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ORIGINAL ARTICLE

Effect of Zinc and Chromium on Protein Profile of the Fresh Water Fish *Channa punctatus* Bloch.

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ABSTRACT

The fish, which sub serves the growing demands of food and is also the best source of protein and mineral has also been facing the havocs caused by environmental contamination. The present study is to determine the toxicity of zinc and chromium and its toxicological effects on the protein profile of freshwater fish Channa punctatus. Significant decrements are shown in the total Protein, Albumin, Globulin and Albumin- Globulin ratio after 15days, 30days, 45 days and 60 days exposure of zinc and chromium in comparison to control. Present study shown toxic effects of zinc and chromium induced deleterious effect at protein profile in freshwater fish, Channa punctatus.

Key words: *Zinc, Chromium, Channa punctatus, Total Protein, Albumin, Globulin, Albumin- Globulin ratio*

INTRODUCTION

Water pollution is generally associated with heavy industrialization and dense pollution and is generally one of the major ecological problems, industries are responsible for adding pollutants in our environment as a number of industrial effluents and emission especially toxic substances and gases are spread in the environment. Aquatic pollution due to metals needs considerable attention because of its harmful effects on the aquatic organism which affects the fishing industries. Even sub-lethal concentrations of pesticides or metal may still cause fish mortality in the exposed population after a sufficiently long time of exposure. The use of pesticides made from various metals for the control of disease in agriculture and aquaculture has increased enormously during the last decades. These metals eventually reach the aquatic ecosystem and a considerable magnitude of threat has been imposed on the aquatic biota, amongst them fishes are the worst victims. Since a major part of the world's food is supplied from fish source, so it is secure the health of fish.

Fish are known to be the richest sources of high quality protein. Fish and other aquatic biotas may be harmed by metal contaminated water. Metals surface runoff into rivers and streams can be highly lethal to aquatic life, sometimes killing all the fish in a particular stream. Application of metal containing herbicides to water bodies can cause fish kills when the dead plants root and use up the water's oxygen, suffocating the fish. The metals like arsenic, Chromium, Zinc, lead are regularly used in agricultural pest management for food production but through their excessive and indiscriminate use in agriculture pest management and public health operations. The rapidly increasing use of these metal containing pollutants in agriculture possesses serious hazards to aquatic animals. The fish, which sub serves the growing demands of food and is also a best source of protein and mineral salts has also been facing the havocs caused by environmental contamination. Keeping all these points in view and in light of various sources of literature, the present study is designed to investigate the effect of metals Zinc and Chromium in form of soluble compounds on fresh water fish *Channa punctatuts* in terms of protein profile containing total protein, albumin, globulin and albumin- globulin ratio which will definitely set an asset in fish toxicity studies.

MATERIAL AND METHODS

1. TOTAL PROTEIN:

The total protein was estimated by the modified method of Duma (1971).

Principle-

Protein in serum reacts with the copper of Biuret Reagent in an alkaline medium to form a blue purple complex with absorption maxima at 550 nm.

Procedure-

Three test tubes were taken and marked as 'Blank', 'Standard' and 'Test'. 3ml of biuret reagent was taken into each test tube. 0.05ml of protein standard was added into a test tube which was marked as 'Standard'. 0.05ml of serum sample was then added into test tube was marked as 'Test', then mixed well and allowed to stand at room temperature for 5 minutes. (34) The optical density of 'Standard' and 'Test' was read against blank on colorimeter at 550nm.

Calculation-

$$\text{Total protein (g/100ml)} = \frac{\text{O.D. of Test}}{\text{O.D. of Standard}} \text{ X } 7.2$$

2. ALBUMIN, GLOBULIN AND ALBUMIN/GLOBULIN RATIO:

Serum albumin was estimated by the modified method of Duma (1971).

Principle -

Albumin in serum binds with the dye Bromocresol green at pH 3.68 to form a green coloured complex, the absorbance of which is measured at 600 nm.

Procedure-

Three test tubes were taken marked as 'blank', 'standard' and 'Test'. 3.0ml of Buffered Dye Reagent was taken in each test tube. 0.02ml of protein standard was taken in a test tube marked as 'Standard'. 0.02ml of serum sample was taken in test (35) tube marked as 'Test'. The test tubes were mixed well and allowed tubes to stand at room temperature for 1 minute. The Optical density of standard (S) and test (T) was measured with a red filter against blank (B).

Calculation-

$$\text{Serum Albumin (g/100ml)} = \frac{\text{O.D. of Test}}{\text{O.D. of Standard}} \times 50$$

Serum globulin (g/100ml) = Serum total proteins – serum albumin

$$\text{Serum Albumin Albumin/Globulin Ratio} = \frac{\text{Serum Albumin}}{\text{Serum Globulin}}$$

OBSERVATION

1. TOTAL PROTEIN:

Control-

The total protein has been observed 35.46±0.80 mg/dl in the control set (Fig. 1).

Potassium Dichromate Treated-

The total protein has been observed 31.40±0.90 mg/dl after 15 days treatment; 28.60±0.50 mg/dl after 30 days treatment; 25.20±0.85 mg/dl after 45 days treatment; 21.30±0.75 mg/dl after 60 days treatment of Potassium dichromate.

The values are significant ($p<0.05$) to highly significantly ($p<0.01$) different after treatment with Potassium dichromate when compared to the control set (Fig. 1).

Zinc Sulphate Treated-

The total protein has been observed 30.10±0.70 mg/dl after 15 days treatment; 24.90±0.66 mg/dl after 30 days treatment; 22.10±0.80 mg/dl after 45 days treatment; 17.50±0.70 mg/dl after 60 days treatment of Zinc Sulphate.

The values are significant ($p<0.05$) to highly significantly ($p<0.01$) different after treatment with Zinc Sulphate when compared to the control set (Fig. 1).

2. ALBUMIN:

Control-

The albumin has been observed 20.50±0.55 mg/dl in the control set (Fig. 2).

Potassium Dichromate Treated-

The albumin has been observed 18.35±0.40 mg/dl after 15 days treatment; 16.30±0.25 mg/dl after 30 days treatment; 14.20±0.33 mg/dl after 45 days treatment; 12.05±0.22 mg/dl after 60 days treatment of Potassium dichromate.

The values are significant ($p<0.05$) to highly significantly ($p<0.01$) different after treatment with Potassium dichromate when compared to the control set (Fig. 2).

Zinc Sulphate Treated-

The albumin has been observed 15.65±0.35 mg/dl after 15 days treatment; 12.80±0.20 mg/dl after 30 days treatment; 10.34±0.28 mg/dl after 45 days treatment; 09.60±0.20 mg/dl after 60 days treatment of Zinc Sulphate.

The values are significant ($p<0.05$) to highly significantly ($p<0.01$) different after treatment with Zinc Sulphate when compared to the control set (Fig. 2).

3. GLOBULIN:

Control-

The globulin has been observed 15.60±0.30 mg/dl in the control set (Fig. 3).

Potassium Dichromate Treated-

The globulin has been observed 13.75±0.15 mg/dl after 15 days treatment; 11.80±0.10 mg/dl after 30 days treatment; 09.60±0.25 mg/dl after 45 days treatment; 08.10±0.10 mg/dl after 60 days treatment of Potassium dichromate.

(66)The values are significant ($p<0.05$) to highly significantly ($p<0.01$) different after treatment with Potassium dichromate when compared to the control set (Fig. 3).

Zinc Sulphate Treated-

The globulin has been observed 12.46±0.10 mg/dl after 15 days treatment; 10.35±0.14 mg/dl after 30 days treatment; 08.12±0.10 mg/dl after 45 days treatment; 06.36±0.12 mg/dl after 60 days treatment of Zinc Sulphate.

The values are significant ($p<0.05$) to highly significantly ($p<0.01$) different after treatment with Zinc Sulphate when compared to the control set (Fig. 3).

4. ALBUMIN/GLOBULIN RATIO (A/G):

Control-

The albumin/globulin ratio has been observed 1.32±0.10 in the control set (Fig. 4).

Potassium Dichromate Treated-

The albumin/globulin ratio has been observed 1.25±0.12 after 15 days treatment; 1.22±0.10 after 30 days treatment; 1.21±0.13 after 45 days treatment; 1.18±0.11 after 60 days treatment of Potassium dichromate.

The values are significant ($p<0.05$) to highly significantly ($p<0.01$) different after treatment with Potassium dichromate when compared to the control set (Fig. 4).

Zinc Sulphate Treated-

The albumin/globulin ratio has been observed 1.22±0.14 after 15 days treatment; 1.20±0.11 after 30 days treatment; 1.17±0.09 after 45 days treatment; 1.12±0.09 after 60 days treatment of Zinc Sulphate.

The values are significant ($p<0.05$) to highly significantly ($p<0.01$) different after treatment with Zinc Sulphate when compared to the control set (Fig. 4).

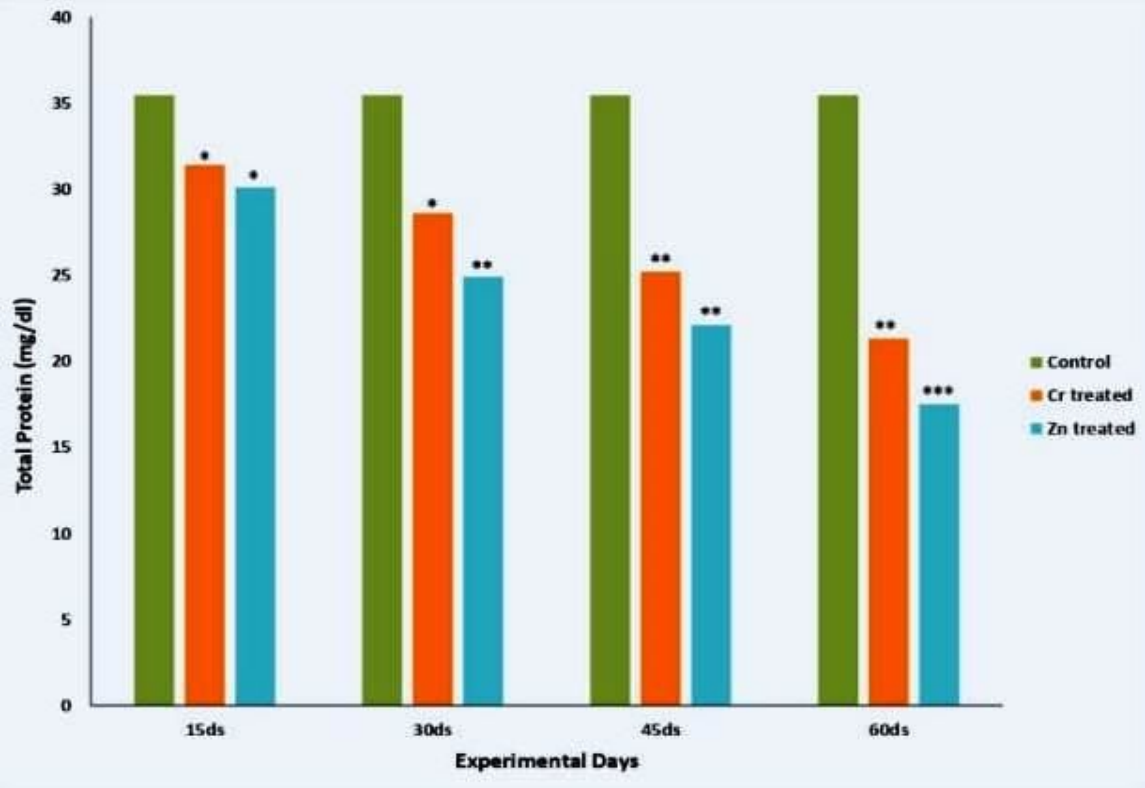

Fig. 1: Effect of Zn and Cr on Total Protein (mg/dl) in *Channa punctatus* (Bloch.) after 15, 30, 45 and 60 days exposure

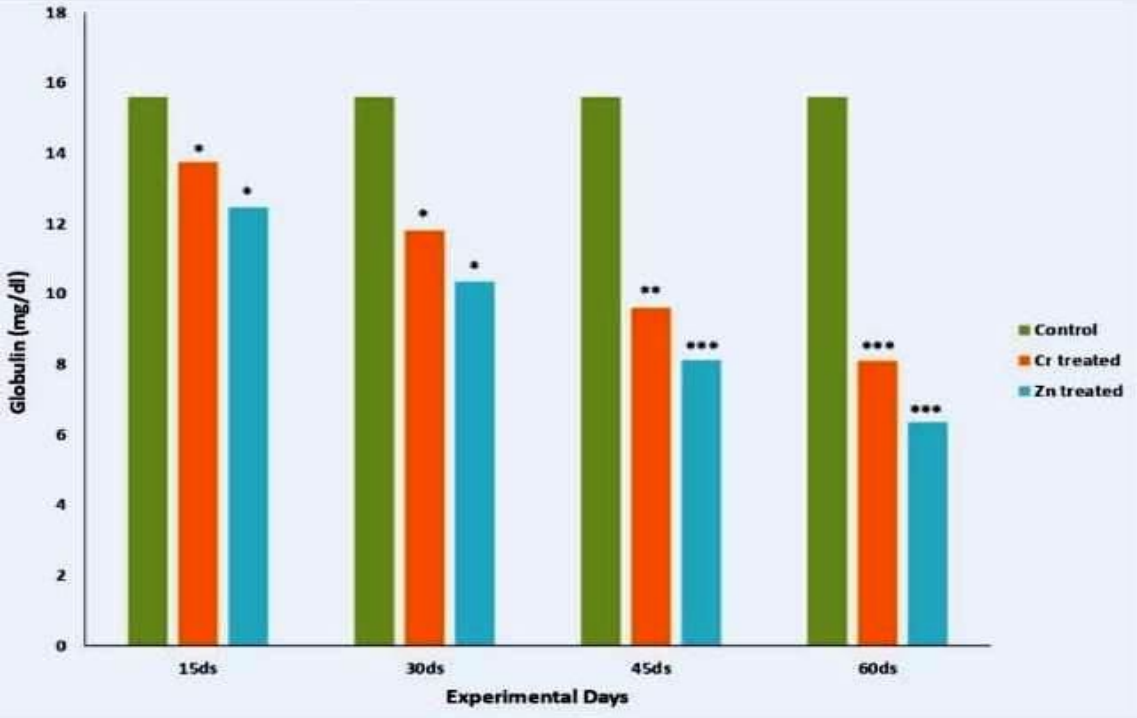

Fig. 2: Effect of Zn and Cr on Globulin (mg/dl) in *Channa punctatus* (Bloch.) after 15, 30, 45 and 60 days exposure

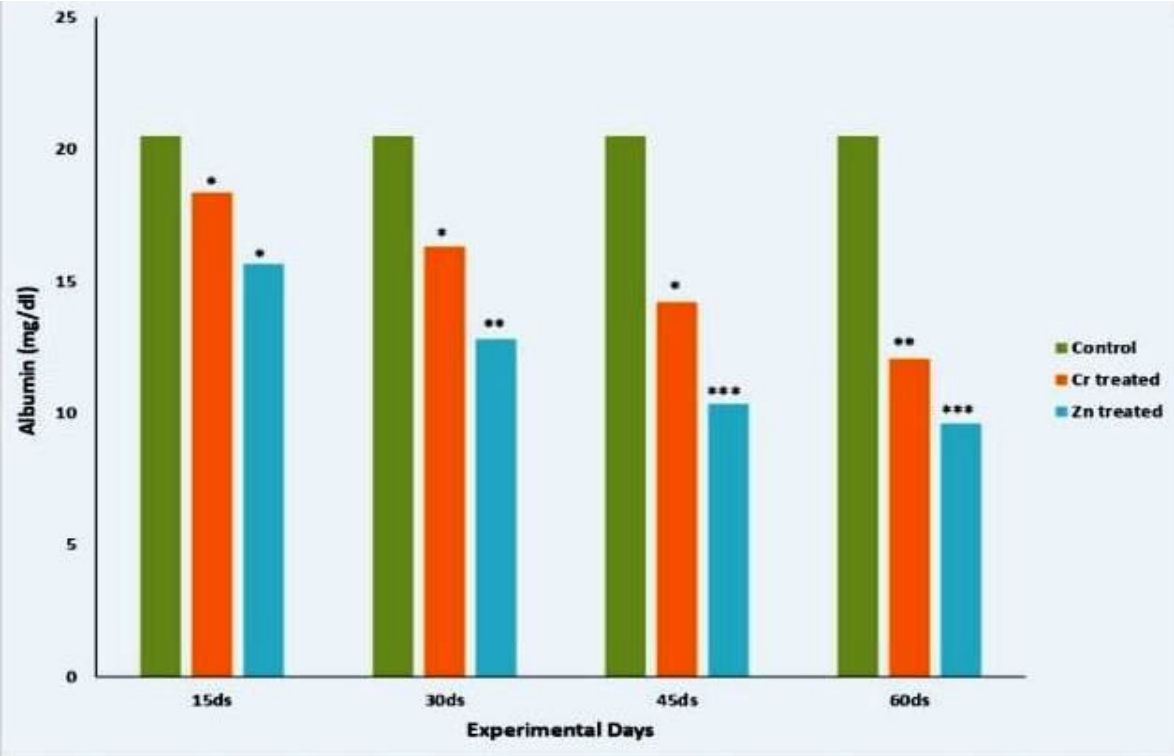

Fig. 3: Effect of Zn and Cr on Albumin (mg/dl) in *Channa punctatus* (Bloch.) after 15, 30, 45 and 60 days exposure

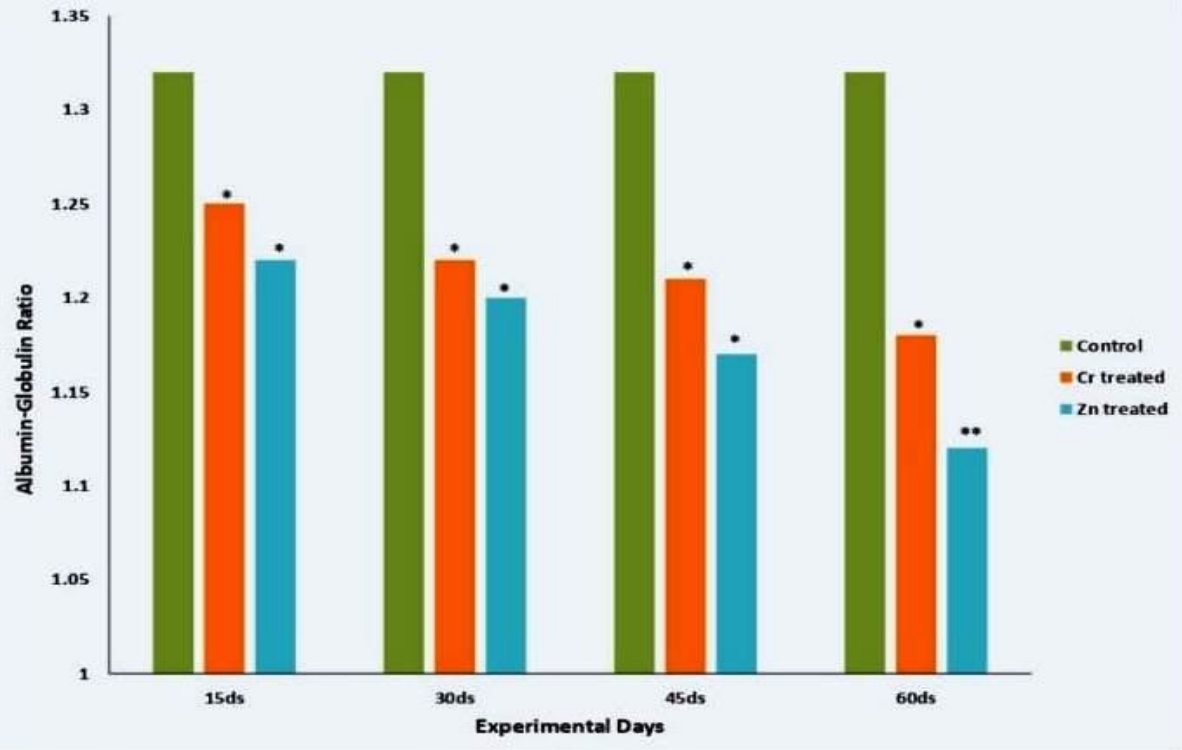

Fig. 4: Effect of Zn and Cr on Albumin/Globulin (mg/dl) in *Channa punctatus* (Bloch.) after 15, 30, 45 and 60 days exposure

DISCUSSION

In the present study Chromium and Zinc produce significant molecular level toxicity prevailing to decrease in total protein, albumin, globulin contents and albumin-globulin ratio after treatment with Potassium dichromate and Zinc Sulphate for 15 days, 30 days, 45 days and 60 days in freshwater fish *Channa punctatus* (Bloch.). The toxic effect was more in Zinc Sulphate treated sets of fishes. In a protein profile, protein is complex organic compound and is main component of every living cell and all body fluids except bile and urine. The basic structure of proteins is a chain of amino acids that are described as essential and nonessential protein or amino acids. Total protein equals to albumins plus globulin. Abnormal tissue protein is usually due to dehydration acute burns nephritic syndromes, chronic liver diseases and other side effects. Albumin is a major component of protein and is synthesized in the liver at the rate of approximately 12 to 14 gram at every 24 hours. Albumin is extremely sensitive to liver damage. There is no storage of albumin by the liver and the daily synthetic rate of albumin represents 50% of the total amount present in the body and a very strong predictor of health. The abnormal decrease in albumin causes hypoalbuminemia. The concentration of albumin drops when the liver is damaged, with kidney disease (nephritic syndrome) and malnutrition. Albumin increases when a person is dehydrated. The investigation of albumin has so much importance not only in the case of edema but also the state of liver function and of protein nutrition.

Total protein measures the combined amount of two classes of proteins albumin and globulin. Globulin is the protein that includes gamma globulins (antibodies) and plenty of enzymes and more than 500 other carrier transport proteins.

There are four major groups that can be identified: gamma globulins, beta globulins, alpha-2 globulins and alpha -1 globulins. Antibodies are produced mature T3- lymphocytes, while most of the other proteins in the A and G fractions are made in the liver globulin antibody deficiency should always causes when globulin level is low. The proteins are important building of all cells and tissues,they are important for body growth and health. The ratio of albumin to globulin (A/G ratio) is calculated from values obtained by direct measurement of total protein and albumin. It represents the relative amounts of albumin than globulins, giving a normal albumin to globulin ratio of slightly over- 1. A reversed albumin to globulin ratio may be a helpful indicator. The proper albumin to globulin ratio is 2: 1 when < 1.7. There may be a need for increasing stomach acidity, while 73.5 there may be need for stomach acidity and pepsin. Abnormal A/G ratio is indicating hypothyroidism, hypogammaglobulinemia and liver dysfunction. In the present investigation protein profile in fish *Channa punctatus* (Bloch.) decreased after Zinc Sulphate and Potassium dichromate exposure with the increased exposure periods. It is assumed that loss of total protein in fish tissues may be associated with intensive proteolysis and inhibition of protein degradation product for mobilization under the influence of metals, it has been incorporated to TCA cycle through transaminase system to cope with the excess demand of energy. The decline protein level may also be due to impaired food intake and increase energy cost of homeostatic and tissue detoxification during stress condition in the fish *Channa punctatus* due to decrease in the availability of energy required for the synthesis of proteins as a consequent of the decreased level of key enzyme (ICDH and MDH) and due to enhanced catabolism of amino acids as a consequence of increased activity as glutamate dehydrogenase. These findings are in affirmation with the findings of Sridhar *et al.* (2002) reported that the protein content of whole body and gut was significantly decreased at higher concentration of Chromium; Vutukuru *at al.* (2003) reported that the total lipid and total protein decrease highly significantly after Chromium induced alteration in *Labeo rohita;*

Pazhanisamy and Indra (2007) reported the reduction in the lives and muscles protein concentrations after inhalation of arsenic; Poornima *et al.* (2007) studied that the total protein and lipid concentration decrease after exposure of cadmium chloride on *Catla catla*; Remyla *et al.* (2007) noticed the plasma protein level decreased under the influence of Zinc and cadmium in *Catla catla*; Srivastava and Srivastava (2008) studied that the total protein and total lipid and cholesterol level decline significantly after chronic exposure of Zn on *Channa punctatus*; Velma and Tchounwou (2010) illustrated the decreased level of total protein at 5% LC50 while increased level at the 10 % of LC50 after intoxication of haxavalent Chromium in *Carassius auratus*; Ranbhare and

Bakare (2012) examine the decline concentration of total protein and total lipids on *Cyprinus carpio and Labeo rohita* after heavy metal exposure*;* Shwetha *et al.* (2012) observed that protein decreased while ammonia level increases respectively at all exposure periods; Tripathi *et al.* (2012) noticed that the protein decreased significantly with sublethal dose of ZnS04; Akan *et al.* (2012) bioaccumulation of some heavy metals in fish sample from river in Vinikliang, Adamawa State, Nigeria; Omar *et al.* (2013) risk reassessment and toxic effects of metal pollution in two cultured and wild fish species from highly degraded aquatic habitats; Pereira *et al*. (2013) studied on the Hematological and biochemical alterations in the fish *Prochilodus lineatus* caused by the herbicide clomazone; Dhanalakshmi and Chitra (2014) reported that the total protein decrease in all the tissues significantly; Shokr (2015) examine the decrease concentration of total protein, creatinine and the activity of aspartate transaminase, alanine transaminase also decrease after Zinc intoxication in *Nile tilapia*; Ranjan *et al.* (2016) examined the decrement in the total protein and lipid in Zinc oxide nanoparticle exposed fish in comparison to control group; Kumar and Banerjee (2016) investigated that the concentration of serum protein lowers at the intoxication of arsenic in *Clarias batrachus*; Palaniappan and Muthulingam (2016) discussed the decrease concentration of protein in kidney, gills and liver at different time intervals after exposure of Chromium in *Channa striatus;* Aslam and Yousafzai (2017) observed the decrease concentration of protein, lipids and glycogen significantly after Chromium toxicity in fish; Batool et al. (2018) reported that the protein level decreased with comparison to control after water born cadmium exposure on the liver of Carnivorous Fish, *Wallago attu*.

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