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PHOTOSENSITIVITY OF HILL STREAM FISHES WITH RETENE PHOTOSENSITIZER

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ABSTRACT

Increase in UVR as a result of global emissions of ozone depleting substances has caused a devastating effect on the climate change and weather conditions. Solar radiation is essential to life on earth but its UV component may also damage both living organisms and non- living matter. Cold water fishes refer to those members of the family cyprinidae which inhabit streams, rivers, upland lakes and reservoirs having water temperature below 25 ºC. UV radiation is one of the most common environmental health hazards that cause highly toxic effects in most living organism due to destruction of ozone layer which result in UV induced problems. Ultraviolet radiation is the most photochemically reactive wavelength of solar energy reaching the surface of the earth, and has a broad range of effects on aquatic biota, and ecosystems. Natural photosensitizer in environment may get into aquatic ecosystem and the combined effect with UV rays cause deleterious effects. Therefore a study has been designed to see the effect of retene on selected hillstream fishes in presence of solar and artificial UV-B radiation. Biochemical and enzymological parameter were recorded which includes motality, glutathione, catalase, glucose-6-phosphatase and lipid peroxidation. Phototoxicity of retene with ultraviolet is attributed to generation of reactive oxygen species and hydrogen peroxide radicals. Artificial UV-B is more toxic than solar radiation. *Tor tor* was found more sensitive than *Barilius bendelisis* followed by *Nemacheilus rupicola* as indicated by mortality rate, lipidperoxidation and enzymological parameters. Enhanced UV radiation and pollution indirectly affect hill stream fishes, aquatic biodiversity and mountain ecosystem productivity.

KEYWORDS: Phototoxicity, UVR, Retene, *Barilius*, *Nemacheilus*, *Tor*, Aquatic Ecosystem

Ultraviolet radiation (UVR) is electromagnetic radiation is emitted by the sun and artificial devices, including sunbeds or sunlamps. Broad-spectrum UVR is known to be a human carcinogen based on sufficient evidence of carcinogenicity from studies in humans. Evidence that the broad-spectrum UVR wavelength (100-400 nm) component of solar radiation is carcinogenic comes from studies of human cancer associated with exposure to devices that emit artificial broad-spectrum UVR, the fact that tumors develop at the same tissue sites in humans exposed to sunlight and in animals exposed to artificial sources. (Panich et al., 2016).

Solar radiation includes most of the electromagnetic spectrum. In the solar UV energy reaching the equator, 95% is UVA and 5% is UVB. No measurable UVC from solar radiation reaches the earth's surface, because the shortest UV wavelengths are completely absorbed by ozone, molecular oxygen and water vapor in the upper atmosphere. The UVR wavelengths to which an individual is exposed vary considerably with latitude, altitude, time of day, and season. People also vary in their length of outdoor exposure, the parts of the body they expose, and the shapes of their bodies. Mechanistic studies with human tissue have demonstrated that the UVB component in solar radiation causes DNA damage (Lim & Lee 2016). Aquatic ecosystems are exposed to excessive input of pollutants and contaminants from various sources like domestic and industrial sewage, agricultural processes, heavy metals and others. The uncontrolled discharge of these compounds into the water directly affects aquatic organisms, including fish, which are considered a bioindicator of environmental contamination. Changes in organs of fish, such as the gills, are good biomarkers of water contamination. Biomarkers are biological indicators showing the effects resulting from exposure to a stressor, and can be identified by biochemical, cellular, histological and behavioral changes. PAH occur as natural products in plants, microbes, released from volcanic activity and forest related precursors produced by fungi, plants and animals. Major sources of PAH in surface waters are oil spills, industrial processes, fossil fuel combustion and other pyrolytic processes attributed to human activity. Retene, (methyl isopropyl phenanthrene- C_{18} H_{18}) is polycyclic aromatic hydrocarbon present in coal tar fraction boiling above 360 ºC. It occurs naturally in the tars obtained by distillation of resinous woods. Retene is derived by degeneration of specific diterpenoids biologically produced by conifer trees.

Barilius bendelisis (Hamilton), commonly known as Indian Hill Trout and *Nemacheilus rupicola* is an upland water fish of South East Asia. It belongs to the family cyprinidae and dwells in shallow, clear and cold water. The fish plays significant role in the capture fishery in several parts of the Himalayan region of Uttarakhand, inhabiting

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shallow lotic and seasonal lentic water bodies where Indian major carps and exotic carps cannot be raised successfully (Mir et al., 2015). The mahseer "The Anglers delight" is one of the important cold water fish groups that inhabit the pristine streams with high content of dissolved Oxygen. *Tor tor* is a widely distributed species in south and SE Asia with a restricted area of occurrence. The species has been reported from across the Himalayan region and elsewhere in South and SE Asia, ranging from Afghanistan, Pakistan, India, Nepal, Bangladesh, Bhutan, Myanmar, Sri Lanka, Western Iran to Thailand. The studies on fish larvae in the presence of UV radiation has been little studied although the UV radiation directed hanges in arthropods and planktonic organism have been documented with retene and riboflavin (Kumar and Kumari., 2011). In this study impact of solar and artificial ultraviolet-B radiation has been observed on *Barilius bendelisis*, *Nemacheilus* and *Tor tor* fishes with retene photosensitizer.

METHODOLOGY

Solar UV was measured with the help of Kipps and Zonen radiometer. The fish collection was done from four sites i.e. Sahastradhara, Song, Maldevta and Gular Ghati. Fish larvae were collected and cultured by the method (Tisher and Songlake, 2001). Fishes were collected and were placed in glass aquariums and maintained the physicochemical parameters for acclimation for about a week. Experimental protocol was prepared for the morphological, phototoxicological and biochemical studies in which the fishes were divided randomly into six groups as. 10 fishes were placed in each group. Group one was control, Group two was exposed to retene. Group three was exposed to solar radiation, Group four was exposed to artificial UV-B radiation. Group five was treated with retene and exposed to solar UV-B radiations and Group six was treated with retene and artificial UV-B. Artificial radiation was given with Philips UV-B Lamps. Experiments were performed on fishes *Barilius bendelisis*, *Nemacheilus rupicola* and *Tor tor* using three replicates. Solar and artificial radiation was given for three hours daily. UV- B radiation intensity used was 0.750 – 800 mw/cm^2. Experiment was performed for 30 days.

Lipidperoxidation was assayed by measuring of malondialdehyde level in fish gills by using thiobarbiturc acid (TBA) through the method (Smith and Anderson, 1987). The absorbance was recorded using UV/ visible spectrophotometer at 532 nm. 1, 1, 3 tetramethoxy propane (Wako, Japan) was used as the standard. Reduced glutathione (GSH) was measured following the method (Beutler et al., 1963) using dithiobis nitrobenzoic acid (DTNB). Absorbance was absorbed at 412 nm with the help of UV/ visible spectrophotometer. Catalase (E. C. 1. 1. 11. 6) H_2O_2- H_2O_2 oxidoreductase enzyme activity in the tissue was determined by breakdown of hydrogen peroxide using titration method (Takahara, 1960). Glucose- 6- Phosphatase was measured following the method (Fishman et al., 1967). Statistical inferences were drawn by using Students't' test (Fisher, 1963).

RESULTS AND DISCUSSION

Results on mortality rate on fish within 30 days with 3 hours of exposure to solar, artificial UV-B and retene indicate highest mortality in *Tor tor* with artificial UV-B followed by natural Solar UV-B. Lowest mortality was observed in *Nemacheilus rupicola* (Table, 1). Reduced Glutathione level decreased subsequently in all the groups in comparision to control. Minimal value of GSH level was observed after treatment of artificial UV-B + retene *Barilius bendelisis* followed by *Tor tor* and *Nemacheilus rupicola* (Table, 2). Malondialdehyde level a marker of lipid peroxidation and cell injury indicated an increase in lipid peroxidation level in comparison to control. Non-significant change was observed after exposure of individual retene and natural solar UV. An increase was observed after exposure to artificial UV-B and solar UV radiation in the presence of retene. MDA level was highest in *Tor tor* than *Barilius* and Nemacheilus (Table 3). Antioxidative enzyme catalase indicates decrease in antioxidant potential which decreased in all the groups in comparision to control. Non- significant change was observed after individual treatment with retene and solar UV. Significant decrease in catalase level was found after artificial UV-B and retene (Table, 4). Glucose- 6-Phosphatase activity decreased in all groups in comparision to control. Maximum reduction was observed after treatments with retene and artificial UV-B (Table, 5).

Table 1: Mortality rate in hill stream fishes with UV radiations and retene

Group	Treatment	*Barilius*	*Nemacheilus*	*Tor*
1	Control	3.5 ± 0.4	3 ± 0.4	3.5 ± 0.4
2	Retene	5 ± 0.6 NS	4±0.1 NS	6 ± 0.6 NS
3	Solar UV-B	6 ± 0.5 NS	4.5 ± 0.5 NS	7 ± 0.6 *
4	Artificial UV-B	7 ± 0.6 *	6 ± 0.3 *	10 ± 0.5 *
5	Retene + Solar UV-B	16 ± 1.2 *	14 ± 0.9*	21 ± 1+.3*
6	Retene + Artificial UV-B	22 ± 2.1**	20 ± 1.8**	26 ± 2.7 **

Results are mean ± S.E. of 5 observations in each group. P value *0.05 *0.01, NS not Significant.

Table 2: Reduced glutathione (GSH) mg/g of tissue fishes with UV radiation and retene

Group	Treatment	*Barilius*	*Nemacheilus*	*Tor*
1	Control	975 ± 2.80	973 ± 1.00	990 ± 2.70
2	Retene	950 ± 3.31 NS	950 ± 2.01 NS	978 ± 4.34
3	Solar UV	928 ± 5.13 NS	920 ± 4.13 NS	945 ± 6.25 NS
4	Artificial UV-B	830 ± 4.02*	830 ± 2.02*	850 ± 3.20*
5	Retene + Solar UV-B	785 ± 1.78*	768 ± 1.35*	790 ± 2.01*
6	Retene + Artificial UV-B	660 ± 2.10**	685 ± 1.14**	670 ± 3.29**

Results are mean ± S.E. of 5 observations in each group. P value *0.05 *0.01, NS not Significant.

Table 3: Lipid peroxidation (MDA µ mole/ 100 g protein) in fishes with solar & artificial UV-B radiation and retene photosensitizer

Group	**Treatment**	***Barilius***	***Nemacheilus***	***Tor***
1.	Control	7.40±0.38	7.35±0.65	7.55±0.72
2.	Retene	8.03±0.62 NS	7.40±0.72 NS	8.20±0.85 NS
3.	Solar UV-B	7.95±0.68 NS	7.72±0.85 NS	8.25±0.85 NS
4.	Artificial UV-B	8.85±0.63*	8.42±0.63*	9.05±0.74*
5.	Retene + Solar UV-B	9.20±0.45*	8.30±0.72*	9.15±0.78*
6.	Retene + Artificial UV-B	10.18±0.73**	9.70±0.91**	10.58±0.89**

Results are mean ± S.E. of 5 observations in each group. P value *0.05 *0.01, NS not Significant.

Table 4: Catalase level (mg/100g tissue) in fishes after exposure to solar and artificial UV radiations with retene

Group	**Treatment**	***Barilius***	***Nemacheilus***	***Tor***
1.	Control	82.2 ± 1.42	79.0 ± 1.22	86.3 ± 1.54
2.	Retene	77.7 ± 1.84 NS	75.1 ± 1.64 NS	80.5 ± 1.56 NS
3.	Solar UV-B	76.7 ± 1.16 NS	74.1 ± 1.06 NS	79.6 ± 1.36 NS
4.	Artificial UV-B	70.6 ± 2.16*	73.6 ± 2.16*	76.4 ± 1.16*
5.	Retene + Solar UV-B	69.5 ± 1.75*	71.7 ± 1.65*	72.4 ± 1.25*
6.	Retene + Artificial UV-B	63 ± 1.97**	62.8 ± 1.97**	68.4 ± 1.78**

Results are mean ± S.E. of 5 observations in each group. P value *0.05 *0.01, NS not Significant.

Table 5: Glucose- 6- phosphatase (G- 6- P) activity in fishes in presence of UV radiation with retene

Group	Treatment	*Barilius*	*Nemacheilus*	*Tor*
1.	Control	13.14 ± 0.95	13.04 ± 0.23	14.11 ± 0.67
2.	Retene	12.10 ± 0.92 ^{NS}	12.11 ± 0.86 ^{NS}	14.90 ± 0.9 ^{NS}
3.	Solar radiation	12.11 ± 1.23^{NS}	12.01 ± 1.01 ^{NS}	15.30 ± 1.12 ^{NS}
4.	Artificial UV-B	11.09 ± 1.90*	10.98 ± 1.65*	13.90 ± 1.60*
5.	Retene + Solar Radiation	9.14 ± 0.10*	9.02 ± 0.07*	11.20 ± 1.25*
6.	Retene + Artificial UV-B	8.60 ± 0.24**	8.901 ± 0.11**	9.35 ± 0.56**

Results are mean ± S. E. of 5 observation in each group. P value *0.05 *0.01. NS not significant.

At present concentration of the ozone depleting substances in the atmosphere are decreasing and according to recent models the stratospheric ozone is recovering.The amount of UV-B reaching the surface of earth depends on the thickness of the ozone layer and atmosphere. UV radiation is a major causative factor in the development of skin cancer. It is well known that an increased incidence of malignant skin melanomas has been attributed to severe sunburn and exposure to excessive sunlight at an early age. UV-B also participates in indirect damage to macromolecules, provokes free radical production and induces a significant decrease in antioxidants (David and Davies., 2006). UVR is absorbed by biologically important molecules such as DNA, proteins, chromatophores which leads to wide ranging effects on organisms (Bhandari and Sharma, 2010). The extent and duration of periods of ice and snow cover on oceanic and inland waters have been decreasing in recent decades, altering the underwater light environment and potentially resulting in direct exposure of the aquatic environment to higher UV radiation (Clark et al., 2013).

Barilius bendelisis commonly known as Indian Hill Trout is an upland water fish of south East Asia, plays significant role in the capture fishery in several parts of Himalayan region of Uttarakhand (Sah et al., 2011). Among Indian mahseers, *Tor tor* (Hamilton, 1822) is the most important food and game fish of India. It constitutes an outstanding fishery in the Himalayan region of India. However, the building of dams across certain rivers has created reservoirs that have destroyed the natural breeding grounds of the fish and caused mortality of brood and juvenile fish indiscriminately. The mahseer fishery of India is further declining as a result of low recruitment of the fish. Stocking rivers and reservoirs with mahseer is therefore essential to restore the fishery (Desai, 2003).

Fishes are susceptible to UV- B radiation with sensitivity varying within groups. Eggs and larvae also have limited behavioral capabilities to avoid UV-B exposure due to their reduced mobility and some species cannot detect UV-B radiation in the early developmental stages (Olson et al., 2006). Studies with numerous fish species have shown that UV-B radiation can be detrimental to fish especially at embryo and larval stages. The most severe effects of UV-B radiation impair larval development and decrease offspring recruitment (Asta et al., 2011). Retene is a natural chemical present in the plants. Aquatic animals are naturally exposed to retene due to its presence in water bodies. Fish larva exposed to artificial UV-B with retene show maximum mortality and lipid peroxidation indicating that enhanced solar UV-B exposure could be lethal to fish fauna. Artificial UV-B had a strong damaging effect than natural solar radiation and become highly toxic in presence of retene. *Tor tor* is more sensitive than *Barilius bendelisis* and *Nemacheilus rupicola* due to enhanced UV-B. Retene photoproducts generate reactive oxygen species which leads to cell injury and mortality. Solar terrestrial UV monitoring data are useful for future planning of weather, climate, human health and biodiversity conservation. At present, concentration of the ozone depleting substances in the atmosphere are decreasing and stratospheric ozone is recovering (Mc Kenzie et al., 2007). Results indicate that climate change and enhanced ultraviolet radiation affect mountain ecosystem and fish biodiversity.

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