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RESEARCH ARTICLE

HISTOCHEMICAL STUDIES ON THE LIVER AND INTESTINE OF *OREOCHROMIS MOSSAMBICUS* (CICHLID FISH) INDUCED BY CADMIUM SULPHATE

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ABSTRACT

In order to see the changes in Protein and Carbohydrate content in Liver and Intestine of *oreochromis mossambicus,* the tissue were stained with Mercuric Bromophenol Blue (MBB) and Periodic Acid Schiff's reagent (PAS). Observations of the histochemical studies were dependent entirely upon the intensity of the coloured chemical constituents. The amount of protein in liver was found decreased in a dose dependent manner with increase in concentration after 10 and 20 days for Cadmium sulphate, as the stains were lighter in hepatocytes. Similarly, the amount of carbohydrate in liver showed marked decrease in carbohydrate content after 10 days. The purple colour of the stain was still lighter showing decrease in carbohydrate content after 20 days. In terms of carbohydrate utilization, the effect showed less carbohydrate content. The intestine section from control fish showed good intensity of blue colour in the area specific to endothelial cells and villi. The amount of protein decreased in a dose dependent manner with increase in concentration of cadmium and the intensity of blue colour was still light and in no way comparable to control for all the tested concentration. In case of carbohydrate content, villi and microvilli took good amount of stain and no marked changes were observed after 10 and 20 days of exposure.

Key words: Histochemical, Hepatocytes, *Oreochromis mossambicus*, Carbohydrate, Protein

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INTRODUCTION

Industrial effluents are major source of water pollution. A variety of contaminants including toxic heavy metals (cadmium, copper, mercury and zinc) are reported to be present in rivers, reservoirs and are disadvantageous for aquatic organisms (Olsson, 1998). Cadmium is widely used in industrialized societies and thus growing the risk of artificially increasing the amount of this element in the environment (Fleischer *et al*., 1974). Cadmium is produced as an inevitable by-product of zinc (or occasionally lead) refining, since these metals occur naturally within the raw ore. However, once collected the cadmium is relatively easy to recycle. The most significant use of cadmium is in nickel/cadmium batteries, as rechargeable or secondary power sources exhibiting high output, long life, low maintenance and high tolerance to physical and electrical stress. Among the heavy metals, Cd is one of the most toxic, non-essential heavy metal known for its corrosive nature and is widely used in paints and dyes, cement and phosphate fertilizers (Jarrup L,). Bio enhancement of Cd transfer along a food chain was studied by (Seebaugh *et al.,* 2005) and fish were reported to be used as biological indicators to assess water pollution (Rashed MN., 2001). The present study is taken up with the context of heavy metal pollution of the aquatic bodies and the aquaculture ponds in India. Since *Oreochromis mossambicus* has secured its place as fastest growing edible fish of economic importance in India, it is necessary to investigate the effects of heavy metal concentrations on this fish. Cadmium was chosen as a heavy metal representative for the present study.

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MATERIALS AND METHODS

Test chemical

The Cadmium Sulphate ($3CdSO_4.8H_2O$) from Central Drug House (Batch no. 13059) of varying concentrations 50, 75 and 100mg/L was used for histochemical studies on Liver and Intestine of *Oreochromis mossambicus.*

Test animal

Oreochromis mossambicus, weight ranging from 50-100 grams were obtained from local pond namely Supatal, Jabalpur. These fishes were then treated with 0.1% potassium

permanganate solution for 15 minutes to get rid of dermal infections. The fishes were acclimatized for 2 weeks in de-chlorinated water of 30-50L glass aquariums under laboratory conditions.

Experimental exposure and tissue processing

The fishes were exposed to the sub lethal concentrations of Cadmium (50mg/l, 75mg/l and 100mg/l) each for 10 and 20 days of exposure for histochemical studies. After exposure period, the fishes were sacrificed and the desired tissues were dissected and kept in a Neutral formalin for 24hrs (Baker, 1958). Paraffin blocks of liver and intestine were cut at 6 micron thickness and stretched on sterilized glass slides. After deparaffinization and dehydration, sections were stained with Bromophenol blue and PAS staining methods. Histochemical changes induced by treatments in the tissues were photographed using Censico photomicroscope.

RESULT AND DISCUSSION

In order to see the changes in protein and carbohydrate content liver and intestinal tissue of *O. mossambicus*, were stained with Mercuric Bromophenol Blue (MBB) and PAS stain, in which proteins are stained as blue colour and carbohydrates as rose to purplish red colour on histochemical staining. Observations of the histochemical studies were dependent entirely upon the intensity of the coloured chemical constituent and do not represent the amount of the substances present in the section.

Changes in protein content in liver

The effect of cadmium sulphate at initial concentrations on proteins in the hepatocytes of liver of *O. mossambicus* in control as well as in fishes exposed for 10 days took good amount of stain and is shown as dark blue cells. The amount of protein was found decreased in a dose dependent manner with increase in concentration of cadmium sulphate after 10 days of exposure time, as the stain was lighter in hepatocytes in comparison to control showing marked decrease in protein as shown in Fig. 1.

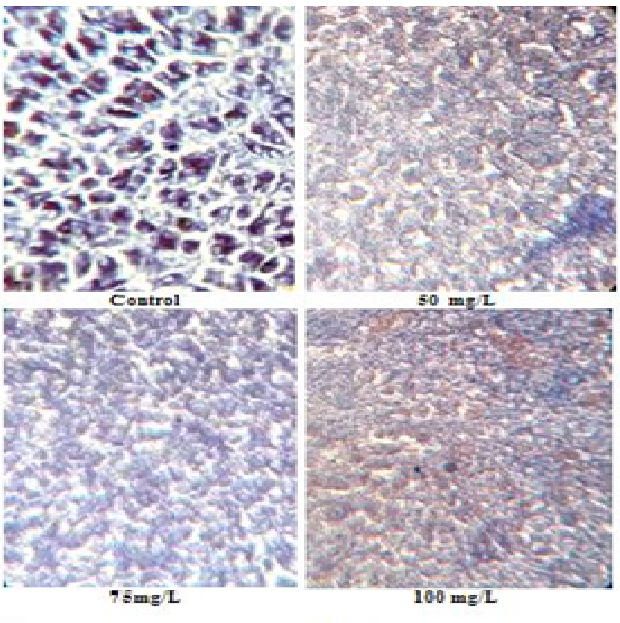


Fig 1: Effect of cadmium sulphate of different concentrations on proteins in the liver of *Oreochromis mossambicus* after 10 days of exposure as is shown by Bromophenol Blue staining (400x)

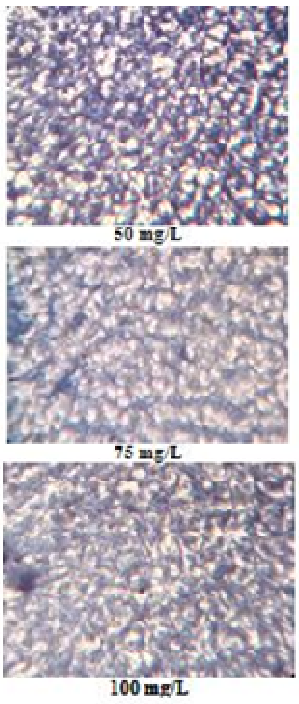
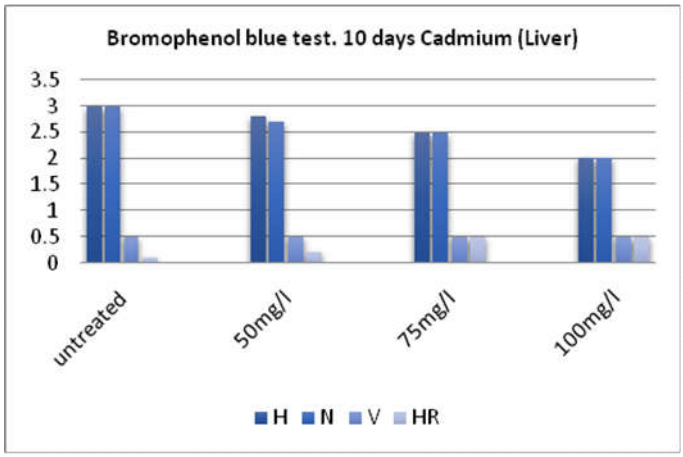
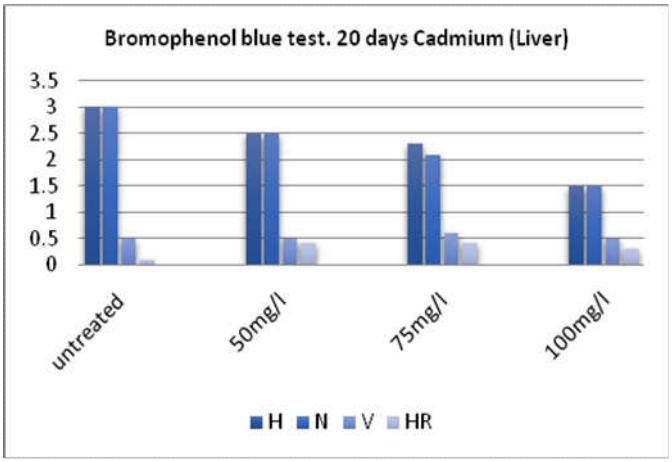


Fig 2: Effect of cadmium sulphate of different concentrations on proteins in the liver of *Oreochromis mossambicus* after 10 days of exposure as is shown by Bromophenol Blue staining (400x)



4- STRONG POSITIVE, 3- POSITIVE, 2- MODERATE POSITIVE, 1-WEAK POSITIVE, 0-NEGATIVE



H- Hepatic cells, N- Nucleus, V- Vaculation, HR- Haemorrhage

Cadmium sulphate at different concentrations on proteins in the liver of *O. mossambicus* in fishes exposed for 20 days shows decreased intensity of blue colour (amount of protein) in

a dose dependent manner. The light blue colour of the stain showed marked decline in protein concentrations in the liver for all concentrations.Fig.2

Changes in carbohydrate contents in Liver

Periodic acid–Schiff (PAS) staining method was used to detect carbohydrate content such as glycogen. Most of these substrates were attained as rose to purplish red colour on histochemical staining. The liver of *O. mossambicus* unexposed to cadmium sulphate showed dark stained hepatocytes, showing presence of carbohydrate substances in liver. The 50 mg/L concentration also was comparable to control; however, the higher concentrations showed decline in purple colour, showing marked decrease in carbohydrate content in liver after 10 days of exposure to cadmium sulphate as shown in Fig 3. The effect of cadmium sulphate after 20 days of exposure with dose of 50 mg/L still showed dark purple colour (glycogen), showing no or less effect of cadmium. The purple colour of the stain was still lighter in all the areas of slides of liver section in other concentrations, showing decrease in carbohydrate concen trations due to the exposure to cadmium sulphate after 20 days as shown in Fig 4.

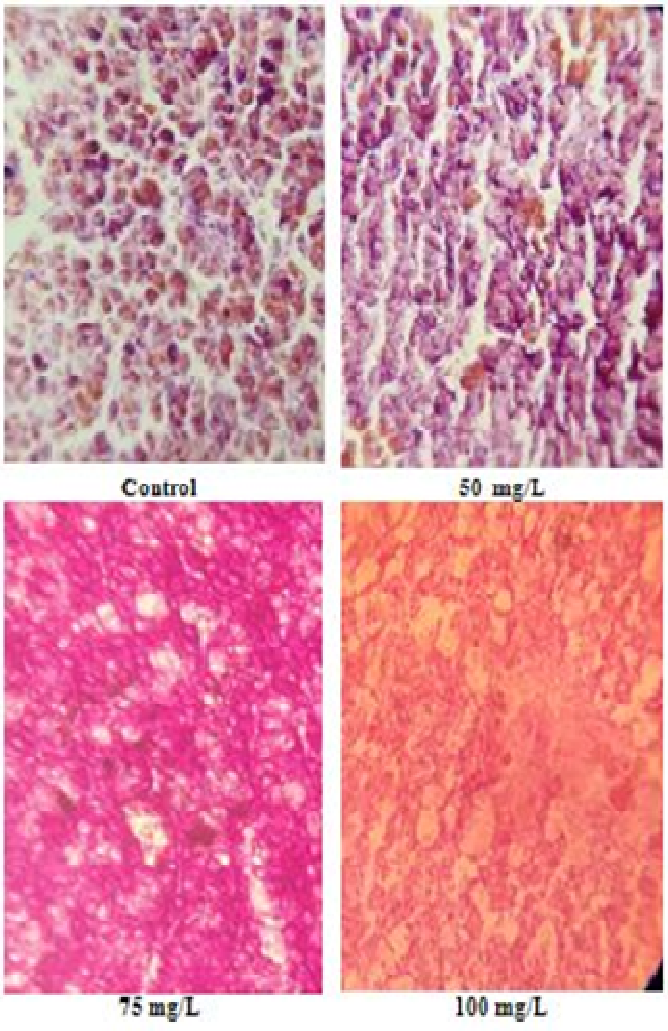


Fig 3: Effect of cadmium sulphate of different concentrations on carbohydrates in the liver of *Oreochromis mossambicus* after 10 days of exposure as is shown by Periodic Acid Schiff's (PAS) staining (400x)

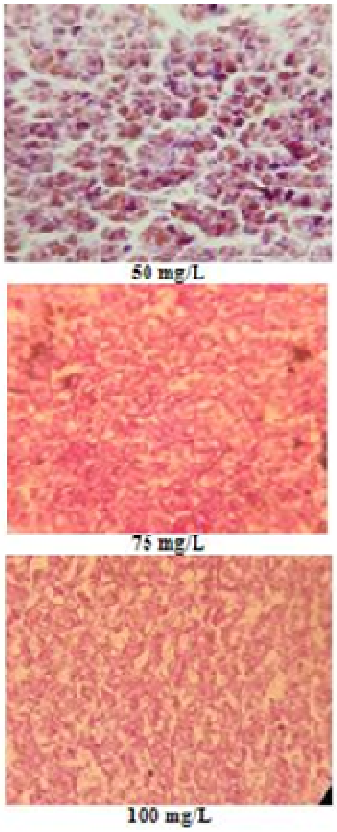
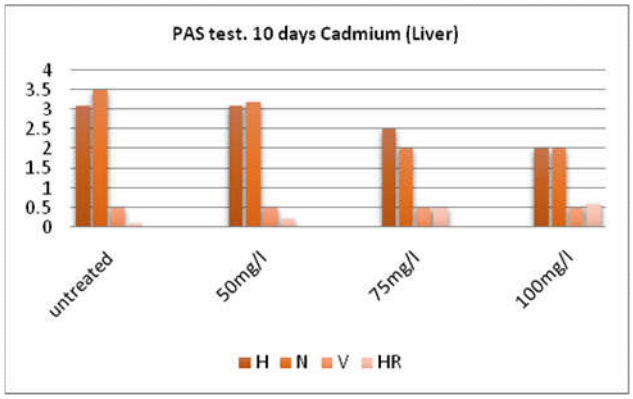
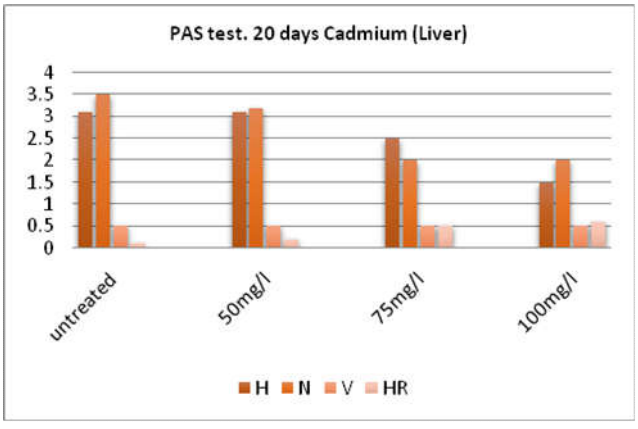


Fig 4: Effect of cadmium sulphate of different concentrations on carbohydrates in the liver of *Oreochromis mossambicus* after 20 days of exposure as is shown by Periodic Acid Schiff's (PAS) staining (400x)



4- STRONG POSITIVE , 3- POSITIVE, 2- MODERATE POSITIVE, 1- WEAK POSITIVE, 0-NEGATIVE



H- Hepatic cells, N- Nucleus, V- Vaculation, HR- Haemorrhage

Changes in protein content in Intestine

Effect of Cadmium sulphate at different concentrations on proteins in intestine of *O. mossambicus* in control as well as in fishes exposed for 10 days shows good intensity of blue colour (proteins) in the areas specific to endothelial cells and villi. The amount of protein decreased in a dose dependent manner with increase in concentration of cadmium sulphate after 10 days of exposure time in all areas of the intestine. The blue colour of the stain was lighter in villi and all the other areas of intestine showing marked decrease in protein concentrations due to the exposure to sub lethal doses of cadmium sulphate. Fig 5.

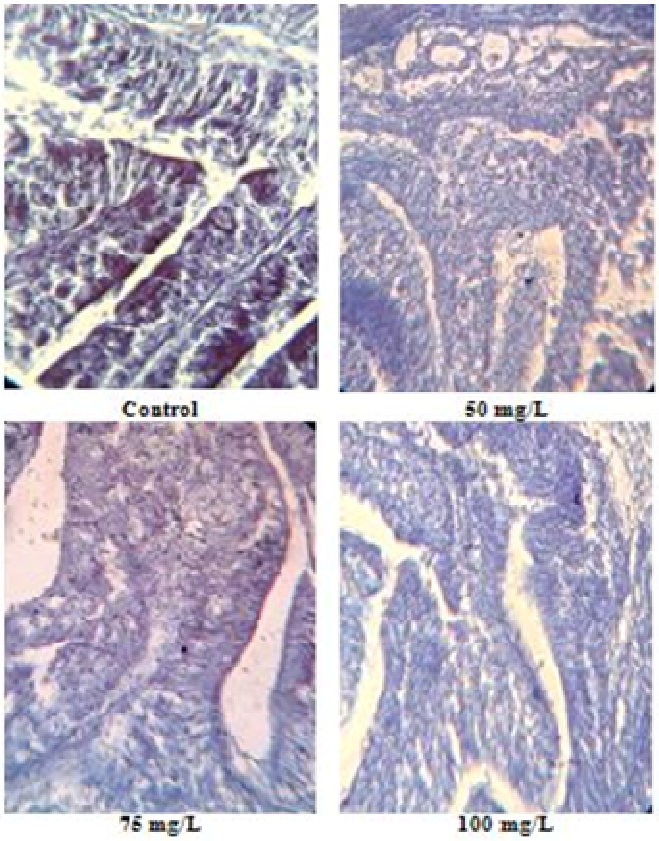
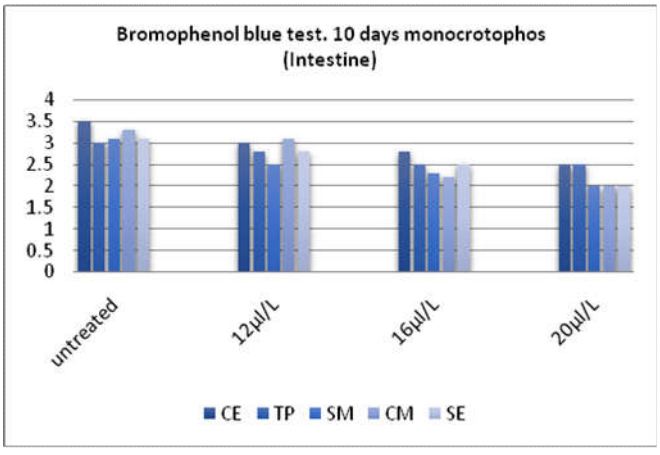


Fig 5: Effect of cadmium sulphate of different concentrations on proteins in the intestine of *Oreochromis mossambicus* after 10 days of exposure as is shown by Bromophenol Blue staining (400x).



CE- Columnar epithelium, TP- Tunica Propria, SM- Sub Mucosa, CM-Circular Muscles, SE-Serosa

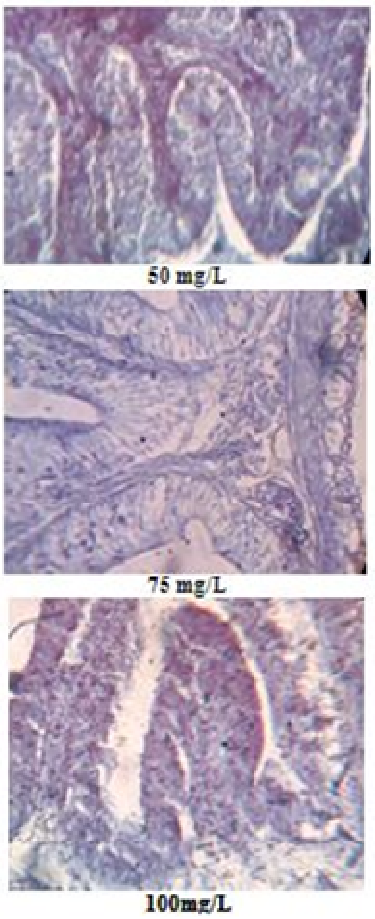
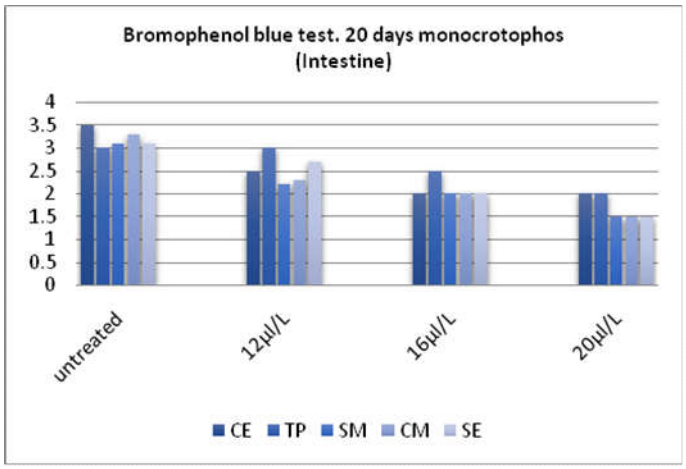


Fig 6: Effect of cadmium sulphate of different concentrations on proteins in the intestine of *Oreochromis mossambicus* after 20 days of exposure as is shown by Bromophenol Blue staining (400x)



After the impact of exposure of 20 days, the intensity of blue colour (amount of protein) was found to be decreased in a dose dependent manner with increase in concentration of cadmium sulphate. The blue colour of the stain was lighter in all the areas of intestine showing marked decrease in protein concentrations.

Changes in carbohydrate contents in Intestine

The effect of cadmium sulphate at different concentrations on carbohydrates in intestine of *O. mossambicus* in control as well as in fishes exposed for 10 days show good intensity of purple colour (glycogen). The villai and microvilli took good amount

of stain showing presence of carbohydrates. No marked change was observed with 50 mg/L concentration, although the colour of the stain was lighter in other concentrations showing marked decrease in carbohydrate concentrations due to the exposure to cadmium sulphate at these concentrations after 10 days of exposure (Fig.7)

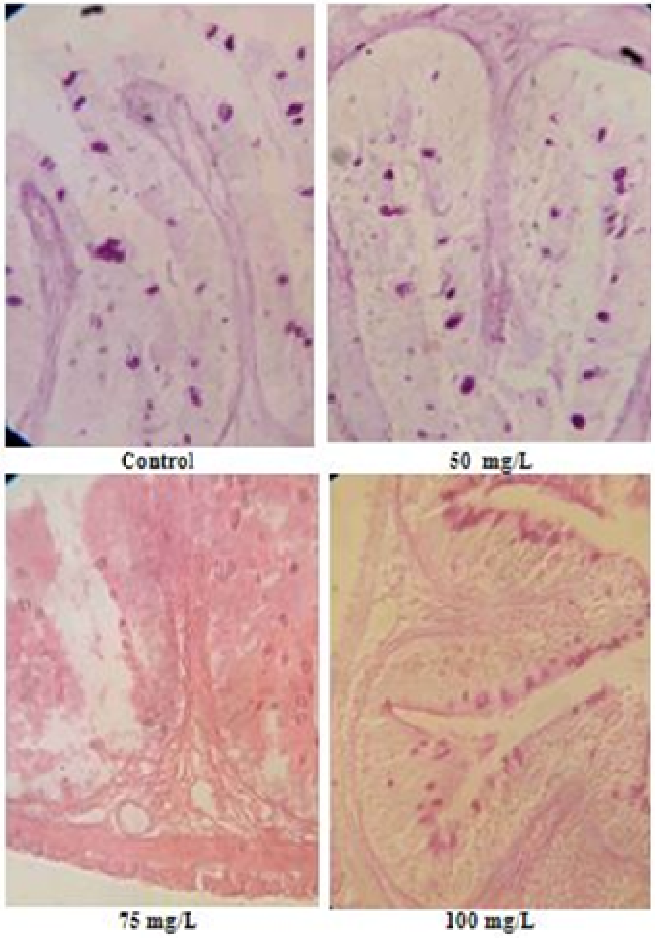
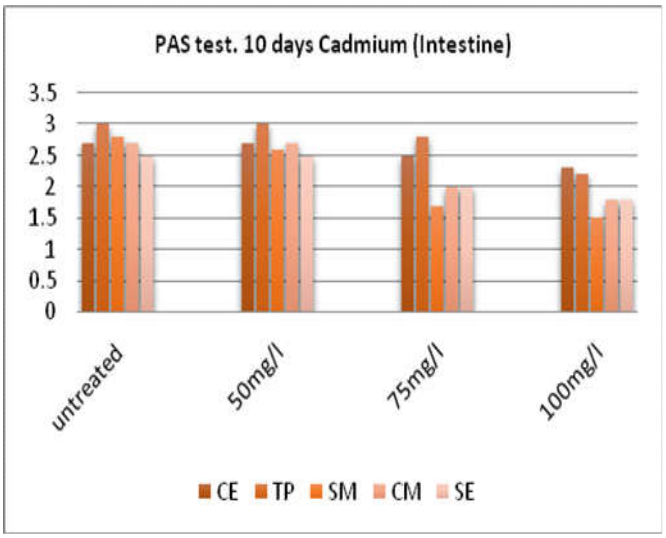


Fig 7: Effect of cadmium sulphate of different concentrations on carbohydrates in the intestine of *Oreochromis mossambicus* after 10 days of exposure as is shown by Periodic Acid Schiff's (PAS) staining (400x)



CE- Columnar epithelium, TP- Tunica Propria, SM- Sub Mucosa, CM-Circular Muscles, SE-Serosa

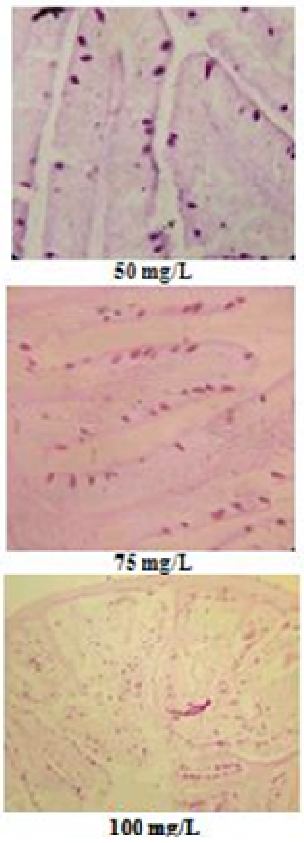
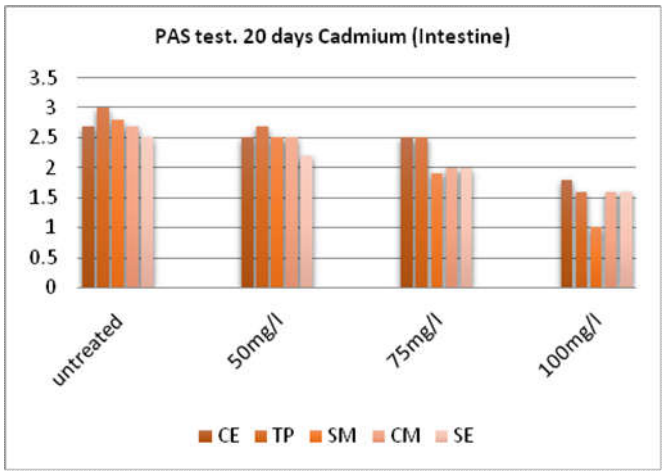


Fig 8: Effect of cadmium sulphate of different concentrations on carbohydrates in the intestine of *Oreochromis mossambicus* after 20 days of exposure as is shown by Periodic Acid Schiff's (PAS) staining (400x)



The fishes exposed to 50 mg/l concentration after 20 days were still comparable to control. The sections from other concentrations that is 75mg/l and 100mg/l of cadmium sulphate showed decreased intensity of the PAS stain. (Fig.8) The present study showed remarkable depletion of protein content in liver and intestine of *O. mossambicus* after exposure to cadmium sulphate in varying concentrations. Present findings are supported by the work of Almeida *et al*., (2001) who studied the environmental cadmium exposure and metabolic responses of the Nile tilapia, *Oreochromis niloticus.* The authors showed that cadmium significantly decrease protein concentrations in liver and Intestine. Our study is similar with the results of Lakshmanan *et al*., (2013) In their

study they showed that when *O. mossambicus* is treated with sub lethel doses of Dichlorvos for all the exposure period, it shows a significant decrease in protein content. Similar results were found by Vincent *et al.,* (1995) who reported in liver and intestine that, protein and carbohydrate contents decrease with increase in exposure period as well as concentration of the chromium in *Catla catla*. Similar study was also made by Dhakad and Sharma (1995) and Jones and Kumar (1996). The depletion in protein from tissue may be due to the excretion of proteins through kidney under toxicant stress. The depletion in tissue proteins of fish may be due to impaired or low rate of protein synthesis under metallic stress or due to their utilization in the formation of mucoproteins, which are eliminated in the form of mucous (Nagai and Ikeda, 1971). During our study, PAS staining showed depletion in glycogen levels in liver and intestine of *O. mossambicus.* Glycogen depletion in liver and muscle after toxic stress has been reported in several studies with aquatic animals (Bhavan and Geraldine, 1997; Aguiar *et al.,* 2004). (Rao, 2006) showed that the glycogen levels of different tissues showed a decreasing trend as exposure progressed. More or less similar results were found by Garg *et al*., (2009) who studied effect of cadmium on Indian major carps and found that heavy metal treatments in general showed significant reduction in carbohydrate and lipid contents in muscles as well as in gills in all the fish species. Other authors who have found similar results were Viswaranjan *et al.,* (1998), (Neeraja and Rao, 1998) and palanichamy *et al*., (1989) who have studied that the chemical effluents on the body components like proteins and carbohydrates content of muscles, liver, gill and intestine decreased with the increased concentration of effluents.

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