Impact of UV-B Radiation on the Physiology of Freshwater Carp *Labeo rohita* Larvae and Evaluation of UV-B Protective Properties of Seeds of *Achyranthes aspera* and Vitamin C

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Abstract Effect of harmful UV-B radiation and their remedial measures have been studied in *Labeo rohita* (rohu) larvae. Larvae were fed with four different diets: D1 and D2 contained 0.1 and 0.5 % *Achyranthes aspera* seeds, respectively; D3 contained 800 mg vitamin C/kg diet, and D4 contained control diet. After 50 days, all groups were exposed to UV-B radiation ($80 \mu\text{W}/\text{cm}^2$). One batch of control diet-fed fish was exposed to UV-B (D4Ex), and the other group remained unexposed (D4UEx). Survival rate of rohu was significantly ($P < 0.05$) higher in D2 when compared to others. Among the exposed groups, highest average weight was recorded in D2 (103 ± 4 mg). Total tissue protein concentration was higher in exposed groups compared to the unexposed one. Glutamic oxaloacetic transaminase (GOT) and glutamate pyruvate transminase (GPT) levels were minimum in unexposed control diet-fed fish. Vitamin C provided protection against lipid peroxidation which was evident from the lowest level of thiobarbituric acid reactive substance in exposed fish (D3). Significantly ($P < 0.05$) higher nitric oxide synthase level in fish fed with *Achyranthes aspera* seeds (0.5 %) supplemented diet showed the immunomodulatory property of the seeds. A combination of vitamin C and seed may be more UV-B protective for carp larvae.

Keywords Rohu · UV-B radiation · Survival · Immunomodulation · *Achyranthes aspera* · *Labeo rohita*

Introduction

Ultraviolet (UV) radiation is a potent environmental stressor to aquatic organisms that are exposed to it. In freshwater ecosystem, UV radiation can easily penetrate as the water is transparent and consequently the effect of radiation is more pronounced [26]. The harmful effect of UV-B includes damage that compromises the physiology, biochemistry, reproduction, and growth of the exposed animals [1, 19, 25, 37]. The early developmental stage is more prone to UV-B damage as the less pigmented skin is very transparent, and therefore, vulnerable to the harmful radiation. The embryos

and larvae of many species are more sensitive to environmental stressors like UV-B compared to their later life stages [2, 7]. Sharma et al. [33] reported that exposure of UV-B affected the survival rate of catla, *Catla catla* larvae under laboratory conditions. UV-B radiation affected the skin and eyes of ayu, *Plecoglossus altivelius* larvae, and gills of catla larvae [31, 32].

In recent years, aquaculture industry is suffering from acute disease problem. In India, carp seed is produced mainly in nursery with optimum water depth, and sunlight shows increasing trend during breeding season (especially in May–June). Therefore, the larvae are more prone to the exposure of harmful UV-B radiation. UV-B exposure induces pronounced immunomodulation in cyprinids [22]. The digestive physiology and immune system of catla were affected by UV-B radiation [34]. Biological protection from UV-B may be achieved by specific responses, like the intensification of melanin pigmentation and antioxidant defenses, and by improvement of cell-repair mechanisms [4, 7, 11, 38]. However, these responses give only incomplete protection and UV-B

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may cause molecular damage to lipids, proteins, and nucleic acids, besides inducing oxidative stress on living organisms [18, 19]. Photoprotective pigments (MAAs, carotenoids), antioxidant defense compounds (flavonoids, vitamins C and E), and cell cycle development genes are some molecules involved in UV radiation defense [10]. We have observed the damaging effect of UV-B on freshwater carp larvae in our earlier studies [31, 33, 34]. Dietary supplementation of antioxidant vitamin C and plant ingredient may be beneficial for fish larvae to face the challenge of exposure of harmful UV-B irradiation. *Achyranthes aspera*, a herb belonging to the family Amaranthaceae has showed immunostimulatory effect in carps [5, 28]. *Labeo rohita* (Family: Cyprinidae), commonly known as ‘rohu’, is an omnivorous species, contributing considerably in Indian aquaculture production. Outbreak of disease is reported in this species. The present investigation was carried out to study the effect of dietary supplementation of seeds of *Achyranthes aspera* and vitamin C (L-ascorbate-2-triphosphate calcium salt) separately on the physiology of UV-B-irradiated rohu larvae. The ability of larvae to face the challenge of UV-B radiation was evaluated.

Materials and Methods

Experimental Diets

Four different diets were prepared for the experimental trial (Table 1). Two diets D1 and D2 contained 0.1 and 0.5 % *Achyranthes aspera* seeds, respectively; diet 3 (D3) contained 800 mg vitamin C (L-ascorbate-2-triphosphate calcium salt, Himedia Laboratory Pvt. Ltd., Mumbai, India)/kg of diet, and diet 4 (D4) was control diet without seeds and vitamin C. Food was given at the rate of 5 % of body weight throughout the study period.

Culture of Fish and Exposure to UV-B Radiation

Larvae of *Labeo rohita*, rohu were obtained from Chatterjee Brothers’ fish farm, Mogra, West Bengal having

induced breeding facility. Larvae (1.0 ± 0.01 mg) were acclimated for 10 days, and then randomly distributed in glass aquarium (15 l each) kept under indoor condition. The stocking density was 50 larvae/aquarium. Feeding with experimental diets started from day 11.

After 50 days of culture, fish were challenged with UV-B radiation. The dose was $80 \mu\text{W}/\text{cm}^2$ [31]. All fish fed with vitamin C and seed-incorporated diets were exposed to the UV-B radiation, whereas, control diet-fed fish were subdivided into two batches—one batch was exposed to UV-B (D4Ex) and the other remained unexposed (D4UEX). Four replicates (aquaria) were used for each treatment. Fish were exposed to UV-B radiation every day at 12.00 p.m. for 10 min, and this was continued for 40 days. Total duration of experiment was 90 days. Then the experiment was terminated, survival rate was recorded, and weight of individual fish was measured. Dechlorinated, transparent water was used and the depth of water in the aquarium was 20 cm. During experiment, different physico-chemical parameters like temperature, pH, and dissolved oxygen level were monitored regularly. Water temperature and pH ranged from 30 to 31 °C and 7.5 to 8.1, respectively, throughout the study period. Dissolved oxygen level was always maintained above 5 mg/l with the help of aerators.

The source of UV-B (280–320 nm) was a Philips tube light (TL 20/12 RS, Holland) fixed above each aquarium. All tubes were preburned for 100 h to give a stable output. The spectral output of the tubes, as defined by the manufacturer has the maximum emission at 313 nm, with negligible energy above 320 [3]. UV-B tubes were covered with cellulose acetate which absorbs wavelength of <280 nm. Florescent lamp (Philips 20 W) was used as ambient light source for both treated and control fish. The UV-B radiation was measured by Microtops II, Sunphotometer, Solar Light Company, Philadelphia, USA.

Biochemical Assay

Fish were euthanized, tissue was collected without scales, head, tail, and bones from individual fish. Tissue sample

Table 1 Composition of experimental and control diets

Ingredients (g/kg)	Experimental diet			Control diet
	D1	D2	D3	D4
Dry fish powder	583.3	583.3	583.3	583.3
Wheat flour	401.7	397.7	394.7	402.7
Cod liver oil	10.0	10.0	10.0	10.0
Vitamin–mineral premix	4.00	4.00	4.00	4.00
<i>Achyranthes aspera</i> seeds	1.00	5.00	0.0	0.0
Vitamin C	0.0	0.0	8.00	0.0

D1 diet contained 0.1 % seeds, D2 diet contained 0.5 % seeds, D3 diet contained vitamin C, D4 control diet

obtained from each aquarium was pooled. Then, the samples were preserved at -80°C . Tissue (100 mg) was homogenized with 1 ml of chilled phosphate buffer; then spun down at $10,000\times g$ for 10 min, and supernatant was immediately used for assay. Four replicates were used for each assay.

Total tissue protein was measured according to the method of Lowry et al. [21]. The absorbance was recorded at 750 nm using Microplate Reader (BioTek, Synergy HT, New York, USA).

Tissue glutamic oxaloacetic transaminase (GOT) and glutamate pyruvate transaminase (GPT) were assayed using diagnostic kits (Siemens Healthcare Diagnostics Ltd., Baroda, India). The absorbance was recorded at 340 nm. GOT and GPT levels were expressed as IU/l.

Thiobarbituric acid reactive substance (TBARS) was measured according to the method of Ohkawa et al. [27]. The absorbance was measured at 532 nm. The standard was prepared using 1,1,3,3-tetramethoxy propane, and the result was expressed as $\mu\text{mol MDA/mg protein}$.

Nitric oxide synthase (NOS) was measured according to the method of Lee et al. [17]. The absorbance was recorded at 540 nm. The nitrite concentration was determined from the nitrite standard curve and was expressed as mol/mg tissue .

Specific Growth Rate (SGR)

The specific growth rate was calculated using the formula:

$$\text{SGR} = (\ln W_t - \ln W_i) / t \times 100$$

where W_i and W_t were the initial and final body weight and t , the time in days.

Statistical Analysis

Results are presented as mean $\pm$ SE. Data were analyzed using one way analysis of variance (ANOVA) and Duncan's multiple range (DMR) test [24]. The significance level was accepted at $P < 0.05$.

Results

Survival rate of rohu was significantly ($P < 0.05$) higher in 0.5 % *Achyranthes aspera* seed-supplemented diet fed (D2) exposed group compared to the other exposed and unexposed groups (Table 2). This group was followed by 0.1 % seed-supplemented diet fed one.

Among the exposed groups, the highest average weight was recorded in D2 diet-fed fish (103 ± 4 mg), but there was no significant ($P > 0.05$) difference in the average weight between D2 and D3 diet-fed rohu. The average

weight of control diet-fed unexposed fish (D4UEx) was 10.67 % higher compared to the 0.5 % seed-incorporated diet (D2) fed exposed fish. But the average weight of the latter group was 12 % higher compared to the control diet-fed exposed fish (D4 Ex). SGR value was maximum in D4 UEx and D2 treatments (Table 2). Total tissue protein concentration was higher in all exposed fish (regardless of diets supplied) compared to the unexposed control diet-fed group (Table 2). Tissue protein was maximum in 0.5 % seed-incorporated diet (D2) fed group (0.111 ± 0.009 mg/mg tissue). Among the exposed fish, there was no significant ($P > 0.05$) difference in the concentration of tissue protein between 0.5 % seed- and vitamin C-supplemented diet-fed groups, and between 0.1 % seed-supplemented and control diet-fed exposed groups. Supplementation of vitamin C and seed of *Achyranthes aspera* (0.5 %) in diets enhanced the tissue protein level.

GOT and GPT levels were minimum in unexposed control diet-fed fish. There was no significant ($P > 0.05$) difference in the GOT level among the UV-B exposed treated and control diet-fed rohu (Fig. 1). Among the exposed fish, GPT level was maximum and minimum in vitamin C- and 0.5 % seed-supplemented diet-fed groups, respectively. TBARS level was significantly ($P < 0.05$) lower in vitamin C-supplemented diet-fed fish compared to the other groups. Among the UV-B-irradiated fish, two to sevenfold higher TBARS level was found in control diet-fed fish compared to others (Fig. 2a). NOS level was significantly ($P < 0.05$) higher in 0.5 % seed-incorporated diet (D2)- fed fish compared to other groups (Fig. 2b). This was 1.24- to 2.8-fold higher in D2 diet-fed fish compared to the other groups.

Discussion

The environmental stressor UV-B radiation disturbs the physiology of fish. Substances having UV-B protective properties may help to overcome the harmful effect of irradiation. In the present study, the UV-B protective role of seeds of *Achyranthes aspera* is very clear. The survival rate was higher in two plant-incorporated diet-fed UV-B challenged rohu compared to the other treatments, even higher than that of the unexposed control diet-fed group. Similar result was found by Rao et al. [29] in rohu challenged with *Aeromonas hydrophila*. Supplementation of seeds of *Achyranthes aspera* increased survival rate and growth of rohu. The presence of a number of oleonic acids, ecdysterone, and various amino acids [14] confirmed the nutritional quality of seeds. *Achyranthes aspera* seeds contained protein with calorific value of 3.92/g. The amount of leucine, isoleucine, phenylalanine, and valine were similar to the Bengal gram, while tryptophane, methionine, and cystine contents were

Table 2 Effects of seeds of *Achyranthes aspera* and vitamin C on the survival, average weight, SGR and total tissue protein level of UV-B irradiated *Labeo rohita* larvae

Diets					
Parameters	D1	D2	D3	D4Ex	D4UEx
Survival rate (%)	79.5 ± 4.35b	91.33 ± 1.29a	59.33 ± 4.50e	70 ± 3.74d	74.5 ± 2.06c
Average weight (mg)	92.0 ± 7c	103.0 ± 4b	100.0 ± 11b	92.0 ± 25c	114.0 ± 18.6a
SGR (%)	5.02 ± 0.08b	5.15 ± 0.05a	5.09 ± 0.13b	4.93 ± 0.28c	5.23 ± 0.15a
Total protein (mg/mg tissue)	0.075 ± 0.004b	0.111 ± 0.009a	0.105 ± 0.008a	0.073 ± 0.004b	0.06 ± 0.006c

Mean ($n = 4$) sharing different letters in the same column are significantly ($P < 0.05$) different

D1 diet contained 0.1 % seeds, D2 diet contained 0.5 % seeds, D3 diet contained vitamin C, D4 control diet (Ex UV-B exposed, UEx UV-B unexposed groups)

Fig. 1 GOT and GPT levels found in UV-B-irradiated rohu larvae fed with seeds of *Achyranthes aspera* and vitamin C-supplemented diets. Vertical bars with different superscripts are significantly ($P < 0.05$) different

higher than the most pulses [15]. Chakrabarti et al. [6] reported that the presence of ecdysterone in the raw seeds of *Achyranthes aspera* enhanced the growth rate of *Cyprinus carpio* common carp. Similarly, in the present study, ecdysterone served as the growth-stimulating component for rohu larvae. In this study, vitamin C also enhanced the growth of rohu. Vitamin C is an essential nutrient for optimum growth and maintenance [9, 13, 16, 20]. Total tissue protein concentration was higher in exposed fish fed with seeds (0.5 %) and vitamin C. Ascorbic acid plays important role in certain aspects of protein metabolism [8, 35]. It acts as a cofactor in the enzymatic hydroxylation of proline residues of collagen and connective tissue of vertebrates. It enhanced the growth of parrot fish *Oplegnathus fasciatus* [39].

Higher levels of GOT and GTP account for the damage level of tissue. Significantly higher levels of GOT and GPT were recorded in UV-B-exposed catla larvae [34]. Similar result was found with rohu in the present investigation. Low GPT level found in 0.5 % seed-incorporated diet-fed fish indicated that seeds helped the fish to fight against the

Fig. 2 (a) Effect of UV-B radiation on thiobarbituric acid reactive substance (TBARS) and (b) nitric oxide synthase (NOS) levels of rohu larvae fed with seeds of *Achyranthes aspera* and vitamin C-supplemented diets. Vertical bars with different superscripts are significantly ($P < 0.05$) different

irradiation. Vitamin C was not found to be effective in reducing GOT and GPT levels in UV-B-irradiated rohu.

Higher level of TBARS is an indicator of lipid peroxidation. In the present investigation, among the UV-B-irradiated groups, significantly ($P < 0.05$) higher level of TBARS was recorded in control diet-fed fish compared to others. Vitamin C provided highest protection against lipid peroxidation to the UV-B-irradiated rohu. Seeds of *Achyranthes aspera* (0.5 %) also provided protection to the fish. Fish tissue contains large quantities of polyunsaturated fatty acids (PUFAs) essential for membrane function [23]. Lipid peroxidation is the process of oxidative degradation

of PUFA, and its occurrence in biological membranes causes impaired membrane function, structural integrity and inactivation of several membrane-bound enzymes [12]. Vitamin C is a potent antioxidant and protects tissues from oxidative stress. Loading cells with vitamin C reduce oxidative cell death, inhibits FAS-induced apoptosis and confers genomic protection through the quenching of intracellular reactive oxygen species [30].

High level of NOS in fish fed with 0.5 % *Achyranthes aspera* seed-supplemented diet showed the stronger defense system of rohu challenged with *Aeromonas hydrophila* [36]. The present investigation with UV-B-irradiated rohu confirmed the earlier study. NOS are a family of enzymes that catalyze the production of cellular signaling molecule nitric oxide. This nitric oxide plays vital role in many biological processes. The inducible isoform iNOS produces a large amount of nitric oxide as a defense mechanism. The presence of long-chain polyunsaturated fatty acids such as linolenic and oleic acids have been reported in the seeds of *Achyranthes aspera*. The immunostimulating effect of seeds may be due to the presence of essential fatty acids [6].

Conclusions

The results showed that feeding of seeds of *Achyranthes aspera* at 0.5 % level helped rohu larvae to face the challenge of UV-B irradiation. Higher survival and growth of larvae are positive indicators of the health status of the larvae. Vitamin C is able to protect the cells from oxidative stress during UV-B irradiation. A combination of vitamin C and seeds may be more UV-B protective for carp larvae.

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Conflict of interest The authors declared that they have no conflicts of interest

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