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Toxicological effect of cobalt chloride on freshwater fish *Oreochromis mossambicus*

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Abstract
The toxicity of cobalt chloride (30, 60, 90, 120 and 150 ppm) on freshwater fish *Oreochromis mossambicus* were investigated (96 hrs). LC_{50} was observed at 340 ppm. Micronucleus (MN) was observed in fish erythrocytes for cytogenetic abnormalities. MN frequencies were significantly increased in different dose levels. Haemoglobin and RBC's count were found higher in control than exposed groups and WBC's count found higher in 150 ppm group. Tissues such as gill, liver (histology) and muscle (biochemical) were analyzed. Carbohydrate, Cholesterol and Protein levels were gradually reduced in treated concentrations (30 > 60 > 90 > 120 > 150 ppm) than control. Gill and liver tissues of treated groups showed several histopathologies than control. These alterations could affect vital physiological functions of gill and liver such as respiration, osmotic and ionic regulations, nitrogenous waste excretion and absorption, storage and secretion of various enzymes could ultimately affect the survival and growth of *O. mossambicus*. These observations strongly suggested that an even low concentration (ppm) of Cobalt Chloride was toxic; and seriously alters the major haematological, biochemical and histoarchitecture of fishes. Thus, all possible remedial measures should be adopted to prevent the occurrence of Cobalt Chloride exceeding permissible limit in the aquatic environment.

Keywords: *Oreochromis mossambicus*, Cobalt Chloride, LC_{50}, Haematology, Biochemical, Histology.

1. Introduction
The natural aquatic systems were the ultimate recipient of the pollutants (Fleeger *et al.*, 2003) [25]. Aquatic ecosystems were contaminated with a wide range of pollutants has become a matter of concern over the last few decades (Vutukuru, 2005) [63]. The accumulation and persistence of pollutants by contaminants and toxicants, released from weathering of geological matrix, or from anthropogenic sources, such as industrial effluents and mining wastes (Ebrahimpour and Mushrifah, 2010) [19] represents a major threat to the biological life (Fleeger *et al.*, 2003) [25].
Aquatic animals were the keystone species in many ecosystems (Lonsdale *et al*., 2009) [39]. Fishes are one of the most widely distributed organisms in the aquatic ecosystem and reflect the biological effects of environmental pollution. The contamination of aquatic system was attracted the attention of researchers all over the world (Dutta and Dalal 2008) [18].
Cobalt is the 33rd most abundant (ATSDR, 2004) [6] oligo-element which is essential for the formation of vitamin B_{12} and other cobalamines (Garoui *et al.,* 2011) [28]. It is naturally present in aquatic environment as low concentrations. The permissible limits of cobalt in surface, irrigation and livestock wastewaters were 1 ppm, 0.05 ppm and 1.0 ppm respectively (Comhaire *et al.,* 1998) [13]. It is used in alloy metals, as colorant for paints, glass and ceramics industries, as additives in agricultural and medical products (ATSDR, 2004) [6].
Tilapia (*Oreochromis mossambicus*) is one of the most popular fresh water fish consumed in several countries (Alwan *et al.,* 2009) [7] and well-known for its ability to tolerate many types of environmental stressors (Stickney, 1986) [56] and act as a good biological model for toxicological studies due to diverse characteristics, great resistance to diseases and for handling practices, good tolerance to a wide variety of environmental conditions (Fontainhas-Fernandes, 1998) [27].
Blood tissues reflected the physical and chemical changes of treated organisms (Parveen and Shadab, 2011) [49]. Monitoring of blood parameters has considerable diagnostic value in assessing early warning signs of pesticide poisoning and also acts as pathophysiological reflector of the whole body while important in diagnosing the structural and functional status of fish exposed to toxicants (Adhikari *et al.*, 2004) [4].

Application of the erythrocytes (fish) micronucleus assay in the assessment of genotoxic pollutants, acts as a valuable biomarker in field surveys, in monitoring studies and in comparing different levels of pollutants (Omar *et al*., 2012) [48] and acts as a bioindicator for environmental mutagenicity studies over other cytogenetic techniques (Anbumani and Mohankumar, 2011) [8]. The micronucleus test detects both clastogenic and aneugenic effects and therefore can detect the genotoxicity of a wide range compounds (Heddle *et al*., 1991) [31].

Biochemical changes are the most sensitive indices of the stress state of an organism (Ramesh *et al*., 2009) [51]. High concentrations of biochemical molecules in blood indicate that the fish was in stress and it was intensively used their energy reserves i.e., glycogen in liver and muscles (Vijayavel *et al*., 2006) [62].

Histopathological studies are very sensitive and crucial parameter reflects the effect of toxicants on organ (Abdel-Warith *et al*., 2011) [2]. Histological analysis was used to be a cost-effective tool to determine the health of fish populations. Tissue changes in test organisms exposed to a sub-lethal concentration of toxicant were functional response of organisms, which provides information on the nature of the toxicant (Das and Mukherjee, 2000) [15].

Gills were frequently used in the assessment of impact of aquatic pollutants in freshwater habitats (Fernandes *et al*., 2007; Jimenez *et al*., 2007) [22, 36]. Liver plays an important role in vital functions in basic metabolism; major organ of accumulation, biotransformation and excretion of contaminants in fish (Figueiredo-Fernandes *et al*., 2006) [24]. Co^{2+} binds with gill and obstructs the ionic regulation, acid base balance, gas transfer and nitrogenous waste excretion (Evans, 1987; Wood, 1992) [20, 65]. Many workers have assessed the effect of various chemicals on the behaviors and haematological responses of various species of fish (Benarji and Rajendranath, 1990; Svoboda *et al*., 2001) [10, 58]. In this study, the effect of $CoCl_2$ on freshwater fish *O. mossambicus* was studied.

2. Materials and methods

2.1. Fish maintenance

Fresh water fishes *Oreochromis mossambicus* were collected from the Cauvery River (lat. 11°29'; long. 79°50'E), Tiruchirappalli District, Tamil Nadu (South India). Fishes were acclimatized to laboratory conditions in Environmental Research Laboratory, Jamal Mohamed College (Autonomous), Tiruchirappalli. After two weeks, the fishes were divided into groups (n=10) and kept in aquaria (30 L) under light-dark (12:12 hrs) cycle. As per the APHA (1998) [9], the water parameters were maintained throughout the experiment (Table 1) and aerated. Fish food pellets were provided ad libitum (Affonso *et al.,* 2002) [5], but the fishes were fastened for at least 24 hrs prior to the experiments.

Table 1: Physiochemical parameters maintained in aqueous medium

Parameters	Values
Temperature (1 °C)	28–32
Salinity (ppt)	1.4
Total Hardness (mg/L)	255.0
pH	8.2
Nitrate (mg/L)	1.6
Chloride (mg/L)	27.0
Ammonia (mg/L)	0.058
Dissolved Oxygen (DO) (mg/L)	6.7
Biological Oxygen Demand (BOD) (mg/L)	5.8
Chemical Oxygen Demand (COD) (mg/L)	14.7
Total solids (g/L)	1.7

2.2. Acute lethal studies

The chemical cobalt chloride purchased from nice chemicals Pvt. Ltd. (Kochi, India). Based on the literature, the different concentrations of cobalt chloride (50, 100, 200, 300, 400, 500 and 600 ppm) were exposed to seven groups and one group maintained as control, the results observed at 24, 48, 72, 96 hrs time intervals. For confirmatory results, twelve groups of fishes were exposed to twelve concentrations (300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410 ppm) of Cobalt Chloride.

2.3. Experimental design

The five groups were exposed to 30, 60, 90, 120 and 150 ppm concentrations of cobalt chloride. Control group was maintained separately. After 96 hrs, blood and organs were collected from control and treated fishes.

2.4. Micronuclei (MN) assay

Fish peripheral blood smear is fixed in methanol for 10 min and left to air-dry at room temperature and finally stained with 5% Giemsa in Sorenson buffer (pH 6.9) for 20 min. The slides were randomly selected and total of 1000 erythrocytes were examined for MN in each group under the light microscope (Leica microscope, Switzerland) (Fenech *et al.,* 2003) [21]. Only the cells clearly isolated from the surrounding cells were scored (Cavas *et al.,* 2005) [11]. The criteria for the identification of micronuclei were as follows:

1. MN must be smaller than one-third of the main nuclei
2. MN must be separated from the main nuclei or marginally overlap with main nuclei
3. MN must be same plane of focus and have same colour (stain)

$$MN\ (\%) = \frac{\text{Number of cells containing micronuclei}}{\text{Total number of cells counted}} \times 100$$

2.5. Haematological parameters

The blood samples were collected from fish by cardinal vein puncture (Schmit *et al*., 1999) [53] technique using a heparinized syringe and red blood cell (RBC) (Shah and Altindag, 2004a) [54] and white blood cell (WBC) (Mgbenka and Oluah, 2003) [42] cells were counted by using an improved Neubauer Haemocytometer. Haemoglobin (Hb) was determined by cyanmethemoglobin method (Drabkin, 1946) [17].

2.6. Histochemical analysis

Gill, liver and muscle tissues from fishes in each group were examined for protein, carbohydrate and lipid contents. The methods adopted for analysis of protein, carbohydrates and lipid were Lowry *et al*. (1951) [40], Roe (1955) [52], Folch *et al*. (1957) [26] respectively.

2.7. Tissue preparation for histological observations

The gill and liver tissues were immediately fixed in Bouin's (Gurr, 1962) [30] fixative (48h) and dehydrated in an alcohol series, cleared in xylene, infiltrated with liquid paraffin at 58 °C, and finally embedded in paraffin blocks. The blocks were trimmed and sectioned at 5 µm thick cut on a rotary microtome (Weswox MT Chennai, India). The fixed sections were stained with Hematoxylin & Eosin stain, observed under a light microscope (Leica microscope, Switzerland).

2.8. Statistical analysis

For LC_{50} calculation, probit analysis tool was used. The observed data were presented as mean ± SD. Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Post Hoc (Duncan's) test using the SPSS (17.0 version).

3. Results

3.1. Acute lethal studies

The Cobalt Chloride exposure with high intervals of concentrations (50, 100, 200, 300, 400, 500 and 600 ppm) showed mortality between 300 - 400 ppm. In confirmatory studies, the lethal concentration (LC_{50}, 96 hrs) of cobalt chloride in *O. mossambicus* fishes were attained at 340 ppm in three replicates respectively (Figure 1).

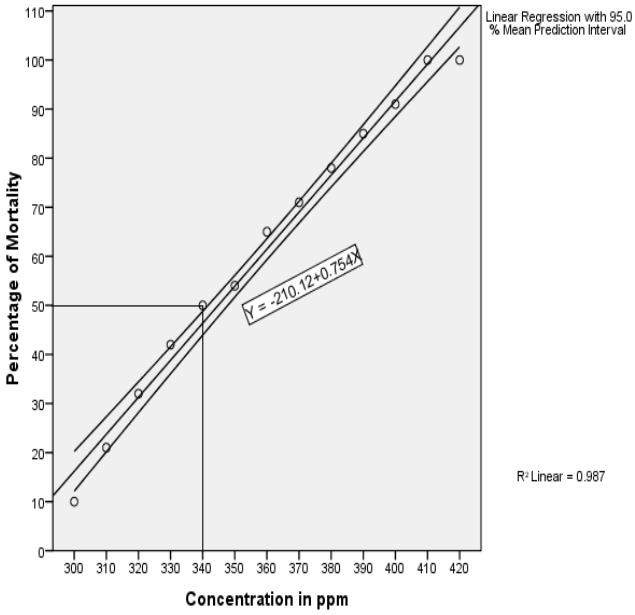

Fig 1: The acute lethal concentrations of cobalt chloride in *O. mossambicus* for 96hrs

3.2. Micronuclei assay

Frequency of micronuclei in control group was recorded as 1.98 ± 0.11 (Table 2). Micronuclei frequencies revealed (96 hrs) that higher values were obtained in 150 ppm (9.06 ± 0.15) > 120 ppm (7.43 ± 0.20) > 90 ppm (5.80 ± 0.39) > 60 ppm (4.32 ± 0.32) > 30 ppm (3.17 ± 0.16). The increased MN frequency of erythrocytes is dose dependent (Figure 2).

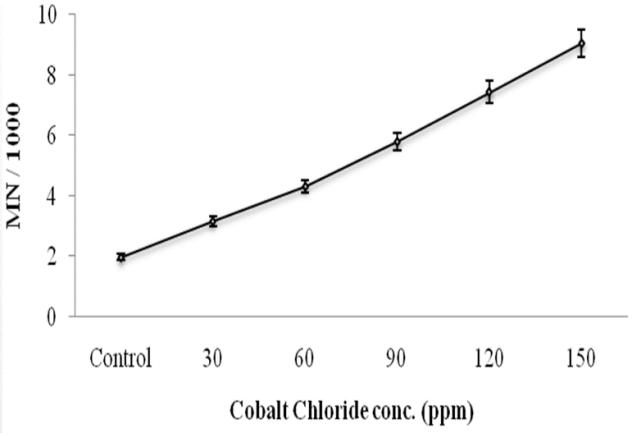

Fig 2: Micronuclei frequency comparison in cobalt chloride exposed groups with control in 96hrs after exposure.

Table 2: Frequency of micronuclei in peripheral erythrocytes of *O. mossambicus* exposed to cobalt chloride (n = 10)

Concentration of $CoCl_2$ (ppm)	Total no. of counted cell	MN/1000 (mean ± SD)
Control	5335	1.98 ± 0.11
30	5192	3.17 ± 0.16
60	5223	4.32 ± 0.32
90	5219	5.80 ± 0.39
120	5432	7.43 ± 0.20
150	5163	9.06 ± 0.15

3.3. Haematological parameters

RBC's in control and treated groups were counted on every 24 hrs. Average (96 hrs) value (mean ± SD) of control group was 1.39 ± 0.08 $\times 10^6/mm^3$; exposed groups were 1.05 ± 0.05, 0.72 ± 0.14, 0.54 ± 0.09, 0.45 ± 0.05 and 0.38 ± 0.06 $\times 10^6/mm^3$ for 30, 60, 90, 120 and 150 ppm concentrations respectively. Treated groups were found to inflict a drastic reduction in their RBC values. The RBC reduction was dose and duration dependent; the exposed group values were showed a significant decrease when compared to control group (Table 3; Figure 3).

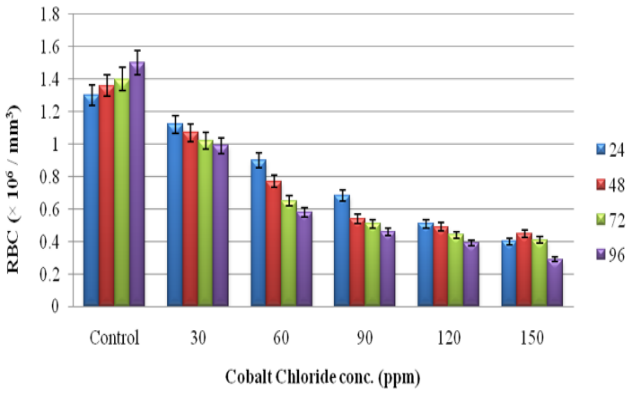

Fig 3: Comparison of red blood cells (mean ± SD) of cobalt chloride exposed groups with control in different sampling periods.

WBC counting in control group showed 6.21 ± 0.07 $\times 10^3/mm^3$. In exposed groups, the WBC count found as 11.20 ± 2.46, 16.69 ± 2.52, 22.34 ± 3.27, 32.56 ± 3.89 and 39.72 ± 4.63 $\times 10^3/mm^3$ for 30, 60, 90, 120 and 150 ppm respectively. After 96 hrs, the values of exposed groups were seems to have significant increase them control (Table 3; Figure 4).

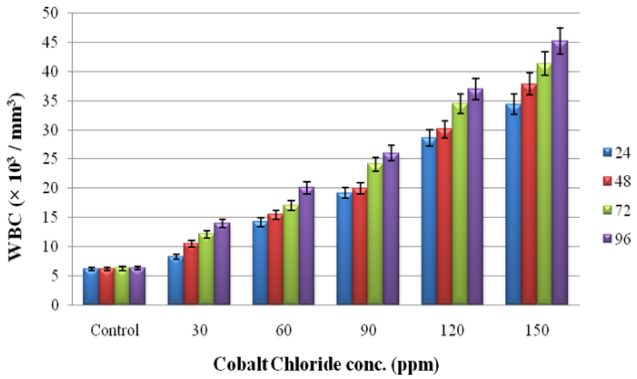

Fig 4: Comparison of white blood cells (mean± SD) of cobalt chloride exposed groups with control in different sampling periods.

Hb level in control group was 6.24 ± 0.87 g/dL. Values of exposed groups were 7.35 ± 1.33, 9.96 ± 3.25, 7.39 ± 1.60, 7.35 ± 1.50 and 7.07 ± 2.55 g/dL for 30, 60, 90, 120 and 150 ppm respectively (Table 3; Figure 5). In 60 ppm exposed group, the Hb level showed a significant difference when compared with 30, 90, 120, 150 ppm and control group.

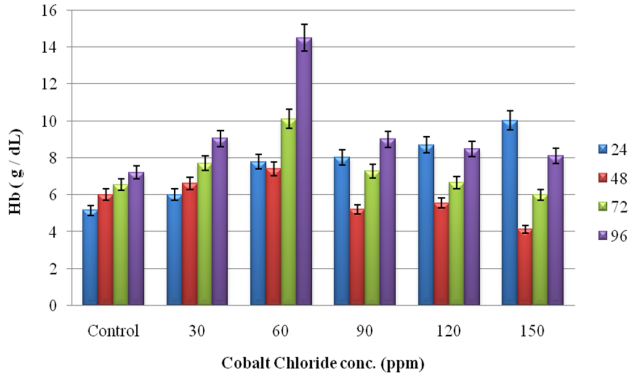

Fig 5: Comparison of average haemoglobin (Hb) level of cobalt chloride exposed groups with control in different sampling periods.

Table 3: Levels of RBC, WBC and Hb values in control and cobalt chloride exposed groups

Conc. of $CoCl_2$ (ppm)	Total RBC count ($\times 10^6/mm^3$)	Total WBC count ($\times 10^3/mm^3$)	Haemoglobin (g/dL)
Control	1.50	6.29	7.22
30	0.99	14.01	9.05
60	0.58	20.06	14.5
90	0.46	26.05	9.01
120	0.39	37.04	8.48
150	0.29	45.20	8.11

3.4. Biochemical analysis

3.4.1 Protein

The protein concentrations was found to be higher in Muscle (70.4 ±1.77 g/dL) > Liver (49.9 ± 1.06 g/dL) > Gill (30.2 ± 1.14 g/dL) (Figure 6). However, after 96h of exposure, the protein content in exposed groups were reduced than control. 150ppm group values found as 46.5 ±1.61 g/dL in muscle; 35.94 ±1.98 g/dL in liver; 19.5 ±1.35 g/dL in gills (Table 4). Same kind of Scenario was observed in other two bio compounds analysis too. The carbohydrate and cholesterol content were high in control and it gradually reduced, when increased concentration of $CoCl_2$, lower values were observed in 150 ppm exposed group.

3.4.2. Carbohydrate

Among the three tissues, the carbohydrate content was found to be high in the order of Liver > Muscle > Gill (Figure 6). The carbohydrate content was found to be lower in all exposed groups, particularly in 150 ppm group contains lower level of carbohydrate as gills, muscle and liver tissues were 86.5 ± 1.78 mg/dL, 115.76 ± 1.39 mg/dL, 127.64 ± 1.74mg/dL respectively (Table 4). Increased dose concentrations influenced the carbohydrate content in fishes.

3.4.3. Cholesterol

The level of cholesterol was found to be higher in Liver (197.94 ± 1.13 mg/dL) > Gill (133.5 ± 1.38 mg/dL) > Muscle (102.54 ± 1.24 mg/dL) (Figure 6). However, after 96 hrs of exposure, the cholesterol content of exposed fishes is low than control. The exposed group (150 ppm) contains values of 99.38 ± 1.47 mg/dL in liver, 87.34 ± 1.36 mg/dL in muscle and 78.52 ± 1.59 mg/dL in gills (Table 4).

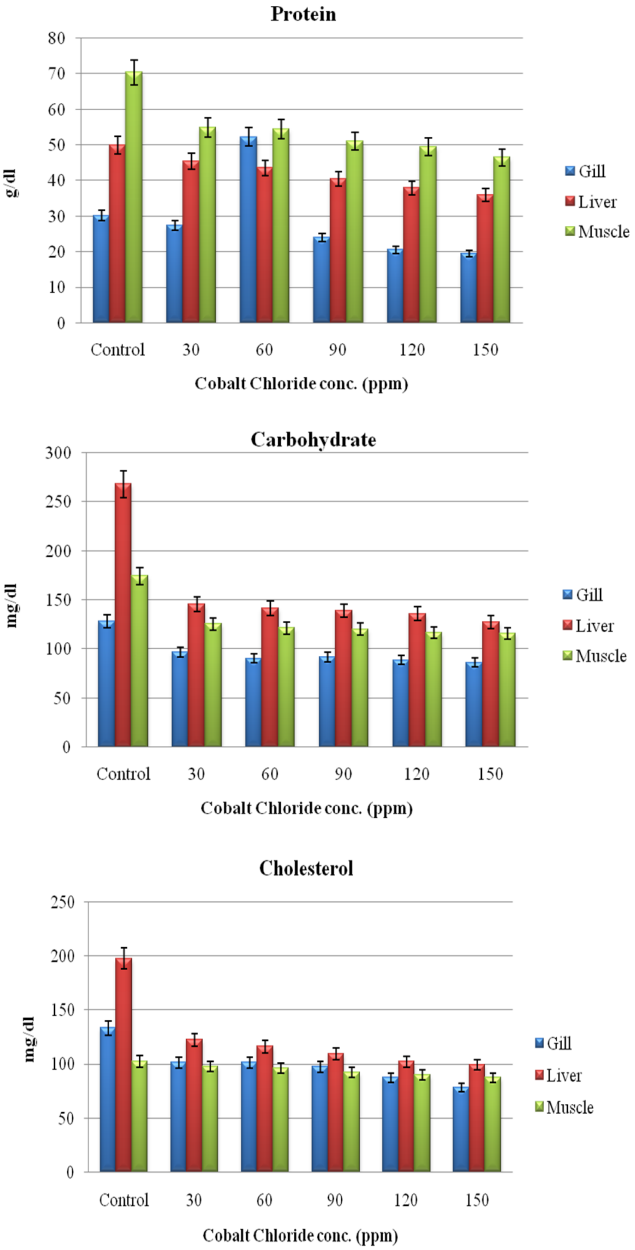

Fig 6: Biochemical analysis of freshwater fish *O. mossambicus* exposed to cobalt chloride.

Table 4: Effect of different concentrations of cobalt chloride on protein, carbohydrate and cholesterol of different tissues of *O. mossambicus* exposed for 96hrs.

Groups	Protein (g/dL)			Carbohydrate (mg/dL)			Cholesterol (mg/dL)		
	Gill	Liver	Muscle	Gill	Liver	Muscle	Gill	Liver	Muscle
Control	30.2 ±1.14	49.9 ±1.06	70.4 ±1.77	128.46 ±1.98	268 ±1.75	174.34 ±1.50	133.5 ±1.38	197.94 ±1.13	102.54 ±1.24
30ppm	27.4 ±1.54	45.42 ±1.24	54.9 ±1.65	96.84 ±1.98	145.66 ±1.68	125.46 ±1.62	101.64 ±1.59	122.42 ±1.60	97.68 ±1.42
60ppm	52.26 ±1.69	43.56 ±1.61	54.4 ±1.40	90.52 ±1.39	141.62 ±1.39	121.52 ±1.75	101.7 ±1.54	116.44 ±1.62	96.06 ±1.85
90ppm	24 ±1.10	40.4 ±1.75	51.1 ±1.41	91.9 ±1.90	139.06 ±1.01	120.3 ±1.23	97.54 ±1.17	109.42 ±1.85	92.48 ±1.48
120ppm	20.6 ±1.49	37.9 ±1.21	49.5 ±1.31	88.9 ±1.95	136.08 ±1.36	116.52 ±1.40	87.56 ±1.73	102.44 ±1.30	90.06 ±1.17
150ppm	19.5 ±1.35	35.94 ±1.98	46.5 ±1.61	86.5 ±1.78	127.64 ±1.74	115.76 ±1.39	78.52 ±1.59	99.38 ±1.47	87.34 ±1.36

3.5. Histological analysis

3.5.1. Gill

In the control group, no pathological changes were observed in the gills. Observations showed the uniform arrangement of gill lamellae (L) with inter lamellar space (ILS), surface of each filament lamellae (F) was covered by a monolayer of epithelial cell, primary gill lamellae (PL), secondary gill lamellae (SL) (Figure 7A). The normal structure of Gill was lost in 30 ppm cobalt chloride exposed group with mucus (M) formation, lamellar debris (LD), fused lamellae (FL) (Figure 7B). In 60 ppm cobalt chloride exposed group lifting of lamellar epithelium (LLE), Necrotic Lamellae (NL), Fused Lamellae and increased mucus cells (Figure 7C) were observed. Proliferated chloride cells (PCC), increased lifting of lamellar epithelium and necrotic lamellae were observed in 90 ppm cobalt chloride exposed group (Figure 7D). Fused lamellae, lifted lamellar epithelium, Necrotic Lamellae, increased mucus cells and Proliferated chloride cells were observed in 120 and 150 ppm cobalt chloride exposed groups (Figure 7E; Figure 7F).

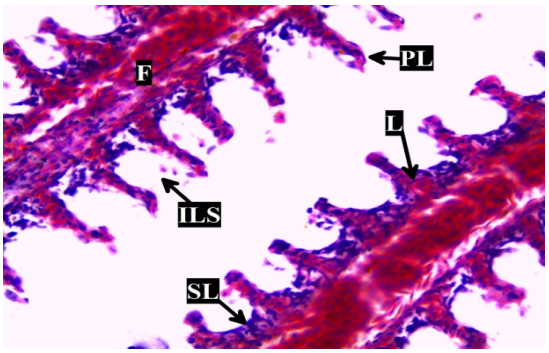

Fig 7A: Gill Control

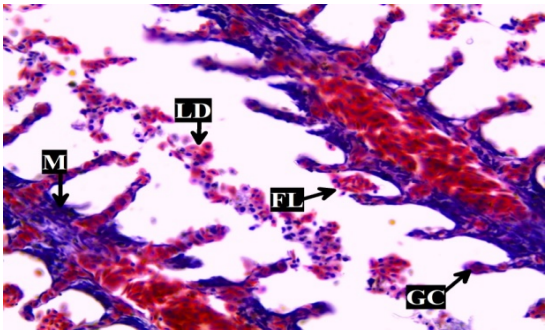

Fig 7B: Gill exposed to 30ppm

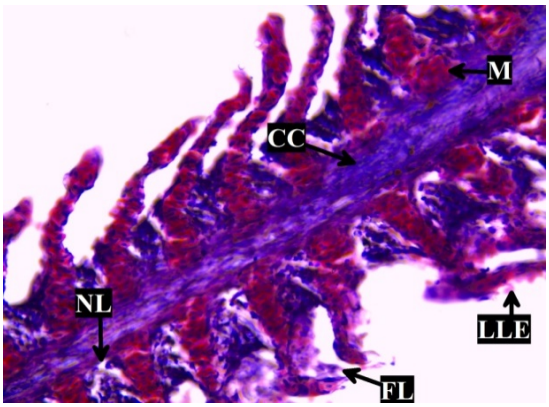

Fig 7C: Gill exposed to 60ppm

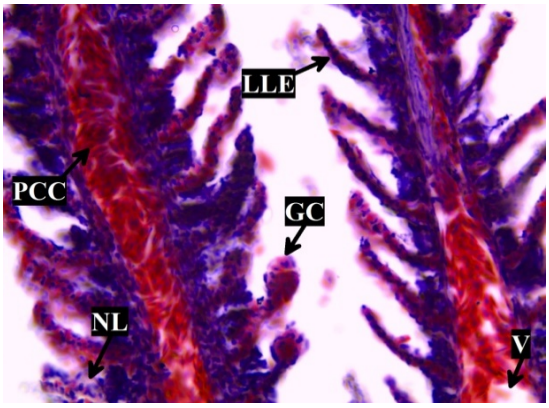

Fig 7D: Gill exposed to 90ppm

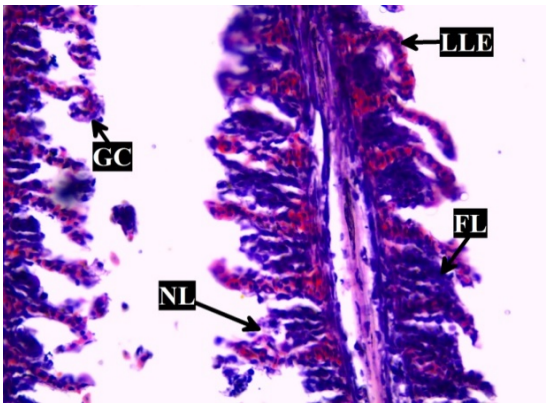

Fig 7E: Gill exposed to 120ppm

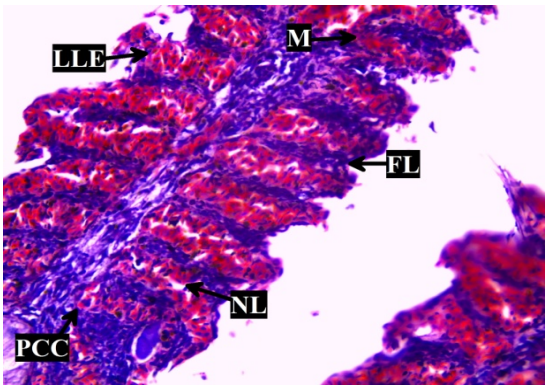

Fig 7F: Gill exposed to 150ppm

Fig 7: Histopathological changes of gill in *Oreochromis mossambicus* exposed to different concentrations of Cobalt Chloride.

3.5.2. Liver

The normal histology of liver structure was observed in control group with pancreatic tissue (PT), portal vein (PV) and hepatocytes with nucleus (N) (Figure 8A). The normal structure of liver was lost in 30 ppm cobalt chloride exposed group, the anomalies such as necrotic liver tissue (NL), elongated pancreatic tissue (PT) and conjuncted portal vein (PV) were observed (Figure 8B). High level of structural alteration such as blood conjunction (BC), increased necrotic liver tissue (NL) and formation of vacuolation (V) were observed in 60 ppm cobalt chloride exposed group (Figure 8C). Histopathological changes in 90, 120 and 150 ppm were necrotic liver tissue (NL) / (NT), elongated pancreatic tissue, conjunction of blood portal vein (PV) and formation of vacuolation (V) (Figure 8D; Figure 8E; Figure 8F).

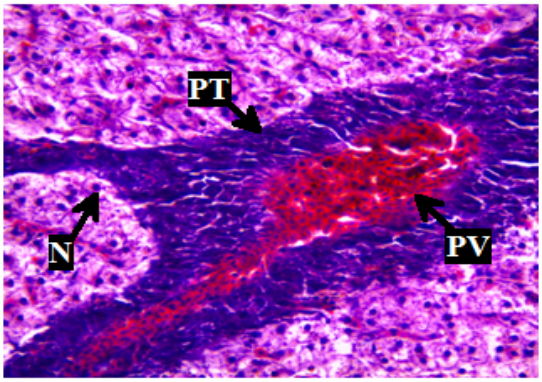

Fig 8A: Control Liver

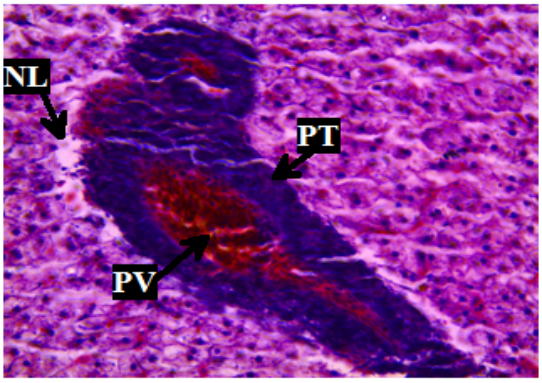

Fig 8B: Liver exposed to 30ppm

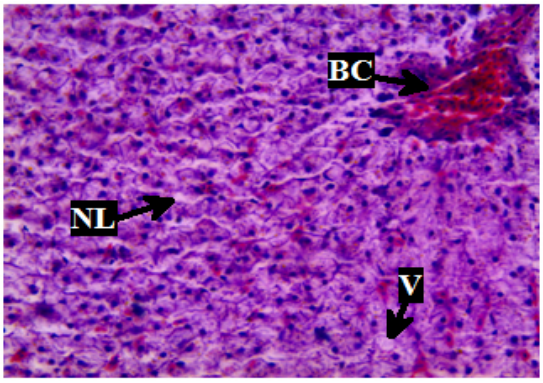

Fig 8C: Liver exposed to 60ppm

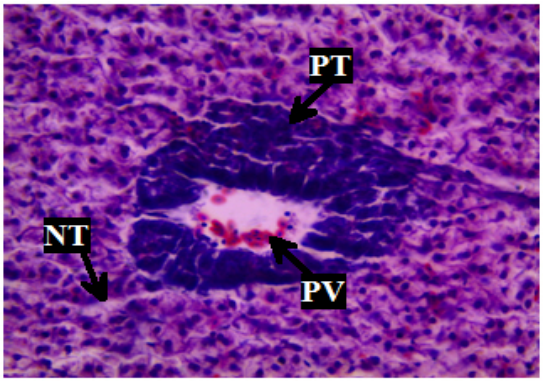

Fig 8D: Liver exposed to 90ppm

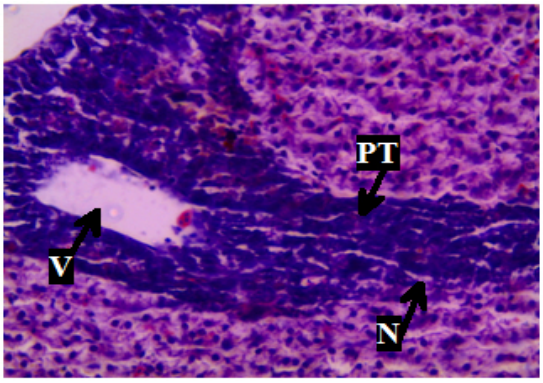

Fig 8E: Liver exposed to 120 ppm

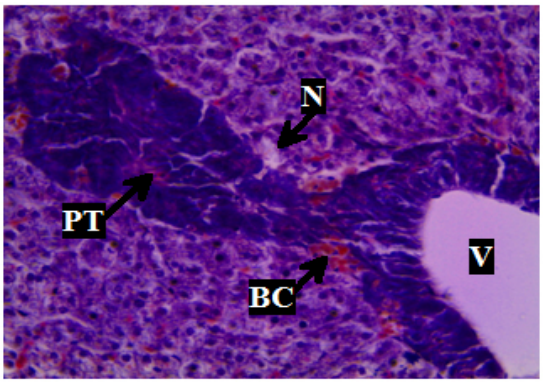

Fig 8F: Liver exposed to 150ppm

Fig 8: Histopathological changes of liver in freshwater fish *O. mossambicus* exposed to different concentrations of Cobalt Chloride.

4. Discussion

Mortality (LC_{50}) and impaired hatching were observed in $CoCl_2$ exposed common carp embryo at 96 mg/L (96 hrs). The adult giant Gouramis exposed to 232.8 mg/L (96 hrs), fathead minnow 48 mg/L (96 hrs) and *Olyzias latipes* to 620 ppm (48 hrs) were evidenced LC_{50} for $CoCl_2$ (DOSE, 1993; NPIS, 1998) [16, 46] in other species. The LC_{50} (96 hrs) for gold fish exposed to cobalt chloride were determined at 333 ppm (Das and Kaviraj, 1994); for *Cyprinus carpio* at 327 ppm and 328 ppm in two replicates respectively (Naji *et al*., 2007) [45].

The MN frequency shows increased mean value at 96 hrs exposure period in all treated groups, but in control group it was found to be lesser. Yadav and Trivedi (2009) [66] reported gradual increase of MN frequency upto 96 hrs (15 ± 1.414) in *Channa punctatus* exposed to AS_2O_3. Hooftman and Raat (1982) [32] concluded that based on sampling time, there was a time-dependent increase in MN induction in Ethyl Methane sulfonate exposed peripheral blood of *Umbra pygmaea*, an effect corroborated by the present work.

Haematological abnormalities were studied in various toxicants exposed fishes such as *Channa punctatus* to lead (Hymavathi and Rao, 2000) [34], *Cyprinus carpio* to carbofuran (Chandra *et al.,* 2001) [12], *C. punctatus* to cadmium (Karuppasamy *et al.,* 2005) [37] and *Labeo rohita* to synthetic detergents and sublethal levels of nitrite (Acharya *et al.,* 2005) [3]. Hb level was found to higher in all treated groups than control. Sukhover khov (1967) [57] revealed the importance of cobalt chloride in the formation of erythrocytes and synthesis of haemoglobin in *Cyprinus carpio* by incorporating it along with food. Accumulation and elimination patterns of cobalt concentration in *C. fusca* tissues were liver > gill > muscle > skin and gill > liver > skin > muscle respectively at the end of 30 days of exposure (Mansouri *et al.,* 2011) [41].

Sublethal concentrations of certain pesticides cause biochemical changes in the liver (Lal and Singh, 1986) [38]. During stress condition, the stored energy (protein, carbohydrate, lipid) in various vital organs (gills, liver, muscle etc.) where released or utilized and there was an increased concentration of biochemical compounds in plasma and decreased level in tissues. Cobalt binds with insulin and reduces plasma glucose levels (Watanabe *et al.,* 1997) [64]. Reduced lipid and protein levels were observed in the pesticide exposed fish liver (Mustafa and Zofair, 1985) [44]. Protein level was reduced in Pesticide or heavy metal exposed fishes (Jana and Bandyopadhyaya, 1987) [35]. Reduced protein level was reported in *Saccobranchus fossilis* exposed to chlordane (Verma *et al*., 1979) [61]. The lipids stored in the vital organs were oxidized by lipases to release energy to meet demand under stress, lipid level were declined in tissues (Vijayavel *et al*., 2006) [62]. Changes in carbohydrate metabolism can be used as general stress indicators in fish. Reduction in carbohydrate levels after exposure to toxicants appears to be caused by hypoxic conditions leading to an excess utilization of stored carbohydrates (Ramesh *et al.,* 2009) [51]. Hussein *et al*. (1996) [33] found significant decrease in serum glucose.

The alterations in liver due to toxicity impact were often associated with a degenerative necrotic condition (Olojo *et al.*, 2005) [47]. The changes induced by chromium in the liver hepatocytes such as vacuolization, necrosis and nuclear condensation were also reported for copper exposure (Figueiredo-Fernandes *et al.,* 2007) [23].

In this study different concentrations of cobalt chloride (30, 60, 90, 120 and 150 ppm) were exposed to fish *O. mossambicus* and their impact on blood (MN, hematological), tissues (histological) and organs (biochemical) were studied. Similar kind of toxicity affects were noticed in fish exposed to other toxicants including heavy metals (Olojo *et al*., 2005; Figueiredo-Fernandes *et al*., 2007) [47, 23].

Atrophy and necrotic hepatic cells, nucleus and nucleoli size decreased, indistinguishable cell membranes were observed in cadmium exposed (liver) *Cyprinus carpio* (Morsey and Protasowicki, 1990) [43]. Hypertrophy, hyperplasia, bulged secondary gill lamellae and separated epithelial layers were observed in cadmium exposed (gill) *Oreochromis mossambicus* (Usha Rani, 1999) [59]. Singh (1993) [55] reported fused and loss of secondary lamellae in gills due to exposure of pesticides in *C. carpio.*

Histopathological changes were observed in zinc exposed (gill) *Tilapia sparrmanii* (Grobler *et al.,* 1989) [29]; cadmium and zinc exposed (liver) *Oreochromis mossambicus* (Van Dyk *et al.,* 2007) [60]; environmental copper on Nile tilapia (Abdel-Tawwab *et al*., 2007) [1]. Increased WBC whereas decreased RBC, Hb and protein levels in Chlorpyrifos treated *Cyprinus carpio* (Ramesh and saravanan, 2008) [50] which was similar to the present study results except increased Hb level. Vutukuru (2005) [63] reported that there was a decreased trend in different biochemical constituents in various tissues in fresh water fish *Labeo rohita* under chromium stress.

5. Conclusion

The freshwater fishes *O. mossambicus* were exposed to different concentrations of Cobalt Chloride showed various haematological, biochemical and histological abnormalities. RBC level was drastically reduced in 150 ppm exposed group than control. WBC and Hb levels were increased due to the toxicant stress developed and interaction of cobalt in (high) haemoglobin synthesis. Cytogenetic abnormality such as micronuclei frequency was observed. Due to the toxicant stress, to cope up the biological needs, the fish utilized the stored energy in the form of protein, carbohydrate and lipid from vital organs. As a result, the biomolecules found lesser in examined organs (muscle, liver and gills) of exposed groups than control group. The cobalt chloride toxicity alters the normal histology of gill and liver. Thus, all possible remedial measures should be adopted to prevent the occurrence of cobalt chloride exceeding permissible limit in the aquatic environment.

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