*International Journal of Engineering Science Invention*
*ISSN (Online): 2319 – 6734, ISSN (Print): 2319 – 6726*
*www.ijesi.org Volume 3 Issue 6‖ June 2014 ‖ PP.44-52*

# An Experimental Studies on the Effect of Heavy Metals on Lymphoid Organ of *Channa gachua*

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***ABSTRACT :*** *In this study, the morphological, histological and cytological impacts of heavy metals on lymphoid organ of Channa gachua were investigated to understand the impact of selected heavy metals on lymphoid organ of fish which ultimately affects the immune response. The fish was treated with two different concentrations of three heavy metals viz. 0.22 and 0.32 mg/l of Cr, 0.22 and 0.32 of Cu and 0.22 and 0.32 of Co, below $LC_5$o dose. Red spots, loosening of scales and decolourisation of skin were noted morphologically while decrease in total red blood cell count, white blood cell count and heamocrite value was noted under hematological investigation. In differential WBC count, increase 'in number of granulocytes excluding neutrophils and decrease in number of agranulocytes and neutrophils were observed. In microscopic and electron microscopic examination, different grades of tissue destruction was noticed in ground matrix as well as in lymphoid follicles. All the changes noted under the present study clearly depict various levels of damage in lymphoid organs due to exposure of selected heavy metals and the maximum damage observed in case of cobalt.*

***KEYWORDS:*** *fish lymphoid follicle, Blood cells, lymphoid follicles, Histopathology, Electron microscopic study*

## I. INTRODUCTION

Fishes are frequently exposed to multiple, sequential or concurrent stressors. It appears that the physiological response to such events is adaptive in terms of resisting the stressors but could be maladaptive in terms of allostatic load [1,2]. Healthy fish exhibit both nonspecific and specific immune responses depending directly on environmental temperature. Pollution of the natural aquatic environment with industrial or agricultural sewage is an important immunosuppressing factor resulting in higher susceptibility to infectious diseases. Usually, the possible immunotoxicity of a substance is evaluated using quantification of humoral factors like lysozyme, complement, C-reactive protein or total immunoglobulins but less often using functional assays. Furthermore, most of the functional assays (phagocytosis, respiratory burst, proliferative response) are based on the measurement of the response of resting but not of specific activated immune cells. However, the physiological responses of the immune system to an infection are based on a complex, stepwise activation and proliferation, especially of the specific immune functions after first contact to the microorganisms.

The nonantigenic material is trapped mainly in three locations, splenic ellipsoids [3], sinusoidal blood vessels, macrophages and reticular cells of the kidney [3,4] and ventricular endothelial cells and atrialendocardial macrophages of the heart [5]. There are enough evidences to support the cytological damage in animals including fish also, due to higher concentration of heavy metals in aquatic environment [6-13]. The rapidly increasing pollutions in the aquatics system due effluents discharge from various sources, made it mandatory to understand the impact of these pollutants on the health of aquatic organisms. Thus, the present work is conducted to analyze the impact of different concentrations of heavy metals; Cr (VI), Cu (II) and Co (II) are evaluated on lymphoid cells of head kidney which are very prominent in the aquatic system. For the purpose of present work, some bottom dwelling fishes were required and thus the *Channa gachua* was suitable candidate for this purpose.

## II. MATERIAL AND METHODS

**Experimental Protocols**

Individuals of air breathing catfish *C. gachua*, were procured from local fish market in living condition and transported to laboratory. They were acclimatized for seven days in laboratory condition during which they were fed on minced goat liver @ 2% of their body weight. Length and weight of fish were measured and the individuals ranging between 14-18 cm in length and 25-35 g in weight were selected for experiment. To eliminate unwanted microflora, water was treated with antiviral drug (Zovirex:Wellcome, India), antibacterial

drugs (Gentamycin, Streptomycin and Penicillin : E-Merck, Germany) and antimycotic drug (Amphotericine B: Sarabhai, India). A group of ten individuals each was kept in two concentrations of copper sulfate ($CuSO_4$ 0.22 and 0.32 mg/1), potassium dichromate ($K_2Cr_2O_7$ 0.22 and 0.32 mg/1) and cobalt sulfate ($CoSO_4$ 0.22 and 0.32 mg/1) in aquaria of size 90x45x45 cm. Observations were made on 24 hourly basis continuously for 15 days and the mortality if any was recorded. The fresh water was used as a medium for control. After the termination of experiment, the individuals were sacrificed for collection of blood and head kidneys were dissected out for histological and cytological details. The temperature throughout the experiment was maintained at 20-24 $^oC$ and the water used was disinfected by using antibiotics and passed through UV light.

**Blood Collection and hematological Parameters**

The haematological parameters of fish exposed to different heavy metals were compared with apparently healthy individuals with no visual disease symptoms. For this purpose, the blood samples were taken from the caudal peduncle and heart with the help of 2 ml disposable syringe. R.B.Cs. and W.B.Cs. were counted with the help of haemocytometer. Blood smears were prepared and stained by the method given by Anderson, [14] for the differential count of W.B.Cs. Hemoglobin concentration was measured by using hemoglobin meter.

**Histopathology**

For the histological examination, dissected head kidneys were preserved in aqueous Bouin's fluid for 48-72 hours. They were then processed routinely and prepared into paraffin blocks. The blocks of the tissue were cut at 4-6 mm in thickness and stained with Delafield's Haematoxylin and Eosin (H.E.) and studied under microscopes on different magnification and photographed.

**Electron Microscopy**

The samples for the electron microscopic studies were prepared as per procedure given by Madeley [15]. Head kidney tissue was fixed in Karnovosky's solution and ultra-thin sections were visualized under transmission electron microscope at the All India Institute of Medical Sciences (AIIMS), New Delhi.

## III. OBSERVATIONS

**Effect on Fish Morphology**

The fish under controlled condition showed no superficial symptoms within 10 days of experiment. Overall reduction in mucus quantity on skin and disappearance of scales at certain places has observed in all most all cases. The fish kept in 0.22-mg/l concentration of chromium exhibited the appearance of red spots on the skin after 11$^{th}$ day of treatment. In case of 0.32-mg/l concentration of chromium the red spots were observed on 9$^{th}$ day of the experiment. The fish 0.22 mg/l concentration of cobalt showed the occurrence of red spots after the 7$^{th}$ day of experiment while those kept in 0.32 mg/1 concentration of cobalt showed the appearance of red spots after 6$^{th}$ day of experiment. The fishes kept in 0.22 mg/l concentration of copper exhibited decolourisation of skin after 6$^{th}$ day, descaling after the 8$^{th}$ day and red spots after 12$^{th}$ day of experiment. While in case of those kept in 0.32-mg/l concentration of copper, the decolourisation appeared after 3$^{rd}$ day, descaling after 4$^{th}$ day and red spots after 10$^{th}$ and haemorrhage after 13$^{th}$ day of the experiment (Tab.1).

Table- 1 Showing the result of experimental trials with heavy metals

| Days of treatment | **Control** | **Concs.of Cr (mg/l)** | | **Concs. of Cu (mg/l)** | | **Concs. of Co (mg/l)** | |
|---|---|---|---|---|---|---|---|
| | Water | 0.22 | 0.32 | 0.22 | 0.32 | 0.22 | 0.32 |
| 1 | - | - | - | - | **dc** | **dc** | - |
| 2 | - | - | **dc** | - | **ds** | - | **dc** |
| 3 | - | - | + | - | + | - | **ds** |
| 4 | - | **dc** | + | **dc** | + | **ds** | **Rs** |
| 5 | - | + | + | + | + | **Rs** | - |
| 6 | - | **ds** | **ds** | **ds** | + | + | + |
| 7 | - | + | **Rs** | + | + | + | + |
| 8 | - | + | + | + | **Rs** | + | + |
| 9 | - | **Rs** | + | + | + | + | + |
| 10 | - | + | + | **Rs** | + | + | + |
| 11 | - | + | + | + | **Hm** | + | + |
| 12 | - | + | + | + | + | + | **Hm** |
| 13 | - | + | + | + | + | + | + |

Hm – haemorrhage, dc – decolouration, Rs – Red spot, ds- descaleing

**Effect on Haematological Parameters**

The hematological parameters in the fishes of controlled set are found to be normal, in which the Hemoglobin count, Pack cell volume (PCV), Total Erythrocyte Count (TEC) and Total Leucocytes count (TLC) are (9.0), 2.4 x $10^6$ ± 0.05, 0.01x $10^3$ ± 0.14 and 42.6% respectively. The decrepitude from the normal, are noticed in all most all cases where the fishes are treated with the heavy metal at the experimental concentrations. The maximum reduction in the hemoglobin concentration in notices in the fishes treated with the set in which the fish is treated with 0.32 mg/l concentration of cobalt and this was 6.3. The values of PCV, TEC and TLC are also reduced significantly in this case and these are 35.8, 1.46x$10^6$±0.16 and 7.5 x$10^3$±0.12. In case of the fishes treated with copper (II), the values of hemoglobin, PCV, TEC, TLC was found to be 7.1-7.8, 37.5, 1.92-1.78 x $10^6$ ±0.16 and 7.9-7.6x$10^3$ ± 0.17 respectively, which are well within the range of the rest two other metals tested under present work (Tab 2).

Table – 2 showing the hematological parameters of healthy and experimentally treated *Channa gachua*

| **Set** | **Conc. of heavy metals (in mg/l)** | **TEC $10^6$/µl** | **TLC $10^3$/µl** | **Hb (%)** | **PCV (%)** |
|---|---|---|---|---|---|
| Control | ------- | 2.4 ± 0.05 | 10.01 ± 0.14 | 9.0 | 42.6 |
| Fishes with Chromium | 0.22 | 2.0 ± 0.12 | 8.13 ± 0.14 | 7.8 | 36 |
| | 0.32 | 1.89 ± 0.16 | 8.01 ± 0.13 | 7.2 | 38 |
| Fishes with copper | 0.22 | 1.92 ± 0.16 | 7.9 ± 0.17 | 7.1 | 37.5 |
| | 0.32 | 1.78 ± 0.16 | 7.6 ± 0.12 | 7.8 | 37.5 |
| Fishes with cobalt | 0.22 | 1.59 ± 0.21 | 7.5 ± 0.11 | 6.9 | 36 |
| | 0.32 | 1.46 ± 0.16 | 7.65 ± 0.13 | 6.3 | 35.8 |

TEC= Total Erythrocytes Count; TLC =Total Leucocytes count; Hb= Hemoglobin; PCV = Pack cell Volum

The differential leukocytes counts also reflected the condition of stress in the fishes, studied under present work. The maximum percentage of eosinophiles was 7.9, recorded in the fishes treated with the 0.22 mg/l concentration of the cobalt while the maximum percentage of neutrophils was 17.5, recorded in same set of fishes. Similarly, the maximum percentages of lymphocytes and monocytes are found to be 61.1 and 33 in set where the fishes treated with the chromium and cobalt respectively (Tab.3).

Table –3 Showing differential leucocytes count in healthy and experimentally treated *Channa gachua*

| **Set** | **Conc. of heavy metals (in mg/l)** | **Eosinophiles** | **Basophiles** | **Neutrophiles** | **Lymphocytes** | **Monocytes** |
|---|---|---|---|---|---|---|
| Control | ------- | 6.90 | 0 | 14.6 | 56.40 | 22.1 |
| Fishes Treated with Chromium | 0.22 | 7.40 | 0 | 7.1 | 59.10 | 26.4 |
| | 0.32 | 6.40 | 0 | 0.6 | 61.10 | 31.9 |
| Fishes treated with copper | 0.22 | 6.90 | 0.5 | 2.6 | 60.40 | 29.6 |
| | 0.32 | 7.10 | 0 | 12.8 | 55.5 | 24.6 |
| Fishes treated with cobalt | 0.22 | 7.90 | 0.5 | 17.5 | 51.2 | 22.9 |
| | 0.32 | 7.80 | 0.5 | 8.92 | 52.95 | 33.63 |

**Effect on Lymphoid Tissues :** The histological observations of kidney of untreated fish revealed two clear-cut parts; one that of ground matrix in which cells are found scattered and the second, which contains encapsulated lymphoid follicles, intermingled with the ground matrix. The lymphoid follicles are encapsulated by dense collagen tissue, from which tuberculae extended up to variable distance into follicle. The parenchyma of follicles consists of an open meshwork of reticuline fiber, which provides support for an ever-changing population of lymphocytes. The cortex consists of densely packed lymphocytes. In the present study, the cortex is not found dividing into outer and inner cortex, but lymphoid follicles are found to be distributed throughout the region without any distinction of cortical region. The lymphoid follicles seem to be comprised of an outer densely cell rich cortex and a pale stained medulla. The deeper cortex or Para cortical zone also densely cellular but has a more homogenous staining appearance. Some lymphoid follicles appear to be located deep in the Para cortex, which might be due to the plane of section. The superficial cortex, the Para cortex and medulla represent

the different centers of immunological activity in the lymph node being dominated by B-lymphocytes, T-lymphocytes and plasma cells, respectively (Fig.1).

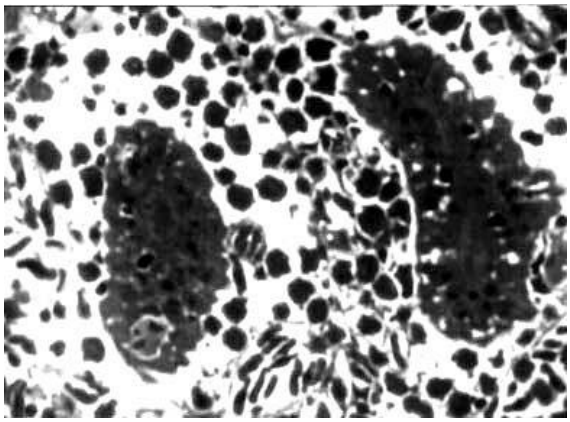

Fig. 1. Micrograph of a c.s. of a cephalokidney of *C.gachua* experimentally treated with Cu (x 450)

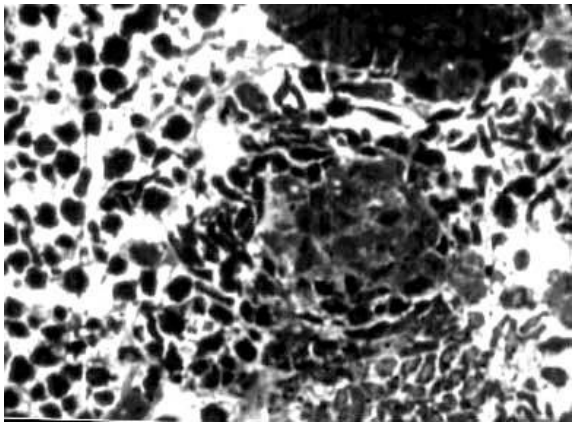

Fig.2. Micrograph of a c.s. of a cephalokidney of *C.gachua* experimentally treated with Cr (x 450)

The head kidney of fishes treated with cobalt sulfate (CoII) revealed conspicuous damage. The histological sections when observed under microscope exhibited great damage in the ground parenchyma, which has got disintegrated at many places resulting in formation of lacunae. Large number of haemopoietic cells is seen scattered in the ground parenchyma while the periphery of the lymphoid follicle is found to be damaged (Fig. 2).

The head kidney of fishes treated with potassium dichromate (Cr VI) also revealed conspicuous damage. Formation of several lacunae is observed in the ground parenchyma due to disintegration of the reticuline fibers but lymphoid follicles are found to be intact. Several crypts are observed in the tissue and huge amount of lymphoid cells is found to be scattered in ground substance due to rupturing of lymphoid follicles at certain places (Fig.3).The head kidney of fishes treated with copper sulfate (CuII) revealed considerable damage. The lymphoid follicles are also found to be considerably damaged and lymphoid cells are observed scattered throughout the ground matrix. Apart from this, the reticuline fibers are also completely damaged and cellular texture has got disintegrated and thus lymphoid follicles are also found to be disintegrated (Figs. 4).

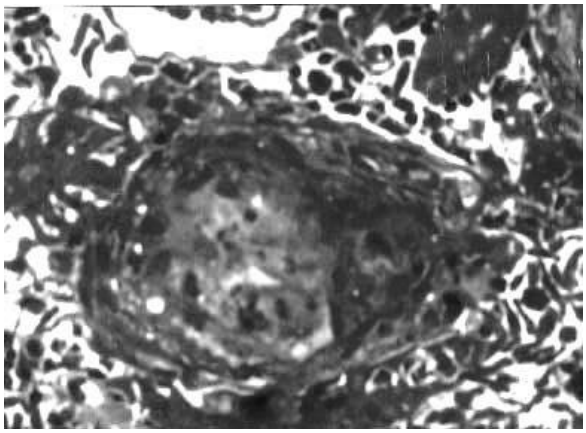

Fig.3 Micrograph of a c.s. of a cephalokidney (ck) of *C.gachua* experimentally treated with Co (x 450)

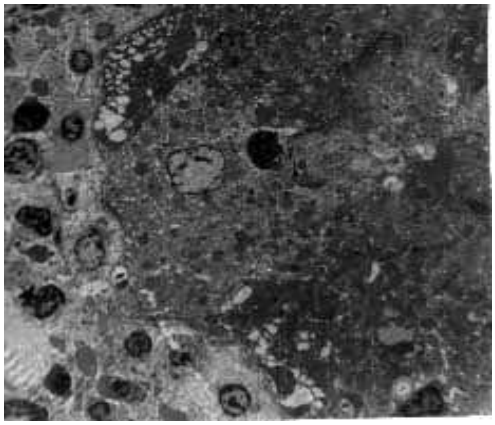

Fig.4 Ultrastructure of c.s. through (ck) of healthy *C.gachua* showing histological details (x 26.1K)

The ultra structural observations of the head kidney of untreated *C. gachua* are exhibited typical teleostean structure. Some lymphocytes seem to occupy defined areas in kidney. The lymphohemopiotic cells are seen scattered randomly throughout stroma of histoenzymatically heterogeneous fibroblastic reticular cells and sinusoidal blood vessels. Every hemopiotic cell lineage seems to be differentiated from cell progenitor Antigen Processing Cells (APC) and B-lymphocytes are also seen to be present in the kidney. The lymphoheamopiotic cells occur in scattered condition or forming pyronimophilic cell clusters. In some cases, various lymphoid cells are found to be scattered at various stages of their maturity including macrophages,

granulocytes and lymphocytes. A single macrophage with some lymphocytes is observed in the renal cortex. Apart from this some lymphoid cells including lymphocytes and granulocytes are observed as single blast cells (Figs. 5-8).The basic architecture of the renal lymphoid organ treated with potassium dichromate (Cr VI) is found to be disturbed but macrophages are observed intact. Many granulocytes are observed to be scattered in the renal matrix. Macrophages are also found to be prominent but compact renal matrix with scattered manufacturing lymphocytes is not observed. At the cellular level, large number of lysosomes is observed. Rough endoplasmic reticulum is not seen and mitochondria are found in deformed in shape. In some cases large number of granulocytes is found to be prominent along with the weakly stained neutrophils (Figs.9-12).

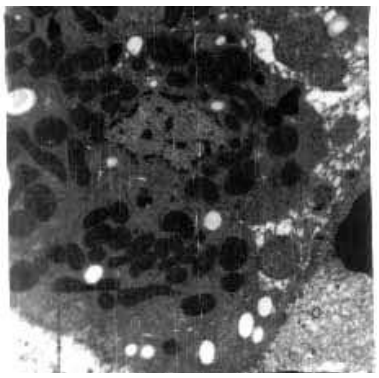

Fig.5. Ultrastructure of c.s. through (ck) of healthy *C.gachua* showing detail structure of lymphoid folical with large number of mitochondria (x13.5 K)

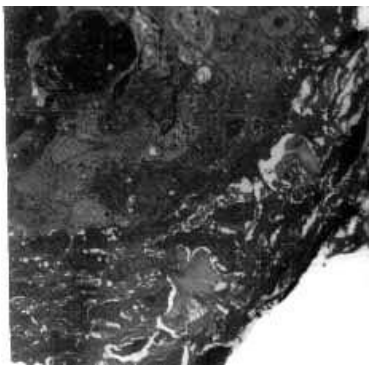

Fig.6. Ultrastructure of c.s. through (ck) of healthy fish showing cellular organization in head kidney (x72 K)

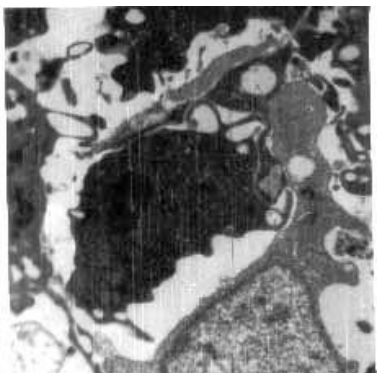

Fig.7. Ultrastructure of c.s. through (ck) of healthy fish with enlarged view of plasma cell and lymphocytes (x 36.9K)

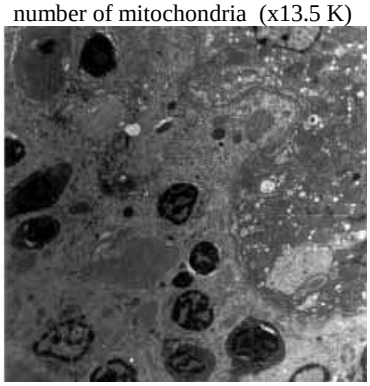

Fig.8. Ultrastructure of c.s. through (ck) of *C.gachua* treated with chromium, showing enlarged view of lymphocytes (x 72.9K)

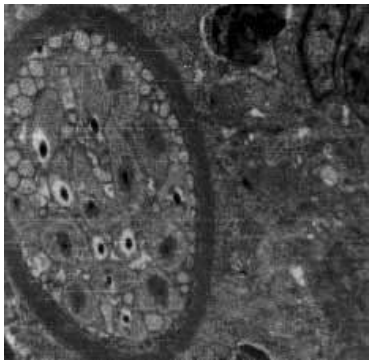

Fig.9. Ultrastructure of c.s. through (ck) of *C.gachua* treated with chromium, showing enlarged view of macrophage with APC (x 13.5K)

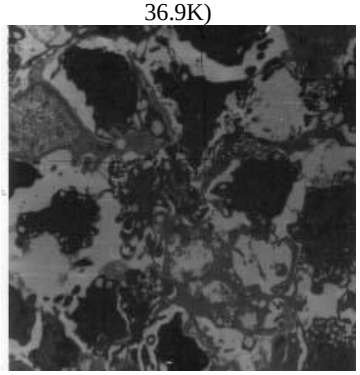

Fig.10. Ultrastructure of c.s. through (ck) treated with Cr, showing the damaged histological architecture (x72K)

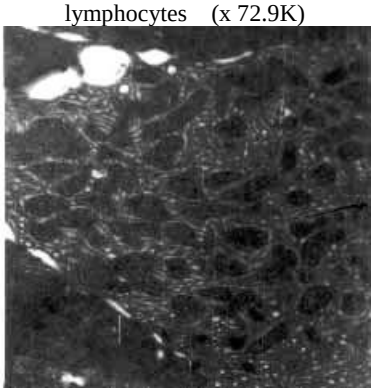

Fig.11. Ultrastructure of c.s. through (ck) of *C.gachua* treated with chromium, showing enlarged view of lymphoid folical (x13.5K)

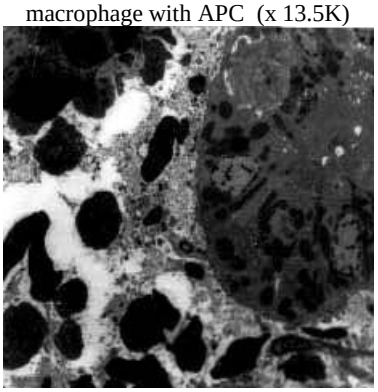

Fig.12. Ultrastructure of c.s. through (ck) of fish treated with Cu, showing damaged histological architecture with centrally located macrophage surrounded by lymphocytes (x13.5 K)

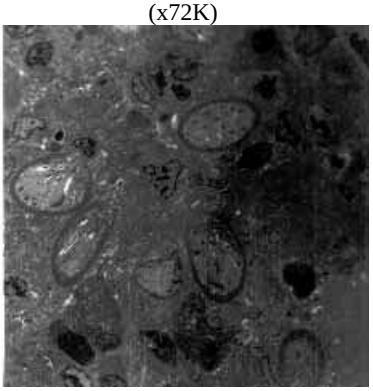

Fig.13. Ultrastructure of c.s. through (ck) of *C.gachua* treated with chromium, showing detailed structure of eosinophil with many granules, lysosomes and mitochondria (x 40.5 K)

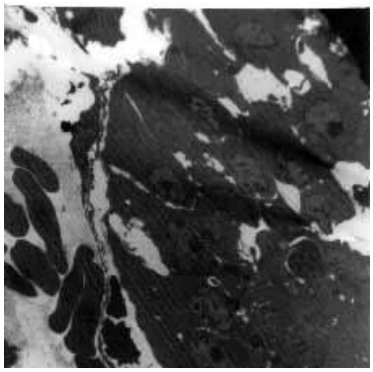

Fig.14. Ultrastructure of c.s. through (ck) of *C.gachua* treated with copper, showing the histological detail of germinal follicle (x 13.5 K)

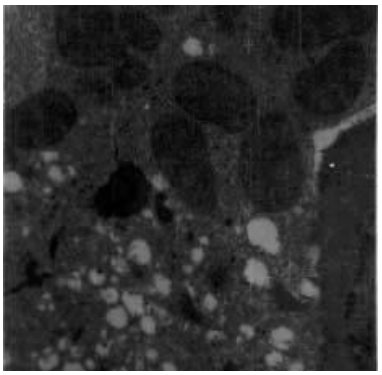

Fig.15. Ultrastructure of c.s. through (ck) of *C.gachua* treated with copper, showing the detail of peripheral zone of a germinal follicle (x 26.1K)

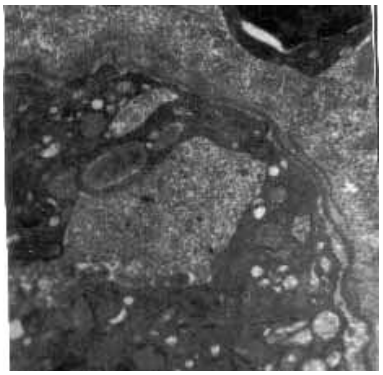

Fig.16. Ultrastructure of c.s. through (ck) of *C.gachua* treated with copper, showing the enlarged view of germinal follicle (x 73.8 K)

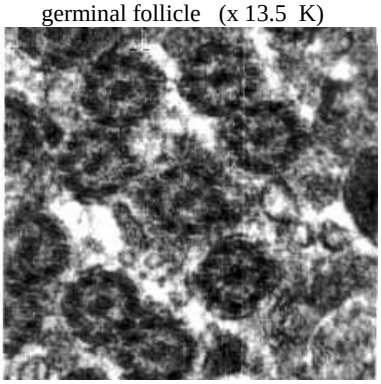

Fig.17. Ultrastructure of c.s. through cephalokidney of *C.gachua* treated with copper, showing the capsulated germinal follicle(x 13.5 K)

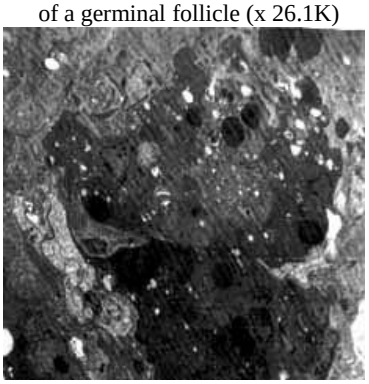

Fig.18. Ultrastructure of c.s. through cephalokidney of *C.gachua* treated with copper, showing the damaged lymphoid follicle with peripherally megakaryocytes (x 37.8 K)

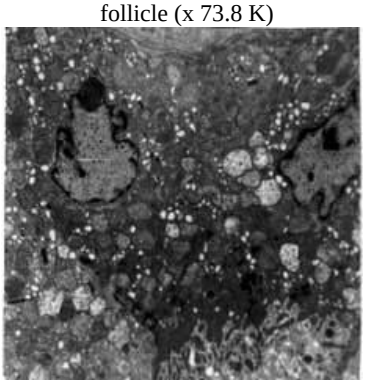

Fig.19 Ultrastructure of c.s. through cephalokidney of *C.gachua* treated with cobalt, showing the germinal follicle with large number of monocytes and APC (x 10.35 K)

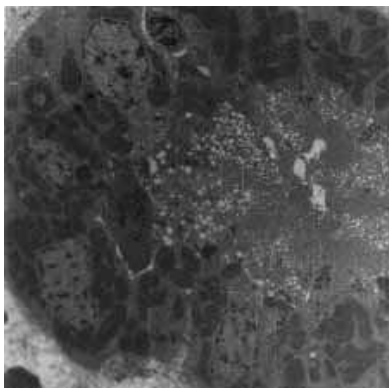

Fig.20. Ultrastructure of c.s. through cephalokidney of *C.gachua* treated with cobalt, showing the enlarged view of germinal follicle with monocytes and plasma cells (x 37.8 K)

In case of fishes treated with copper sulfate (CuII), the basic architecture of lymphoid organs is seen to be damaged. The cells are found loosely arranged and large intracellular spaces are observed in cortex of the renal lymphoid portion. The number of macrophages has become highly decreased and they exhibited large number of pseudopodia. A large number of melanophorocytes are also observed in renal lymphoid matrix along with large melanophorocyte progenitor cells. In this case a large number of lymphocyte at different stage of production is observed along with eosinophils and basophils. Large number of basophils is observed when basic architecture of lymphoid follicle is somewhat intact. Some lymphoid aggregate are also seen as compact mass with small black spaces of fibroblastic reticulo-endothetial cells (Figs. 13-16).

In case of cobalt (CoII) treated fishes, the renal lymphoid architecture is nearly completely destroyed. The renal lymphoid matrix is found with scattered macrophages and lymphocytes. The margins of reticulo-endothetial fibroblastic cell are also found to be loosely arranged along with some lymphoid cells. The haemopiotic cells are also seen to be originated in large number with damaged fibroblastic matrix. At some places, the endothelial lining of the renal matrix contains many lymphocytes at various stages of development (Figs17-19). Under the present work, some cells are seen, differentiated out from the matrix. These are of the size 16-20μ, containing centrally located nucleus surrounded by some unrecognized partials of size 0.4μ and 9 in numbers (Fig.20).

## IV. DISCUSSION

The impact of heavy metals including Al, Cu, Cd, Pb, Ni, and Zn, on fish immune response has been extensively studied on liver, kidney, skin and gill in many fishes and found to cause great damage at architectural and functional level [6-13], but exact role on lymphoid organ is still to be established.Stressful situation results in cascade of events that are transduced via, nervous and endocrine system [11] [15-18] and predispose fish to disease [19, 20]. The catcholamine and cortisol, main teleost renal hormone directly or indirectly result in secondary and tertiary immune response that can be generally classified as those involve in energy metabolism, hydromineral balance, and other major physiological function that may affect disease resistance [16] [20-21].The experimental trials with three different metals viz. potassium dichromate (Cr VI), copper sulfate (Cu II) and Cobalt sulfate (CoII) are conducted to observe their impact on the first line of defense i.e. rupturing the mechanical, physiological and immunological barriers, revealed that all the three metals have their more or less role in breaking of these barriers and great loss in the mucous quantity. The first line of defense in a fish includes physical barriers like scales and skin and bio chemical barriers like mucous which contain bacteriolytic enzymes [22], which are found to be damaged to more or less extent in all the cases. The same type of disorder is reported by Gupta and Rajbanshi, [23] for copper, Khangrot and Tripathi [13] for chromium but no correlation with cobalt has been appeared in the literature. Under the present study, cobalt has been found to be having the maximum deteriorating effects among the metals selected. Till date, no direct correlation is established to correlate the ultra structural disorders of lymphoid organs as related to stresses especially caused by heavy metals. In the present study, the lymphoid organs of treated fish exhibited a marked destruction as compared to that of a normal fish. The basic architecture of lymphoid organ, the natural reticulo-endothelial lining and the matrix of lymphoid tissues are also found to be disturbed and/ or damaged.

The histological study of fishes treated with copper showed considerable damage in histological architecture of lymphoid organs. The histological sections of fishes treated with chromium showed considerable amount of histological damage. The ground parenchyma is noticed damaged due to disintegration of the reticuline fiber but lymphoid follicles are found to be intact. The kidney sustained much histological damage in case of fish exposed to cobalt exhibiting necrosis. The destruction of tissues of the treated fish, as compared to normal one, is reported by Gupta and Rajbanshi [23] and Dalela *et al*. [24].The fishes treated with copper also showed same impact on the ultra structure of fish lymphoid organ. In case of ultra structure of lymphoid organ of fishes treated with cobalt, drastic changes in normal architecture of lymphoid organ have been noticed. The supporting parenchyma matrix of cortex has completely lost. Lymphocytes are found in loose arrangement and the cortex of the lympho-haemopiotic capsule, and all lymphocytes have got converted into dendratic natural killer cells with large number of parapodia and ground parenchymatus tissue has been completely destroyed. Numerous lacunae are observed in the histological section of fish exposed to cobalt. Considerable damage is observed in the histological sections of the tissue when noticed and the ground parenchyma is seen disintegrated at certain places. Large number of haemopoietic cells is found scattered in the ground parenchyma while the periphery of the lymphoid follicles is found to be damaged. Varying degree of damage has been reported in different tissues of fish exposed to copper chromium and cobalt [10][13][25-28].Furthermore, the observed effects on the lymphoid organ of *Channa gachua* can be explained on the basis of their impact caused by heavy metals on cellular metabolism. TbF deleterious effects on cells associated with oxidative stress, such as lipid peroxidation, methenoglobunimia, and DNA oxidation, have also been investigated in aquatic animals [29-48]. This can be explained on the basis of the studies conducted in relation with the impact of heavy metals on cellular metabolism. The correlation of the above facts supports the observations made during the present study.

## V. CONCLUSION

On the basis of above discussion, it is clear that all the three heavy metals, applied for the present work, have their adverse effects on immune system of fishes. These metals weaken the immune system by districting the lymphoid tissues, which in turn decreased the rate of heamatopiosis and ultimately lead to impairment in various immunological reactions in the fishes. Authors also report the maximum deterioration in case of fishes treated with cobalt.

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