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PROTECTIVE ROLE OF *SPIRULINA PLATENSIS* ON HAEMATOLOGICAL PROFILE OF CADMIUM SULPHATE EXPOSED TELEOST, *CLARIAS BATRACHUS*

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ABSTRACT

In the present investigation protective role of *spirulina platensis* was evaluated on heavy metal treated *Clarias batrachus*. 96 hr Lc 50 value for Cadmium sulphate was calculated to be 13.8mg/l. Following parameters were evaluated viz. TLC, TEC, Hb and PCV for the period of 24, 48, 72,96 and 120 hours. Fishes were divided in 4 groups group1: control, group2: treated with cadmium, group 3: treated with cadmium along with *Spirulina platensis* supplemented diet, group 4: supplemented with *Spirulina platensis*. Experimental chemical Cadmium showed decline in all the parameters. *Spirulina platensis* provides protective role and help in improvement in all parameters as *Spirulina* contains pycocynanin, which is potent antioxidant.

KEYWORDS: *Spirulina platensis*, cadmium sulphate, *Clarias batrachus.*

INTRODUCTION

Cadmium, is one of the major contaminate of aquatic environment and is toxic towards aquatic organisms even at concentration found in natural waters, referred as non-essential element with no known biological role. Black listed by 'Black list 'of European Community and is reported as major contaminant of aquatic ecosystem causing adverse effects to aquatic ecosystems.[1] Cadmium is reported to be associated with the effluents of battery, electroplating, fertilizers, mining, metallurgy, paints and dye industries. Fish have been the focus of many ecotoxicological studies since they are located at the top of the aquatic food chain. Blood is highly susceptible to internal and external environment fluctuations because it

is the vehicle for the transport of such pollutants[2] and is currently considered as an essential index to the general health status in a number of fish species. Thus day by day increased use of cadmium is posing great threat to fresh water ecosystem especially fish and fisheries which are highly susceptible to chemicals *Clarias batrachus*, which is important food fish in India is contaminated by heavy metal, Cadmium sulphate and suffers pathological alterations, with consequent inhibition of metabolic processes, alterations in Haematological parameters.

Since microalgae is considered as the natural food for aquatic animals, one of such microalgae is *Spirulina platensis*. A cyanobacterium, used in many countries as nutritional supplement for human and animal consumption rich in proteins, carbohydrates, polyunsaturated fatty acids, Sterols, minerals and vitamins.[3][4][5][6] *Spirulina platensis* has been used as a natural feed supplement for increasing fish growth in various aquaculture practices and has been studied by various workers. It provides phycocyanin, a source of biliverdin which is among the most potent of all intra-cellular antioxidants. The present study will be aimed to evaluate and elucidate the protective role of *Spirulina platensis* against Cadmium ($CdSo_4$) toxicity in the haematological profile of *Clarias batrachus.*

MATERIALS AND METHODS

Test fish: Healthy living specimen of teleost, *Clarias batrachus* were collected from local fish market of Meerut. About 50 fishes, measuring 15 ± 2cm in length and 60 ± 8 gm in weight were selected for the present study. Prior to the experimentation, fishes were thoroughly washed for 5 minutes with ·01% Kmno4 to avoid any dermal infection.

Preparation of basal and supplemented diet: The diet was prepared as method described by worker.[8] The experimental diet was prepared with the ingredients shown in the table.

Diet composition (% of dry weight)

Ingredients	basal diet	SP supplemented diet
Fish meal	51.25	51.25
Wheat flour	36.75	26.75
Cod liver oil	10.00	10.00
Spirulina	_	10.00
Mineral mix	2.00	2.00
Total	100.00	100.00

Determination of Lc-50 for Cadmium Sulphate: 96-hour median tolerance limit (96^{-} hr) Lc 50 was determined (at a static condition) by exposing the fishes to seven (2,4,6,8,10,12,14

mg/l) ascending concentrations of cadmium sulphate cumulative mortality was determined after 96-h, the dead fishes were removed once observed. The 96-h Lc-50 13.8 mg/l for Cadmium Sulphate was determined by graphically plotting the percentage mortality versus concentration of chemicals.

Collection and preservation of blood: The blood samples for haematological estimation were collected from the caudal vein of fish with the help sterilized dry syringe in small glass vials. Anticoagulant di-potassium salt of ethylene di-amine tetra acetic acid. (K_2EDTA) was added to the vials before collecting the blood.

Following methods were used for calculation haematological profile: TLC was recorded with the help of haemocytometer (Superior Marienfeld, Germany) with improved Neubauer ruling and Turks solution. RBC were calculated.[7] Method. Shali's acid haematin method was used for estimation of haemoglobin percent.

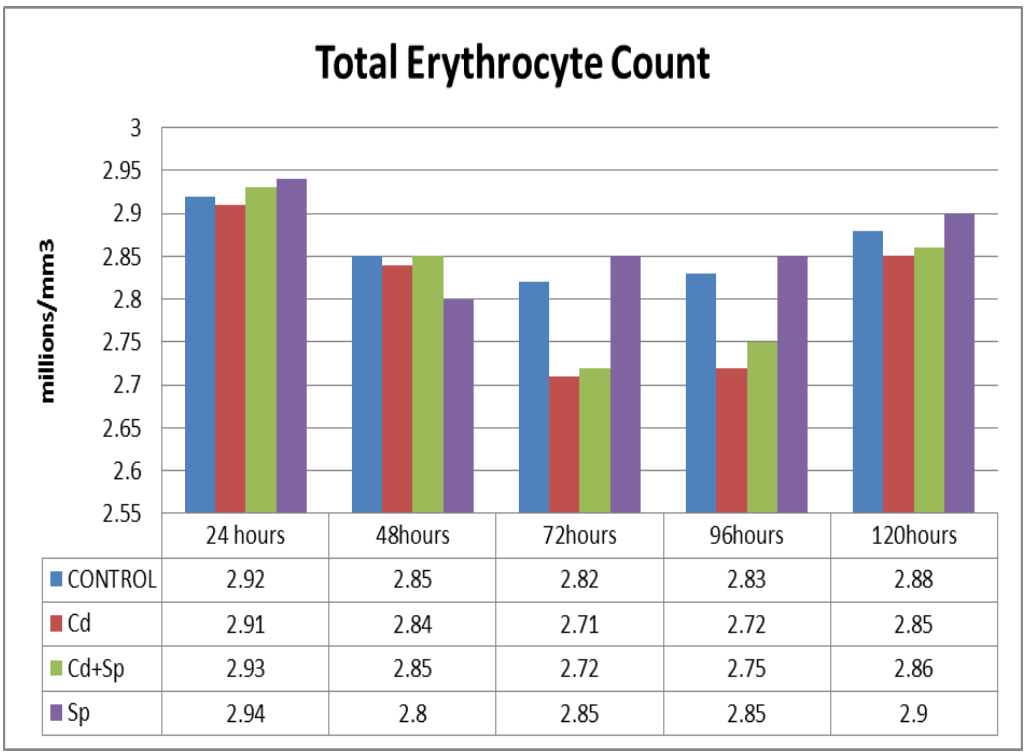
Stastical tests: For Statistical analysis Gen Stat Statistical Analysis software was used to calculate analysis of variance and Ducan Multiple range test was used to test level of significance.

Preparation of supplemented diet: Basal diet was prepared using Proteins (50.00%), carbohydrates (30%), cod liver oil (8.00%), and minerals and vitamins (2%), while supplemented diet was prepared by supplementing 10% SP in to basal diet by replacing same quantity of carbohydrates as described by workers.[8] Fishes were divided in four groups (**group I**) – control group of fishes, (**group II**) fishes treated with cadmium, (**group III**)- fishes exposed to cadmium+ feed with *Spirulina platensis*, (**group IV**) fishes feed with supplemented diet of *Spirulina platensis*.

RESULTS

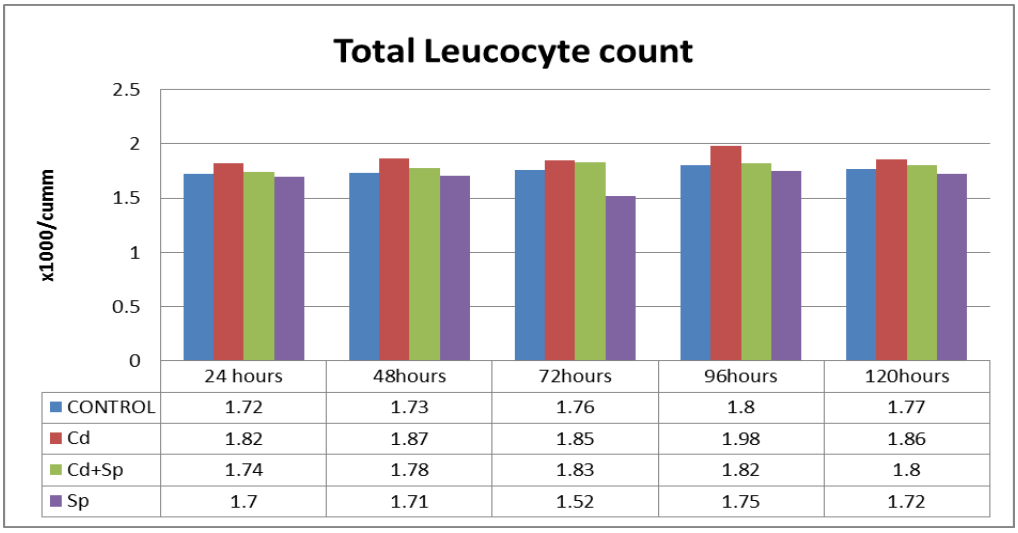
Total Erythrocyte Count showed decline in all the values after post treatment with Cadmium Sulphate, following observations viz.2.91, 2.84,2.71,2.72 and 2.85millions/mm^3 were {fig1}recorded. Further there was observed improvement in this parameter when both cadmium and *Spirulina* supplemented diet is administered together, shows progressive improvement after all exposure periods. The observed value are 2.94, 2.8, 2.85, 2.85 and 2.9.millions/mm^3**.** The control value of WBC count observed were 1.72, 1.73, 1.76, 1.8, 1.77 x1000$mm^{3.}$ Whereas increase in parameter was prominantly observed after treatment with

cadmium and the values observed were 1.82, 1.87, 1.85, 1.98 and 1.86 /1000 mm^3 {fig 2} also there was observed increase in parameter in group III where as group IV showed decline in parameter in only *Spirulina platensis* group but still parameters improves as compared to group II. Packed cell volume showed decline in parameter after treatment with cadmium sulphate i.e 36.8, 28.5, 32.6, 31 and 32.4% {fig3}. However there is observed improvement in parameter after treatment with *Spirulina* supplement diet. The value observed are 37.5, 30.2, 35.8, 32.5 and 33.6%. *Spirulin*a alone showed positive increase in value when the value were observed in group IV. When the experimental fishes were treated with cadmium sulphate Hb count was decreased the observe value are 9.8, 9.6, 9.6, 9.4 and 10 gm/100ml {fig4} these parameter showed increase in group III also. There was noted progressive increase in group IV the value observed are 10.4, 10.2, 10.8, 10.6, 10.8 gm/100ml.



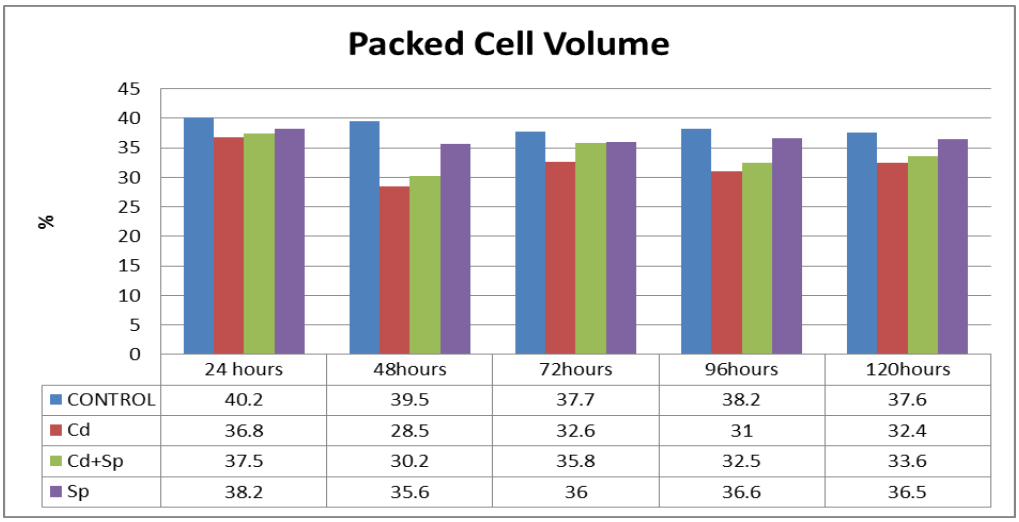
	24 hours	48hours	72hours	96hours	120hours
CONTROL	2.92	2.85	2.82	2.83	2.88
Cd	2.91	2.84	2.71	2.72	2.85
Cd+Sp	2.93	2.85	2.72	2.75	2.86
Sp	2.94	2.8	2.85	2.85	2.9

Figure: 1.



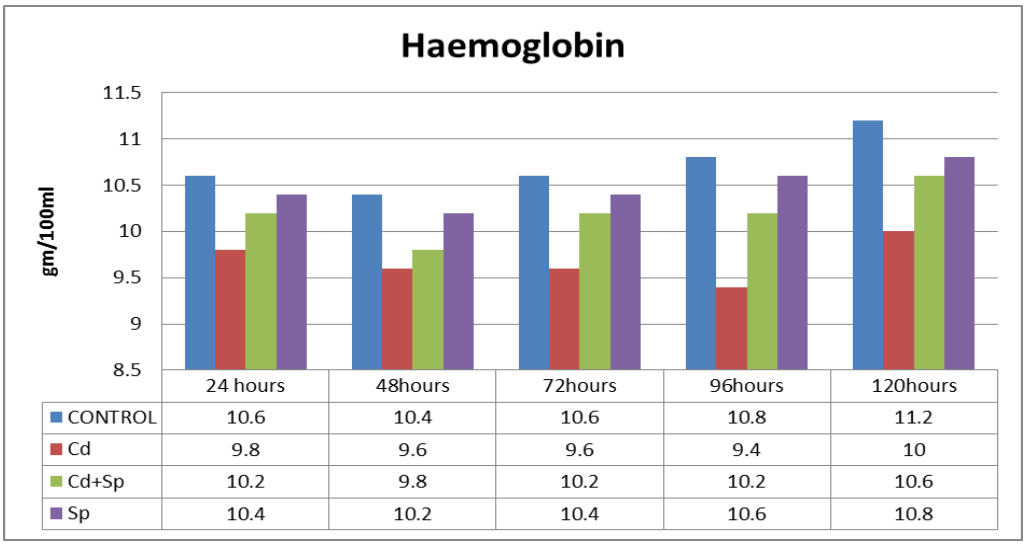
	24 hours	48hours	72hours	96hours	120hours
CONTROL	1.72	1.73	1.76	1.8	1.77
Cd	1.82	1.87	1.85	1.98	1.86
Cd+Sp	1.74	1.78	1.83	1.82	1.8
Sp	1.7	1.71	1.52	1.75	1.72

Figure: 2.



	24 hours	48hours	72hours	96hours	120hours
CONTROL	40.2	39.5	37.7	38.2	37.6
Cd	36.8	28.5	32.6	31	32.4
Cd+Sp	37.5	30.2	35.8	32.5	33.6
Sp	38.2	35.6	36	36.6	36.5

Figure: 3.



	24 hours	48hours	72hours	96hours	120hours
CONTROL	10.6	10.4	10.6	10.8	11.2
Cd	9.8	9.6	9.6	9.4	10
Cd+Sp	10.2	9.8	10.2	10.2	10.6
Sp	10.4	10.2	10.4	10.6	10.8

Figure: 4

DISCUSSION

Total Erythrocyte Count in the present work showed decline after all exposure periods when treated with cadmium sulphate the reason being total erythrocyte count indicates worsening of an organism state due to cadmium sulphate and subsequently the organism develop state of anemia. This work is in conformation with the following workers[8][9][10] on *Pleuronecteus flesus and Oreochromis mossambicus peters* and various workers worked on different aspects.[11][12][13][14] The decreased value indicates acute anemia. The anemia could be due to destruction of RBC.[15][16] After supplementation of diet with *Spirulina* the observed results proved that *Spirulina* contains carotenoids, which increase the ability to fight off infections through the reduction of stress levels.[17] Total Leucocyte Count showed increase in value after treatment with cadmium sulphate when compared with control parameters. The major functions of WBC's are to fight infection and defend the body against foreign organisms and in immune response. Inclination in WBC's counts in treated fish after food shortage stress. These observation are in aggrement with studies conducted by workers[18][19][20][21] in blood of *Acipenser baeri.* The increase in WBCs content recorded in the present investigation after cadmium treatment when compared with control could be due to the attempt of the fish to fight against the antigens (pollutant) and this augmented the production of more WBC to improve the health status of the fishes which agreed with the reports of workers.[22][23] The increased in WBC count can be correlated with an increased in antibody production which helps in survival and recovery of fish exposed to sub-lethal concentration of pesticides[24][25] reported an increased in TLC in *Channa punctatus* exposed fenvalerate.

When *Clarias batrachus* is fed with cadmium and supplemented diet there is improvement in parameter, as the supplemented diet of *Spirulina* alone, brings positive inclination during all the duration period. The investigation is supported by workers.[26][27] These results are in good agreement with earlier work that reported by various workers a significant decrease in RBC's, Haemoglobin and Packed Cell Volume metabolic and hemopoietic activities of fish exposed to sub- lethal concentration of pollutants.[28][29][30] Packed cell volume decline in cadmium treated observations which are in aggrement with earlier work which is reported by[11][13][30][31] on fresh water fish. Feeding dietary *Spirulina* had a significant ($P < 0.05$) increase in the levels of haematocrit[31] recorded that haematocrit value in treated fish was lower than that of control fish. On the other hand, the obtained results revealed that feeding dietary.

Haemoglobin showed observations which are in accordance with reports of workers.[11][13][32][10] The decline in concentration of haemoglobin is same as earlier studies done on blood of eel and perch after exposure to cadmium sulphate by workers[33] observed a decline in haemoglobin in the blood of tilapia (*Oreochromis mossambicus Peters)* depending on the duration of exposure and the concentration of cadmium. Significant reduction in haemoglobin in experimental animals might be destruction (decrease in haemoglobin) has been reported by various workers. Exposure to heavy metals or pesticides leads to reduced haemoglobin content and haematocrit *via* disorders in haemopoietic and accelerated disintegration of erythrocyte cell membrane. These results are in good agreement with earlier work that reported by various workers a significant decrease in RBC's, Haemoglobin and Packed Cell Volume of fresh water fish exposed to heavy metals observed by.[29][30][32][33] This increase could be due to the presence of C-phycocyanin in the *Spirulina* alga, which can help build the immunity capacity supported by work of various workers.

CONCLUSION

The present study showed haematological profile for the *Clarias batrachus* species and conclude, that *Spirulina* feeding improved the haemtological parameters in all exposed groups compared with treated and control groups. Thus using *Spirulina* as immun-inducer and growth factor in food ingredients supplemented in diet can be recommended. Chlorophyll of *Spirulina* acts as a cleansing and detoxifying phytonutrient against the toxic substances and heavy metals. It can also be concluded that *Spirulina's* supplementation in diet is promising for improving the haematological parameters in *Clarias batrachus*, which is exposed to heavy metal cadmium and proves as immun-inducer and as a chelating agent in food ingredients for fish health. *Spirulina* contains phycocynin (14%) cholorophyll (1%) and carotenoid (0.37%) pigments. Carotenoid of *Spirulina* maintains the mucous membrane firmly and thereby entry of toxic elements in to the body is prevented.

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