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High intensity ultraviolet radiation induced changes in aquatic arthropod with retene and riboflavin

Sunil Kumar✉ and Priyanka Kumari

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Abstract

Ozone depletion is resulting into increase in ultraviolet radiation level in the world. Exposure to UV radiation has been found to have negative effects on aquatic and terrestrial organisms. Adverse effect of natural solar and artificial ultraviolet-B and UV-A radiations was observed in crustacean species *Daphnia magna* in presence of retene and riboflavin. *Daphnia magna* exposed to artificial ultraviolet-B with retene causes maximum physiological changes and mortality, indicating that enhanced solar UV-B exposure could be lethal to aquatic fauna. Artificial UV-B had a stronger damaging effect than solar radiation and become highly toxic in presence of retene. Riboflavin is slightly phototoxic in presence of solar and artificial UV radiation. Results on mortality rate indicated highest mortality in retene + ultraviolet-B exposed group followed by riboflavin + artificial ultraviolet - B radiation. A dose and intensity dependent change in mortality rate was observed. Retene and riboflavin photoproducts with ultraviolet radiation generate reactive oxygen species leading to cell injury and mortality thus are threat to aquatic biodiversity.

Keywords: *Aquatic biodiversity, Ozone depletion, phototoxicity, retene, riboflavin, ultraviolet radiation.*

Introduction

The role of stratospheric ozone layer in absorbing biologically harmful UV radiation is well known. Ultraviolet radiation is the most photochemically reactive wavelength of solar energy reaching the earth surface and has a broad range of effects on aquatic and terrestrial ecosystem (Williamson 1996). The intensity of solar (UV- A and UV-B) is increasing due to ozone depletion (Mckemzie *et. al.* 2007). Life of aquatic environments experiences extreme conditions with respect to temperature, food availability and radiation. UV radiation especially UV-B (280-320 nm) is found to be harmful for the aquatic organisms. Aquatic organisms of shallow arctic water must reproduce successfully within a very short breeding season under these extreme environmental conditions.

Daphnia magna occur circumpolar and play an important role in food web. The knowledge of the effect of solar ultraviolet radiation on *Daphnia magna* is of great interest. Most of the irradiation experiments on *Daphnia* and other zooplankton have been conducted under standardization conditions in the laboratory using artificial light sources for irradiation (Borgeraas and Hassen, 2008). Some authors demonstrated that aquatic organisms of shallow habitats such as ponds, with high UV radiation doses or intensities, may be harmed, even killed due to natural irradiation. The effect of solar (UV-A and UV-B) on some sensitive model species i.e. *Tubifex* and *amphibians* has also been studied by (Formicki et. al. 2003). Still there is a lack of information on phototoxicity of endogenous and exogenous chemicals present in our body and in environment. The effects of (UV-A and UV- B) on aquatic organisms depend on the dose of the harmful radiation to which an individual organism is exposed. Ultraviolet radiation penetration in aquatic habitats is modulated by some factors as dissolved organic carbon, suspended particles, phytoplankton and reflection (Daiz *et al.,* 2000, Hargreaves, 2003). Certain chemicals become phototoxic in presence of solar and ultraviolet radiation. Natural photosensiizers are present in many organisms including bacteria, protozoa, plants, invertebrate and vertebrates (Al-Akhras *et al*. 2007, Kumar et. al. 2010). Retene is found naturally in resinous plants and riboflavin is vitamin B-2 commonly present in our body. This study was performed to investigate the adverse

Author's Address

Department of Zoology,
D. A.V. (P.G.) College, Dehradun - 248001,
Uttarakhand, INDIA
Email: sunilkumarddn@yahoo.co.in,

effect of natural and artificial (UV-A and UV-B) on crustacean species *Daphnia magna* in presence of retene and riboflavin.

Materials and Method

Solar terrestrial UV-A and UV- B was recorded by Cole-Parmer radiometer (USA) having, Vilber Laurmat France calibrated UV-A and UV- B sensors with spectral sensitivity 365 and 312 nm. *Daphnia magna* were collected from Doon valley Uttarakhand and cultured in the laboratory by the method of Songlake and Tisher (2001). During the experiment water depth was about 6cm and water transparency was very high. Transmission was measured with a Spectrophotometer.

Daphnia placed in Petri dish with pond water. Twenty five adult *Daphnia,* egg carrying size were selected for each group. Experimental protocol with two replicates was designed and *Daphnia magna* were divided into twelve groups. Group one was control. Group two was exposed with retene, group three was exposed with riboflavin and group four was treated with low intensity of natural solar radiation. Group five was exposed with low intensity of artificial UV-A radiation and group six was exposed with low intensity of artificial UV-B radiation, group seven was exposed with solar radiation + retene, group eight was exposed with solar radiation + riboflavin. Group nine was exposed with artificial UV-A radiation + retene and group ten was exposed with artificial UV-A radiation + riboflavin, group eleven was exposed with UV-B radiation + retene and group twelve was exposed with UV-B radiation + riboflavin. Another experiment was setup with high intensity of solar and artificial UV-A and UV-B radiation having twelve groups. Dose used for retene and riboflavin was 25mg/liter. Low intensity of UV- A and UV-B radiation used was 0.600 mw/ cm^2 and higher intensity was 0.900 mw/ cm^2. Exposure time was 1 hrs and 2hrs through Philips UV-A and UV- B lamps emitting 365 and 312 nm wavelength radiation. During the experiment morphological, physiological and reproductive changes were observed in *Daphnia*. Results were statistically analyzed using students "t" test (Fisher, 1963).

Results and Discussion

Results on mortality rate of *Daphnia magna* indicates that retene and riboflavin is not harmful when given separately but become photo-toxic with solar light and artificial UV-A and UV-B. Non significant changes in mortality were observed with low intensity of ultraviolet radiation exposure. 10% mortality was found with high intensity of natural solar light. Mortality rate in *Daphnia* was found higher i.e. 23% with high intensity of artificial UV-B exposure (Table-1 & 2). Maximum mortality 42% was found in artificial ultraviolet- B radiation with retene. UV-B radiation and retene show maximum changes in physiological activity, growth, movement, mortality and reproduction. UV- A and solar radiation is less toxic in comparison to artificial UV-B (Table-2). Significant increase in mortality rate was observed after high intensity of solar radiation and artificial UV-B exposure. Co-treatment of retene and riboflavin with solar, artificial UV-A and UV-B exposure further increase the mortality showing phototoxic effect. Artificial UV-B was found more toxic than solar radiation and UV-A (Table- 1 & 2). Retene was found more phototoxic than riboflavin. A dose wavelength and intensity dependent change in mortality rate was observed (Fig-1).Retene (7- isopropyl- 1-methylanthrene) is a compound formed from resin by anaerobic microbes. In natural waters, retene is mainly formed anaerobically from resin acids, oleoresinous constituents of coniferous trees. It has been found in sediment particles contaminated by treated pulp and paper mill effluents as well as in the sediments surface in lake areas contaminated by the industry (Leppanen *et. al.* 2000). Riboflavin is vita-B_2 present in the body. Results on mortality rate and behavior of *Daphnia magna* in presence of solar light UV-A, UV- B individually and with retene and riboflavin indicates that retene is not harmful when given separately but, become phototoxic in presence of solar light, artificial UV-A and UV- B. Difference in phototoxicity of retene, riboflavin with UV-A and UV-B was observed through growth, movement and behavioral change. Reversible effect of UV radiation which recovered within 3-4 hr. of withdrawal of exposure was observed (McKim *et. al*. 2001). Results on dose and intensity dependent increase in phototoxicity are supported by our studies on photohemolysis of erythrocytes (Kumar *et. al*. 2009). Lake having high content of dissolved organic material shields and protect the organism from UV radiation. It act as natural sunscreen as it influence water transparency, therefore determine the light penetration. Low vegetation at alpine areas lakes offer less protection from UV to the organism.

Transparency of water maximizes the penetration and effect of UV radiation however; organisms develop adaptation towards increase radiation (Carbol *et. al*. 2004, Rautio and Tartarotti 2010). Shallow Water Lake is more sensitive to UV radiation and could be harmful to flora, fauna including mammals (Laura *et. al*. 2010). Results on crustacean species are supported by the studies on *Metaphire* (Kumar et. al. 2010).

Table - 1: **Effect of low intensity 0.600mw/ cm^2 of natural and artificial UV radiation on mortality rate in *Daphnia magna*.**

Group	Treatment	Mortality %	
		1 Hour	2 Hour
1.	Control	2 ± 0.4	2 ± 0.4
2.	Retene	4 ± 0.4 ^{NS}	4 ± 0.3 ^{NS}
3.	Riboflavin	3 ± 0.5 ^{NS}	3 ± 0.6 ^{NS}
4.	Solar radiation	6 ± 0.5 ^{NS}	8 ± 1.4 ^{NS}
5.	Artificial Ultraviolet-A	6 ± 0.7 ^{NS}	8 ± 1.06 *
6.	Artificial Ultraviolet-B	12 ± 1.12*	14 ± 1.71*
7.	Solar radiation + retene	13 ± 0.8*	18 ± 0.9*
8.	Solar radiation + riboflavin	12 ± 0.7*	16 ± 0.8*
9.	Artificial Ultraviolet-A + retene	18 ± 1.08*	22 ± 3.05**
10.	Artificial Ultraviolet-A + riboflavin	15 ± 1.45*	19 ± 1.92*
11.	Artificial Ultraviolet-B + retene	28 ± 1.2**	32 ± 1.1**
12.	Artificial Ultraviolet-B + riboflavin	26 ± 1.4**	30 ± 0.9**

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

Table - 2: **Effect of high intensity 0.900 mw/ cm^2 of natural and artificial UV radiation on mortality rate in *Daphnia magna*.**

Group	Treatment	Mortality %	
		1 Hour	2 Hour
1.	Control	2 ± 0.4	2 ± 0.4
2.	Retene	4 ± 0.5 ^{NS}	4 ± 0.3 ^{NS}
3.	Riboflavin	3 ± 0.6 ^{NS}	3 ± 0.4 ^{NS}
4.	Solar radiation	7 ± 0.6 ^{NS}	10 ± 1.05 *
5.	Artificial Ultraviolet- A	9 ± 1.65*	10 ± 2.24*
6.	Artificial Ultraviolet-B	20 ± 1.27*	23 ± 1.06*
7.	Solar radiation + retene	24 ± 1.28*	28 ± 2.09*
8.	Solar radiation + riboflavin	22 ± 1.18*	26 ± 3.17*
9.	Artificial Ultraviolet-A + retene	25 ± 1.43**	28 ± 2.16**
10.	Artificial Ultraviolet-A + riboflavin	23 ± 1.43*	25 ± 3.12**
11.	Artificial Ultraviolet-B + retene	38 ± 3.31**	42 ± 2.42**
12.	Artificial Ultraviolet-B + riboflavin	32 ± 2.24**	36 ± 3.19**

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

Riboflavin and retene are important chromophores for photoinduced lethality in *Daphnia*. Retene and riboflavin photoproducts generate reactive oxygen species with UV radiation leading to cell injury and mortality. Those species whose early life stage occur near the surface, there may be circumstances- such as a cloudless sky, lack of wind, calm seas, low nutrient loading- under which the contribution of UV-A and UV- B radiation to the productivity and mortality of a population could be far more significant. Reproductive parameters seem to be very sensitive in determining UV radiation induced damaged. Further increase in solar UV due to stratospheric ozone depletion may leads to significant change in the zooplankton communities and aquatic ecosystem.

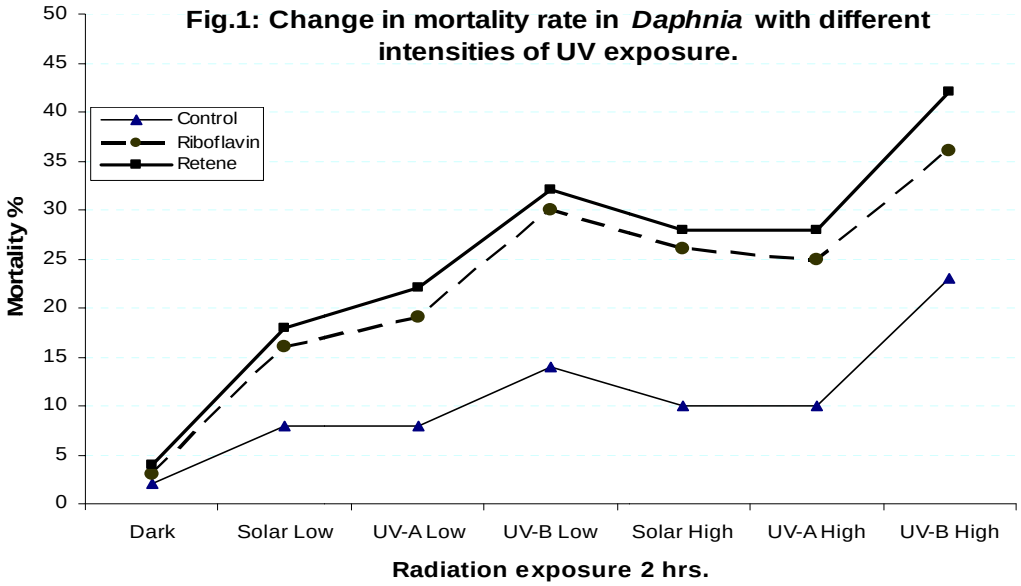

Fig.1: Change in mortality rate in *Daphnia* with different intensities of UV exposure.

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