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Biochemical and histopathological studies on lead nitrate induced toxicity in fresh water fish *grass carp* (*Ctenopharyngodon Idella)*

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ABSTRACT

Fish species were recently suggested as environmental biomarkers. Measuring heavy metals in aquatic organisms may be a bioindicators of their impact on organisms and ecosystem health. From bioaccumulation studies, the proportion of lead was found to be significantly higher in different tissues of fish. In the present study the acute toxicity of lead nitrate was analyzed by the exposure of the lead nitrate to the fresh water fish Grass carp (ctenopharyngodon idella) at the time interval of 24hrs, 48hrs, 72hrs, 96hrs in the concentration of (5.15 mg/l). The vital organs like Gill, Liver, and muscle tissues showed pronounced effect on the activity of enzymes GOT (Glutamate oxaloacetate transaminase), GPT (Glutamate pyruvate transaminase) ALP, and Acid phosphatase (ACP) and Alkaline phosphatase (ALP). The antioxidant activity was also evaluated. A significant decreased in the activity of enzymes GOT, GPT, SOD (superoxide dismutase) and CAT (catalase) was noted. The enzymes ALP and ACP levels were found to be increased in all the tissues. Histopathological examination of liver, gill and muscle revealed the cellular degeneration, inflammation of the organs due to metal induced free radical generation and oxidative stress.

Key words: Lead nitrate; Toxicity; Inflammation; antioxidant; oxidative stress.

INTRODUCTION

The ecosystem and the marine environment is damaged by the continuous discharge of the heavy metals [1]. Heavy metal has the ability to enter the water sources and thus affects the aquatic lives. The natural and anthropogenic activities increase the concentration of metal ions in the aquatic environment. Due to the potential uptake of the metals through the gills and skin, the requirement of metals for fish is much more composite than the mammals. The metal content of the tissues play an important role in the life of aquatic animals. The metabolic activity of fish needs some metals in higher level and some in trace amounts [2]. They must be taken from water, food, and sediments. However, both the essential and nonessential metals enter simultaneously into the fish and accumulated. Accumulation of heavy metals in the various organs of the fish dependent mainly based upon the contamination of water and the time of exposure .The acute toxicity of the heavy metals changes the enzyme activities which often reflects cell or organ damage in specific organs. [3]. By the food chain the heavy metals enter in the human and causing various diseases and destroy the organs [4,5,6]. The liver and kidney are the major target organs to detoxify

the heavy metals induced toxicity [7]. Analysis of Biochemical parameters helps to identify the target organs of toxicity and the general health status of animals [8]. Lead nitrate is an inorganic compound. It affects the human central nervous system. It is a cancer causing agent in the lungs and kidneys. Lead act as a cumulative poison and causes an irreversible behavioral disturbance, neurological damage and other developmental problems on young children and infants [9, 10, 11]. Histopathological studies have been used to evaluate the effects of toxic chemicals on the morphologic and physiologic alterations of fish [12]. The objective of the study was to assess the lead nitrate induced toxicity in the fish Grass carp at the different time interval and to describe the histopathological alterations in the gills, liver and muscles of the specimens.

MATERIALS AND METHODS

Sample Collection and Maintenance

The live specimens of adult fresh water fish G*rass carp, (ctenopharyngodon idella),* body weight 20-60g (20-25 cm in length,) were collected from the pond water located at the Karayamputhoor area in Cuddalore district and used as the test animals. They were acclimatized to the laboratory conditions for few hours in plastic fish tanks. Physiochemical characteristics of the tap water used in experiment were temperature at 26^{o}C and pH-7. The toxicant was dissolved in water and mixed well in the fish tank. The tanks were provided with continuous aeration and were maintained under normal day & night. During acclimatization, the fish were fed with commercial pelleted food for 2-4 times per day. The water content of the tank was renewed after 24hrs, 48hrs, 72hrs, and 96hrs.

Experimental Design:

Toxicity Study:

Group-I : Control (Received only saline)
Group- II : 5.15mg of lead nitrate/l (24hrs).
Group-III : 5.15mg of lead nitrate /l (48hrs-2times at 24hrs time interval).
Group-IV : 5.15mg of lead nitrate /l (72hrs-3times at 24hrs time interval).
Group-V : 5.15mg of lead nitrate/l (96hrs-4times at 24hrs time interval).

The toxic substance lead nitrate was changed daily with fresh addition, after 24 hrs, 48hrs, 72hrs and 96hrs, the fishes were sacrificed at the end of the experimental period, the Gill, liver & muscles were excised for histopathological studies and the biochemical parameters were estimated.

Biochemical Analysis

Six fishes from each group were sacrificed at the end of 24hrs, 48hrs, 72hrs, and 96hrs. The vital organs like Gill, Liver, muscle of the fish were taken for the determination of the activity of the four enzymes. The activity of acid phosphatase and alkaline phosphatase, was determined by King and King method [13,14].The enzyme glutamate oxaloacetate transaminase and glutamate pyruvate transaminase activity was determined by Reitman and Frankal method [15] and the catalase activity was determined by the method of Sinha [16]. The Superoxide dismutase activity was estimated by the method of Kakkar et al.[17].

Statistical analysis of data

The data is expressed as mean ± Standard Deviation (SD). Statistical comparisons were performed by one-way analysis of variance followed by Duncan's Multiple Range Test. The results were considered statistically significant if the P values were less than 0.05.

RESULTS AND DICUSSION

Heavy metals are natural components of earth's crust. Large doses of heavy metals can enter the water and thus affect the aquatic organisms. The heavy metal accumulation in fish depends on both the structure and exposure of the tissues and organs to the environment. Alterations in the gills have been reported in the fish exposed to metals.

The purpose of the present study was to investigate the toxic effects of lead nitrate in *grass carp* at the different time interval of 24hrs, 48hrs, 72hrs and 96hrs. A significant decrease on the activities of enzymes such as Glutamate oxaloacetate transaminase (GOT), Glutamate pyruvate transaminase (GPT), and the activity of enzymatic antioxidants Superoxide Dismutase (SOD), and Catalase (CAT) was observed. The activity of Acid and Alkaline phosphatase (ACP & ALP) was gradually increased after the toxicity.

In our study it has been observed that lead nitrate decreased the level of GOT, GPT, activities in the liver ,gill &muscle of *grass carp* after 24hrs, 48hrs, 72hrs and 96hrs exposure (Fig.1 and Fig.2). A pronounced effect was noted on the activity of GOT & GPT level in 96hrs compared to the control value. The decreased activity of GOT & GPT may indicates disturbance in the cell organelles. Such damage to the cell organelles has been reported in various studies [18].

FIGURE 1 Variation in the level of GOT in certain tissues of fresh water fish *Grass carp* after 24, 48, 72, and 96hrs exposure to Lead nitrate

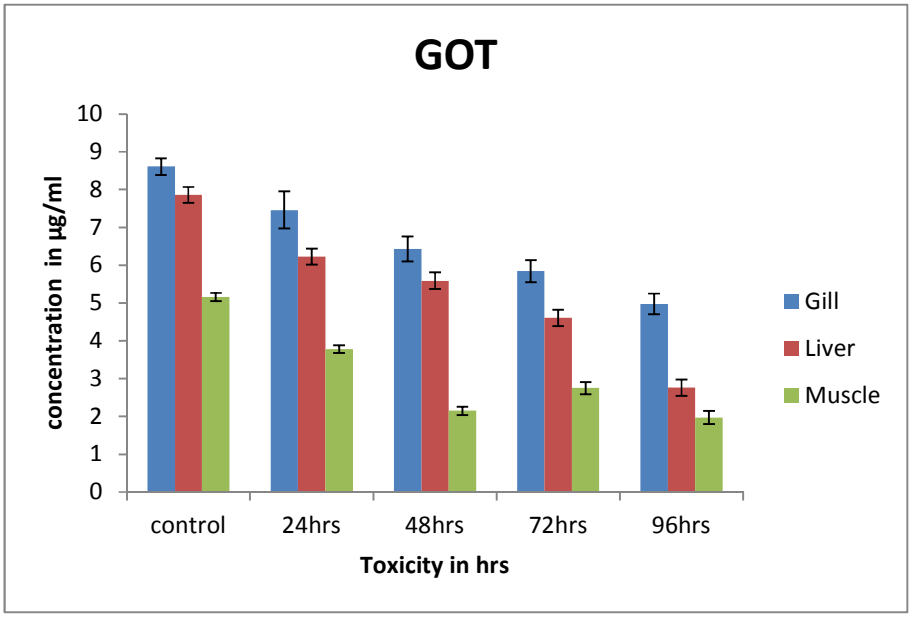
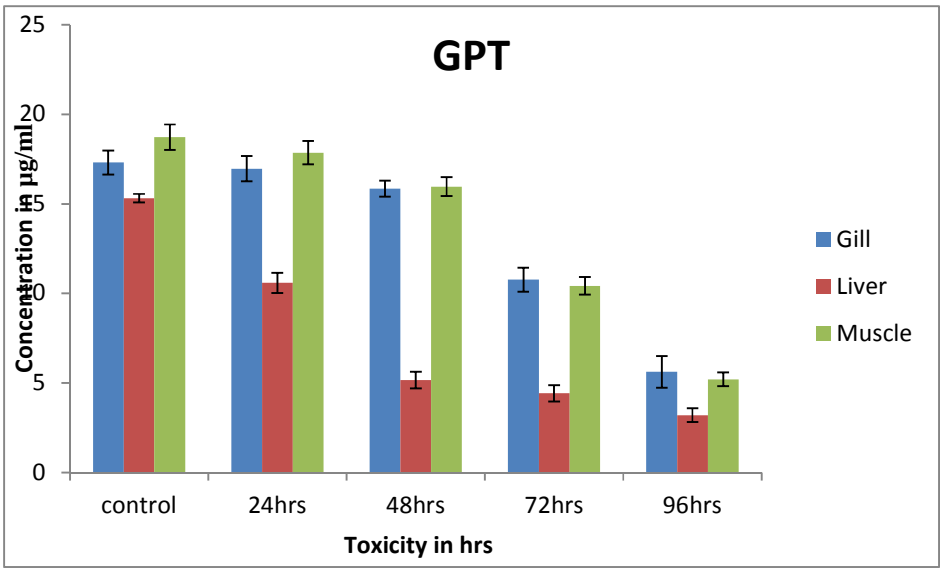


FIGURE 2 Variation in the level of GPT in certain tissues of fresh water fish *Grass carp* after 24, 48, 72, and 96hrs Exposure to lead nitrate



Acid phosphatase is a marker enzyme of lysosome and exist in a latent form. They are characterized by their ability to hydrolyze a large variety of organic phosphate esters. Alteration in the enzyme activity is due to adverse effect of xenobiotics on the cell and its organelles [19]. Alkaline phosphatase is a brush border enzyme mediates membrane transport [20]. It is known to be involved in a variety of metabolic activities such as permeability [21]. In our study

an increase in the level of ACP & ALP was observed in Gill, liver & muscle after the exposure of lead nitrate for 24hrs, 48hrs, 72hrs and 96hrs (Fig.3& Fig 4). Stimulation or inhibition of enzyme can result in the disturbance of metabolism. The elevated level of Alkaline phosphatase may be due to the liver diseases and portal cirrhosis [22]. Striking elevations of plasma ALP are usually associated the cholestatic disorders and is due to in an increased synthesis and reduced biliary excretion of ALP [23].

FIGURE 3 Variation in the ACP level of certain tissues of fresh water fish *Grass carp* after 24, 48, 72, and 96hrs Exposure to Lead nitrate

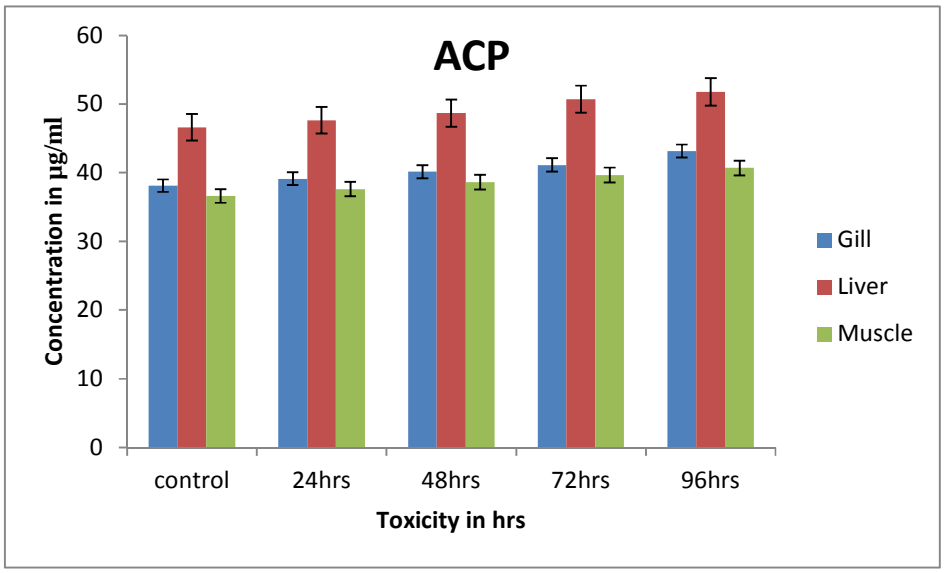
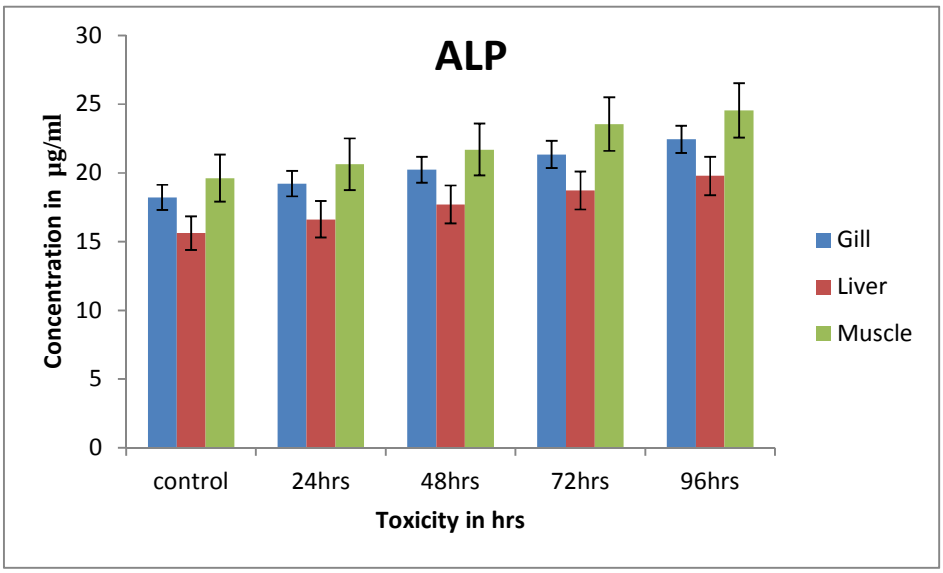


FIGURE 4 Variation in the ALP level of certain tissues of fresh water fish *Grass carp* after 24, 48, 72, and 96hrs Exposure to Lead nitrate



Free radicals and other reactive oxygen species (ROS) have been recently incriminated in the pathogenesis of various metal toxicities [24]. The level of antioxidant enzyme is a good indicator for the impact of pollutants like heavy metals [25]. An antioxidant enzyme such as CAT and SOD prevents oxidative stress [26]. The SOD-CAT system provides the first defense against oxygen toxicity [27]. In our study the activities of CAT and SOD activity was decreased in the gill, liver & muscle tissue of fish exposed to lead nitrate (Fig 5 & Fig 6). The low level of CAT in the liver tissue could be attributed to the high production of peroxide radicals responsible for the oxidative stress

[28]. The decreased level of SOD and CAT indicates that it may play a role in the suppression of oxygen free radical formation in gill, liver & muscle tissue. Similar result was observed by the heavy metal toxication in the fish [29]. The alterations in the activity of the biochemical parameters may be used as a tool in biomonitoring the toxicity of lead nitrate in the aquatic environment

FIGURE: 5 Variation in the SOD level of certain tissues of fresh water fish *Grass carp* after 24, 48, 72, and 96hrs Exposure to Lead nitrate

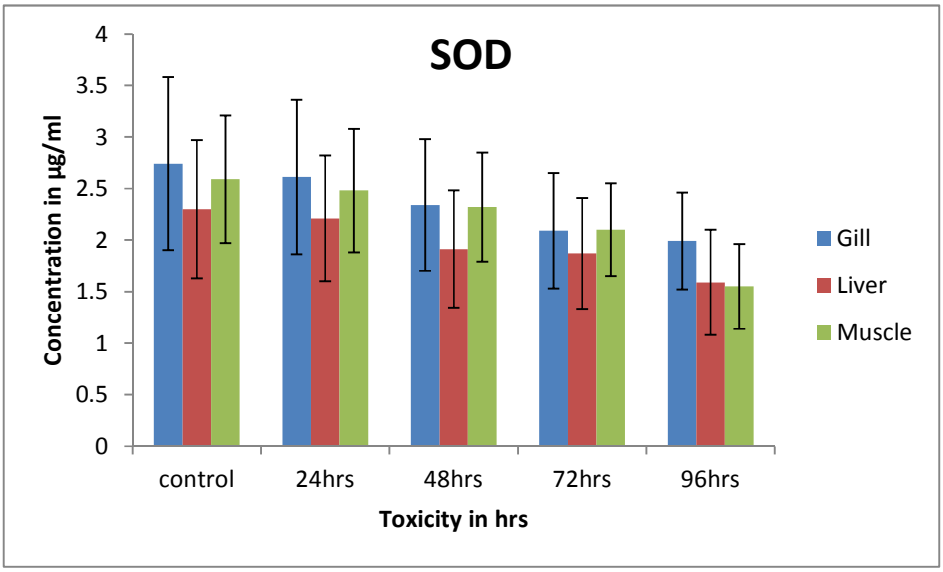
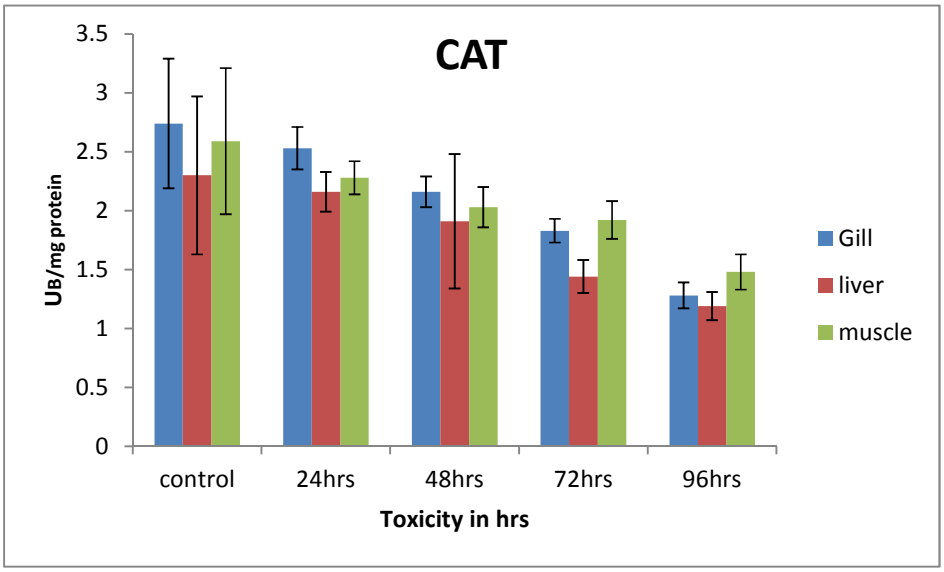


FIGURE: 6 Variation in the CAT level of certain tissues of fresh water fish grass carp after 24, 48, 72, and 96hrs Exposure to Lead nitrate

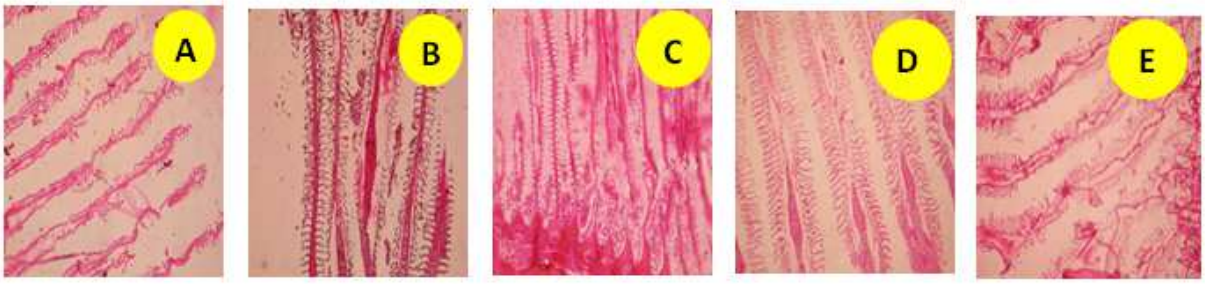


Histopathology

The heavy metal damage is an important factor in many pathological and toxicological processes [25]. Gill is an important tissue because of its direct contact with water and any effect or agency has to go through it to come into the fish body. The lamella epithelial lining reacts to dissolved lead creating tissues osmoregulatory imbalance [30]. The observed changes in gills such as hyperplasia, lamellar fusion, epithelial necrosis and edema were generally attributed to toxic effects of lead. Similar alterations in the gills have also been reported in the fish exposed to metals [31,32,33].

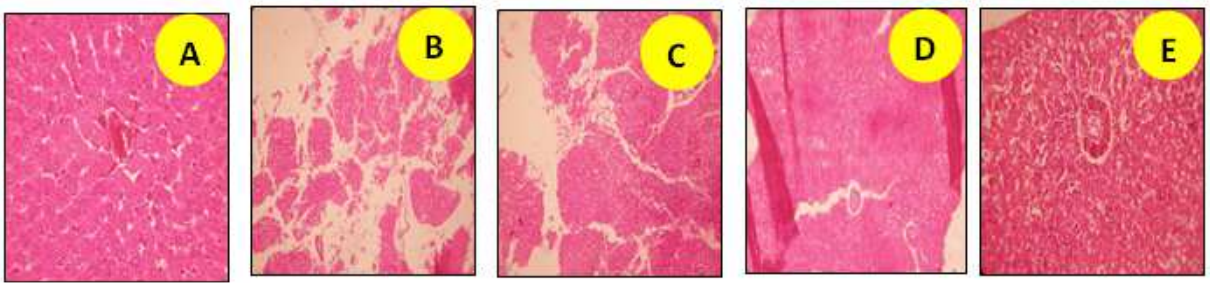
Pathological findings in liver included cytoplasmic vacuolation, cellular degeneration, swelling of hepatocytes, and focal necrosis. Histology of muscles included chronic inflammation, leucocyte infiltration, hyalinized muscle tissue, and edema of muscle bundles. These histopathological alterations confirmed the toxic effect of lead nitrate. In conclusion, lead nitrate induces gills, liver, and muscle damages as indicated by the elevation of histopathological alterations (Fig 6, Fig 7, and Fig 8) the decline of the enzyme and antioxidant activity.

Figure-6: Shows the histopathology of Gill after Lead intoxication of normal, 24, 48, 72 & 96hrs (10X)



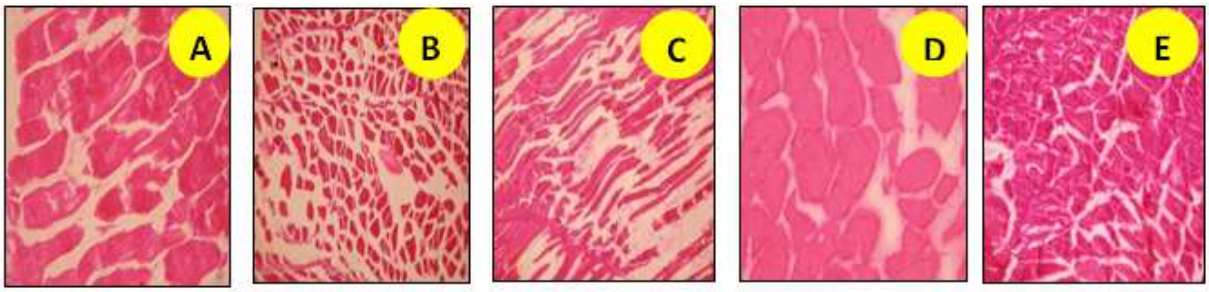
(A)Normal epithelial cell (B)Epithelial hyperplasia (C) Lamellar fusion (D) Epithelial necrosis(E) Edema

Figure-7: Shows the histopathology of Liver after Lead intoxication of normal, 24, 48, 72 & 96hrs (10X)



Liver photomicrograph of studied areas showing: (A) Normal hepatocyte (B) Liver sections showing cellular degeneration (C) Cytoplasmic vaculation. (D) Cloudy swelling of hepatocytes (E) Focal necrosis (H&E, X400).

Figure-8: Shows the histopathology of Muscle after Lead intoxication of normal, 24, 48, 72 & 96hrs (10X)



(A) Muscular bundles (B) Chronic inflammation (C) Leucocytic infiltration (D) Hyalinized muscles tissue (D) Edema of muscle bundles

CONCLUSION

Now days the assessment of environmental pollution cannot be based exclusively on chemical analysis since the toxic effect of pollutant does not provide a clear indication on the aquatic organisms. The unequivocal conclusion of ecosystem health requires the higher aquatic organisms. The present study, the exposure of lead nitrate toxicity to the Grass carp caused considerable alterations in the activities of enzymes and antioxidant activities at the different time interval of 24hrs, 48hrs, 72hrs and 96hrs. A significant decrease on the activities of enzymes such as GOT & GPT, and the activity of enzymatic antioxidants [SOD & CAT] was observed at the end of 96 hrs. The activity of ACP, ALP was gradually increased after the toxicity. In conclusion the lead nitrate toxicity induces gill, liver and

muscle damage as indicated by the histopathological alterations, the decline of the antioxidant activity. Further, the present study pointed out that enzymatic responses in fish exposed to contamination of metal in the environment can be used as indicator for both the tissue damage and alterations of biomarkers in pollution.

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