to be 14 mg/l. Hence, sub lethal concentration ranges comprising of low (0.01, 0.015, 0.02, 0.025%) and higher ranges comprising of 10, 20 and 30% were selected further for short and long term exposure experiments. The amount of phenol present in effluent was estimated to be 5.6 mg/l. This was subsequently diluted with tap water so as to have the before mentioned concentrations (0.01, 0.015, 0.02, 0.025%) and 10, 20 and 30%.

Live, healthy fishes belonging to the same age group were collected from local streams and acclimatized under normal laboratory conditions for 15 days. They were then separated into 7 groups of 16 fishes each. Short term exposures to various concentrations of the Phenolic Effluent along with Normal tap water as control was done in 20 liters glass aquaria for a period of 7 days and day to day observations made at different time points viz.,24,48,72,96,120,144 and 164 hours. Uniform feeding and aeration of the tanks was done throughout the experiment. Long term exposure of fishes to the toxicant at (10%, 20%, 30%) of effluents were done for a duration of 4 weeks and observations on mortality made at weekly intervals.

For the exposure of fishes, two concentration ranges viz., low (0.01, 0.015, 0.02 and 0.025%) and high (10, 20 and 30%) of the whole phenolic effluent were taken after dilution with normal tap water. Normal tap water served as control. The fishes were divided into 8 groups consisting of 28 fishes each. Nine of the groups were individually exposed to Tap water (Control) and low concentrations (0.01, 0.015, 0.02 and 0.025%) of Phenol and Effluent in 20 liters glass aquaria. The remaining 7 groups were similarly exposed to the higher concentration ranges (10, 20, and 30%) Feeding of fishes and aeration of the tanks were done uniformly throughout the experiment.

Collection of tissues: Tissues for histopathology were collected separately from 4 live fishes belonging to

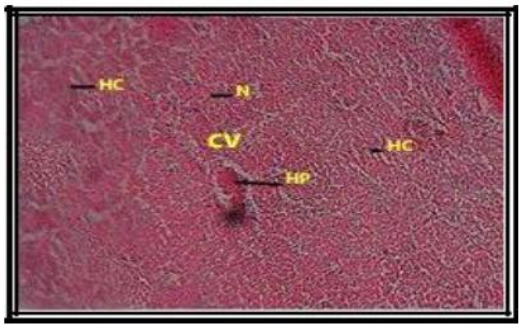
each group at 24,48,72,96,120,144 and 168 hours by mid-ventral incision. Liver of the exposed fishes were taken out by mid incision and put it in normal saline Tissues was Dehydrated through various grade of alcohol series, cleared in xylene and embedded in Paraffin wax Sections of 8 µ thickness were cut and ribbons obtained were gently placed on slides smeared with Mayer's albumin Deparaffinization of sections was done in Xylene followed by hydration through descending grades of alcohol. After hydration through various grades of Alcohol in descending order, the sections were then brought to water and stained by haematoxyline-eosin stain and examined under light microscope.

Results and Discussion

Histopathological changes were studied in liver cell exposed in both control and treated groups indicated incidence of damage in tissues after exposure to phenolic effluent for short term and long terms in low and high concentration. Liver from control fishes were observed to be darker in colour and exhibited a normal compact architecture with polygonal hepatocytes presenting a homogenous cytoplasm and clearly visible central blood vessels from first to 4th week of exposure [Plate 1]. Although, no lesions were observed in response to short term exposure to both low and high concentrations of toxicants, however remarkable changes in liver structure were observed in response to long term exposure.

No visible changes were observed upon exposure to 10% effluent after Ist and IInd however, degenerative changes in liver tissue were observed in the 3rd week after exposure [PLATE 2C] followed by vascular congestion, hemorrhage and degeneration in the 4th week after exposure [PLATE 2 (D)]. Similar changes are also observed in response to 20% effluent [PLATE 3(A to 3 D)]. Exposure to 30% effluent [PLATE 4(A-D)] shows the presence of leucocytic infiltration, vacuolated cells and hemorrhage in the 1st week followed by degenerative changes in the 2nd week and 3rd week. Fatty degeneration and necrotic changes were observed in the 4th week.

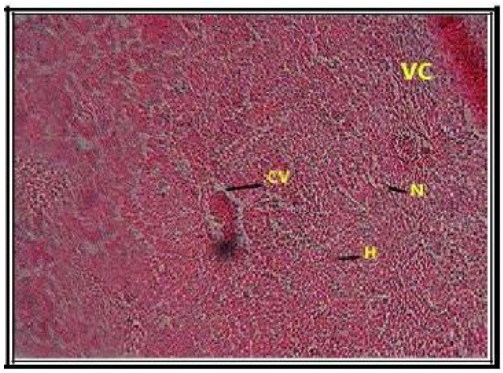
Control (Plate -1)



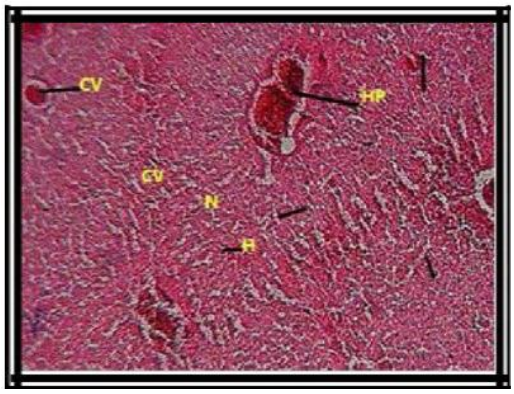
Showing no visible changes

Exposed-Effluent 10% (Plate 2)

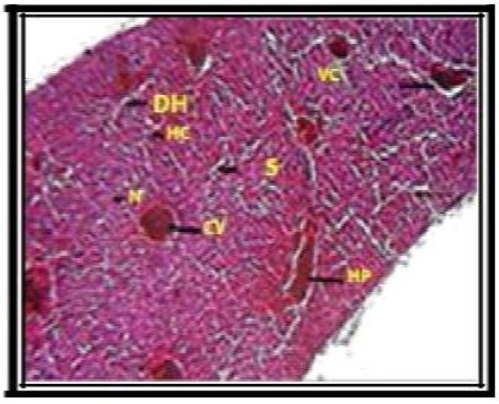
(Plate 2-1) Week 1- Showing no visible changes



(Plate 2-2) Week 2 Showing no visible changes



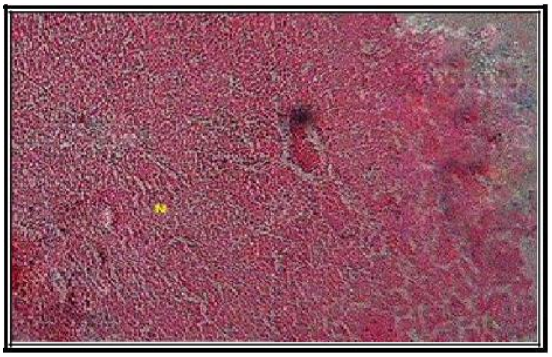
(Plate 2-3) Week 3 Showing degenerated



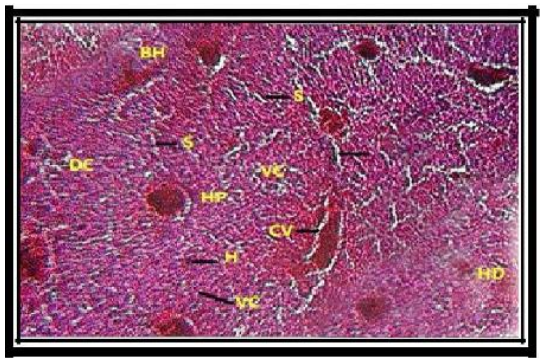
(Plate 2-4) Week 4 Showing vascular hemorrhageand degeneration

Exposed-Effluent 20% ((Plate 3)

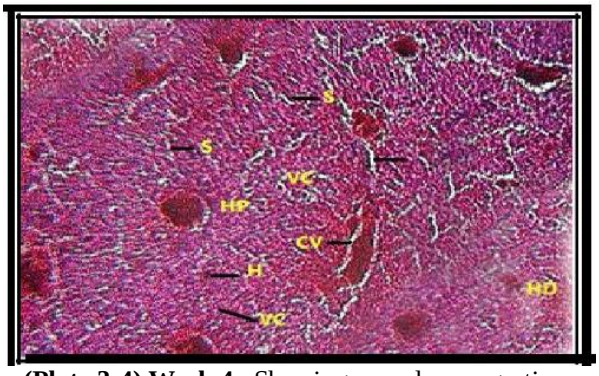
(Plate 3-1)Week 1- Showing no visible changes



(Plate 3-2) Week 2- Showing vacuolization in cell

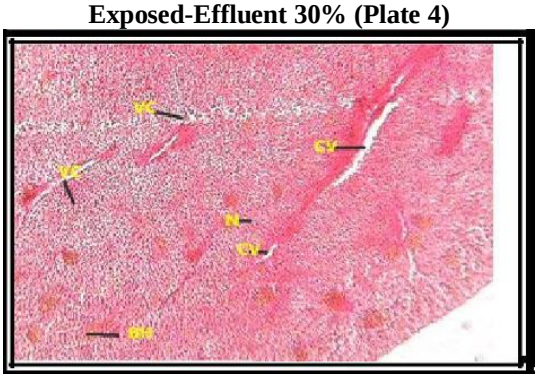


(Plate 3-3)Week 3 Showing vacuolar degeneration and hemorrhage

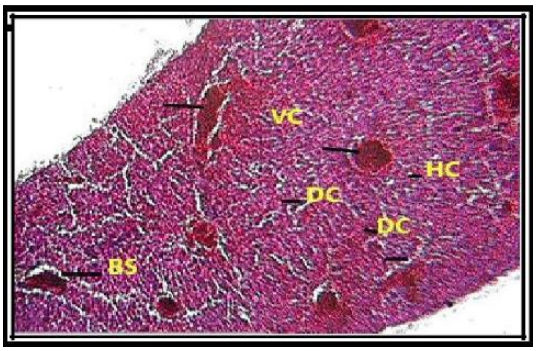


(Plate 3-4) Week 4 –Showing vascular congestion, hemorrhage and tissue degeneration

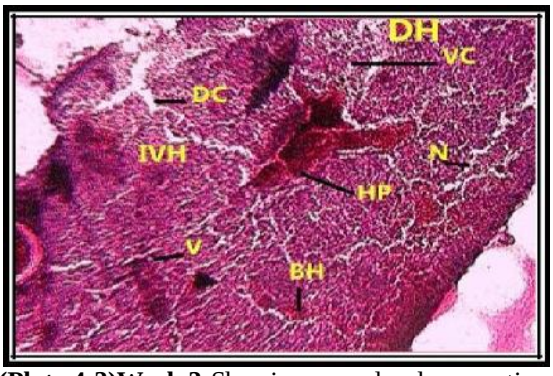
Exposed-Effluent 30% (Plate 4)



(PLATE 4-1)Week 1 Showing leucocytic infiltration degeneration and hemorrhage



(Plate 4-2) Week 2 Showing vacuolar and degeneration hemorrhage



(Plate 4-3)Week 3-Showing vacuolar degeneration and necrosis formation

(Plate 4-4) Week 4- Showing svascular congestion, hydrops fatty degeneration and necrosis

Although, no lesions were observed in response to short term exposure to both low and high concentrations of the toxicants, however similar but remarkable changes in liver structure were observed in response to long term exposures in different concentration of effluent. These included onset of nuclear and cytoplasmic degenerations in the form of hydropic change followed by leucocytic infiltration, vascular congestion, hemorrhage, fatty degeneration and necrotic changes which were observed to be accelerated with increasing concentration of the toxicant which proves beyond doubt that hepatocytes are the primary targets of toxic lesions. Presence of shrunk and pyknotic nuclei may be indicative of functional degradation of liver cells.

Similar histopathological changes in the form of vacuolar degeneration of hepatocytes, focal necrosis, thrombosis of central vessels, dilation and congestion of blood sinusoids and fibrosis in liver of *Tillapia zilli* and *Solea vulgaris* have been reported by Fatma (2009) who attributed this to direct action of pollutants. Similarly, congestion of central vein, vacoulation of hepatocyte, oedema, cellular infiltration and cellular necrosis in *Clarias gariepinus* exposed to detergent effluent was also observed[14-16] also reported cellular vacuolization with hypertrophy of hepatocytes in advanced cases of Lindane toxicity. Cloudy swelling of hepatocytes, congestion, vacuolar degeneration, karyolysis, karyohexis, dilation of sinusoids and nuclear hypertrophy have been reported in response to Dichlorvos[17,18] also studied the histopathological effect of phenol on liver of *Oreochromis aureus* juveniles and reported the presence of inflammation, central necrosis and cell degeneration.

Cytopathological alterations in hepatocytes of fish following exposure to xenobiotic compounds represent a powerful tool to reveal sublethal effects of chemicals and to elucidate underlying modes of action. Liver and hepatocytes are considered as efficient biomarkers of contaminant exposure because the liver is central to numerous vital functions in basic metabolism[19-21]. It is also the major site of accumulation, biotransformation and excretion of xenobiotic compounds, besides being a major site of Cyt P450 mediated mixed fraction oxygenase system[22]. Bile produced in hepatocytes and released into the proximal portion of intestine serves as a carrier of conjugated toxins[23]. Besides it is also a site of exogenous vitellogenesis[24] enabling transfer and storage of toxicants in the yolk of embryo. Thus hepatocytes are primary targets of toxic lesions[25].

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