INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES
IJPAES
Volume-6, Issue-1, Jan-Mar-2016 Coden: IJPAJX-CAS-USA, Copyrights@2016 ISSN-2231-4490
Received: 5th Jan-2016 ***Revised: 17th Jan 2016*** ***Accepted: 19th Jan 2016***
Research article

SILK GLAND SOMATIC INDEX AND TISSUE BIOCHEMICAL CONTENTS OF UV-B IRRADIATED ERI SILKWORM, *PHILOSAMIA RICINI* (H.)

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ABSTRACT: The depletion of stratospheric ozone layer and the resulting increase in UV- B radiation reaching the earth is of worldwide concern. Silk producing insects are terrestrial in habits and more prone to UV- B radiation exposure. The growth and development of silk gland depends on the health of the silk worm and the silk industries in turn depend on silk production by healthy silk worms. Hence, an attempt was made to study the effect of UV- B irradiation of different time duration; 30, 60, and 120 minutes on silk gland, its development, and tissue biochemical content of 5th instar eri silkworm *Philosamia ricini*. Further, the SGTSI which indicates the percentage of silk gland in a silk worm larva was also calculated. The study revealed that the SGTSI of UV-B irradiated silk worms decreased significantly from day 1 to day 5 of 5th insar (at $p< 0.05$) as compared to control. The total protein and carbohydrate content (g%) of silk gland were also significantly decreased (at $p < 0.001$) as compared to control. This indicates that UV-B light alters the growth and metabolism of eri silk worm which is undesirable from economic point of view.
Key words: UV-B, *Philosamia ricini*, Silk Gland, SGTSI, Biochemical content

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INTRODUCTION

Eri silkworm *Philosamia ricini* is a non mulberry silkworm [1]. The growth and development of silk gland depends on the healthy silkworm and silk production which is related to the silk industries. The silk gland exhibits enormous growth during 5th instar [2].Silk gland is an exocrine gland secreting large amount of silk protein. It is a paired organ consisting of modified labial/salivary gland situated ventero-lateral side of the body extending mouth to anus [3]. The somatic index of silk gland (SGTSI) is the ratio between silk gland weight and the body weight [4]. Silk gland weight was positively correlated with larval weight [5]. Also it was observed that cocoon shell weight was positively correlated with silk gland weight.

The depletion of stratospheric ozone layer and the resulting increase in UV- B radiation reaching the earth is worldwide concern. Insects are terrestrial in habits and more prone to be affected by UV-B. The increase in UV- B radiation impelled researchers to study the effect of UV radiation on insects. The effect of UV rays on hatching and adult emergence in *Tribolium cataneum*, *Tribbolium confusum* and *Cadra cautella* eggs of various stages was determined by Faruki et al. [6]. The effect of UV radiation on the larvae of lesser mealworm and their progeny was studied by Faruki et al. [7]. Okamoto [8] observed the lethal effect of UV radiation on adult German cockroach. The effect of UV radiation on the American cockroach, *Pereplaneta americana* has also been studied by Wharton [9]. The radio sensitivity varies according to species, strain, and individual and even at different developmental stages of the individual [10].

Some pioneers worker observed the effect of UV radiation on some commercially relevant traits of first, second and third instars larvae of two multivoltine strains of silkworm *Bombyx mori*, and the effect of ultraviolet (UV) radiation on some economic traits of the silkworm, *Bombyx mori* L. variety and they found that UV radiation had significant effects on different characters of *Bombyx mori* [7, 11].

The macromolecular profile and digestive enzymes of silkworm *Bombyx mori* fed with UV treated mulberry leaves was studied by Sadiq et al. [12]. The effect of UV light on biochemical parameters of posterior silk gland of *B. mori* fed with artificial diet was studied by Bharathi and Yungen [13]. Very less work has been done on silk gland development and biochemical content of silk gland related to UV-B irradiation. In this study an attempt has been made to assess the silk gland somatic index and biochemical content analysis of silk gland tissue of eri silkworm.

MATERIALS AND METHODS

Collection and Culture of Eri Silkworm *P. ricini*

The eggs of *P. ricini* were collected from the Department of Sericulture Government of Odisha, Deogarh unit and were kept in temperature control room i.e. Eri culture lab of School of Life Sciences, Sambalpur University for hatching. The eri culture lab and rearing appliances were disinfected with 2 % formaldehyde. After hatching the larvae were fed with leaves of castor collected from nearby locality.

Protocol for UV Irradiation

Larvae of *P. ricini* were taken in three replicates (50/replicates).After the first instar they were irradiated with 20 watt UV B tube with constant intensity in three exposure durations i.e.30,60,120mins.UV exposure was given each day in each replicate with the same duration till the end of fifth instar before feeding and also control was maintained without irradiation.

Body Weight and Silk Gland Development

This was done by taking the body weight and silk gland weight of UV irradiated fifth instar larvae of *P. ricini* five days of fifth instar as per [13]. SGTSI was calculated as a percentage of body weight [4].

Estimation of Total Protein

Total protein content was estimated by the method of [14]. For this a known amount of silk gland was taken and homogenized with 10ml of TCA. The homogenate was centrifuged at 4000x g for 10 min. The residue was washed with 10% TCA and centrifuged at 4000x g for 5 min. The residue was dissolved in 1N NaOH. The total volume of the extract was measured and diluted with 0.3N NaOH solutions for colorimetric estimation. The volume of the extract was recorded and made up to 1 ml by distilled water. Then 3 ml of protein reagent was added and incubated for 30min.Then Folin's reagent was added and incubated for 20 min. The absorbance was measured at 750nm.Standard graph was plotted taking bovine serum albumin (BSA standard-1mg/1ml).Protein content was calculated and expressed as g%.

Estimation of Carbohydrate

Total carbohydrate was estimated by phenol sulphuric acid method of [15].Silk gland of fifth instar larvae was weighed and homogenized into smooth paste in distilled water with the help of mortar and pestle. The homogenate was centrifuged at 4000 x g for 10 min. and the supernatant was collected. The residue was washed with small amount of distilled water and centrifuged. After pooling the both supernatants volume was measured and diluted for spectroscopic analysis. Concentrated H_2SO_4 was added to the test tubes containing phenol, water and glucose standard (10mg/100ml), shaken well and allowed to stand for 20 min. Absorbance was measured at 470nm and standard graph was plotted taking absorbance and concentration of glucose standard. Then absorbance sample (unknown) was cross matched with the standard graph to find out the concentration and the amount of carbohydrate was expressed as g%.

Statistical Analysis

The data obtained were subjected to t-test and analysis of variance.

RESULTS

The body weight and silk gland weight (g) and silk gland somatic index (SGTSI) of eri silkworm *P. ricini* were presented in the Table1 and 2. Protein and carbohydrate content of eri silk gland was showed in the Figure 1and 2 respectively.

Body Weight and Silk Gland Weight (g) of *Philosamia ricini*

Table1 indicates the mean body weight and silk gland weight (g) of UV-B irradiated (30, 60 and 120min) fifth instar larvae (N=10) for 5 consecutive days. The body weight and silk gland weight UV-B irradiated for different duration showed significant decrease from day 1 to day 5 at $p<0.05$ as compared to control. The mean body weight of fifth instar larvae (N=10) irradiated for 30 min for five consecutive days was (6.63, 8.59, 10.78, 12.38, and 11.61); silk gland weight was (0.6, 0.74, 1.29, 1.69, 1.46).Body weight of 60min irradiated silkworm was (6.17, 8.73, 10.33, 12.60, 10.92) and silk gland weight was (0.68, 0.94, 1.14, 1.54, 1.83) respectively. Body weight of 120 min irradiated silkworm was (6.73, 9.07, 10.69, 12.03, 10.80) and silk gland weight was (0.55,0.71,1.14,1.48,1.38).Similarly, the body weight of larvae which was not irradiated with UV-B taken as control (7.02,11.33,13.07,13.08,12.91) and silk gland weight was (1.49,1.88,1.96,2.6,3.05) respectively.

Table 1. Body weight and silk gland weight (g) of UV- B irradiated and control eri silkworm larvae.

Days	Body weight(g)				Silk gland weight(g)			
	Control Mean ±SEM	30min Mean ±SEM	60min Mean ±SEM	120min Mean ±SEM	Control Mean ±SEM	30min Mean ±SEM	60min Mean ±SEM	120min Mean ±SEM
1	7.02 ±0.11	6.63 ±0.1*	6.17 ±0.12*	6.73 ±0.21*	1.49 ±0.11	0.6 ±0.06*	0.68 ±0.07*	0.55 ±0.08*
2	11.33 ±0.18	8.59 ±0.37*	8.73 ±0.32*	9.07 ±0.51*	1.88 ±0.05	0.74 ±0.06*	0.94 ±0.1*	0.71 ±0.08*
3	13.07 ±0.50	10.78 ±0.63*	10.33 ±0.51*	10.69 ±0.53*	1.96 ±0.11	1.29 ±0.18*	1.41 ±0.12*	1.14 ±0.06*
4	13.08 ±0.17	12.38 ±0.17*	11.60 ±0.50*	12.03 ±0.20*	2.60 ±0.17	1.69 ±0.13*	1.54 ±0.05*	1.48 ±0.09*
5	12.91 ±0.77	11.61 ±0.12*	10.92 ±0.17*	10.80 ±0.30*	3.05 ±0.01	1.46 ±0.05*	1.83 ±0.16*	1.38 ±0.08*

*t is significant at p 0.05

The difference of above studied parameters between treated eri silkworm and the silkworm not exposed to UV radiation were found to be significant t at $p<0.05$.

Silk Gland Tissue Somatic Index (SGTSI) of *Philosamia ricini*

The SGTSI was expressed as silk gland percentage. The Silk gland tissue somatic index was significantly decreased as compared to control from 1st to 5th day of fifth instar larvae (Table 2). The result revealed that SGTSI was (9.05, 8.62, 11.97, 13.65, 12.58); (11.02, 10.78, 13.64, 13.28, 16.76) and (8.17, 7.83, 10.66, 12.30, 12.78) in larvae irradiated with 30, 60 and 120min respectively; whereas in control it was found as (21.33, 16.59, 14.99, 19.88, 23.63).

Table 2. Silk gland somatic index of UV-B irradiated and control eri silkworm larvae.

	Larval weight(g)	Silk gland weight(g)	Silk gland (%)
Control	7.02	1.49	21.23
	11.33	1.88	16.59
	13.07	1.96	14.99
	13.08	2.60	19.88
	12.91	3.05	23.63
30 min	6.63	0.6	9.05
	8.59	0.74	8.61
	10.78	1.29	11.97
	12.38	1.69	13.65
	11.61	1.46	12.58
60 min	6.17	0.68	11.02
	8.73	0.94	10.78
	10.33	1.41	13.64
	11.60	1.54	13.28
	10.92	1.83	16.76
120 min	6.73	0.55	8.17
	9.07	0.71	7.83
	10.69	1.14	10.66
	12.03	1.48	12.30
	10.92	1.38	12.64

Total Protein Content (g%)

Figure 1 represents the total protein content of silk gland of fifth instar larvae of *P.ricini* UV- B irradiated for 30,60,120 min and control of six consecutive days. Silk gland protein content of UV-B irradiated silkworm was significantly decreased as compared to control. It was indicated that total protein content of 30 min UV-B irradiated was (0.52±0.03, 0.68±0.04, 1.02±0.05, 1.08±0.12, 3.42±0.29, 5.47±0.18); (0.49±0.04, 0.49±0.04, 0.67±0.02, 0.88±0.03, 1.47±0.21, 2.58±0.19) for 60 min and (0.24±0.03, 0.26±0.01, 0.38±0.02, 0.67±0.02, 0.95±0.04, 1.10±0.04) for 120 min, whereas in control it was (0.60±0.03, 0.89±0.02, 1.27±0.12, 2.28±0.05,

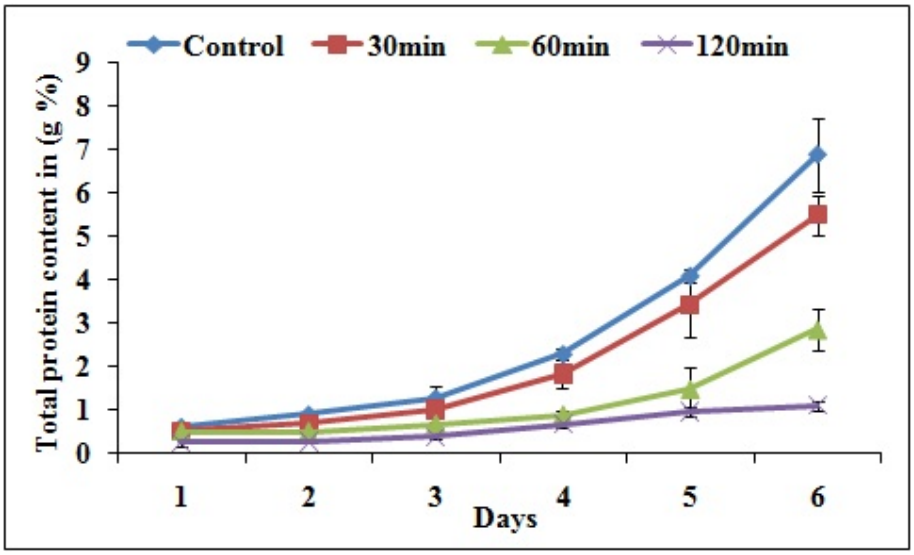

Figure 1. Silk gland protein content (g%) of UV-B irradiated and control eri silkworm.

4.08±0.07, 6.88±0.32) respectively. From two way ANOVA with replication it was confirmed that there was significant variation in silk gland protein content of UV-B irradiated and control larvae of six consecutive days at p 0.001 (F=179.15 between days and 157.05 duration of UV-B irradiation).

Total Carbohydrate Content (g%)

The silk gland total carbohydrate content of fifth instar larvae of *P.ricini* UV-B irradiated for 30,60,120min and control of six consecutive days was given in the Figure 2. The total carbohydrate content of silk gland significantly decreased as compared to control. It was indicated that total carbohydrate content of 30 min UV-B irradiated was (0.52±0.02, 0.55±0.02, 0.65±0.03, 1.02±0.05, 2.26±0.06, 2.99±0.11) for 60 min and (0.36±0.02, 0.52±0.04, 0.57±0.01, 0.77±0.03, 1.06±0.03, 1.52±0.15) and for 120 min (0.21±0.02, 0.25±0.01, 0.26±0.02, 0.38±0.02, 0.67±0.05, 0.86±0.02) where as in control it was (0.58±0.02, 0.74±0.03, 0.93±0.02, 1.49±0.15, 3.59±0.12, 4.92±0.09 respectively). A significant variation of silk gland carbohydrate content was observed (at p 0.001) in UV-B irradiated and control eri silkworm larvae (F=149.95 between days and 188.80 duration of UV-B irradiation).

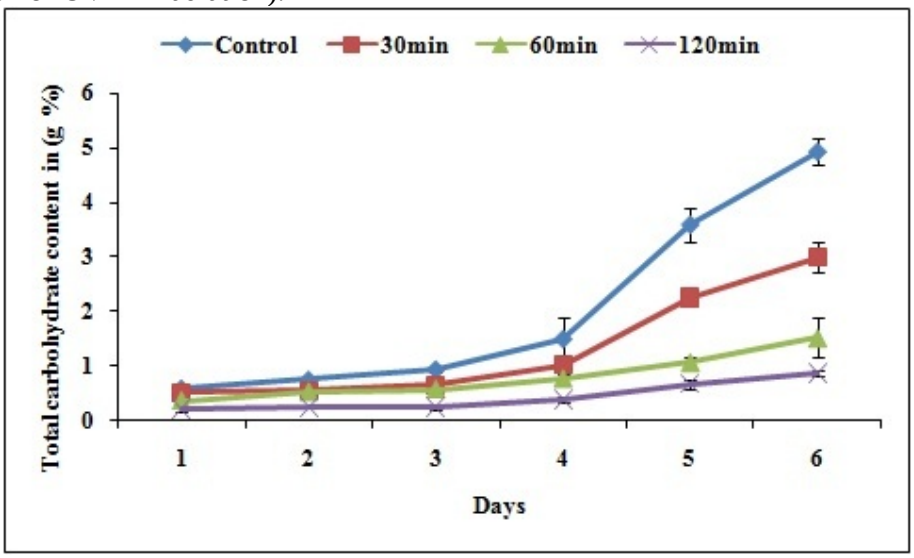

Figure 2. Silk gland carbohydrate content (g %) of UV-B irradiated and control eri silkworm.

DISCUSSION

Silk gland is a typical exocrine gland secreting large amount of silk protein. It is paired organ consisting of modified labial/salivary gland situated on the ventro- lateral side of the body extending from mouth to anus.

The silk gland is made up of huge polyploidy cells, each with an extremely ramified nucleus containing numerous nucleoli. Nuclear ramification develops gradually as the larvae grow and reach conspicuous size in the 4^{th} and 5^{th} instars [3].

The present study reveals that the body weight and silk weight of UV- B irradiated for different duration; 30, 60, and 120 min show significant decrease (at $p<0.05$) as compared to control. This indicates UV irradiation reduced the growth and development of silk gland. The findings are slightly varied from the findings of Bharathi and Yungen [13].From the literature related to *Bombyx mori* showed that UV treatment produced insignificant effect at 30 min on the silk gland growth. Also analysis of protein and carbohydrate levels in UV- B irradiated eri larvae decreased significantly compared to control. A positive correlation between SGTSI, cocoon weight and shell weight in *Bombyx mori* was observed by [17]. Further, it was observed that protein and carbohydrate content on different days increased progressively from the first day. The significant decline in biochemical constituents in UV-B treated eri larvae may be due to decreased biosynthetic activities associated with formation of cocoon, shell and silk protein. [13] found that UV treatment produced significant decrease in the level of protein, free amino acids, RNA and carbohydrates at 60 and 120 min irradiation of fifth instar (5-8) at $p<0.05$. UV-irradiation altered the growth and metabolism of silkworms in a time-dependent manner. The decreased weight of mature larvae, pupae and adults with increased radiation doses showed negative effects on economic traits in *B. mori* due to UV-irradiation exposed at larval stage [15]. This indicates that UV- B light altered the growth and metabolism. The decrease in total protein content indicated decreased metabolic activities and enhancement of proteolysis of silk gland. Carbohydrate being an important dietary constituent in most of insects, difference in total carbohydrate content of silk gland suggests that UV- B irradiation altered the extent of utility of these energy sources necessary for the function of silk gland.

CONCLUSION

The overall study indicates that UV radiation disturbs the growth, biosynthesis of silk gland, decrease cocoon production supposed to affect economic status. Further, experimentations with different doses on various developmental stages of *Philosamia ricini* are highly recommended.

ACKNOWLEDGEMENT

Authors are thankful to the department of School of Life Sciences, Sambalpur University and the Department of Sericulture Odisha, Deogarh Unit.

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INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

ISSN 2231-4490

International Journal of Plant, Animal and Environmental Sciences