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Studies on biochemical components of the larval haemolymph, fat body and silkgland of tropical tasar silkworm, *Anthereae mylitta Drury (Daba T.V)* under cold stress condition

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ABSTRACT

Anthereae mylitta Drury(Daba T.V.) produces tasar silk and is an endemic species of the Indian subcontinent exposed to low temperatures during winter season. The present study has been carried out on cold-stressed 5th instar Daba T.V larvae to analyze the mortality rate, variations in the biomolecules like protein, carbohydrate and enzymes like Glutamic pyruvic transaminase (GPT) and Glutamic Oxaloacetic Transaminase (GOT) in haemolymph, fat body and silk gland under 3,5 and 7 days of cold stress.The results revealed that exposure to low temperatures ($10^oC \pm 1^oC$) for seven days leads to 100% mortality, five days exposure caused 54% mortality and two days exposure resulted in 22% mortality after returning the larvae to normal temperature. The exposure of Daba T.V larvae to low temperatures for different durations resulted in increased protein content (21-67%) in the haemolymph and decreased in fat body (8-27%),silk gland(10-27%) in comparison with control group reared at $28^oC \pm 2^oC$.The cold stressed larvae has shown a reduction in the carbohydrate content(11-35%) in haemolymph and fat body(5-13%) whereas increased in the silk gland(13-43%).The activities of GPT(19-48%) and GOT(17-57%) decreased in haemolymph but GPT increased in fat body(9-20%) and silk gland(59-196%).Whereas GOT activity decreased in the fat body (4-11%) and increased in silk gland(29-122%).

Keywords: Haemolymph ,Fatbody, Silkgland, proteins, carbohydrates, Glutamicpyruvictransaminases, Glutamic Oxaloacetic Transaminase, cold stress, *Daba T.V.*

INTRODUCTION

It is well known that temperature plays a major role in the physiological behavior of the insects. Being a poikilothermic organism temperature decides the fate of the development in *Anthereae mylitta drury*.The insects will get acclimatized to the low temperatures by the production of various cryoprotectants like glycerol, trehalose, sorbitol etc. with a drastic change in the components like protein,carbohydrates, pyruvate, total free amino acids, total lipids, phospholipids and triglycerols [12].During rapid cold hardening and high temperature exposure heat shock proteins were synthesized which protect against thermal injury [2,4].In insects,the growth and development is associated with protein metabolism [16].Studies on cold acclimation of insects have shown that carbohydrates undergo profound metabolic changes and sugars of low molecular mass get accumulated[7].In silkworm,protein metabolism in the tissues like fat body, silk gland and haemolymph is regulated by the enzymes like glutamic

pyruvic transaminase,glutamic oxaloacetic transaminase whose activity will vary under cold stress conditions[1].The exposure of the insect larvae to low temperature is expected to effect the survival and also lead some changes in its biochemical constituents. Hence, the present experiment was conducted to estimate the mortality rate and also the levels of proteins,carbohydrate and enzymes like Glutamic pyruvic transaminase (GPT) and Glutamic Oxaloacetic Transaminase (GOT) in haemolymph, fat body and silk gland of fifth instar larvae of *Antherea mylitta drury(Daba T.V.)* during cold stress conditions.

MATERIALS AND METHODS

Newly hatched larvae of *Daba T.V.* (250) were reared on tender fresh leaves of *Terminalia arjuna* in the laboratory,Department of Biotechnology,Gokaraju Rangaraju Institute Of Engineering and Technology, Hyderabad, Andhra Pradesh, at $28^oC\pm 2^oC$ and humidity 70% - 75% until fifth instar. Removal of faecal matter, diseased worms and bed cleaning was done at regular intervals.To study the effect of temperature alterations on the biochemical components, fifth instar larvae were divided into four batches each containing 50 larvae.Batch at $28^oC\pm 2^oC$ –T1 (Control),Batch at $10^oC \pm 1^oC$ for 3 days – T2,Batch at $10^oC \pm 1^oC$ for 5 days – T3 and Batch at $10^oC \pm 1^oC$ for 7days – T4.The larval mortality rate was recorded during different durations of cold stress and also after returning back to normal temperature. After cold stress, larvae were selected from all the four barches separately and the haemolymph was collected in the test tubes and stored in the deep freezer. The fat body and silk gland were isolated in cold condition by using Bodenstein's Ringer solution.20% (W/V) homogenates of fat body and silkgland were prepared in 50mM Tris -HCl Buffer(pH- 7.0) and was centrifuged at 10,000 rpm for 20 minutes. Supernatant was collected for quantitative estimation of biochemical components.Total protein was estimated according to the standard procedure [9] and carbohydrate was estimated according [17].The enzymes Glutamic pyruvic transaminase (GPT) and Glutamic Oxaloacetic Transaminase (GOT) were estimated by the method[14].

STATISTICAL ANALYSIS

Each assay was replicated 3 times. Values were expressed as mean ± SE and Student's t-test was applied to locate significant ($P \leq 0.05$) differences between treated and control groups.

RESULTS AND DISCUSSION

Table 1 explains the mortality of *Daba T.V* larvae which were incubated at low temperature (10°C± 1°C) for varying treatment durations. After returning to control temperature, *Daba T.V.* larvae for 7 days cold stress resulted in high rate of mortality with 40% on 8th day and total mortality at the end of the 10th day. *Philosamia ricini* larvae kept under cold stress conditions for 7 days have shown 100% mortality after returning to room temperature [1].Prolonged exposure to low temperature, the organisms finally die from the exhaustion of energy reserves [15]. When returned to the control temperature after 5 days exposure to 10°C± 1°C, 54% of larvae died on seventh, eigth,ninth and tenth days. Low temperature exposure of *Daba T.V* larvae for 2 days has shown 22 % mortality in fourth, fifth and sixth days after returning the larvae to normal temperature.

The results shown in Table 2 indicate that cold stress profoundly affects the levels of proteins and carbohydrates in haemolymph of the insect ($p \leq 0.05$).When compared with the control, the levels of total proteins were found to be increased (21-67%) in the haemolymph of cold-stressed larvae and recorded very high under 7 days of cold exposure.Low temperature exposure stimulates accumulation of low molecular weight cryoprotectants and synthesis of antifreeze proteins[3,6].Reduced intake of amount of food at low temperature is one of the factor for increase in protein content[1].Proteins are not a source of energy in colder environment but involve in lowering the super cooling and freezing points and protects the larvae from injury, this supports the present results [11,19]. When compared with the control the results show that there was a significant decrease (11-35%) in the carbohydrate content present in the haemolymph and recorded very low in the larvae under 7 days of cold stress.Carbohydrate is the energy requirements in case of organisms under low temperature acclimatization [8,10].The data suggest that the impact of the cold stress on the total proteins and carbohydrates of the insect larvae was dependent on the duration of treatment.

The biochemical analysis carried out on fat body and silk gland of cold-stressed 5th instar larvae showed a remarkable decrease ($p \leq 0.05$) in the level of proteins (8-27%) after the treatment duration (Table 3,4).A drastic reduction in the protein content was recorded in the silk gland of the larvae exposed to low temperature for 7 days.Thus in comparison with the fat body and silk gland the protein content of haemolymph was found to be in the

reverse order.Low transaminase activity or high proteolytic activity results in high amino acid content and low protein[18].*Philosamia ricini* larvae under cold stress conditions have shown the decrease in protein content in the fat body and silk gland[1].Studies on *Chironomus riparius* exposed to anoxia had shown a decrease in total protein content due to degradation into aminoacids as they contribute to energy in insect[5].When compared with the control the results show that there was a significant decrease in the carbohydrate content (5.2-13%) in the fat body (Table 3) whereas in case of silkgland it was recorded in the reverse order (13-43%)with a significant increase in the silk gland of larvae under 7 days cold stress(Table 4).

Table 5 shows a significant decrease ($p \leq 0.05$) in the enzyme activities under cold stress condition. When compared with the control the activities of the enzymes were decreased in the range of GPT (19-48%) and GOT (17-57%) in the haemolymph of fifth instar larvae.The percentage decrease in enzyme activity was very high in 7 days cold stressed larvae.The transaminases like GOT and GPT which have an important role in protein synthesis were seriously reduced in the *philosamia ricini* larvae under cold stress[13].The activities of GPT and GOT present in haemolymph and fatbody will be depressed in low temperatures[5].

The results presented in Table 6 and 7 show an increase in the activities of GPT (9-20%) in the fat body and a drastic increase in the silk gland (59-196%) over the control. An insignificant decrease in the activity of GOT was recorded (4-11%) in the fat body whereas a significant elevation (29-122%) in the activity in the silk gland (Table 6,7). Thus the results denotes that cold stress treatment had increased the activation of GPT and GOT with the increased duration of exposure.The increase in activity of GPT and GOT in turn increases the supply of precursors to Krebscycle which finally results in energy production[1].

Thus in conclusion the mortality rate of *Daba T.V.* larvae increases as the duration of cold stress increases.The protein content found to be increased in the haemolymph whereas it was in the reverse order in fat body and silk gland. A siginificant decrease in carbohydrate was noted in haemolymph,fat body and increased in silk gland. The activity of the enzymes like GPT and GOT decreased in the haemolymph and increased in fat body and silk gland.

TABLE 1.Mortality (in number) of *A.mylitta.D*(*Daba T.V)* larvae exposed to low temperature for different durations after returning back to control temperature ($28^{o}C \pm 2^{o}C$)

Day of rearing at Control Temp after cold exposure	No. of larvae died on 3 days exposure (T2) (50 larvae)	No. of larvae died on 5 days exposure (T3) (50 larvae)	No. of larvae died on 7 days exposure (T4) (50 larvae)
First	-	-	20
Second	5	10	18
Third	3	8	12
Fourth	3	5	-
Fifth	-	4	-
Sixth	-	-	-
Seventh	-	-	-
Eigth	-	-	-

TABLE 2. Impact of cold stress on biomolecules present in the haemolymph of fifth instar larvae of *A. mylitta .D(Daba T.V.)*

Component	Control (mg/ml)	3 DaysExposure (mg/ml)	5 Days Exposure (mg/ml)	7 Days Exposure (mg/ml)
Protein	17.8±0.32	21.5±0.28 (+20.78)	25.2±0.22 (+41.57)	29.8±0.53* (+67.41)
Carbohydrate	14.3±0.27	12.7±0.23* (11.19)	10.5±0.17 (26.58)	9.3±0.21 (34.97)

The values in parentheses indicate percent increase (+) or (-) decrease over control.
** indicate significantly difference at $P \leq 0.05$(student's t -test)*

TABLE 3. Impact of cold stress on biomolecules present in the fat body of fifth instar larvae of *A.mylitta .D(Daba T.V.)*

Component	Control (mg/g wet wt.tissue)	3 Days Exposure (mg/g wet wt.tissue	5 Days Exposure (mg/g wet wt.tissue	7 Days Exposure (mg/g wet wt.tissue
Protein	95.3±0.48	87.5±0.32 (8.19)	79.6±0.27* (16.48)	69.7±0.21 (26.87)
Carbohydrate	55.2±0.31	52.3±0.2 (5.26)	50.7±0.18* (8.16)	48.2±0.13 (12.69)

The values in parentheses indicate percent increase (+) or (-) decrease over control.
** indicate significantly difference at $P \leq 0.05$(student's t -test)*

TABLE 4. Impact of cold stress on biomolecules present in the silk gland of fifth instar larvae of *A.mylitta .D(Daba T.V.)*

Component	Control (mg/g wet wt.tissue	3 Days Exposure (mg/g wet wt.tissue	5 Days Exposure (mg/g wet wt.tissue	7 Days Exposure (mg/g wet wt.tissue
Protein	113.2±0.6	101.3±0.48 (10.52)	93.3±0.37* (17.58)	82.5±0.28 (27.13)
Carbohydrate	51.3±0.27	57.8±0.42 (+12.67)	63.7±0.41 (+24.17)	73.5±0.31* (+43.27)

The values in parentheses indicate percent increase (+) or (-) decrease over control.
** indicate significantly difference at $P \leq 0.05$(student's t -test)*

TABLE 5. Impact of cold stress on enzymes present in the haemolymph of fifth instar larvae of *A.mylitta.D(Daba T.V.)*

Enzymes	Control (units/mg protein)	3 Days Exposure (units/mg protein)	5 Days Exposure (units/mg protein)	7 Days Exposure (units/mg protein)
GPT	26.4±1.71	21.3±0.97 (19.32)	18.6±1.14* (29.55)	13.7±1.25 (48.1)
GOT	98.7±1.85	81.8±0.93 (17.13)	53.5±1.87 (45.8)	41.6±1.38* (57.86)

The values in parentheses indicate percent increase (+) or (-) decrease over control.
** indicate significantly difference at $P \leq 0.05$(student's t -test)*

TABLE 6. Impact of cold stress on enzymes present in the fat body of fifth instar larvae of *A.mylitta .D(Daba T.V.)*

Enzymes	Control (units/mg protein)	3 Days Exposure (units/mg protein)	5 Days Exposure (units/mg protein)	7 Days Exposure (units/mg protein)
GPT	18.6±1.21	20.3±1.31 (+9.13)	21.2±1.38* (+13.97)	22.3±1.41 (+19.89)
GOT	48.3±1.86	46.3±1.55 (4.15)	43.2±1.48* (10.56)	42.8±1.31 (11.39)

The values in parentheses indicate percent increase (+) or (-) decrease over control.
** indicate significantly difference at $P \leq 0.05$(student's t -test)*

TABLE 7.Impact of cold stress on enzymes present in the silk gland of fifth instar larvae of *A.mylitta .D(Daba T.V.)*

Enzymes	Control (units/mg protein)	3 Days Exposure (units/mg protein)	5 Days Exposure (units/mg protein)	7 Days Exposure (units/mg protein)
GPT	34.3±2.12	54.7±1.86 (+59.47)	69.3±1.17* (+102.04)	101.5±1.3 (+195.91)
GOT	30.5±1.37	39.3±1.25 (+28.85)	52.7±1.42 (+72.78)	67.8±1.36* (+122.29)

*The values in parentheses indicate percent increase (+) or (-) decrease over control.*indicate significantly difference at $P \leq 0.05$(student's t -test)*

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