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Solar and artificial ultraviolet-B induced erythrocytes hemolysis with photosensitizers

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Aim of this study was to monitor the solar ultraviolet-B intensity and to compare the phototoxic effect of different intensity of natural and artificial ultraviolet-B on human red blood cells in presence of compounds as riboflavin and chloroquine. Photohemolysis of erythrocytes was studied under natural solar radiation and artificial ultraviolet-B radiation of 312 nm. Monitoring of solar ultraviolet-B radiation was performed in Garhwal region of Uttarakhand, India. Level of solar ultraviolet-B measured show seasonal and altitudinal variations. Monthly average of solar UV-B intensity was minimum in the month of December and January (0.299 mw/cm^2) and maximum in the month of July and August (1.027 mw/cm^2). Natural solar radiation intensities 0.402 mw/cm^2 and 0.824 mw/cm^2 of the month of January and June were used in the photohemolysis experiment. Two intensities of artificial UV-B i.e. 0.824 mw/cm^2 and a double intensity 1.65 mw/cm^2 were also used. Results on human erythrocytes hemolysis indicate that haemolysis was highest i.e. 71% in chloroquine + artificial ultraviolet-B intensity (1.65 mw/cm^2) followed by 62% in chloroquine + artificial ultraviolet-B (0.824 mw/cm^2) exposed groups and 54% in natural solar radiation intensity 0.824 mw/cm^2 + chloroquine. Natural solar UV-B alone caused 17% hemolysis and show dose response relationship. A difference in phototoxicity was observed in natural solar and artificial UV-B of same intensity. Artificial UV-B was found more toxic. Riboflavin was more phototoxic in presence of solar light, while chloroquine was more phototoxic with artificial UV-B.

Keywords: Chloroquine, Ozone depletion, Photohemolysis, Riboflavin, Ultraviolet-B

UV radiation is recognized as an important biological stressor for many species[1]. Stratospheric ozone naturally reflects UV-C and most of the UV-B, so only UV-A and little of the UV-B reach the earth[2]. Organisms on earth are therefore evolutionarily adapted to UV-A, but might not be adapted to UV-B[3]. During the past decades researchers have conclusive evidence that man-made chemicals chlorofluoro-carbons (CFCs) and other environmental agents are destroying the stratospheric ozone (O_3) layer[4]. A major depletion in ozone thickness is increasing the penetration of UV-B light to the earth's surface, with toxic effects on all form of life[5]. The intensity of solar UV radiation especially of the short wavelength of UV-B (290-320 nm) is increasing due to ozone depletion[6]. Increased UV-B intensity has been registered in terrestrial station mainly in Antarctica and Arctic and great deal of attention has been focused on UV induced photodamage in organisms[7]. A 10% decrease in the total stratospheric ozone would increase the amount of UV-B reaching the earth's surface up to 20%[8]. Effects caused by UV-B including DNA damage, melanogenesis, skin erythema, skin cancer and damage to eyes, have been studied[9]. Photohemolysis of the red blood cells is one of the method for the study of phototoxicity *in vitro* and for elucidation of the mechanism on phototoxic action. Hemolysis of human erythrocytes by hypochlorous acid and its modulation by amino acid, antioxidants has been studied[10].

Phototoxic irradiation experiments on *Daphnia* and other zooplankton have been conducted under standardized condition in the laboratory using artificial light sources for irradiation[11]. Effects of solar ultraviolet radiation on some sensitive model species i.e. *Metaphire posthuma* and *Daphnia magna* have been reported[12,13]. Riboflavin is naturally present in our body as vitamin B-2 and chloroquine is used as antimalarial drug. There is a lack of information on enhanced natural solar ultraviolet-B radiations and its impact with chemicals present in the environment. Artificial UV-B selected in order to compare the effect of natural solar UV light and consequences of

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increasing intensity of solar UV-B due to ozone depletion. Erythrocytes were exposed with different intensities of natural and artificial ultraviolet-B radiation with and without riboflavin and chloroquine.

Materials and Methods

For monitoring, solar terrestrial UV-B was recorded by Cole-Parmer radiometer (USA) having, Vilber Laurmat France calibrated UV-B sensors with spectral sensitivity 312 nm. Monitoring of solar UV-B was performed from January to December 2008 between 12:00 - 13:00 hrs for one year, at Dehradun (latitude 30° 19′ N, longitude 78° 04′ E and altitude 639 msl) and at Haridwar station at (latitude 29° 58′ N, longitude 78° 13′ E and altitude 294 msl). Data were collected weekly and results are mean of four observations of each month. UV-B dose in natural solar light selected for experiment in the month of January and June on clear sunny day were 0.402 and 0.824 mw/cm^2 respectively. Experimental artificial UV-B lamp intensity selected were 0.824 mw/cm^2 equal to solar radiation and double intensity 1.65 mw/cm^2 for studying the dose response relationship.

Two experimental protocols were designed and experiment was carried out between January to June. In first experimental setup, group I was wrapped with aluminum foil and kept as dark control. Group II was exposed with natural solar radiation UV-B dose 0.402 mw/cm^2 and group III was exposed with natural solar radiation UV-B dose 0.824 mw/cm^2. Group IV was exposed with artificial UV-B radiation intensity 0.824 mw/cm^2 and group V was exposed with artificial UV-B radiation intensity 1.65 mw/cm^2. Group VI was exposed with riboflavin, group VII was exposed with solar radiation intensity 0.402 mw/cm^2 + riboflavin, group VIII was exposed with solar radiation intensity 0.824 mw/cm^2 + riboflavin and group IX was exposed with artificial UV-B radiation intensity 0.824 mw/cm^2 + riboflavin and group X was exposed with artificial radiation intensity 1.65 mw/cm^2 + riboflavin. In second protocol group 1-5 were same and in group six, seven, eight, nine and ten riboflavin was replaced by chloroquine. Three replicates were prepared. Natural solar radiation was given between 11:00–13:00; hrs for one hour per day. Experiment was performed during clear weather condition. Replicate I was exposed for 1 hr first day and hemolysis percentage was calculated. Replicate II was used for second day also having total two hrs of exposure and replicate III was carried up to third day having total three hrs of exposure and hemolysis percentage was calculated. Artificial UV-B was given by Philips UV-B lamp with wavelength 312 nm. Riboflavin and chloroquine concentrations used were 50 mg/l.

Photohemolysis of human erythrocytes was performed by collecting heparinized blood of human and procedure of Hetherington and Johnson[14] was followed. Blood was centrifuged at 3000 rpm for 5 min and erythrocytes were washed three times with saline buffer (0.85% NaCl in 0.01 *M* phosphate buffer, *p*H-7.4). A 20 ml sample containing a 1% erythrocyte suspension was exposed to artificial UV-B and natural sunlight as per protocol in petri dishes without cover. Temperature was kept between 20 ± 5ºC by keeping the petri dishes in cold water tray. The irradiated suspension was centrifuged at 3000 rpm for 20 min. The supernatant (2 ml) was mixed with 2 ml Drabkins reagent (KCN, 0.05 g + $K_3Fe(CN)_6$. 0.2 g in 1 liter H_2O) and absorbance at 420 nm was recorded. Measurement of methemoglobin in blood was carried out by the methodology described by Dacie and Lewis[15]. Chemicals and reagents used throughout the investigation were purchased from Sigma Chemical Company (USA), Aldrich Chemical Company, (Milkwaukee, WI USA) and BDH (Poole, Dorset. UK). Results were statistically analyzed, standard error was calculated and inter group comparisons were made using the Student's "*t*" test[16].

Results and Discussion

Result on monitoring of solar UV-B show seasonal and altitudinal variations. Solar UV-B was found lowest in the month of December and January and highest in the month of July, August and September. When comparing the results between two sites, value was higher at high altitude at Dehradun than Haridwar (Fig. 1). Results on photohemolysis of human erythrocyte in presence of solar light and artificial UV-B with riboflavin indicates that, riboflavin is not harmful when given separately but it become phototoxic in presence of solar light. Riboflavin is none or slightly phototoxic with ultraviolet-B radiation. Artificial UV-B intensity-1.65 mw/cm^2 alone and with riboflavin causes almost same 34% and 33% hemolysis, while it shows phototoxic effect with solar radiation (Table 1). Chloroquine is toxic in it and further show phototoxic effect in presence of solar light and UV-B both. Chloroquine phototoxicity

is more with UV-B radiation than solar radiation (Table 2). Haemolysis was found highest i.e. 71% after 3 hr exposure of artificial UV-B intensity-1.65 mw/cm^2 exposure with chloroquine followed by 62% on artificial UV-B (0.824 mw/cm^2) with chloroquine and 54% with same dose of solar UV-B +

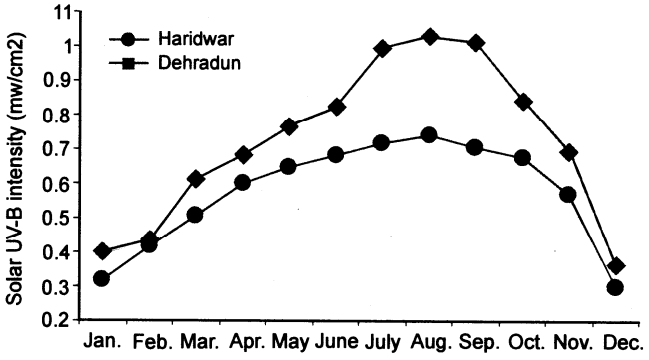

Fig. 1 — Seasonal variation in solar UV-B radiation at different altitudes and location of Uttarakhand, India

chloroquine exposure. When comparing with equal intensity (0.824 mw/cm^2) of solar and artificial UV-B, artificial UV-B was found more phototoxic (Table 2). Low dose of natural sunlight show non-significant change in hemolysis percentage but higher doses have significant changes in erythrocyte haemolysis percentage, causing greater stress to erythrocytes membrane and showing dose response relationship (Tables 1 & 2, Fig. 2).

Results are supported by our own studies on erythrocytes of different animal species and study on *Daphnia magna* with ultraviolet radiation[17,18.]. Low dose UV-B exposure resulted in low haemolysis in all treatments. This is in agreement with Kumar and Joshi[17] and Lurling and Vandonk[18] who reported no effect on survival in *Daphnia pulex* exposed to UV-B in a deep pond for several days in poor weather condition. Considerable variations in hemolysis rate in presence of photosensitizers and ultraviolet

Table 1 — Effect of different doses of solar and artificial UV-B on hemolysis percentage of erythrocytes with riboflavin

[Values are mean ± SE of 5 independent observations in each group.]

Group	Treatment	Hemolysis (%)		
		1 h	2 h	3 h
1	Dark control	3 ± 0.18	4 ± 0.28	4.8 ± 0.32
2	Solar radiation 0.402	5 ± 0.34^{NS}	6 ± 0.47^{NS}	8 ± 0.48*
3	Solar radiation 0.824	8 ± 0.54^{NS}	11 ± 0.38*	17 ± 0.48*
4	Artificial UV-B 0.824	9.6 ± 1.12*	14 ± 1.71*	20 ± 1.42*
5	Artificial UV-B 1.65	18 ± 1.11*	27 ± 1.19*	34 ± 1.54**
6	Riboflavin	3.6 ±0.27^{NS}	5 ± 0.42^{NS}	5.8 ± 0.52^{NS}
7	Solar radiation 0.402 + riboflavin	8 ± 0.56^{NS}	21 ± 1.25*	28 ± 2.31**
8	Solar radiation 0.824 + riboflavin	10 ± 0.32*	25 ± 1.32**	32 ± 2.00**
9	Artificial UV-B 0.824 + riboflavin	9 ± 1.40*	16 ± 2.03*	26 ± 2.51**
10	Artificial UV-B 1.65 + riboflavin	18 ± 1.10*	29 ± 1.14*	33 ± 1.13**

P values : < * 0.05, ** 0.01, NS-non-significant (between control and experimental group)

Table 2 — Effect of different doses of solar radiation and artificial UV-B on hemolysis percentage of human erythrocytes with chloroquine

[Values are mean ± SE of 5 independent observations in each group]

Group	Treatment	Hemolysis (%)		
		1 h	2 h	3 h
1	Dark control	3 ± 0.18	4 ± 0.28	4.8 ± 0.32
2	Solar radiation 0.402	5 ± 0.34^{NS}	6 ± 0.47^{NS}	8 ± 0.48*
3	Solar radiation 0.824	8 ± 0.54^{NS}	11 ±0.38*	17 ± 0.48*
4	Artificial UV-B 0.824	9.6± 1.12*	14 ± 1.71*	20 ± 1.42*
5	Artificial UV-B 1.65	18 ± 1.11*	27 ± 1.19*	34 ± 1.54**
6	Chloroquine	14 ±1.20*	22 ± 1.50*	30 ± 1.83**
7	Solar radiation 0.402 + chloroquine	15 ±1.82**	32 ± 3.19**	40 ± 3.20**
8	Solar radiation 0.824 + chloroquine	31 ± 0.59**	43 ± 2.34**	54 ± 1.23**
9	Artificial UV-B 0.824 + chloroquine	36 ± 1.81**	45 ± 1.75**	62 ± 3.18***
10	Artificial UV-B 1.65 + chloroquine	41 ± 2.03**	63 ± 2.18***	71 ± 2.56***

P values: < * 0.05, ** 0.01, *** 0.005, NS-non-significant (between control and experimental group)

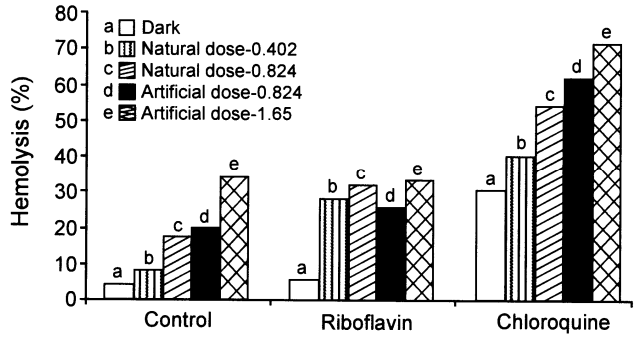

Fig. 2 — Effect of different intensities of natural and artificial UV-B on hemolysis with riboflavin and chloroquine

radiation and photosensitizers alone suggesting the photo reactivity of riboflavin and chloroquine. Riboflavin and chloroquine are important chromosphores for photo induced lethality and hemolysis.

Phototoxicity of riboflavin and chloroquine is due to radiation of solar light and UV-B attributable to generation of reactive oxygen species and hydrogen peroxide radicals[19]. Riboflavin and chloroquine principal photoproduct are lumichrome, lumiflavin and quinines[20]. Quinones are toxic intermediate which create toxicity and variety of hazardous effect *in vivo*. Solar radiation is the primary source of human exposure to UV radiation[21]. It was demonstrated that solar light can cause severe damage to human health and biota. Interaction of therapeutic dose of UV radiation and their absorption through blood proteins, heme components of hemoglobin, UV absorption and luminescence have been reported[22].

Results indicate 17% haemolysis in natural solar light, 20% with same dose of artificial UV-B and 34% with double dose of artificial UV-B showing wave length specific dose dependent relationship. Some interaction among solar radiations (visible, solar UV-A and UV-B) appear which might show protective effect as solar radiation has less toxic effect than pure artificial UV-B. Riboflavin and chloroquine are phototoxic and photosensitivity is wave length specific. Small increment in solar UV-B radiation caused by stratospheric ozone depletion may lead to significant changes in the human health and disease pattern. Further field investigation on *in vivo* and *in vitro* studies under natural radiation conditions are necessary to gain a better understanding of the effect of solar UV radiation on organism.

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