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Protective effects of dietary spirulina against cadmium chloride exposed histoarchitectural changes in the liver of freshwater catfish *Clarias batrachus* (Linnaeus, 1758)

BILAL AHMAD DAR[1], RUMYSA KHALIQ[2], G. N. JHA[1], PINKY KOUR[3] AND T. A. QURESHI[3]

[1]*316, Project Implementation Unit, National Agricultural Innovation Project (NAIP), Indian Council of Agricultural Research (ICAR), Pusa, New Delhi - 110 012, India*

[2]*Department of Biotechnology, Saifia Science College, Bhopal - 462 026, India*

[3]*Department of Zoology and Applied Aquaculture, Barkatullah University, Bhopal - 462 026, Madhya Pradesh, India*

e-mail: bilalaqua@gmail.com

ABSTRACT

Hepatoprotective and antioxidant effects of dietary supplementation of *Spirulina platensis* (SP) against cadmium chloride ($CdCl_2$) exposed catfish, *Clarias batrachus* was elucidated during sixty days experiment and the histoarchitectural changes in the liver were assessed. One hundred fishes (average weight 90±6 g and average length 20±4 cm) were randomly distributed (20 in each tank) into 5 treatment groups *viz*., T_0 (control), T_1 and T_2 (exposed to 4 ppm $CdCl_2$) and T_3 and T_4 (exposed to 8 ppm $CdCl_2$). Fishes of T_0, T_1 and T_3 groups were fed with normal (basal) diet, while of T_2 and T_4 fed with 10% SP supplemented diet. Deshaping of hepatocytes, eccentric position of nuclei, enucleation, and development of vacuoles in cell cytoplasm, erythrocyte infiltration into blood sinusoids and necrosis of hepatic tissue were common features of T_1 and T_3 groups of fishes, while the lesions were less severe in case of T_2 and T_4 groups of fishes fed with SP supplemented diet. Addition of spirulina at 10% level in diet was found to be beneficial to mitigates histopathological disorders of liver due to cadmium chloride exposure, and proved the protective effect of SP against liver alteration in fishes due to heavy metal toxicity. The results of the present study indicate that dietary spirulina can be recommended as a protective agent against hepatotoxicity in fishes.

Keywords: Cadmium chloride, *Clarias batrachus*, Histopathology, Liver, *Spirulina platensis*

Introduction

Hepatotoxicity is considered as one of the most common pathological effect in vertebrates. Food additives, alcohol, fungal toxins (aflatoxins), toxic industrial chemicals, other air and water pollutants are the major risk factors of liver toxicity (Farazi *et al.,* 2006) as most of the chemicals are metabolised by liver (ICES, 1991). An increasing number of chemicals have shown the potential to induce lesions in the liver of fish (Moutou *et al.,* 1997). A condition of fish disease called ‘‘liver and gall syndrome”, with the symptoms of liver enlargement and colour change, has frequently been reported and caused dramatic loss world over, which is reported to be a non-infectious disease (Meng and Ding, 2004). Neither pathogenic bacteria nor viruses have been isolated, and it was proposed that xenobiotic challenges due to drug abuse and environmental pollution may be one of the most important causes of the diseases. So far, no effective method has been found for the treatment of ‘‘the liver and gall syndrome’’, and much attention has been focused on the use of medicinal herbs to prevent and control this disease (Yin *et al.,* 2011).

During last few decades, pollution of aquatic environment by heavy metals is an extremely imperative and serious problem and has attracted the attention of researchers (Marcovecchio *et al.,* 2007). Cadmium available in electronic wastes like computers, monitors, television, cathode ray tubes (CRT), telephones, cell phones, chip resisters, infrared detectors, TV screens and semiconductors ultimately causes severe water pollution (U.S. EPA. 2009). In such situation, it becomes highly imperative to alleviate heavy metal pollution in aquatic ecosystem and its amelioration to minimise accumulation in aquatic food chain, using available, low cost antidotes gains importance. Fish contaminated by heavy metals suffers pathological alterations, with consequent inhibition of metabolic processes, hematological changes, and decline in fertility and survival. Hence, a scientific

method of detoxification is essential to improve the health of economic fish in any stressed environmental conditions (Kaoud and El-Dahshan, 2010).

Spirulina platensis (SP) is a cyanobacterium, used in many countries as nutritional supplement for human and animal consumption, labelled as a powerful food, rich in proteins, carbohydrates, polyunsaturated fatty acids, terols, minerals and vitamins. (Piñero *et al.,* 2001; Chamorro *et al.,* 2002; Sarma and Jha 2010, Jha *et al.,* 2012). *S. platensis* is well known for its protective effect against heavy metal toxicity (Amin *et al.,* 2006). Keeping this in mind the property of SP, the study was aimed to elucidate its protective effects against cadmium chloride ($CdCl_2$) toxicity in the hepatocytes of fish and to find out its potential use as antioxidant against liver toxicity in fish.

Materials and methods

Freshwater catfish, *Clarias batrachus* (n=120) with average length of 20±4 cm and weight of 90±6 g were collected in live condition from local fish market of Bhopal, and acclimatised for 15 days to laboratory conditions using tap water (DO_2 7.5±0.4 mg l^{-1}, hardness 30.2±0.6 mg l^{-1}, pH 7.2±0.06 and temperature 25±6°C). Fifty percentage water was changed daily in the morning hours during acclimation and fish were fed with basal pellet diet (containing 35% protein) @ 10% of their body weight in two equal instalments (Sarma and Jha, 2010). Basal diet was prepared using fish meal (51.25%), wheat flour (36.75%), cod liver oil (10.00%), and minerals (2%), while experimental diet was prepared supplementing 10% SP in to basal diet by replacing same quantity of wheat flour as described by James *et al.* (2009) (Table 1).

Table 1. Diet composition (% of dry weight)

Ingredients	Control 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

*Agrimin; Glaxo India Ltd., Mumbai (copper: 3.12%, cobalt: 0.45%, magnesium: 24.14%, iron: 9.79%, iodine: 1.56%, zinc: 21.30%, calcium: 30.00%, phosphorous: 8.25%).

For determination of $LC_{50,}$ acclimated fishes were exposed to different toxic concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120 and 130 ppm) of $CdCl_2$ and rate of mortality was observed for 96 h. A stock solution of $CdCl_2$ prepared by dissolving 2.026 g ($CdCl_2.H_2O$ 98% pure; analytical grade, procured from Sisco Research Laboratories) in one litre of distilled water was used for the experiment (Kumar *et al.,* 2009). Probit analysis was used to calculate 96-h LC_{50} (Litchfield and Wilcoxon, 1949). The 96-h LC_{50} value of $CdCl_2$ for *Clarias batrachus* was 101 ppm and its 95% confidence limits were 1.10 (lower limit) and 1.82 (upper limit). Active and healthy fishes (90±6 g) were taken out and starved for 24 h prior to the commencement of the experiment (Kumar *et al.,* 2009).

Fishes were divided in to five treatment groups *viz*., T_0, T_1, T_2, T_3 and T_4. T_0 was used as control, while T_1 and T_2 exposed to 4 ppm $CdCl_2$ and $T_3$and T_4 were exposed to 8 ppm $CdCl_2$. In the present study 100/12 and 100/24 of the 96 h LC_{50} were selected as sub lethal concentration as suggested by Kumar *et al.* (2009). Fishes of T_0, T_1 and T_3 were fed with basal diet (@10% of their body weight), while of T_2 and T_4 were fed with 10% SP supplemented diet (Table 1) in two equal instalments and the unconsumed feeds were removed by siphoning after one hour of feeding. Fifty percentage of the fishes from each group were scarified by decapitation on the 30th day and remaining after 60th day of experimentation to obtain livers aseptically which were preserved in 10% buffered formalin solution and used for histopathological examination following the method as described by Luna (1968). The histopathological sections were observed under an Olympus research microscope, photomicrographs were taken and were critically analysed.

Results and discussion

Histological sections of the liver of the experimental fishes are shown in Fig. 1 to 9, and compared in Table 2. It is clear that histological section of the liver of normal fish shows normal architecture, characterised by polyhedral shaped hepatocytes, cytoplasm granulated with small uniform nuclei, hepatocytes arranged in well-organised hepatic cords and separated by narrow blood sinusoids as observed in case of T_0 group of fishes (Fig. 1). Histopathological alterations depending on the dose and duration of exposure were seen in the liver of experimental fishes. Loosening of hepatic tissue, cytoplasmic vacuolation along with mild enucleation on 30th day of cadmium exposure (Fig. 2, Table 2), while damaged hepatocytes, erythrocyte infiltration into blood sinusoids, development of vacuoles in cell cytoplasm, enucleation along with moderate loosening of hepatic tissue on 60th day of exposure (Fig. 3, Table 2) were the common histopathological changes observed in case of T_1 group of fishes. The alterations were more severe as extensive loosening and enucleated hepatocytes, development of vacuolated cell cytoplasm, along with severe necrosis of hepatic tissue as observed in case of T_3 group, exposed to 8 ppm $CdCl_2$ for 30 and 60 days (Fig. 6 and 7 respectively, Table 2).

Similar results were seen in case of *Tilapia mossambica* exposed to cadmium chloride at 5 and

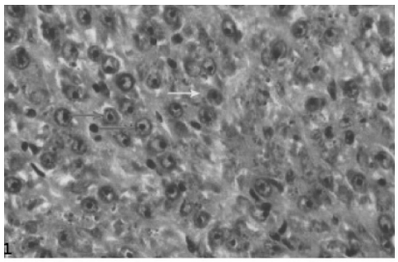


Fig. 1. Liver structure of control fish: showing hepatocytes (H) with granular cytoplasm (yellow arrow) and centrally placed round nuclei (red arrows). H&E X 400.

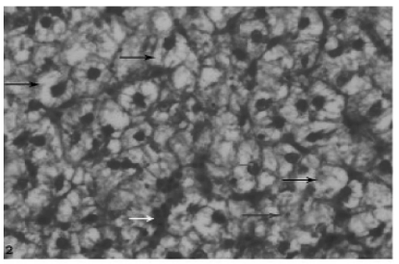


Fig. 2. Liver of fish exposed to 4 ppm $CdCl_2$ for 30 days showing loosening of hepatic tissue (blue arrow), cytoplasmic vacuolation (black arrows), eccentrically situated nuclei (red arrow) and erythrocyte infiltration into blood sinusoids (white arrow). H&E; X 400.

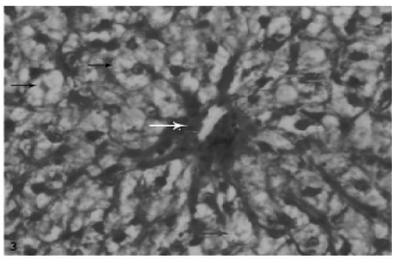


Fig. 3. Liver of fish exposed to 4 ppm $CdCl_2$ for 60 days showing excessive loosening of hepatic tissue (blue arrow), eccentrically situated nuclei (red arrow), cytoplasmic vacuolation (black arrows) and erythrocyte infiltration into blood sinusoids (white arrow). H&E; X 400

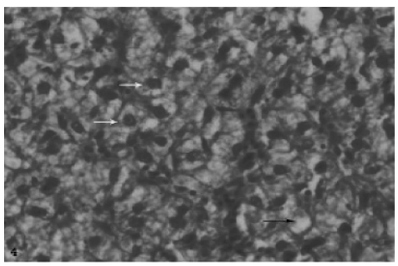


Fig. 4. Liver of fish exposed to 4 ppm $CdCl_2$ and fed @ 10% SP supplemented diet for 30 days showing compactness in hepatic tissue, slight cytoplasmic vacuolation (black arrow) and hepatocytes (yellow arrows) with more or less normal polygonal shape with centrally placed nuclei. H&E; X 400

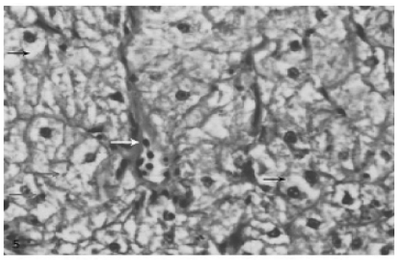


Fig. 5. Liver of fish exposed to 8 ppm $CdCl_2$ and fed @ 10% SP supplemented diet for 30 days showing compactness in hepatic tissue and, hepatocytes with more or less normal shape. However, cytoplasmic vacuolation (black arrows), enucleation (red arrows) and erythrocyte infiltration (white arrow) are also seen. H&E; X 400

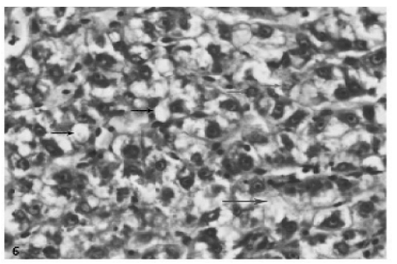


Fig. 6. Liver of fish exposed to 8 ppm $CdCl_2$ for 30 days showing severe loosening of hepatic tissue (blue arrow), cytoplasmic vacuolation (black arrows) and enucleation (red arrows). H&E; X 400

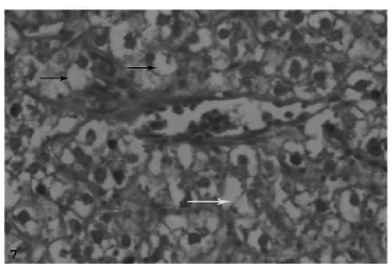
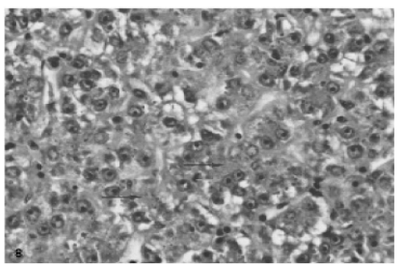


Fig. 7. Liver of fish exposed to 8 ppm $CdCl_2$ for 60 days showing excessive loosening and necrosis of hepatic tissue (yellow arrow), damaged hepatocytes (DH) severe cytoplasmic vacuolation (black arrows) and enucleation (red arrows). H&E; X 400



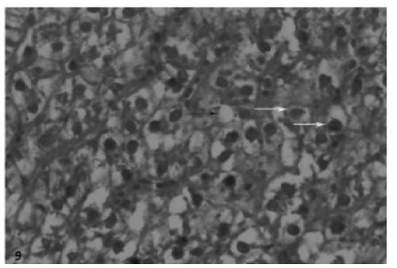


Fig. 8&9. Liver of fish exposed to 4 ppm and 8 ppm $CdCl_2$ and fed @ 10% SP supplemented diet for 30 and 60 days showing compactness in hepatic tissue (blue arrows), slight vacuolation (black arrow) with mild damaged hepatocytes with more or less normal polygonal shape with centrally placed nuclei (yellow arrows). H&E; X 400

50 ppm for 1, 7, 15 and 30 days by Rani and Ramanmurthi (1989). Hepatocyte vacuolation with mild hepatocellular necrosis and shrinkage of hepatocytes due to chronic exposure to sublethal hexavalent chromium in the liver of *Clarias batrachus* was observed by Selvanathan (2013), and swelling in the liver cells along with vacuolation of hepatocytes of cadmium exposed *Oreochromis mossambicus* was reported by Dyk *et al.* (2007). Hepatocytic vacuolation is associated with inhibition of protein synthesis, energy depletion, disaggregation of microtubules, or shifts in substrate utilisation (Hinton and Lauren, 1990), while cellular swelling occurs either directly by denaturation of volume-regulating ATPase or indirectly by disruption of the cellular energy transfer processes required for ionic regulation (Hinton and Lauren, 1990). Atrophy and necrosis of hepatic cells, decrease in the size of the nuclei and nucleoli, and indistinguishable cell membranes in the liver of *Cyprinus carpio* exposed to cadmium is reported by Morsey and Protasowicki (1990). Metals either increase or decrease hepatic enzyme activities and can lead to histopathological hepatic changes, depending on the metal type and concentration,

Table. 2. Histoarchitectural changes in the liver of *Clarias batrachus*

Parameters	Treatments									
	30th day of exposure					60th day of exposure				
	T_0	T_1	T_2	T_3	T_4	T_0	T_1	T_2	T_3	T_4
Vacuolation	-	+	+	+	+	-	++	++	++	+
Loosening of hepatic tissue	-	++	-	++	++	-	++	+	+++	++
Eccentric position of nuclei	-	+	+	++	+	-	++	+	+++	++
Enucleated hepatocytes	-	++	+	++	+	-	++	++	+++	+
Necrosis of hepatic tissue	-	+	+	++	+	-	++	++	+++	+
Deshaped hepatocytes	-	+	-	++	+	-	+	+	++	+

(−) none, (+) mild, (++) moderate, (+++) severe

fish species, period of exposure, and other factors reported by Paris-Palacios *et al.* (2000). There are several pathways by which, Cd is thought to induce oxidative stress. It inhibits the mitochondrial electron-transfer chain reaction, leading to accumulation of semi-ubiquitous toxicants, which enables it to transfer one electron (e−) to molecular oxygen and to form superoxide radicals (Wang *et al.,* 2004). Histopathological changes in the hepatocytes could be attributed to the cumulative effect of metals and the increase in their concentrations in the liver as liver is considered as organ of detoxification, excretion and binding proteins such as metallothioneins (MTs), and the metal-binding proteins present in the nuclei of hepatocytes leads to increase in cell damages as reported by De Smet and Blust (2001).

In contrast, the fishes of group T_2 and T_4 showed better condition of hepatic tissue over that of group T_1 and T_3 with both hepatic tissue and hepatocytes having compactness, more or less, normal shape and size with reduced cell size, reduced enucleation and vacuolation, and the nuclei in more or less central position. Fishes fed with SP supplemented diet showed less histopathological alterations in comparison to that of the other diets (Fig. 4, 5, 8, 9, and Table 2), which indicates the protective effect of SP against cadmium induced hepatotoxicity. Observation of Amin *et al*. (2006) also indicates the protective effect of spirulina against cadmium-induced hepatotoxicity in rats, while Pinero *et al.* (2001) reported that spirulina possess strong antioxidant and free radical scavenging properties, in addition to its strong chelating effect (Chen and Pan, 2005). These characteristics can be attributed to the presence of high levels of antioxidants such as vitamin B1 and B2, selenium, carotenoids, gamma-linolenic acids and phyocyanin in spirulina (Pinero *et al.,* 2001; Sarma and Jha, 2010; Jha *et al.,* 2012).

An improvement in histopathological changes in the liver of albino rats exposed to cadmium toxicity was observed when treated with spirulina by Jeyaprakash and Chinnaswamy (2005) and it is reported that most of the antioxidant capacities of spirulina are due to presence phycocyanin (Bermejo *et al.,* 2008). Dietary spirulina is known for its metal chealating properties as it reduced copper toxicity in *Cirrhinus mrigala* (James *et al.,* 2009). Metal chelating potential of spirulina was reported by Islam *et al.* (2009), who used spirulina as a feed supplement against arsenic induced toxicities in ducks and reduction of tissue arsenic concentration from 80% to 20% was recorded. Luxia *et al.* (1996) reported that β-carotene of spirulina may reduce cell damage, especially damage to DNA molecules, thus playing its role in the repair of regeneration process of damaged cells.

There is lack of literature on the exact quantity of spirulina to be used as protective agent against metal toxicity in fishes, while some of the authors suggested to use 3-20% spirulina in diet (Bermejo *et al.,* 2008; Islam *et al.,* 2009; James *et al.,* 2009) which needs further study. Considering the fact of price escalation of the diet supplemented with spirulina, spirulina was added only at 10% level in the diet prepared for the present study (Sarma and Jha, 2010) and protective effect against $CdCl_2$ exposed histoarchitectural changes in the liver of *C. batrachus* were recorded. There may be different effects of adding spirulina in higher or lower concentration which needs further study.

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