SEQUESTRATION PROPERTY OF *SPIRULINA* AGAINST LEAD INDUCED HISTOLOGICAL CHANGES IN MALE ALBINO WISTER RAT *RATTUS NORVEGICUS*

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ABSTRACT

Lead acetate induces the histological changes in liver and kidney tissues of the male albino Wister rat were observed by light microscopy. The rats were exposed to sublethal concentration of lead acetate 1.896 mg/kg body wt for seven days with parallel untreated control, lead acetate along with *spirulina* 2mg/Kg body wt and *spirulina* 2mg/Kg body wt groups were maintained. Liver and kidney tissue used for histological studies. No pathological changes were observed in the untreated control and *spirulina* alone groups. Lead acetate treated group shows sevier histological changes were noticed and lead acetate along *spirulina* treated group cell recover was observed.

Keywords: lead acetate, *Spirulina*, histology, albino rat.

INTRODUCTION

Heavy metals are extremely toxic and ubiquitous in natural environments and they occur in soil, surface water and plants, which readily mobilized by human activities such as mining and dumping of industrial waste in natural habitats such as forests, rivers, lakes and ocean¹. Metals are non-biodegradable and also considered as major environmental pollutants causing cytotoxic mutagenic and carcinogenic effects in animals². Among the heavy metals lead (Pb) are found at relatively high levels in crops, vegetables and pasturage herbs for animals³. These metals are transported and concentrated through the food chain and can accumulated in the human body and cause chronic poisoning, leading to cancers and other diseases³. Lead toxicity is probably the most common form of heavy metal intoxication. It is well documented that, one of the most dangerous and insidious poisons. It continuous environmental and occupational exposure may contribute to renal, nervous, hepatic, hematological and reproductive disorders in man and animals⁴. Liver and kidney being one of the major organs involved in the storage, biotransformation and detoxification of toxic substances. The observed toxicity induced by lead acetate in rats was similar to those previously reported by^{5,6,7}. Histological changes provide a method to detect effects of irritants, especially chronic, in various tissues and organs⁸. *Spirulina*, a blue green algae known for its rich energy value, is used as a protein supplement approximately by 100 million people worldwide, especially in countries like Japan, USA, Thailand Taiwan, India, etc *Spirulina* has been labeled as a powerful food, rich in proteins, carbohydrates, polyunsaturated fatty acids, sterols and some more vital elements like calcium, iron, zinc, magnesium, manganese and selenium. It is a natural source of vitamin B₁₂, vitamin E, ascorbic acid, tocopherols and whole spectrum of natural mixed carotene and xanthophyll phytopigments⁹. In the present investigation lead induced histological changes in the liver and kidney tissue of Albino Wister rat and ameliorative effect of *Spirulina* on the lead toxicity.

MATERIALS AND METHODS

Animal selection and maintenance

Adult male albino Wister rats (170-200g) maintained in the University animal house. These were maintained on commercial pellet feed, water and libitum. The ethical committee of Raja Muthaiya Medical College, Annamalai University approved. Before experimentation the animals were housed in PVC cages, which were closed by metal grid in the departmental animal room for the acclimation. The animals were maintained under standard laboratory conditions. They were fed standard diet pellets and water was provided *ad libitum*.

Toxicity studies

The heavy metal lead acetated is used as sublethal dose (LD₅₀ value is 1.896 mg of lead/kg by body weight of the animal for 7 days).

Spirulina powder

spirulina in the form of powder was obtained from *spirulina* farm auroville, puducherry.

Drug administration

The rats were treated with lead acetate and *Spirulina* in the following way:

1. Lead acetate was weighed accurately and dissolved in distilled water.
2. The *Spirulina* weighed and dissolved in distilled water.
3. Lead acetate and *Spirulina* were administered orally into the mouth cavity of the experimental rat with the help of syringe whose tip ended with infant feeding tube.

Experimental design

After acclimation the rat were divided into four groups. Each group consisted of 5 animals.

Group I: Control

Group II: Oral administration of lead acetate 1.896 mg/kg body wt for 7 days.

Group III: Co-administration of lead acetate 1.896 mg/kg body wt and *Spirulina* 2 mg/body wt. orally for 7 days.

Group IV: *Spirulina* 2 mg/kg body wt for 7 days.

At the end of the experiment, all the groups were sacrificed liver and kidney tissue of each animal was dissected for histological studies.

Histological studies

To examine the extent of cellular damage caused by lead acetate, the liver and kidney of the control and treated tissues were fixed in Bouin's fluid. After 24 hours, the standard histological technique was followed by the method of Gurr¹⁰.

Liver Tissues

Figure 1: Liver group1

Figure 2: Liver group 2

Figure 3: Liver group 3

Figure 4: Liver group 4

Figure 1 shows control liver slid, No: 2 shows lead acetate treated (group2), No: 3 shows recover (group 3) lead acetate and *Spirulina* treated liver, No: 4 shows *Spirulina* alone treated (group 4) Hepatocyte. HC- Hepatic cells, PN – Pycnotic nucleus, N – Nucleus, V – Vacuolation S – Sinusoids, VA – Vacuoles .

Kidney Tissues

Figure 5: Kidney (group 1)

Figure 6: Kidney (group 2)

Figure 7: Kidney (group 3)

Figure 8: Kidney (group 4)

Figure 1 the control kidney cells (group1), No:2 shows lead acetate teated (group 2) kidney tissues, No:3 shows lead acetate and *Spirulina* treated (group3) kidney tissue, No:4 shows *Spirulina* alone treated (group 4) kidney tissues. D – Distal convoluted tubule, P – Proximal convoluted tubule, E- Epithelial BC – Bowman's capsule, HE – Hemopoitic tissues, R- Renal tubule. RB – Ruptured cell boundary, V – Vacuolation

RESULT AND DISCUSSION

The liver of rat exposed to sublethal concentration of lead acetate shows (Figure 2) drastic changes in the cellular architecture compared to the untreated control (Figure 1). The outer membrane of the liver is ruptured at many points. Variations in the space of hepatocytes are evident in different region of the liver. The blood vessels are destroyed leaving large spaces (vacuoles) with damaged red blood cells (Figure 2). Deterioration of cellular architecture with marked necrosis is also observed. The hepatocytes are found usually in groups with conspicuous spaces between them. Hepatocytes are posses a markedly thickened nuclear and cytoplasmic membrane. This is obliterated at some places. The hepatic ducts have become larger in size. Inter hepatic spaces have been dilated and orientation of nuclei is promiscuous. Nuclear pycnosis, cytoplasmic lysis and karyolysis are observed in different regions. (Figure 2) The degenerated paranchymal cells showed abnormal enlargement and these cells are called hypertropic sized cells, which have increased the volume of the liver. In group III lead acetate along with *Spirulina* administered rat shows slight cytoplasmic vacuolization, lateralization and condensation of the nuclei were also observed (Figure 3). The group IV *Spirulina* alone administered rat showed the histological pattern to near normalcy (Figure 4). In the present study, lead hepatotoxicity manifests itself in disorganization of the hepatic cords, cytoplasmic vacuolization and invading of infiltrative inflammatory cells^{11 12}. Since chronic lead toxicity affect on a range of cellular enzymes particularly those involved in energy

production and associated with massive dilated mitochondria leading to hydropic degeneration appear as cytoplasmic vacuolization¹³. However, mild and to a lesser extent, moderate degrees of hydropic degeneration and mild portal cellular infiltration were seen in 50-60% of rats exposed to Pb + *Spirulina*. The histological examination of the liver tissue of the animals treated with Pb revealed severe histopathological changes typical to those reported in the literature. Similar observations were reported by^{14,17}. When *Spirulina* was given to lead treated rats, the liver had essentially normal appearance in histopathological examination. *Spirulina* exhibit some protective effects against Pb intoxication. Albino rat administered to lead acetate has produced some conspicuous histopathological changes in the kidney. The distal and collecting convoluted tubules of the kidney undergo degeneration and have a large lumen due to hypertrophy (Figure 5). The cells of the kidney are destroyed. Vacuolization, shrinkage and breakage of tissue, degeneration of tubular epithelium and swollen nuclei are seen in the kidney (Figure 5). In the some areas, the cells boundaries are disrupted and hence the cells become indistinct. There is an aggregation of blood cells in some areas (Figure 6). Lead administered along with *Spirulina* exhibited higher protective effects against lead when compared to lead administered rats (Figure 5) while *Spirulina* alone administered rats (Figure 7) showed histological pattern to near normalcy. In group III, lead acetate along with *Spirulina* administered rat shows recovered from the toxic effect of lead (group II) epithelial cells of the renal tubules with the consequent

reduction in tubular lumens (Figure 6). The group IV administered rats' histological pattern to near normal (Figure 8). In the kidneys, lead intoxication causes, among other reactions, intestinal fibrosis, as well as both hyperplasia and gradual atrophy of tubules and glomeruli¹⁸. It is well known that chronic lead exposure also results in glomerular and tubulointerstitial changes that lead to glycosuria, proteinuria and chronic renal failure and hypertension^{19, 20}. Another histological indication of Pb toxicity in the rat kidney shows the karyomegaly of tubular cells²¹. Tubular, intestinal and glomerular damage these findings were supported by the findings of^{22, 23}. Inclusion bodies rapidly disappear with chelation therapy²⁴. This may explain the improvement of histological findings in the kidneys, especially number of cells exhibiting. Karyomegaly and intranuclear inclusions Pb intoxicated rats' supplemented with *Spirulina*. Several studies have focused on physiological properties of some valuable antiviral or antioxidant compounds in blue green alga *Spirulina*^{25, 26, 27}. *Spirulina* species exhibit various biological activities such as antitumour^{28, 29} Antimicrobial³⁰ metalloprotective³¹.

CONCLUSION

In the present investigation leads induce cell damages causes' histological alteration in the cells. Supplementary *spirulina* enhance the cell repairing mechanism and chelating the toxic lead substance to the animal body. This chelating capability may happen due to nutritive elements and vitamins present in the *spirulina*.

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