HISTOPATHOGICAL STUDIES AS POTENTIAL AND DIRECT BIOMARKERS OF POLLUTION

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ABSTRACT
The present investigation is a part of a detailed investigation entitled (Assessment of the river Chambal pollution using biomarker responses). The present part aimed to study the histological changes on the liver, and kidney of albino mice *Muscus albinus* as biomarkers in combination with chemical analysis of the water from the river Chambal. This work provides a general evaluation of the main pathological alterations reported in mice exposed to contaminants. In this study we analyze the histopatholoigcal studies which have been employed as are of promising potential biomarkers in pollution monitoring programmes. The usefulness and applicability of each biomarker were examined and evaluated against a number of objective criteria, including: ecological relevance, sensitivity, and specificity. The aim of these investigations was to perform a histological analysis of different tissues in mice to represent a reliable indicator of the ecological condition of the aquatic ecosystem and the possible negative impact of the surrounding environment.

KEY WORDS: Effluent, Histopathology, Chambal River, Nagda

INTRODUCTION

Histopathology is the microscopic evaluation of disease processes (Giari, *et al.*, 2008). It is a very powerful, subjective tool which may be used to establish primary and secondary disease patterns in populations of fish. Most biomarkers are narrow in their expression whereas pathology is broad in its evaluation [Moore, M.J. and M.S. Myers, 1994]. There is a shortage of literature concerning the critical evaluation of mammal histopathology in environmental effects monitoring. Histocytological responses are relatively easy to determine, and can be related to health and fitness of individuals which, in turn, allows further extrapolation to population/community effects. A wide range of histocytological alterations in fish and bivalves have been developed and recommended as biomarkers for monitoring the effects of pollution. But such studies in mammals are lacking. Therefore, histopatholoigcal evaluation remains an important part of the assessment of the adverse effects of xenobiotics on the whole organism.

Histopatholoigcal changes in animals tissues are powerful indicators of prior exposure to environment stressors and are net result of adverse biochemical and physiological changes in organisms. For filed assessment histopathology is often the easiest method assessing both short and long term toxic effects (Hinton and Lauren, 1990). Histopatholoigcal biomarkers can be indicators of the effects on organisms of various anthropogenic pollutants and are a reflection of the overall health of the entire population in the ecosystem. The alterations in cells and tissues in vertebrate fish are recurrently used biomarkers in many studies, but such changes occur in all vertebrates and invertebrates inhabiting aquatic basins. Histopatholoigcal biomarkers embody tissue lesions arising as a result of a previous or current exposure of the organism to one or more toxins. Well-documented lesions based on experimental data in liver, kidney, gill, ovary, skeleton system and skin have been used as biomarkers to date (Hinton *et al*., 1985). Histopatholoigcal biomarkers are closely related to other biomarkers of stress since many pollutants have to undergo metabolic activation in order to be able to provoke cellular change in the affected organism. For example, the mechanism of action of several xenobiotics could initiate the formation of a specific enzyme that causes changes in metabolism, further leading to cellular intoxication and death, at a cellular level, whereas this manifests as necrosis, i.e. histopatholoigcal biomarker on a tissue level. As well as from chemical insult, histopatholoigcal lesions may arise from infectious diseases and parasites, provoking necrotic and degenerative alterations to which the organism responds with an inflammatory, defensive reaction (Velvoka, 2002; Roganovic, *et al*., 2003). Jawale *et al.,* (2010) reported hematological changes in the fresh water fish, *Cyprinus carpio* exposed to sub-lethal concentration of piscicidal compounds.

Histopathology is the gold standard when defining toxicological effects, but it is invasive, time consuming and expensive. Using biomarkers linked to distinct, defined cell types and tissues may provide a direct link to histopathology without its drawbacks and it also provides increased sensitivity and specificity. Furthermore, as histological testing is often impractical in human subjects, using biomarkers with a known histological distribution may fill the need of localizing toxic injury to distinct organs or tissues. This paper discusses how, by using biomarkers with a known cellular origin (histologically defined biomarkers), toxic effects may be found earlier and at lower doses of compound, leading to potential savings in drug development. Using of pathology is well established as an essential section of the set of 'biomarkers' applied in biological effects of variety pollutants monitoring programs in Europe such as International Council for the Exploration of the Sea (ICES). Use of histopathological techniques allows investigators to examine specific target organs and cells as they are affected by exposure to environmental chemicals. Moreover, it offers a means of detecting acute and chronic harmful effects of exposure in the tissues and organs of individual animals.

MATERIALS AND METHODS

Study Area:
Chambal River in Nagda is very close to tropic of cancer at 23'27 N and 75'25 and 517 meters above MSL. More than one lakh of residents in and around the Nagda rely on water from Chambal River for public use, industrial supplies, power plant cooling and waste water treatment. The river receives water from different Industrial units including municipal sewage from Nagda town. Waste after coming from the factory complex runs in a channel for about 3 km and joins River Chambal near Juna Nagda.

Sampling:
Effluents were collected in sterilized phosphate free cleaned polythene bottles, near Mukteswar temple at Juna Nagda where discharges of industrial complex and domestic waste are drained into this station. The samples after collection were immediately placed in dark boxes and processed for physico chemical analysis like pH, electrical conductivity (EC), total dissolved solids (TDS), total hardness (TH) dissolved oxygen (DO), Biochemical oxygen demand (BOD) and chemical oxygen demand (COD).The procedure for analysis followed 'Standard methods of analysis of water and waste water. Chemical used of analytical grade and instruments are of precise accuracy. Double distilled water and borosil glassware were used throughout the work.

Experimental design:
For the present experiment 30 healthy six months old Swiss albino male mice *Musculus albinus* weighing (30+2 gm) were procured from the veterinary college, Mhow. These mice were acclimated for two weeks before experimentation in well-ventilated animal cages (270 X 220 X 140mcm.) and under standard laboratory conditions (photoperiod and temperature). The acute toxicity of industrial effluent to *Muscus albinos* was determined using a standard 24-h static renewal technique. (USEPA, 1975) and it was found to be 35%.

The persistent mice were divided into 3 groups .Group (I) represented the control group was fed on diet free from any other additions with normal drinking water, Group (II) was given same diet with 10%v/v of effluent as drinking water whereas the group (III) was given also the same diet with 15%v/v of effluent. The experiments were terminated after 28 days. The mice of experimental groups and along with control group animals were sacrificed and liver and kidney was separated and processed for histomorphology investigation.

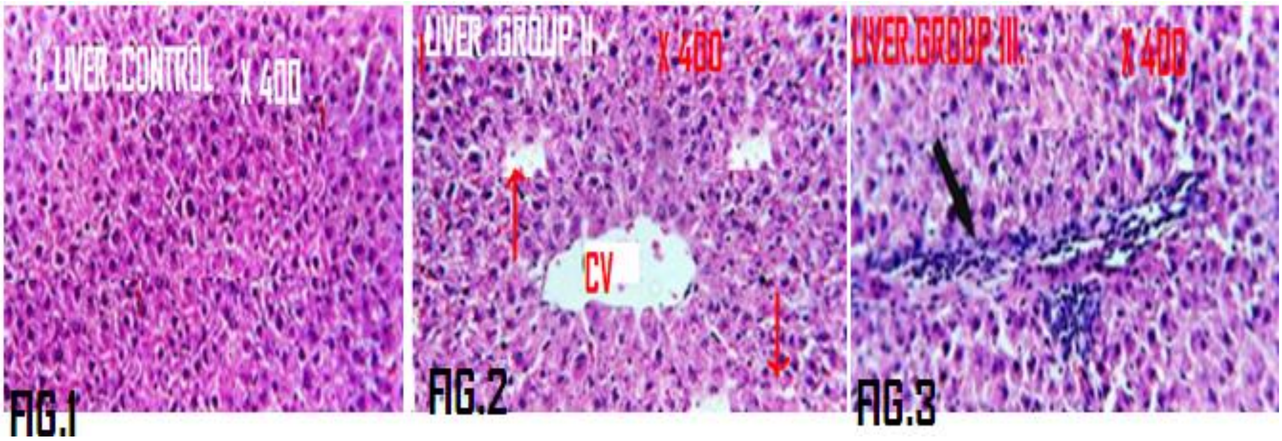
Histological Studies:
The liver was fixed in formal saline at room temperature for 24 h before being dehydrated and embedded in paraffin wax (melting point 65oC). The tissues were sectioned at 5u and routinely stained with haematoxylin and eosin (H&E). Histological structure of the liver was evaluated under a light microscope (NIKON ECLIPSE E 400, USA) and photographed using digital camera attached to the microscope.

RESULTS AND DISCUSSION

Water analysis:
A summary of physico chemical parameters obtained in Chambal River from non-point source for all are shown in Table.1. Results clearly indicate that the physico chemical parameters showed high levels of BOD, COD, TDS, TSS, and EC and low DO and exceed the limits WHO standards. This must have been as a result of the nature of effluents discharged from the industries. (Table 1).

The 96 h LC50 value for effluent was found to be 37.5%. The mortality ranged from 20% to 53.5% and increased with a corresponding increase in the effluent concentration and also duration of the exposure demonstrating both time and concentration dependent responses. Darting movements and loss of equilibrium associated with convulsions were observed. These observations were more pronounced in higher concentrations effluent.

The organ most associated with detoxification and biotransformation process is the liver and due to its function, position and blood supply it is also one of the organs most affected by the contaminants in the water[8]. Liver carries out essential body functions including the regulation of metabolism, synthesis of plasma proteins, energy storage of certain vitamins and trace metals and detoxication of xenobiotics. In general liver is target organ due to large blood supply that causes noticeable toxicant exposure, accumulation and causes metabolic disturbances. Therefore studies on liver histopathology in fish have increasingly been incorporated in national marine biological effects monitoring programs in both Europe and the USA. However, using of liver histopathology as a biomarker of contamination exposure may not be a highly cost-effective method for pollution screening because it needs much time and effort to prepare liver samples and expected pathologists are also required to distinguish hepatological alterations (Au, D.W.T., 2004).



PLATE; 1. Histopathology of liver of *Muscus albinus.* Control mice showing normal structure. B. liver from Group II showing swelling and necrosis C. Histology of liver of *Muscus albinus* from Group III showing acute cellular swelling, hypertrophy and pyknosis Secondary infections in between hepatocytes are also seen .(H and E Stained x400).

Table 1. Physico-chemical analysis of effluent from non-point source of Chambal River at Nagda

pH	9.4±**0.02
Temperature ‘C	23.1±0.44
TDS mg/L	448.1±6.3**
TSS mg/L	148.1±9.6**
EC,umho/cm	180±12.1
DO mg/L	5.1±0.8
BOD, mg/L	54.4±4.2**
COD mg/L	38.0± 2.6**

In our results, Sections of control group (I) show the normal histological structure but Group II treated with 10% effluent show mild swelling and degeneration of hepatocytes. In GroupIII the micro sections of liver show degeneration of hepatocytes, hypertrophy of kupfercells and proliferation of bile ducts was observed .Abnormalities such as irregular shaped hepatocytes, cytoplasm vacuolation and nucleus in a lateral position .Lesions including hepatocellular cytoplasmic vacuolization, leucocytes infiltrations, blood congestion necrosis and fatty infiltrations were found mainly from the mice of Group III. The same changes were reported by Teh *et al*. 2005] in the liver of 7-day-old larvae of the fish *Sarcamento splittail* exposed to sub lethal concentrations of esfenvalerate for one week. (PLATE1. Figure 1-3).

Result of our present study clearly revealed that mice fed with effluent manifest histopatholoigcal changes in liver. It is possible that the pathological alterations in liver studied could be a direct result of the heavy metals, pesticides, fertilizers, salts , which are entered to the river with drainage water (Reddy and Baghel,2012). The histopatholoigcal alterations studied in this mice are in agreement with those observed by many investigators who have studied the effects of different pollutants (Depledge *et al,* 1993; Decaprio, 1997; Adams; 2002).These changes may be attributed to direct toxic effects of pollutants on hepatocytes, since the liver is the site of detoxification of all types of toxins and chemicals (NRC, 1997). The vacuolization of hepatocytes might indicate an imbalance between the rate of synthesis of substances in parenchymal cells and the rate of their release into the circulatory system.

Histopatholoigcal Changes of Kidney; Kidney is the primary organ for water elimination and is especially vital for ion reabsorption, minimize water loss and to eliminate of divalent ions [Nishimura, H. and M. Imai, 1982].

Our results clearly show that (Plate II) the kidney of Group I show normal structure. But the kidney of Group II and III show structural abnormalities like necrosis, vacuolation, dilation of Bowman’s capsule, hyper plasma and fibrosis. These symptoms are more common in the kidney of mice treated with 20% effluent (Group III). Hydropic vacuolation, presence of proteinaceous droplets and necrosis of the tubule epithelial cells have been documented in the kidney exposed to various pollutants. In addition, glomerulus lesions including dilation of Bowman’s space, hyperplasia and fibrosis of the glomerular rate and thickening of basement membrane were also reported by many workers.

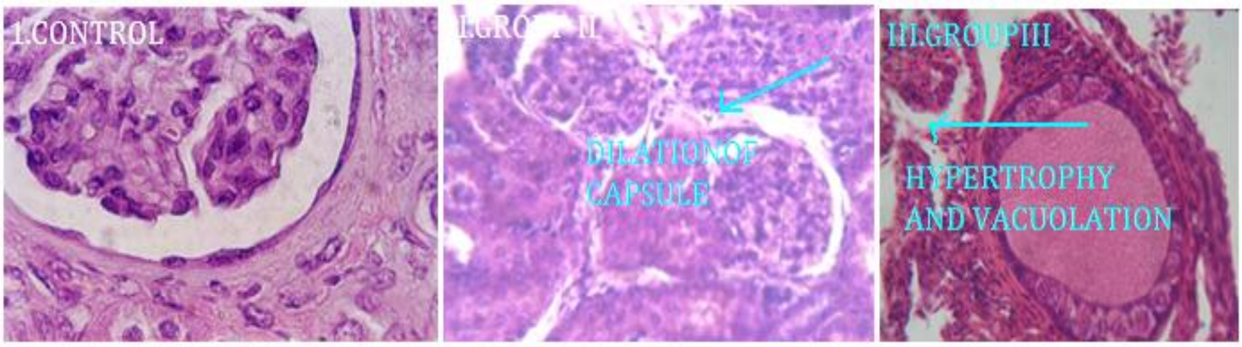


PLATE II. Histopatholgy of kidney of *Muscus albinus.* Control mice showing normal structure. B. Kidney from Group II showing dilation of Bowman's capsule and necrosis of epithelium .C. Histology of kidney of *Muscus albinus* from Group III showing acute cellular swelling, hypertrophy and vacuolation Secondary infections in between renal capsules are also seen .(H and E Stained x400).

Although the renal alterations can provide evidence of toxic affront by themselves but in combination with the other organs pathological change they could provide stronger indications of xenobiotic effect (Rhodes, L.D, 1987). The work presented here only threatened the chemical quality of the effluent, but it is equally important to extend the study to include a) measurement of a range of biological as well as physicochemical properties of soil which receive this polluted water for irrigation purposes b) identification and chemical analysis of plants grown on soils receiving this water and c) microbial analysis of soil (Sail *et al.*, 2006).

CONCLUSION

On the basis of the information presented in different studies, there is no doubt that the application of histopatholoigcal changes as a biomarker of organism exposure to contaminated sites, offers important information that can contribute to environmental monitoring programs designed for surveillance, hazard assessment or regulatory compliance. One of the most important benefits of the use of histopatholoigcal biomarkers in environmental screening is possibility of examining specific target organs including kidney and liver. This information verifies that histopatholoigcal changes are valuable biomarkers for field evaluation, especially in tropical regions that are naturally affected by variety of environmental variations. It should be highlighted that histopathology is able to assess the initial effects and reactions to acute exposure to chemical stressors.

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