Effect of digoxin treated mulberry leaves on Protein profiles in fifth instar larvae of Silkworm, Bombyx mori (L) (PM x CSR2)

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Abstract

The tablets of digoxin (Lanoxin) were to dissolved in water to prepare ten part per million (ten mg per litre) solution. The mulberry leaves were soaked in the stock solution of digoxin for half an hour. The Digoxin treated leaves were drained and fed to the fifth instar larvae of multivoltine cross breed (PM x CSR2) for the second, third, fourth and fifth day (from 48 to 120 hours after the forth moult). The Larvae fed with untreated and water treated mulberry leaves were also maintained. Biochemical estimation of proteins (Soluble and total) was carried out at 120 hours after the fourth moult.

Feeding the larvae with digoxin treated mulberry leaves were found variously reflected into improvement in protein profile of whole body, haemolymph and silk glands. Pattern of increase in soluble proteins and total proteins in whole body and haemolymph was found similar (36.584; 47.87; and 87.963, 91.428 percent respectively). Increased level of tissue proteins of silk glands was found 54.776 (soluble) and 43.373 (Total) percent. Improvement of protein profiles in the larvae fed with digoxin treated mulberry leaves may be explained away as due accelerated rate of digestion, absorption in the alimentary canal. Digoxin titre in the larval body may influence the development of tissue especially the silk glands that causes to accelerate the protein accumulation. Digoxins, the glycoside, exert acceleratory influence, especially on the midgut- glucosidase activity. Digitoxose, the glycone moiety of digoxin deserve cardiotonic activity. It may be improving cardiac physiology in silk worm. Feasible method for using digoxin for rearing larvae of silk worm should be established.

Introduction

Life cycle of insects like silkworm have significant growth and metamorphosis in insects governed by various enzymes, hormones, and important biochemical moieties like proteins, fats, carbohydrates and nucleic acids[1]. Interplay of moulting hormone (MH) and juvenile hormone (JH) in the larval stage of insect serves to orchestrate the progression from one developmental stage to the next, with moulting hormone regulating the onset and timing of moulting cycle and JH regulating the quality of moult[2,3,4]. Plant material could have been the factor of growth and metamorphosis in phytophgus insects[5]. The compounds (plant derived, animal derived and synthetic) exhibiting the action of natural juvenile hormone (JH) are said to be juvenoids or juvenile harmone analogues (JHA)[6]. Natural products with juvenile hormone activity that have been isolated from

animals and plants (and micro-organisms too) represent a rather small but important fraction when compared to the synthetic juvenoids. The biomolecule with previlage of being called the 'first-Juvenoid' ever known was farnesol. The juvenoid of purely plant origin (juvabione) isolated by Bowers and his coworkers was from the wood of the Canadian balsam fir (Abies balsamia Mill). Structurally related dehydrojuvabione was isolated by Cerny et al[7] from the same plant. Juvabiol and Juvabiol esters are the compounds related to Juvabione and belong to various coniferous trees[8]. Juvenogens are water soluable juvabione (Plant derived

juvenoids) significantly active in phytophagous insects like silkworm, Bombyx mori (L). Digoxin, the product of Digitalis lanata occupies position in the list of juvenogens. Feeding the fifth instared larvae of silkworm, Bombyx mori (L) (PM x CSR_2) with mulberry leaves soaked in various aqueous solutions of Digoxin (with various concentrations) was found reflected into significant improvement of economic parameters (characters of cocoon and silk filament) and increase in the midgut glucosidase activity. Hence study was carried out to analyse the reflection of digoxin treated mulberry leaves on the profiles of proteins in silkworm, Bombyx mori (L).

Material and Methods

The larvae of silkworm, Bombyx mori (L) belong to polyvoltine crossbreed (PM x CSR_2) race were reared in laboratory through standard methods prescribed by Krishnaswami et al[9]. Soon after the fourth moult the larvae were divided into three groups each consisting hundred individuals. The tablets of digoxin were dissolved in distilled water and solution of 10 ppm strength was prepared. 100grams of fresh mulberry leaves were soaked in 400 ml of digoxin stock solution for half an hour before feeding. The soaked leaves were then drained and fed to the fifth instar larvae at 48, 54, 60, 72, 78, 84, 90, 102, 108, 114 hours after the fourth moult (table – 1: feeding schedule). 400 gm of mulberry leaves were used to feed the group of 100 lravae for each time. The larvae fed with water treated mulberry leaves were also maintained. The untreated control group of larvae was supplied with untreated mulberry leaves. At 120 hours after the fourth moult, the biochemeical estimation was carried out.

Preparation of Assay Sample: The fifth instar larvae of 120 hour age were used for biochemical estimation of proteins. Ten larvae from each group were selected randomly. They were anesthetized with little quantity of chloroform soaked cotton pad. The larvae were dissected in 0.9 percent saline solution. Two salivary glands from each larva were separated, blotted and weighed on electronic balance. The salivary glands were homogenized in distilled water using mortar and pestle. The homogenate was centrifuged at 1000 rpm for 10 minutes. The supernatant was used as assay sample.

For whole body proteins, ten larvae from each group were selected randomly. They were anaesthetized, fragmented and homogenized in distilled water. The homogenate was centrifuged at 1000rpm for ten minutes and the supernatant was used as assay sample.

For haemolymph, 40 larvae from each group were selected randomly. They were weighed on electronic balance. The larvae were anaesthetized and their prothorasic prolegs were punctured with sterile scissors. The haemolymph was collected from each larva in the vial precoated with thiourea (thiourea prevents blackening/clotting of haemolymph). The vials with haemolymph were weighed on electronic balance. The haemolymph was subjected for centrifugation at 1000 rpm for 10 minutes and the supernatant was used as assay sample.

Estimation of Soluble Proteins: Bioassay of soluble proteins was carried out according to the method prescribed by Lowery et al[10]. The bioassay was carried out in triplicate set. 5.0 ml of Lowery's solution "C" was mixed in 1.00 ml of Assay sample. The content was mixed well and allowed for formation of copper protein complex. 0.5 ml of Folin's phenol was added in the content of copper-protein complex. The set was kept for half an hour (for colour complex formation). The solution was used for reading the optical density on spectrophotometer at 660 nm.

Estimation of Total Proteins: For the purpose of total proteins, another set of estimation was carried out. Addition of 5.00 ml Trichloroacetic acid (TCA) was made in each of 0.5 ml. assay sample. The content was well mixed and subjected for centrifugation at 3000 rpm for ten minutes. The supernatant was separated and mixed with 2 ml of 0.1 NaOH solution. The content was then processed through the method described for soluble proteins.

The quantity of proteins (soluble and total) of assay sample was calculated by referring the optical density readings obtained for respective assay sample, using the standard graph of Bovine

serum albumen and expressed in the unit of micrograms per mg. tissue. The experimentation was repeated for three times. The data was subjected for statistical analysis[11].

Results and Discussion

Feeding the fifth instar larvae of silkworm, Bombyx mori (L) (race : PM x CSR_2) with mulberry leaves soaked in the aqueous solution of digoxin was found variously reflected into increase in the soluble and total proteins of silk glands, haemolymph and whole body (table- 2). Soluble and total proteins of silk glands in the larvae of control group were found measured 95.187 (± 16.483) and 496.58 (± 47.788) units respectively. The Proteins were found increased by 43.373 percent (total protein) and 54.768 percent (soluable proteins) in the group of larvae fed with digoxin treated mulberry leaves. Both of them (total and soluble proteins were significant at $p < 0.01$ in comparison with control group. The haemolymph proteins were found elevated in the treated group. Soluble proteins of haemolymph were 87.9 percent increased over the control. Most significant quantitative improvement in the total proteins ($P < 0.001$) were observed in haemolymph of the larvae fed with digoxin soaked mulberry leaves. Whole body proteins content of the fifth instar larvae was found influnced by digoxin treated mulberry leaves. The percent increase in the soluble and total proteins of whole larval body of treated group was found measured 36.584 and 47.87 percent respectively. In comparison with protein content of silk gland and haemolymph; the total proteins of whole body seems to be least significant (($P<0.05$).

Profiles of proteins in the fifth instar larvae of silkworm, Bombyx mori (L) (PM x CSR_2) recipient of the digoxin soaked mulberry leaves recorded in the study, establish positive effect, which is in agreement with the result on jurenoid treatement in the same insect[12-20]. Improvement in the protein profile in the digoxin receipient silkworm larvae in the study may be explained away as due to accelerated rate of digestion (through the activation of enzymes like β – Glucosidase)[21], absorption and protein turn over[22]. According to Shigematsu[23] the juvenoid compound, in early age of larva help to accelerate the deposition of organic constituents like protein, lipid, Carbohydrates. According to Sen[24], the juvenoids enhance the synthesis of poly RNA for the major silk protein, the fibroin. Stimulation of specific transcription is one of the very fine responses of silk glands to exogenous juvenoid material in insects. It means that the exogenous juvenoid (digoxin) may be caused the silk glands reversion from accumulation phase to the preparatory phase. Treating mulberry with digoxin and feeding the larvae of silkworm possibly, gear the overall protein constituency during the larval development. Digoxin may affect on appetite, nutrition and protein metabolisim. For the fortification of results, especially on the juvenogen nature of digoxin and it's influence on the improvement of biochemical moieties (like the proteins) in larval body of silkworm, Bombyx mori (L), further work (on the body wall chitin, the enzymes like chitinase, dehydrogenases, phosphatases and esterase) should be carried. This will establish the confirmed juvenoid label to digoxin.

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References

1. Agarell I.P.S. and Lundguist A.M., Physiology and biochemical changes during insect development in: The physiology of insects, Academic press New York, (2) **1, (1971)**

2. Riddiford L.M., Hormone action at the cellular level. In: Comprehensive Insect Physiology Biochemistry and Pharmacology Eds. G. A. Kerkut and L.I. Gilbert, Pergamon Press Oxford, **8,** 37-84 **(1985)**

3. Riddiford L.M., Cellular and molecular actions of juvenile hormone: General consideration and premetamorphic actions, *J. Adv. Insect physiology*, **24**, 213 – 214 **(1994)**

4. Sehnal F. and Rambold, Brain stimulation of juvenile hormone production in insect larvae, *Experientia*, **41,** 684 – 685 **(1985)**

5. Bowers W.S., Fales V.M., Thomson M.J. and Uebel B., Juvenile and gonadotropic activity of 10 11 – epoxyfarnesoic acid methyl ester, *Life Science*, **4,** 2323 – 2331 **(1966)**

6. Slama K., Plant as a source material with insect hormone activity, *Ent. Exp. Appl.*, **12,** 721-728 **(1969)**

7. Karlson P. and Schmialek P., Nachweis der Exkretion von juvenilhormonen Z. Naturf. **14** b: 821 **(1959)**

8. Manville J. F., Gregus L., Slama K. and Von Rudoloff E., Occurrence of Juvabione type/ epijuvabione type insects juvenile hormone analogues in three Czechoslovakian firs., Coll. Czech. Chem. Commun., **42,** 3658- 3666 **(1977)**

9. Krishnaswami S., Narasimhana M. N., Suryanarayana S. K. and Kumararaj S., Sericulture Manual- II: Silkworm Rearing, FAO United Nations Rome, 131 **(1973)** and **(1978)**

10. Lowery O.H., Rosenbrough N.J., Far A.L. and Randall R.J., Protein measurement with folin phenol reagent., *J. Biol. Chem.,* **193**, 265-275 **(1951)**

11. Norman T.J. and Baily, Statistical methods in Biology **(1955)**

12. Benchamin K.V., Jolly M.S., Benjamin D.A.I. and Ratnasen, The commercial application of juvenile hormone analogue 'Manta 'in silkworm Bombyx mori (L) and the economics, In: National seminar on silk research and Development, Central Silk Board Banglore, Page–73 **(1983)**

13. Subba Rao G., Das S. K., Nandi S., Sompou R. and Sen S.K., *Sericologia,* **28** (a), 543-548 **(1988)**

14. Rajashekhargouda R., Studies on methods to increase the silk yield of Bombyx mori (L), Ph. D. Thesis, Tamil Nadu Agricultural University, **243**, **(1991)**

15. Mamatha D.N., Nagalakshamma K. and Rajeshwara Rao M., Impact of selected Juvenile Hormone mimics on the organic constituents of silkworm Bombyx mori (L), Proceedings of NSTS – 99, 185 – 186 **(1999)**

16. Vitthalrao B. Khyade, Patil S. B., Khyade S. V., and Bhawane G. P., Influence of acetone maceratives of Vitis vinifera on the larval parameters of silkworm Bombyx mori (L)., *Indian Journal of Comparative Animal Physiology*, **20**, 14-18 **(2002)**

17. Vitthalrao B. Khyade, Patil S.B., Khyade S.V. and Bhawane G.P., Influence of acetone maceratives Vitis vinifera on the economic parameters of silkworm Bombyx mori (L), *Indian Journal of Comparative Animal Physiology*, **21**, 28-32, **(2003)**

18. Vitthalrao B. Khyade, Influence of Juvenoids on silkworm Bombyx mori (L), Ph. D.Thesis, Shivaji University Kolhapur India **(2004)**

19. Vitthalrao B. Khyade and Ganga V. Mhamane,, Vividh Vanaspati Arkancha Tuti Reshim Kitak Sangopanasathi Upyojana, Krishi Vidnyan, **4**, 18-22 **(2005)**

20. Vitthalrao B. Khyade and Jiwan P. Sarwade, Protein profiles in the fifth instar larvae of silkworm Bombyx mori (L), *The Bioscan*, **4(1)**, 41-44 **(2009)**

21. Ghantaloo U.S., Influence of Digoxin on silkworm Bombyx mori (L), M.Phil Thesis, Alagappa University Karaikudi (Tamil Nadu) India **(2007)**

22. Jagtap S.G., Effect of plant juvenoids on consumption and utilization of mulburrey leaves by silkworm Bombyx mori (L) M.Phil Thesis, Algappa University Karaikudi, Tamil Nadu (India) **(2007)**

23. Shigematsu H., The nature of juvenoid on growth and Changes of protein amino acid and nucleic acid content in silkworm Bombyx mori (L) in the case of application of excessive dose, *J. seric. Japan,* **47,** 292-300 **(1978)**

24. Sen S. K., Sericologia, **28** (a), 543–548 **(1988)**

25. Doane W.W., Role of hormone in insect's developmental systems: Insects Ed. S.J., Counce Wadingaton, London/ New York: Academic Press, **1,** 291 – 497. **(1973)**

26. Cerny V., Doles L., Labler L., Sorm F. and Slama K., Dehydrojuvobione: a new compound with juvenile hormone activity from balsam fir, *Tetrahedron Lett*., **12,** 1053 – 1057 **(1967)**

27. Vinod D. Rangari, Glycosides. In: Pharmacognasy and Phytochemistry, First edition, Career Publication Nashik, 238-241 **(2009)**

28. Williams C.M., The Juvenile Hormone of Insects, *Nature,* **178**, 212- 213 **(1956)**

29. Williams C.M., The Juvenile Hormone- I Endocrine activity and corpora allata of the adult Cecropia silkworm, *Biol. Bull Woods Hole*, **116**, 323 – 338 **(1959)**

30. Zaoral M. and Slama K., Peptides with juvenile hormone activity, *Science*, **170**, 92-93 **(1970)**

Table – 1: Schedule of feeding the fifth instar larvae of silkworm, Bombyx mori (L) (PM x CSR2)

Group → / Hour after fourth moult ↓	Untreated control	Water treated control	Digoxin treated
48	+	⊕	√
54	+	⊕	√
60	+	⊕	√
66	+	⊕	√
72	+	⊕	√
78	+	⊕	√
84	+	⊕	√
90	+	⊕	√
96	+	⊕	√
102	+	⊕	√
108	+	⊕	√
114	+	⊕	√
120	-	-	-

Stock solution of digoxin = 10 mg / lit.
Soaking the mulberry leaves = 100 gm in 400 ml Digoxin solution for 30 min.
Feeding = 400 gm mulberry leaves for 100 larvae for each time,
+ = feeding the larvae with untreated mulberry leaves

⊕ = Feeding the larvae with water treated mulberry leaves,
√ = Feeding the larvae with digoxin treated mulberry leaves,
- = No feeding.

Table – 2: Protein profiles of fifth instar larvae of silkworm, Bombyx mori (L) (Race: PM x CSR2) fed with digoxin treated mulberry leaves.

Group → / Tissue ↓	Control		Experimental	
	Soluble proteins	**Total proteins**	**Soluble proteins**	**Total proteins**
Silk glands	95.187 (± 16.483)	496.58 (± 47.786)	* * 147.32 (± 19.037) 54.769	* * 711.96 (± 68.639) 43.373
Haemolymph	83.817 (± 13.576)	358.57 (± 17.252)	* * 157.54 (± 21.573) 87.963	* * * 686.4 (± 48.838) 91.428
Whole body	189.63 (± 46.695)	629.21 (± 72.834)	* * 259.01 (± 54.491) 36.584	* 930.41 (±147.78) 47.87

Unit for protein = micrograms per mg tissue,
Each figure is the mean of three replication,
The figure with ± sign in parenthesis is the standard deviation,
The figure below the standard deviation is the percent change (increase) over the Control.

* = $p < 0.05$ (Significancy), * * = $p < 0.01$ (Significancy), *** = $p < 0.001$ (Significancy)