

THERAPEUTIC AND RESTORATIVE POTENCY OF *SPIRULINA PLATENSIS* IN BIOCHEMICAL PROFILE OF CADMIUM SULPHATE EXPOSED TELEOST., *CLARIAS BATRACHUS*

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ABSTRACT

Present investigation is carried out to study role of *Spirulina platensis* on cadmium treated *Clarias batrachus* as fish health depends upon important biochemical parameters such as Protein, Cholesterol, Glucose, Acid phosphatase and Alkaline phosphatase, which get altered after Cadmium exposure and damages the fish tissue. It interacts with the Calcium metabolism of animal and in fishes causing hypocalcemia, nephrotoxicity, induces oxidative stress, immunotoxicity and also causes functional and structural damages to various organs of fishes. Contaminated food is the most common source of Cadmium toxicity in humans, its regular consumption have increased the exposure in the people. *Spirulina platensis* has shown therapeutic potency against heavy metal cadmium sulphate.

INTRODUCTION

In India increased activities of industrialization as textile, chemicals, synthetic goods, petrochemical and pesticides etc. discharge their chemical hazardous wastes in the rivers and sea which affect the life of important edible fishes. These heavy metals gets dissolved in water and therefore absorbed by the aquatic organism and get transferred to higher animals through the food chain by the process of bioaccumulation altering the normal physiological process and causing damage to the tissue of organism **Malik and Maurya 2014**.

Studies of workers have shown that the Cadmium pollution in aquatic water which is found higher than the WHO permissible limit in some western region of U.P. Comparative study of acute toxicity test of 63 heavy metals, Cadmium is the most toxic metal in unpolluted water, it's concentration is generally less than 1µg/l or part per billion (ppb) **Nordberg et al., 2007; Borgmann et al., 1999**. Cadmium gets biomagnified in the food chain and can accumulate in the human. It enters the fresh water due to various anthropogenic activities **Edmond and Peplow, 2000**. Fishes are the main target organism to study the implication of chemical on aquatic pollution for any toxicological study because of its direct exposure to water bodies as it is easily connected to humans in the form of food chain **Au 2004**. In aquatic ecosystem fishes are the important biological indicators of aquatic pollution. Alterations of antioxidant and biochemical changes are important parameters for toxicity study **Bashir and Zuhair 2008**.

Spirulina platensis is multicellular, symbiotic and filamentous blue green microalgae .It is fresh water algae of the class Cyanobacteria which is good source of protein, energy and used as a less expensive alternative of regular fish feed in place of other chemical based feed stuffs in aquaculture diet **Harel et al., 2002**.The chelating property of *Spirulina platensis* is because of phycocyanin pigment (14%), which stimulates the erythropoiesis hormone production for haematopoiesis and regulate the production of RBC when bone marrow stem cells are damaged by toxic element reported by **Henrikson,1994**. The present study was aimed to evaluate and elucidate the protective role of *Spirulina platensis* against Cadmium Sulphate (CdSO₄) toxicity in the Biochemical parameters of *Clarias batrachus*.

EXPERIMENTAL DESIGN: Present investigation was conducted on catfish, *Clarias batrachus*. Experimental protocols were divided into following heads.

Experimental fish - *Clarias batrachus*.

Experimental chemical - Cadmium Sulphate and *Spirulina platensis*. All these parameters were evaluated after control 30 and 45 days of exposure period according to experimental design.

| Ingredients | basal diet | <i>Spirulina platensis</i> supplemented diet |
|------------------|------------|--|
| Fish meal | 51.25 | 51.25 |
| Wheat flour | 36.75 | 26.75 |
| Cod liver oil | 10.00 | 10.00 |
| <i>Spirulina</i> | – | 10.00 |
| Mineral mix | 2.00 | 2.00 |
| Total | 100.00 | 100.00 |

Experimental groups: The fishes were feed with basal and supplemented diet @ 10% of body weight in control and treated group of fishes. Healthy living 90 specimens of teleost, *Clarias batrachus* were collected from local fish market of Meerut, Hastinapur and nearby areas.

The fishes were divided into following three groups

I- First group of 30 fishes were served as control and feed with basal diet.

II -Second group of 30 fishes were treated with Cadmium Sulphate and feed basal diet.

III-Third group of 30 fishes were treated with Cadmium Sulphate and feed *Spirulina platensis* supplemented diet.

Chemical used: In organic salt of heavy metal Cadmium Sulphate of 99% purity was purchased from Hi media Chemicals Pvt. Limited Mumbai. *Spirulina platensis*, was purchased from market which is Manufactured by E.I.D. parry (India) Ltd.

Preparation of basal and supplemented diet: The diet was prepared as method described by James *et al* (2009). The experimental diet was prepared with the ingredients shown in the table.

Following parameters were studied during the course of study

Biochemical studies

1. Serum Protein- Total Serum protein was estimated by the Biuret method, end point kit method.
2. Serum Cholesterol- Serum cholesterol was estimated by kit method (Chod-pap method (with LCF), end point).
3. Serum Glucose - Serum glucose was estimated by the kit method (God-Pod Method, End point) from Erba Company (Transasia Bio-medicals LTD)
4. Acid phosphatase- Serum Alkaline phosphatase was determined by the kit method.
5. Alkaline phosphatase- Serum Alkaline phosphatase was determined by the kit method.

OBSERVATIONS

BIOCHEMICAL STUDY- Biochemical parameters of the experimental and control group of fishes, *Clarias batrachus* has been studied after 30 and 45 days of exposure of time.

GROUP I - CONTROL

Group I served as control and the fish, *Clarias batrachus* feed with Basal diet.

Serum Protein- The Serum Protein in *Clarias batrachus*, fishes of control group after 30 days, post treatment period were observed to be 4.00 gm/dl. This observed value was slightly decreased when ($p<0.05$) compared to control group values after 45 days.

Serum Cholesterol - Serum Cholesterol in *Clarias batrachus*, fishes of control group after 30 days, post treatment period were observed to be 166.75 mg/dl. This observed value was increased when ($p<0.05$) compared to control group values after 45 days.

Serum Glucose - Serum Glucose in *Clarias batrachus*, fishes of the control group after 30 days, post treatment period were observed to be 57.83 mg/dl. This observed value showed decline, after 45 days.

Acid Phosphatase - Acid Phosphatase in *Clarias batrachus*, fishes of the control group after 30 days, post treatment period were calculated to be 7.79 U/L. This observed value was slightly decreased, when ($p<0.05$) compared to control group values after 45 days.

Alkaline Phosphatase - Alkaline Phosphatase in *Clarias batrachus*, fishes of the control group after 30 days, post treatment period were calculated to be 12.38 U/L. This observed value was slightly decreased, after 45 days.

Table 1.

| Parameters | 30 days | | | 45 days | | |
|-------------------------|-------------------------------|--|--|---------------------------------|--|--|
| | Control | Treated with Cadmium Sulphate and Basal diet | Treated with Cadmium Sulphate and <i>Spirulina platensis</i> supplemented diet | Control | Treated with Cadmium Sulphate and Basal diet | Treated with Cadmium Sulphate and <i>Spirulina platensis</i> supplemented diet |
| Protein | 4.0000 ±.66144 ±.38188 | 3.2333 ±.10408 ±.06009 | 3.6200 ±.12530 ±.07234 | 3.6633 ±.12097 ±.06984 | 2.7233 ±.17616 ±.10171 | 3.2033 ±.27392 ±.15815 |
| cholesterol | 166.752 ±4.1335 ±2.3865 | 190.182 ±7.97476 ±4.60423 | 175.932 ±5.86504 ±3.38618 | 180.672 ±5.1316 ±2.9627 | 202.332 ±8.08290 ±4.66667 | 193.672 ±7.09460 ±4.09607 |
| Glucose | 57.3833 ±1.3188 ±.76145 | 59.0000 ±1.44375 ±.83355 | 58.5767 ±.86118 ±.49720 | 54.4633 ±3.04714 ±1.75926 | 77.2167 ±5.28617 ±3.05197 | 64.3700 ±4.57625 ±2.64210 |
| Acid phosphatase | 7.7933 ±.15535 ±.08969 | 8.5900 ±.07937 ±.04583 | 7.5267 ±.49662 ±.28672 | 7.7467 ±.31005 ±.17901 | 8.7100 ±.21000 ±.12124 | 7.5233 ±.18610 ±.10745 |
| Alkaline | 12.3833 | 11.3467 | 11.4000 | 12.1067 | 10.3200 | 11.0267 |

| | | | | | | |
|-------------|---------|---------|---------|---------|---------|---------|
| phosphatase | ±.41235 | ±.19502 | ±.33045 | ±.53304 | ±.62960 | ±.56889 |
| | ±.23807 | ±.11260 | ±.19079 | ±.30775 | ±.36350 | ±.32845 |

GROUP II - TREATED WITH CADMIUM SULPHATE AND BASAL DIET BIOCHEMICAL STUDY

Biochemical study of the group II *Clarias batrachus*, fishes treated with Cadmium Sulphate and Basal diet has been observed after 30 and 45 days of exposure time.

Serum Protein

1(a) The Serum Protein in Group II *Clarias batrachus*, fishes treated with Cadmium Sulphate and Basal diet, after 30 days were observed to be 3.23 gm/dl. This observed value was decreased, when ($P < 0.05$) compared to control value after 30 days.

1(b) The Serum Protein in Group II *Clarias batrachus*, fishes treated with Cadmium Sulphate and Basal diet, after 45 days were observed to be 2.72 mg/dl. This observed value was decreased, when ($P < 0.05$) compared to control value after 45 days.

Serum Cholesterol

1(a) Serum Cholesterol in Group II *Clarias batrachus*, fishes treated with Cadmium Sulphate and Basal diet, after 30 days were observed to be 190 gm/dl. This observed value was increased, when ($P < 0.05$) compared to control value after 30 days.

1(b) Serum Cholesterol in Group II *Clarias batrachus*, fishes treated with Cadmium Sulphate and Basal diet, after 45 days were observed to be 202 gm/dl. This observed value was increased, when ($P < 0.05$) compared to control value after 45 days.

Serum Glucose

1(a) Serum Glucose in Group II *Clarias batrachus*, fishes treated with Cadmium Sulphate and Basal diet, after 30 days were observed to be 59.00 gm/dl. This observed value was increased, when ($P < 0.05$) compared to control value after 30 days.

1(b) Serum Glucose in Group II *Clarias batrachus*, fishes treated with Cadmium Sulphate and Basal diet, after 45 days were observed to be 77.2 gm/dl. This observed value was increased, when ($P < 0.05$) compared to control value after 45 days.

Acid Phosphatase

1(a) Acid phosphatase in Group II *Clarias batrachus*, fishes treated with Cadmium Sulphate and Basal diet, after 30 days were observed to be 8.59 U/L. This observed value was found to be increased, when ($P < 0.05$) compared to control value after 30 days.

1(b) Acid phosphatase in Group II *Clarias batrachus*, fishes treated with Cadmium Sulphate and Basal diet, after 45 days were observed to be 8.71 U/L. This observed value was increased, when ($P<0.05$) compared to control value after 45 days.

Alkaline Phosphatase

1(a) Alkaline Phosphatase in Group II *Clarias batrachus*, fishes treated with Cadmium Sulphate and Basal diet, after 30 days were observed to be 11.34 U/L. This observed value was decreased, when ($P<0.05$) compared to control value after 30 days.

1(b) Alkaline Phosphatase in Group II *Clarias batrachus*, fishes treated with Cadmium Sulphate and Basal diet, after 45 days were observed to be 10.32 U/L. This observed value was decreased, when ($P<0.05$) compared to control value after 45 days.

GROUP III - TREATED WITH CADMIUM SULPHATE AND *SPIRULINA PLATENSIS* SUPPLEMENTED DIET

Biochemical Study - Biochemical study of the group III *Clarias batrachus*, fishes treated with Cadmium Sulphate and *Spirulina platensis* supplemented diet has been observed after 30 and 45 of exposure time. (Table 3)

Serum Protein

1(a) The Serum Protein in Group III *Clarias batrachus*, fishes treated with Cadmium Sulphate and *Spirulina platensis* supplemented diet, after 30 days were observed to be 3.62 mg/dl. This observed value was increased, when ($P<0.05$) compared to Group II value after 30 days.

1(b) The Serum Protein in Group III *Clarias batrachus*, fishes treated with Cadmium Sulphate and *Spirulina platensis* supplemented diet, after 45 days were observed to be 3.20 mg/dl. This observed value was decreased, when ($P<0.05$) compared to Group II value after 45 days.

Serum Cholestrol

1(a) Serum Cholestrol in Group III *Clarias batrachus*, fishes treated with Cadmium Sulphate and *Spirulina platensis* supplemented diet, after 30 days were observed to be 175.93 mg/dl. This observed value was decreased, when ($P<0.05$) compared to Group II value after 30 days.

1(b) Serum Cholestrol in Group III *Clarias batrachus* fishes treated with Cadmium Sulphate and *Spirulina platensis* supplemented diet, after 45 days were observed to be 193.67 mg/dl. This observed value was decreased, when ($P<0.05$) compared to Group II value after 45 days.

Serum Glucose

1(a) Serum Glucose in Group III *Clarias batrachus*, fishes treated with Cadmium Sulphate and *Spirulina platensis* supplemented diet, after 30 days were observed to be 58.57 mg/dl. This observed value was decreased, when ($P < 0.05$) compared to Group II value after 30 days.

1(b) Serum Glucose in Group III *Clarias batrachus*, fishes treated with Cadmium Sulphate and *Spirulina platensis* supplemented diet, after 45 days were observed to be 64.37 mg/dl. This observed value was decreased, when ($P < 0.05$) compared to Group II value after 45 days.

Acid Phosphatase

1(a) Acid Phosphatase in Group III *Clarias batrachus*, fishes treated with Cadmium Sulphate and *Spirulina platensis* supplemented diet, after 30 days were observed to be 7.52 U/L. This observed value was decreased, when ($P < 0.05$) compared to Group II value after 30 days. and

1(b) Acid Phosphatase in Group III *Clarias batrachus*, fishes treated with Cadmium Sulphate and *Spirulina platensis* supplemented diet, after 45 days were observed to be 7.52 U/L. This observed value was decreased, when ($P < 0.05$) compared to Group II value after 45 days.

Alkaline Phosphatase

1(a) Alkaline Phosphatase in Group III *Clarias batrachus*, fishes treated with Cadmium Sulphate and *Spirulina platensis* supplemented diet, after 30 days were observed to be 11.40 U/L. This observed value was increased, when ($P < 0.05$) compared to Group II value after 30 days.

1(b) Alkaline Phosphatase in Group III *Clarias batrachus*, fishes treated with Cadmium Sulphate and *Spirulina platensis* supplemented diet, after 45 days were observed to be 11.02 U/L. This observed value was increased, when ($P < 0.05$) compared to Group II value after 45 days.

DISCUSSION

Protein is the most abundant biological macromolecules and involves major physiological events. It is the main part of the architecture of the cell. During present investigation Total serum protein showed normal condition in all the three groups after 30 and 45 days of post treatment period. Total serum protein after 30 and 45 days were observed to be 3.23 gm/dl and 2.72 gm/dl in Cadmium sulphate treated fish in group II. These results showed reduction with increment of time. These parameter showed reduction when compared to control group of fishes. During Cadmium stress, the proteolysis was intended to increase the role of proteins in the energy production and also decreases in the level of protein and amino acid

content (Smet and Blust 2001 and Sobha *et al.*, 2007). Pattanyak and Behera 2020 observed the similar results, and mentioned the decrease in the protein content after 14 days post treatment period in *Clarias batrachus*. Reduction in the serum protein level has been reported by Garg *et al.*, 1990 in *Clarias batrachus* against heavy metal. Many other workers also reported similar findings such as Garg *et al.*, 1989. Singh and Reddy *et al.*, 1990 and Matty *et al.*, 1988 reported identical findings in the fish, *Sarotherodon mossambicus*.

Total Serum Protein in fishes treated with *Spirulina platensis* supplemented diet with Cadmium sulphate diet was observed to be 3.62 gm/dl and 3.20 gm/dl, 3.43 gm/dl after 30 and 45 days of post treatment time. These values showed increase in comparison to Group II Cadmium Sulphate treated fish. These results are in agreement with the Makhbatly *et al.*, 2020, in *Clarias batrachus* against Chlorpyrifos toxicity. Priya and Remya 2016 and Vettrivel *et al.*, 2013 also provided the account that the *Spirulina* supplemented diet increase the protein content in the muscle of fish in *Ctenopharygdon idealla* and *Cyprinus carpio* against Cadmium and Lead induced toxicity. Priya *et al.*, 2018 find out the *Spirulina platensis* diet increase the protein content in the liver tissue against Cadmium induced toxicity in *Oreochromis mossambicus*. Sakthivel *et al.*, 2017 also reported that *Spirulina* supplemented feed increase the protein content in the muscle of Gill, Liver and kidney of fish. In the present investigation Serum cholesterol showed increase in group II when compared to control group I. Serum cholesterol in the Group II treated fishes with Cadmium Sulphate and Basal diet observed to be 190.18 gm/dl and 193.67gm/dl. These values showed increment with the time after 30 and 45 days of post treatment period. These results were also in agreement with the Murrey *et al.*, 1991 evident from the present study that hypercholesterolemia observed in *Clarias batracus* may be due to the impairment of liver and inhibitions of enzyme. These observation also showed some similarity with Yang and Chen, 2003. Cholesterol concentration in the Group III fishes treated with *Spirulina platensis* diet with Cadmium Sulphate observed to be 175.93 gm/dl and 193.67 gm/ after 30 and 45 days. These values showed reduction in the parameters in comparison to Cadmium Sulphate treated fishes in group II. There is no significant study is related to this parameter. In the present study the glucose content was observed to be 59.00 mg/dl, 77.21 mg/dl and 80.41 mg/dl after 30 and 45 days interval of time in group II. These value showed increment in comparison to control group of fishes. Pattanayak and Behora, 2020 find out the related work in *Clarias batrachus* against Cadmium toxicity and proved that Cadmium reduced the glucose level in the tissues of fishes. Gill and Pant, 1983 reported similar observation against Cadmium

induced toxicity.

Glucose concentration in the Group III fishes treated with *Spirulina platensis* supplemented diet with Cadmium Sulphate showed decrease in the parameter in comparison to Cadmium Sulphate treated fishes in the group II with Basal diet and the observed values were noted to be 58.57 mg/dl and 64.37 mg/dl after 30 and 45 days of post treatment period. These finding are supported by **Makhbatly et al., 2020** in *Clarias gariepinus* against Cadmium induced toxicity *Spirulina platensis* supplemented diet also decrease the amount of glucose in the Gill and liver tissue against Lead Acetate toxicity in fish *Cyprinus carpio*. The value of Acid Phosphatase in Group II fishes treated with Cadmium Sulphate with Basal diet were observed to be 8.59 U/L and 8.71 U/L after 30, 45 days respectively these value showed increment in the parameter when compared to group I control fishes. Acid phosphatase value in Group III treated with *Spirulina platensis* diet with Cadmium Sulphate were observed to be 7.52 U/L and 7.52 U/L after 30, 45 days respectively. These observations showed decrease when compared to group II Cadmium Sulphate treated fish.

The value of Alkaline phosphatase or ALP in group II were observed to be 11.34 U/L and 10.32 U/L after 30 and 45 days respectively. The values showed reduction in the parameter in comparison to control. Similar kind of reduction were reported in the earlier study in the hepatic ALP of *Heteropneustes fossilis* against Cadmium Sulphate after 15, 30 and 60 days of post treatment period by **Sastry and Subhadra 1985**. also reported significant decline in the hepatic ALP of *Sarotherodon messambicus* after an acute exposure of Mercury.

The value of ALP in Group III treated with *Spirulina platensis* diet and Cadmium Sulphate observed 11.49 U/L after 30 days and 45 days the ALP value were observed to be 11.02 U/L in this group. These values showed reduction in the parameter in comparison to Group II Cadmium Sulphate treated fishes there is no significant similar study found to relate to this work. The metallo- protective role of *Spirulina* may be attributed to the presence of Beta-carotene **Prescott 1978 and Seshadri and Jeji bai 1992**

CONCLUSION

Biochemical parameters are important biomarkers for assessing health of fishes particularly edible fish *Clarias batrachus*. Cadmium exposure caused alterations in all parameters Serum protein content increased in the group III fishes treated with *Spirulina platensis* supplemented diet with Cadmium Sulphate. Fishes in Group III treated with *Spirulina platensis*

supplemented diet with Cadmium Sulphate decreased in the parameter as compare to Group II Cadmium Sulphate treated fishes. Serum Glucose concentration decreased in the Group III fishes treated with *Spirulina platensis* supplemented diet with Cadmium Sulphate in comparison to Cadmium Sulphate treated fish in Group II. Acid Phosphatase increased in Cadmium Sulphate treated fishes in Group II after 30 and 45 days post treatment period. Group III fishes treated with *Spirulina platensis* supplemented diet with Cadmium Sulphate showed reduction in the parameter as compare to Cadmium treated fishes. Alkaline Phosphatase (ALP) increased in the group III fishes treated with *Spirulina platensis* supplemented diet along with Cadmium Sulphate in Comparison to Cadmium Sulphate treated fishes Group II. Supplementation of diet with *Spirulina platensis* brings positive outcomes in present study.

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