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Anomaly in Blood Cells of Fish *Labeo rohita* Resulting from Cobalt Sulphate Toxicity

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ABSTRACT: Heavy metals and its compounds have severely affected the aquatic ecosystem and human health. They are naturally occurring substances which are present in the environment at low and tolerable levels. In large amount they prove to be hazardous. The study carried out in this paper is of heavy metal Cobalt. Cobalt Sulphate is ingested by aquatic life and human beings through (drinking or eating) or inhalation (breathing). Working in or living near on industrial site increases the risk of exposure. The impact of toxic effect of Cobalt Sulphate was experimentally studied in fish "*Labeo rohita*". This metal affects reticule endothelial system and haematopoiesis. They change osmotic resistance of erythrocytes. It has been found out that Red and White blood cells at different stages of the pathological process are subjected to quantitative and qualitative changes, deformations, large scale destructions, cytoplasm (swelling, changes in granulation and degree of coloration, vacuolization etc.) and nucleus (changes in structure color, partial or complete discoloration of nuclear matter, division, extrusion of nuclei etc.). In human beings also this effect is observed. Liver, kidney, heart, joints etc. are damaged. The liver and kidneys produce metallothioneins and cause toxicity which is hazardous to human life.

KEY WORDS: Cobalt toxicity, Hematological studies, Cobalt sulphate, *Labeo rohita*, Heavy metal effect on human life.

I. INTRODUCTION

Heavy metals have a great negative impact on the marine organisms. This effect ranges from behavioral and ecological to physiological changes which in turn reflect on economically, nutritionally and culturally important fish species. Heavy metals are soft but highly toxic as they compete for binding with essential metals [1, 2, 3, 4] and consequently they interfere with sulphhydryl groups that play an important role for normal function of enzymes and structural proteins.

Inhalation of cobalt dust leads to health problems like severe asthma, blood abnormalities, infections in the lungs, inflammation of the lungs and the nasal tract; and fibrosis formation in and around vital organs of the body. Such affected people are those involved in cobalt mining operations and those involved with diamond polishing works. Carcinogenesis of lungs is the deadly result of cobalt inhalation from the dust generated by mining and the adhesives that contain cobalt on the polishing disc of the diamond polishing machine. Use of safety gear to minimize the cobalt dust inhalation is the only way to safeguard people involved in these trades. Tobacco smokers and chewers are at a greater risk of fatal consequences when exposed to an atmosphere charged with cobalt particles [16].

Heavy metals are diluted and affected by various surface water components (carbonate, sulphate, organic compounds – humic, fulvic, amino acids) after entering natural water bodies which induces formation of insoluble salts or complexes. These salts and complexes are predicted to be not harmful to aquatic organisms [5]. Any alteration in the chemical composition of natural aquatic environment usually induces changes in the biochemical aspects of the inhabitants particularly fishes. These alterations in body metabolism due to organochlorine intoxication have been extensively studied [6, 7].

Heavy metals are beneficial for living organisms under normal conditions but they tend to be toxic when their concentrations cross the established safe limits [8, 9]. Presently, heavy metal environmental pollution becomes an alarming signal and global issue for man, as it may cause deleterious and adverse effects on the existing natural

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ecological balance of the recipient environment [10]. Various toxic chemicals produced during industrial and agricultural activities contaminate aquatic ecosystems [11]. Aquatic pollution with metals is a global environment problem. Among various pollutants, heavy metal are unique in their action due to their non-biodegradability [12], prosperity of bio-magnification in food chain [13] and their effects on the ecological equilibrium of the recipient aquatic body and diversity of aquatic organisms [14].

II. MATERIALS AND METHODS

A. PROCESSING OF THE FRESH WATER FINGERLINGS MAJOR CARP *LABEO ROHITA* FOR STUDY:

Live and healthy fresh water fingerlings major carp *Labeo rohita* of both sexes were collected from the local fish farms. The fingerlings were transported in polythene bag containing O₂ saturated water and brought to the aquarium of the size of 4' x 2' x 2' nearly 125 to 160 fingerlings of *Labeo rohita* and were kept as stock. Aquarium containing normal fresh water, commercial fish food was given twice a day. Dissolved Oxygen level and pH of water was maintained in the laboratory. Experiments were commenced after acclimatizing the fishes to the lab condition for a period ranging from 15 to 20 days. Different concentrations of Cobalt were made by dissolving appropriate amount of analytical grade Cobalt sulphate in the fresh water. Live and healthy fresh water fingerlings were exposed to different concentration of Cobalt sulphate. After exposing to different concentration for different durations, 2–3 fingerlings were removed and sacrificed for blood smear study. The thin blood smears were prepared for observing the cellular alterations in the blood cells. The smears were stained with Leishmann's stain and the observations were noted in a tabular form.

B. PREPARATION OF HEAVY METAL SAMPLES AND PROCESSING OF FINGERLINGS:

Different concentration of Cobalt was made by dissolving appropriate amount of analytical grade Cobalt sulphate in the fresh water. These sets of different concentration of Cobalt sulphate (10, 20, 30 ppm) were prepared for treatment. The fish were divided into different group of 3 individuals each in separate polythene boxes. Commercially available fish food was provided twice a day and water medium with Cobalt sulphate was changed every alternate day.

C. PREPARATION OF BLOOD SMEAR:

Live and healthy fresh water fish subjected to different concentration of Cobalt sulphate (10, 20, 30 ppm). The blood smear was prepared for observation. After an interval of 15 days fish were removed from each of container and the blood was drawn from the dorsal aorta or from pressing of gills. A drop of blood was taken on a glass slide and a thin smear was made. It was allowed to dry at room temperature. The slide was then stained with Leishmann's stain for 10 minutes. Then it was washed with distilled water and allowed to dry at room temperature and studied under light microscope.

III. RESULT

Effect of varying concentrations of Cobalt sulphate on the normal blood cells of *Labeo rohita*:

Concentration of Cobalt sulphate	Effect on RBC on Exposure of		
	15 days	30 days	45 days
10 ppm	The cell membrane is damaged with an enlarged nucleus.	The nucleus shifts in position.	The nucleus shifts in position with damage to the cell membrane.
20 ppm	The nucleus is enlarged and damaged.	The nucleus is enlarged with vacullation.	The cell membrane is damaged with vacullation.
30 ppm	The cell membrane is damaged and nucleus enlarged.	The nucleus is enlarged and shifts in position.	The nucleus is damaged and with wrinkled cell membrane.

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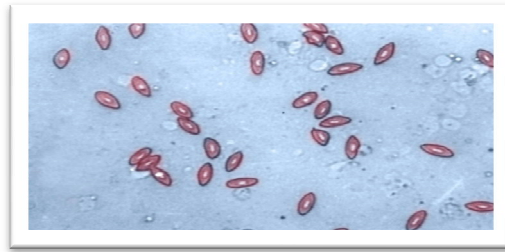


Figure : 1

Normal blood smear of *Labeo rohita*

Figure 1: The figure depicts all the cells in equal size and symmetrical shape. The cell membrane of RBC is intact, vacuolation is unseen. The nucleus is centrally located and is normal in size.

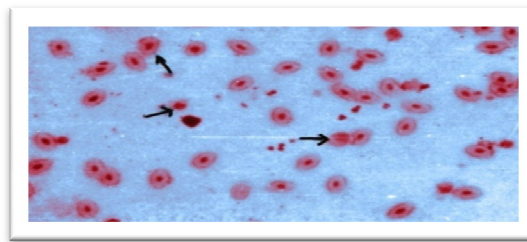


Figure : 2

Damaged blood smear of *Labeo rohita* with vacuolation

Figure 2: This figure shows the Fish *Labeo rohita* showing the cell membrane of RBC's damaged. Vacuolation has also been observed which indicates permanent damage to the cell. Here there is no change in the size and the location of the nucleus.

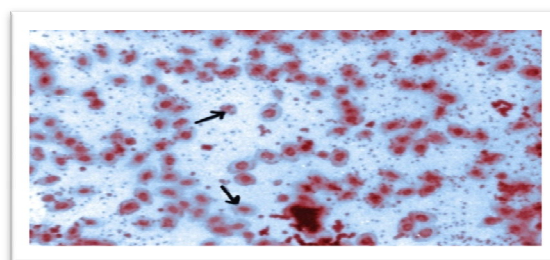


Figure : 3

Damaged blood smear of *Labeo rohita* showing change in the size of the nucleus without vacuolation

Figure 3: This figure is of the blood smear of Fish *Labeo rohita* showing the cell membrane of RBC damaged with considerable enlargement in the size of the nucleus. in this case vacillation is predominantly absent.

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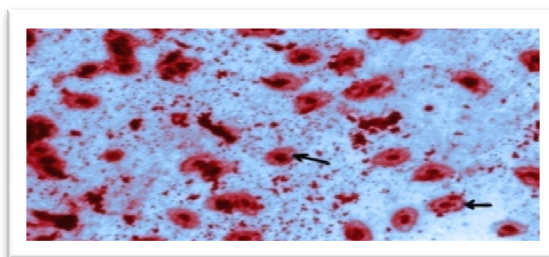


Figure : 4

Damaged blood smear of *Labeo rohita* showing change in the size of the nucleus with vacuolation

Figure 4: In this figure of the blood smear of Fish *Labeo rohita*, nucleus of RBC have been enlarged with dislocation of the nucleus from its central position. Also, the cell membrane has been damaged.

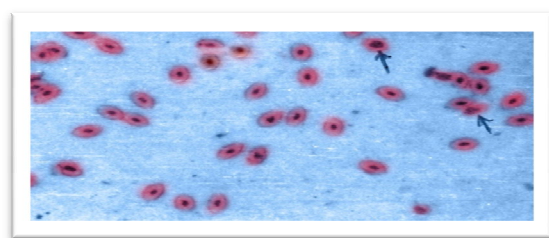


Figure : 5

Damaged blood smear of *Labeo rohita* showing change in the size of the nucleus with vacuolation

Figure 5: This figure of the blood smear of Fish *Labeo rohita* shows the nucleus of RBC to be damaged and the cell membrane wrinkled.

During the study of Cobalt sulphate on RBCs at 10 ppm concentration exposed to fish on 15 days duration, the cell membrane was damaged and nucleus enlarged but the nucleus shifting in position was comparatively less, or even nil. Arrows in the image point out to the changes in the cell membranes. During the study of Cobalt sulphate on RBCs 10 ppm concentration exposed to fish on further exposure of 30 days duration, the nucleus shift in position was also more. Cell membrane damage was also high and vacuolation was also more. This has led to increased commercial losses. Actually, lesser cell membranes were observed in the image, which indicated loss of membrane content. During the study of Cobalt sulphate on RBCs 10 ppm concentration exposed fish on 45 days the cell membrane was damaged and the nucleus shift in position was very high. Economic viability is less in such affected fish as it suffers from more weight loss also.

20 ppm of Cobalt sulphate concentration leads to more nucleus enlargement and damaged even in short term exposure of 15 days. This was comparatively higher compared to lower ppm dosage studied earlier for the same time duration. The arrows in the above figure points to the damaged nucleus with vacillation. 20 ppm of Cobalt sulphate concentration leads to the nucleus enlarged and damage on 30 days exposure the nucleus was more enlarged and vacuolation prominently observed. Cell membrane damage is also prominent in this exposure level. Final losses are on the economic and health fronts for the traders and the end users. 20 ppm of Cobalt sulphate concentration at 45 days exposure has led to an extreme nucleus enlargement and consequent damage. The exposure also led to cell membrane damage and vacuolation observed. End user is the major sufferer in case of such affected fish finding their way to their diet.

30 ppm concentration of Cobalt sulphate leads to cell membrane damage and nucleus enlargement 15 days duration. This damage was higher compared to that observed in lower concentration studies. Affected fish loose body weight very quickly and their survival levels are also low. 30 ppm concentration of Cobalt sulphate for 30 days exposure the

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nucleus has led to greater enlargement and shift in position of the nucleus. Vacuolation is also more and fish muscles were more affected. Survival of such affected fish is at stake, leading to losses for users.

30 ppm concentration of Cobalt sulphate leads to complete cell membrane damage and vacillation on 45 days duration. Nucleus enlargement and its shift in position were also extremely high, and in many cases destruction was total. This aspect of damage clearly implied lowering of its nutritional value in particular and commercial losses in general. Such fish when consumed by human leads to health related severe problems.

These types of condition like wrinkled cell membrane, damaged nucleus or enlarged nucleus, vacuolation lead either to death of cell in long run or are responsible for anemic condition of animals. Anemic conditions and less iron content have been reported by several workers in fish, bird and mammal following exposure to pollutants. It is also reported by [15] that RBCs count was declined by higher concentration of endosulfan subjected to fish. [15] postulated that the reduction of RBCs count might be due to inhibition of RBCs production and destruction of RBCs by pollutant. Heavy metals effect on reticule-endothelial system and haematopoeisis.

IV. CONCLUSION

A wide range of pollutants either physical – chemical – biological and radiological have been observed in the aquatic biota due to urbanization – industrialization and new technological development. Heavy metals are traced in some areas in sufficient concentration and physico – chemical forms that might create pollution problems. Sources of heavy metals in aquatic environment are from different industrial operation particularly South Gujarat region, which due to alarming industrialization will cause serious depletion in fisheries catch. During last decades, parallel with rapidly developing technology, increasing population and urbanization we have been witnessing alarming phenomena of pollution all over South Gujarat region.

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