Available online at www.ijpab.com

ISSN: 2320 – 7051
Int. J. Pure App. Biosci. **1 (4):** 37-41 (2013)
Research Article

International Journal of Pure & Applied Bioscience

Haemolymph Glucose level of F_1 progeny raised from Ethyl Methanesulfonate treated Silkworm *Bombyx mori* L.

H. B. Mahesha[1]* and P.H. Thejaswini [2]

[1]Department of Sericulture, Yuvaraja's College, University of Mysore, Mysore-570005, India.
[2]Department of Biotechnology, Maharani's Science College, Mysore-570005, India.
*Corresponding Author Email: hbmseri@yahoo.com

ABSTRACT

Two pure breeds of mulberry silkworm namely Pure Mysore and NB_4D_2, at the age of fifth instar first day were treated with different doses of ethyl methanesulfonate (EMS) by oral injection. The treated larvae along with their respective controls were allowed to continue development. The F_1 progenies were raised by selfing the moths emerged from the ethyl methanesulfonate treated and control sets separately. The control as well as F_1 progeny raised from the treated silkworm larvae were collected daily with a regular interval of 24 h. for haemolymph collection. The collected haemolymph was subjected for glucose assay. The experimental results clearly indicated the significant variation in blood glucose level between control and experimental sets as well as among experimental sets.

Key words: *Silkworm, Bombyx mori, ethyl methanesulfonate, mutation, inheritance, haemolymph glucose level.*

INTRODUCTION

Mulberry silkworm, *Bombyx mori* L. is the most important insect being used for commercial production of silk in sericulture industry. Studies on the mutagenesis in silkworm, *Bombyx mori* L., have been in progress for the last few decades to synthesize new gene combinations with improved commercial qualities. Majority of the work has been carried out to induce mutations in silkworm through physical mutagens rather than chemical mutagens. Of the physical mutagens, radiations have been extensively exploited to bring a change in the genotype of silkworm [1]. Tanaka [2] was the first one to induce mutations in silkworm after the popular work of the artificial transmutation of the gene by Muller [3] in 1927. Since then, many efforts have been made by various scientists like Kogure, Tazima, Aruga, Takasaki, Chikushi and Tsujita [4] and numerous interesting results have been accumulated. In recent years Datta *et al.* [5], Iyengar *et al.* [6] and Subramanya and Sreeramareddy [7] have succeeded in the induction of beneficial mutations in the silkworm, *Bombyx mori* L., using radiations. Bhoopathy and Muthukrishan [8] studied the effect of a chemical mutagen, diethylsulfate on growth, economic character and mutagenicity in mulberry silkworm. Mahesha and Honnaian [9] studied the effect of EMS on meiotic chromosomes of silkworm *Bombyx mori*. In 2000 Mahesha *et al.* [10] reported the qualitative and quantitative variation in the haemolymph proteins of F_1 progeny raised from ethyl methanesulfonate treated silkworm larvae. Mahesha and Thejaswini [11] studied the effect of ethyl methanesulfonate on vialbility and commectial characters of silkworm *Bombyx mori* and reported that the lower doses of EMS might stimulate vitality and improvement in economic traits. However, studies on the effect of chemical mutagens like EMS on blood glucose level are rather scarce. Therefore, the present investigation was carried out.

MATERIALS AND METHODS

Two mulberry silkworm breeds namely Pure Mysore (multivoltine) and NB_4D_2 (bivoltine), as well as a well known monofunctional ethylating agent, ethyl methanesulfonate (EMS) were used in the present investigation.

The silkworm rearing of both parents as well as F_1 progeny was conducted in the laboratory following the method described by Krishnaswami [12]. In order to select required doses of the chemical various concentrations of EMS like 2.5, 5, 10, 20, 40, 80, 120, 160, 320 and 640 mM were employed. Three different concentrations of EMS like 2.5, 5 and 10 mM were selected after preliminary studies at chromosome level, viability of larvae, hatching percentage and viability of F_1 progeny [11].

The Pure Mysore and NB_4D_2 larvae at the age of fifth instar first day, weighing about 0.577 gm and 1.050 gm respectively were selected. Forty µl of final concentration of EMS freshly prepared in 0.75 % NaCl solution was administered separately to each worm in to the gut by 'oral injection'[9, 10 13]. The control worms received the same amount of NaCl solution only. For each concentration, 60 worms in triplicate were taken. After treatment, the larvae were allowed to continue development, spinning and pupation. Of the cocoons harvested, only uniform and healthy cocoons were selected and processed for the preparation of disease free layings [14, 15, 16]. The layings of NB_4D_2 race were treated with hydrochloric acid to get immediate hatching [14,17]. The rearing of F_1 progeny was conducted as described above. Such F_1 silkworm larvae from first day of fifth instar were collected daily with a regular interval of 24h. till the end of fifth instar. The abdominal legs were punctured and haemolymph was collected in a pre cooled micro centrifuge tubes containing 1 mM thiourea as described by Mahesha *et al.* [10], centrifuged at 3000 rpm for 5 minutes in a cooling centrifuge at 5°C and preserved in a deep freezer at -20°C as stock and it was used whenever required. Protein free filtrate was prepared as described by Oser [18]. Blood glucose level was estimated by following the method of Folin-Wu as described by Oser [18]. The blood glucose level was expressed as mg glucose per ml of haemolymph.

The experimental data obtained were subjected to one way ANOVA [19] and Scheffe's post hoc test [20].

RESULTS AND DISCUSSION

The change in the haemolymph glucose levels is shown in Table 1 and 2 for Pure Mysore and NB_4D_2 strains respectively. The levels of blood glucose in the control of Pure Mysore larvae showed a significant drop from 3rd day till the end of fifth instar except 6th and 7th day; the glucose concentration was increased by about 19.35 and 31.98 % on 6th and 7th day respectively as compared to their respective previous days. In case of EMS treated batches, 2.5mM set showed almost the same pattern of glucose level with significant reduction throughout the fifth instar except 6th and 8th days. As compared to previous days, the blood glucose level was found to be increased by 19.9 and 7.5% on 6th and 8th day respectively during the fifth instar in the larvae treated with 2.5mM of EMS. In 5 and 10mM sets, higher concentration of glucose was observed during the 1st and 2nd day. But, on the 3rd day, significant reduction by about 67.2 and 59.25% over the 2nd day was observed in 5 and 10mM sets respectively. Again, from 4th day, it gradually increased till the end of 5th instar except 6th day. The 10mM set showed almost the same pattern of blood glucose level as in the case of 5mM set, but difference in the quantity of glucose was noticed. The pattern of glucose level in the control was same as in 2.5 mM set. But, 5 and 10mM sets showed altered pattern from that of control set. The quantity of glucose level was high in the control (average glucose level during 5th instar was 3.041 mg/ml) followed by 5mM (2.84 mg/ml), 10mM (2.38 mg/ml) and 2.5 mM (2.32 mg/ml) sets. A well defined change in the quantity of glucose could be observed between the control and treated batches as well as among treated batches also. The results of two way ANOVA revealed that the variation between experimental batches, between age groups and the interaction effect of experimental sets and different age groups are all significant at 0.000 level. Further, the results of post hoc test revealed that the control batch is significantly ($P<0.05$) different from 2.5 and 10mM sets.

The blood glucose level in the control as well as EMS treated batches of NB_4D_2 is significantly different from that of Pure Mysore larvae. In the control of NB_4D_2, it increased gradually from 1st to 6th day. In case of treated batches, 2.5mM set showed almost the same pattern except 3rd day when 24.54% reduction in glucose concentration was noticed, as compared to 2nd day. The set treated with 5mM of EMS also showed 12.19% reduction on 3rd day as compared to 2nd day. The set of 10mM followed the same pattern as in case of control. But, quantitative change in the glucose level was observed. The difference was only in the quantity of glucose between the control and treated batches as well as among treated batches. The quantity of glucose level during fifth instar was high in the control worms (2.625 mg/ml was the average during 5th instar) followed by 2.5mM (2.27 mg/ml), 5mM (2.06 mg/ml) and 10mM (1.68 mg/ml). The results of two way ANOVA revealed that the variation between tested sets, between age groups and the interaction effect between tested sets and age groups are all significant at 0.000 level. Also, results of post hoc test revealed that the control worms are significantly ($P<0.05$) different from that of 10mM set only.

In EMS treated batches the average blood glucose level was lesser than the control worms of both Pure Mysore and NB_4D_2 breeds. Higher blood glucose level was observed only in control sets and lesser blood glucose level was observed in all the EMS treated batches. This difference in blood glucose level might be due to their quicker utilization of blood glucose for the synthesis of fat body and glycogen, or to lesser release of glucose in to blood stream. Former assumption is found to be more probable because the feeding behavior of the treated worms was good in F_1 progeny; such assumption is supported by the fact that the activity levels of amylase enzyme in the midgut was also high in the treated worms as compared to the control worms of both the breeds (Mahesha and Honnaiah 2000). On some days like 5th and 8th days of Pure Mysore silkworms (Table 1) the blood glucose level was higher than in the control larvae. In such cases, it may be presumes that the input on glucose in to the haemolymph more than the consumption of glucose for the synthesis of fat body, glycogen and other metabolism.

Mahesha and Thejaswini [11] reported that the lower doses like 2.5 and 5 mM sets exhibited more productivity over control in almost all characters in all the four generations, whereas higher concentration like 10 mM showed inferiority over the control as well as the batches treated with 2.5 and 5 mM EMS. These observations are also supported by the results of biochemical studies [1, 11, 13]. In addition, it is proved that lower doses of EMS might stimulate silkworm vitality and improves the commercial characters, which has been confirmed by the biochemical studies also [1,13,11]. The present investigations also clearly indicated that the lower doses of EMS might bring about some changes in either the genotype of the silkworm itself or some influencing factor that may be generated due to EMS treatment; perhaps, such factor might be responsible for this alteration. As the EMS is known to be a potent mutagen [21], it might have induced mutation resulting in the changed character, which is passed on from generation to generation in *Bombyx mori*. The enhanced rate of metabolism in the treated sets clearly indicated enhanced digestion, absorption, conversion and productivity. This basic knowledge can be exploited in the sericulture industry during breeding of new strains, which goes a long way to improve the sericulture industry.

Table 1: Amount of glucose (mg/ml) in haemolymph of F_1 progeny raised from EMS treated Pure Mysore silkworm during fifth instar

Conc. of EMS (mM)	Fifth Instar Development (Days)								Average Conc.
	1	2	3	4	5	6	7	8	
0.0	4.48	4.80 (+7.14)	3.62 (-26.33)	2.42 (-33.15)	1.86 (-57.00)	2.22 (+19.35)	2.93 (+31.28)	2.00 (-31.74)	3.010
2.5	2.48 (-44.64)	2.6 (+4.83) (-45.83)	2.42 (-6.92) (-33.15)	2.16 (-10.74) (-10.74)	2.16 (0.00) (+16.75)	2.59 (+19.90) (+16.16)	2.00 (-22.78) (-31.71)	2.15 (+7.5) (+7.5)	2.320
5.0	4.64 (-3.57)	5.0 (+7.76) (+4.16)	1.64 (-67.2) (-54.69)	1.90 (+15.86) (-21.48)	2.29 (+20.53) (+23.78)	2.14 (-6.55) (-3.6)	2.42 (+13.08) (-17.40)	2.72 (12.39) (+36.0)	2.840
10.0	4.03 (-10.04)	3.24 (-19.60) (-32.5)	1.32 (-59.25) (-63.53)	1.70 (+28.78) (-29.78)	2.04 (+20.00) (+10.81)	1.98 (-2.94) (-10.81)	1.91 (-3.53) (-34.81)	2.79 (-46.07) (+39.50)	2.376

Values within parenthesis (1st row) represents percent change over previous day.
Values within parenthesis (2nd row) represents percent change over control.
The variation between experimental sets, between age groups and the interaction effect between experimental sets and age groups are all found to be statistically significant at 0.000 level.

Table 2: Amount of glucose (mg/ml) in haemolymph of F_1 progeny raised from EMS treated NB_4D_2 silkworm during fifth instar

Conc. of EMS (mM)	Fifth Instar Development (Days)						Average Conc.
	1	2	3	4	5	6	
0.0	0.89	0.93 (+4.82)	2.03 (+117.11)	2.19 (+7.88)	2.90 (+32.42)	6.81 (+134.82)	2.625
2.5	2.59 (+190.36)	2.73 (+5.40) (+191.98)	2.06 (-24.54) (+1.48)	2.12 (+2.91) (+3.20)	2.06 (+2.83) (-28.96)	2.05 (-0.48) (-69.89)	2.269
5.0	1.88 (+110.76)	2.05 (+9.04) (+119.25)	1.80 (-12.19) (-11.23)	1.92 (+6.66) (-12.33)	2.22 (+15.62) (-23.45)	2.52 (+13.51) (-62.99)	2.065
10.0	0.790 (-11.43)	0.97 (+22.78) (+3.74)	1.53 (+53.73) (-24.63)	1.63 (+6.53) (-25.57)	1.76 (+7.97) (-39.31)	3.4 (+93.18) (-50.07)	1.680

Values within parenthesis (1st row) represents percent change over previous day.
Values within parenthesis (2nd row) represents percent change over control.
The variation between experimental sets, between age groups and the interaction effect between experimental sets and age groups are all found to be statistically significant at 0.000 level

ACKNOWLEDGMENT

Authors wish to thank the University of Mysore for extending the facilities to carry out this work.

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