



ORIGINAL ARTICLE

STUDIES ON HISTOLOGICAL CHANGES IN FATBODY AND SILK GLAND OF SILKWORM *BOMBYX MORI* L. (LEPIDOPTERA: BOMBYCIDAE) FED WITH V₁, MULBERRY LEAVES AND AG NANOPARTICLES TREATED LEAVES.

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ABSTRACT

The domesticated silkworm, *Bombyx mori* has been the target of intensive scientific study. Since the dawn of human civilization, the silkworm has been used as a source of silk for producing exquisite textiles and dress materials. Because of its industrial importance, the study of this insect has been encouraged by governments in several countries. Keeping in this view, the present study is aimed to find out the histological changes in the fat body, and silk gland of silkworm fed with treated Ag nanoparticle on mulberry V₁, leaves. The present results revealed that characteristic histological changes in the V instars of silkworm, fat body, and silk gland of silkworm fed with treated Ag nanoparticle than control silkworm. Silkworm fatbody consisted of nucleus, cytoplasmic vacuole and granular substances. In control, silk gland showed columnar epithelium with nucleus, vacuoles, and the lumen contained secretory substances. Whereas in Agnps treated V₁ mulberry leaves fed silkworm in the V instar fat body and silk gland showed spectacular changes such as swollen nucleus, with more vacuoles in the fat body, where as, the silk gland showed shrunken nucleus, vacuole in the epithelium, lumen contained less suggested that the mobilization of secretory substance from V instar posterior silk gland to middle silk gland which enhance to store more amount of secretory substances for spinning.

Key words: *Bombyxmori*, Agnanoparticles, Fatbody, Silk gland, V₁ mulberry leaves

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1.INTRODUCTION

Silkworm, a lepidopteron insect has a great economical interest due to its contribution for the synthesis of silk protein. Fat bodies serve as the precursor for metabolism compare to other tissues. The fat body consists of either loosely aggregated or compact masses of mesodermal cells enclosed in a membranous sheath. Mostly, the fat body tissues are found in the abdominal region. They occur in groups of lobes below the integument around the digestive tract and reproductive organs of the insect.

The silk gland is also a kind of the dermal glands. It derives from the invagination of the labial ectoderm. The process of histolysis in posterior silk gland cells of the silkworm during metamorphosis from larva to pupa have been studied by Matsumura *et al* (1968). The gland cells of Lyonet's gland which is accessory to the silk gland in the silkworm larva, is characterized by the presence of complicated canaliculi bearing microvilli on their inner surface, large numbers of mitochondria

and remarkably convoluted basal plasma membrane (Yoshio wakv and Ken-Ichi sumimoto, 1974). The silk gland in the V instar of silk producing lepidopteran larvae are known to pass through four consecutive phase i.e, growth, secretory, regression and degenerative phases and are revealed in *Bombyx mori* (Tashiro *et al.*,1976 and Sehnal *et al.*,1983 Sehnal and Akai,1983 Chenthilnayaki, 2004, Balasundaram, 2008 and Ganesh prabu, 2012).

Silver nano particles are silver precursors like silver citrate, silver acetate and silver nitrate. Silver particles size between 1- nm to 100- nm. While frequently described as being 'silver' some are composed of a large percentage of silver oxide due to their large ratio of surface to bulk silver atoms. Silver precursor were reduced by several reducing agents like chemical (Trisodium Citrate, Sodium Borohydrate etc.,). Plant leaf extracts, plant seed extracts, micro algae extracts micro organisms like bacteria and fungi. From the forgoing literature,

the works in relation to the histological changes of fat body and silk gland of V instars larvae of *Bombyx mori* when fed with V₁ mulberry leaves treated with silver nanoparticles are meagre. Therefore, it has been programmed in the present study to know the histological changes of fatbody and silk gland in *Bombyx mori*.

2.MATERIAL AND METHODS

Silkworm V instar of popular Indian bivoltine hybrid (CSR₂ x CSR₄) silkworm *Bombyx mori* (Local Bivoltine) race were collected from silkworm culture centre at 2nd Agraharam, Salem and Neyveli in Tamilnadu, and they were maintained up to cocoon.

The larvae transported from Salem and Neyveli were transferred to bamboo baskets of size 26 cm diameter and 5cm height as described by Govindan *et al.*,(1981). The bamboo baskets were covered with paraffin paper and placed in an iron stand with ant wells. The larvae were reared simultaneously both in control and experimental groups separately on mulberry leaves dipped in silver nanoparticles solution in the laboratory. The V instar larvae placed at ambient temperature of 25± 27^oc and relative humidity of 70 to 80%. The larvae were reared in card board boxes measuring 22x15x5 cms covered with nylon net and placed in an iron stand with ant wells. The control and silver nanoparticles treated V₁ mulberry (*Morus indica*) leaves were fed to silkworm, *Bombyx mori*.

Mulberry Plant *Morus indica* (V₁, Variety)

This is one of the varieties of mulberry plant. It was selected from faculty of Agriculture, Annamalai University, Annamalai nagar, Tamilnadu, India. This mulberry plant branches are simple, vertical, grayish leaves are light green, unlobed, elliptic. Palmately veined, leathery/smooth/wrinkled. It has good agronomic characters like high rooting ability.

Preparation of Tissue Samples for Histological Study

Control and silver nanoparticle treated V₁, mulberry leaves fed V instar *Bombyx mori* larvae fat body and silk gland (anterior, middle, and posterior) were dissected in insect Ringer's Solution (Ephurussi and Beadle, 1936). Dissected fat body and silk gland were fixed by immersion in Bouin's solution or 10% formalin in separated sterilized sample bottles or vials.

Preparation of Permanent Histological Slides

After 24h of fixation, the fat body and silk gland tissues were processed for dehydration using ascending grades of alcohol. The tissues were gross stained in 70% aqueous eosin to facilitate orientation during embedding. The tissues after dehydration in absolute alcohol and acetone were cleared in xylol and finally embedded in paraffin wax (58-62^oc). Sections were cut at 6μ thickness were deparaffinized using ascending grades of alcohol and stained with heamatoxylin and counter stained with aqueous eosin for microscopical observation and microphotographs were taken (Gurr, 1958).

3.RESULTS

The transverse section of the control fat body of this V instar appeared to be dirty white mass of tissue. The cytoplasm which stained less intensely stained with eosin exhibited granular organizations. The occurrence of large sized vacuoles was a characteristic feature of this stage. The nucleus seems to be an irregularly shaped. These changes indicate that the fat body cells were in a state of less synthetic and secretory activity during this period, suggesting the mobilization of more amounts of substances from this fat body which appeared to be sequestered probably into the silk gland for spinning purpose (Fig.1).

The silk gland is three region, anterior, middle, and posterior. The anterior silk gland of V instar larvae exhibited certain remarkable histological changes than the previous stage such as less packed epithelial layer with shrunken nuclei and less cytoplasmic inclusions and more vacuoles in the cytoplasm, indicating very less synthetic and secretory activity. The lumen contained more amounts of secretory substances, suggesting its less utilization of these substances for the act of spinning. The middle silk gland of V instar larvae showed certain histological architecture such as an occurrence of a thick disorganized, shrunken and thin epithelial layer with less cytoplasmic inclusions, indicating less synthetic and secretory activity by these cells. The lumen contained globular and gelatinous colloidal secretory substances. Comparatively, the secretory substances in the middle silk gland of V instar larvae were more than the IV instar larvae, in this stage, the lumen was completely filled with secretory substances, indicating both the secretory substances of MSG and PSG considered as storage organ. The posterior silk gland was composed of less packed epithelium with large and longitudinal nuclei. It has less packed chromatin materials with more vacuoles in the cytoplasm. The secretory droplets were well evident in the cell as well as in the lumen of PSG in the V instar larvae fed with V₁, mulberry leaf (Fig. 2,3,4).

The Ag nanoparticle treated silk worm exhibited remarkable histological changes in the fat body and silk gland. Such as the occurrence of vacuoles in the cytoplasm and less granular substances, indicating the mobilization of nutrient materials for the enhancement of synthetic and secretory activity of the silk gland. The shrunken nuclei in the fat body cells, indicating the less synthetic and secretory activity. The anterior silk gland of the V instar larvae of *Bombyx mori* exhibited certain histological architecture such as an occurrence of an outer thick epithelial layer which surrounds the lumen. The epithelial layer was appear to be thickened and the cells were very active due to the presence of swollen nuclei, indicating a higher synthetic and secretory activity. The lumen contained secretory substances which were seems to be homogeneous and globular in nature and more compare to control (Fig.5). The middle silk gland of the V instar larvae of *Bombyx mori* showed certain histological changes such as the presence of an outer thin and faint epithelium. The epithelial cells became degenerated and the lumen was completely filled with secretory substances, indicating the dual function of MSG, both secretory and storage of fibroin and sericin from PSG and MSG, respectively. The posterior silk gland of this worm showed certain remarkable

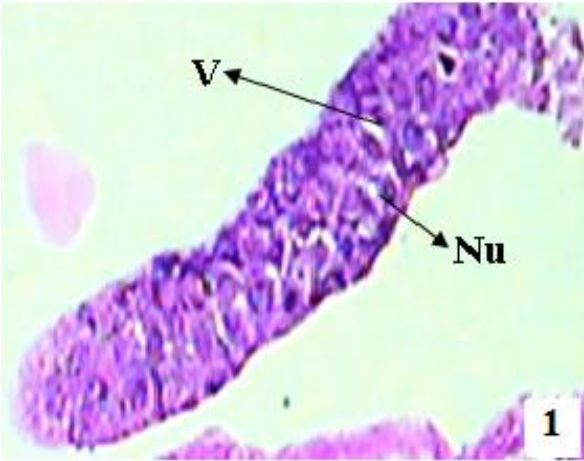


Figure 1: Histological section of V instar *Bombyx mori* larvae fat body (Control)(\times ca 100)
Nu-Nucleus
V-Vacuole

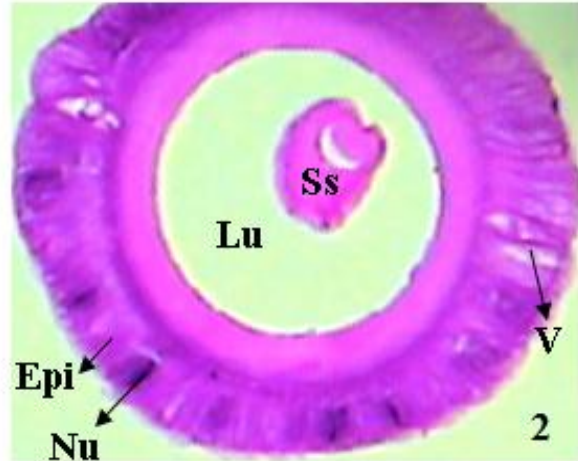


Figure 2: Histological section of V instar *Bombyx mori* larvae anterior silk gland (Control)(\times ca 100)
Lu-Lumen
Ss-Secretory substance
Nu-Nucleus
Epi-Epithelium
V-Vacuole

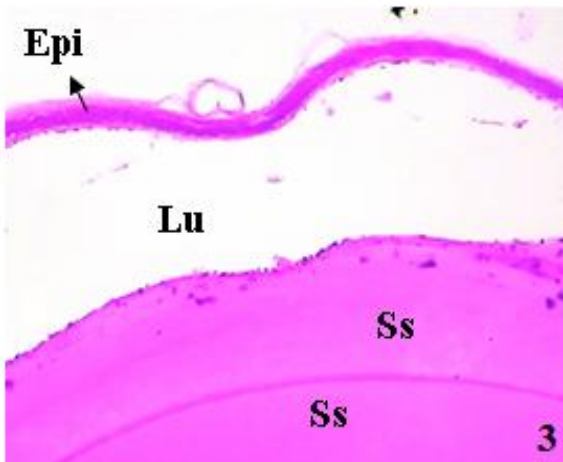


Figure 3: Histological sections of V instar *Bombyx mori* larvae middle silk gland (Control)(\times ca 100)
Lu-Lumen
Ss-Secretory substance
Epi-Epithelium

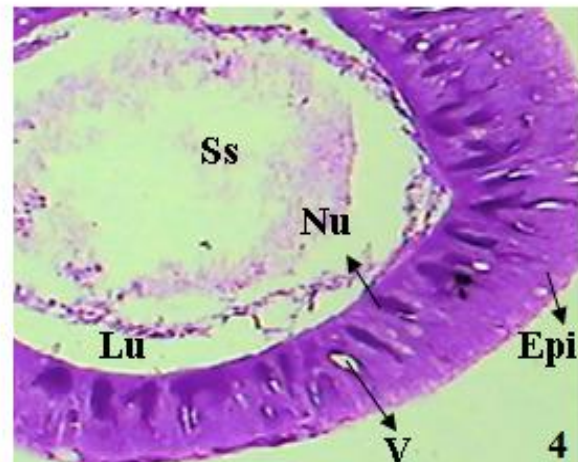


Figure 4: Histological sections of V instar *Bombyx mori* larvae posterior silk gland (Control)(\times ca 100)
Lu-Lumen
Ss-Secretory substance
Nu-Nucleus
Epi-Epithelium
V-Vacuole

changes in the structure of the gland. The outer-epithelium was thick, in each cell contained intact cytoplasm with shrunken nuclei, indicating the less synthetic and secretory activity. The lumen consisted of less amount of secretory substances than the lumen of the same age group of worms when fed with V₁ leaves, indicating that silver nanoparticle seems to stimulate the secretory and synthetic activity of the silk gland. The secretory substances of the lumen consisted of two types, one was homogenous and the other one was globular in appearance where probably the synthesis of fibroin protein from this PSG (Fig.6,7,8).

4.DISCUSSION

In the present observation, it has been shown for *Bombyxmori* that it's fat body undergoes marked histological changes during the period from V instar larvae before spinning. The granular materials identified in the cytoplasm of the cells of fat body with small and large sized cytoplasmic vacuoles seem to increase concomitantly from V instar and also the cytoplasm and nuclei stained very feebly subsequently from V instar larvae of *Bombyx mori* fed with V₁, and silver nanoparticles



Figure 5: Histological sections of V instar *Bombyx mori* larvae of fat body (Treated)(× ca 100)
Nu-Nucleus
Vu -Vacuole

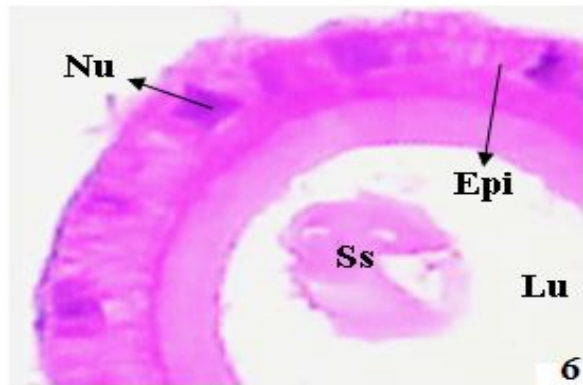


Figure 6: Histological sections of V instar *Bombyx mori* larvae of anterior silk gland (Treated)(× ca 100)
Lu-Lumen
Ss-Secretory substance
Nu-Nucleus
Epi-Epithelium

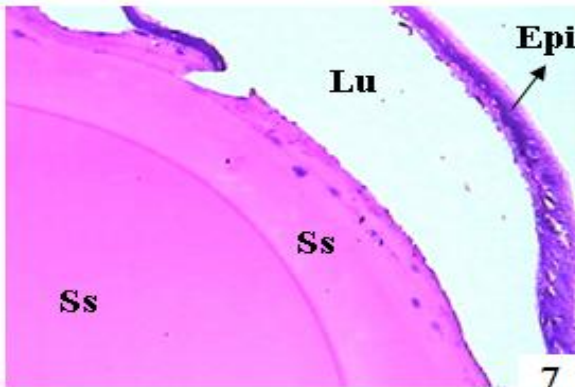


Figure 7: Histological sections of V instar *Bombyx mori* larvae of middle silk gland (Treated)(× ca 100)
Lu-Lumen
Ss-Secretory substance
Epi-Epithelium

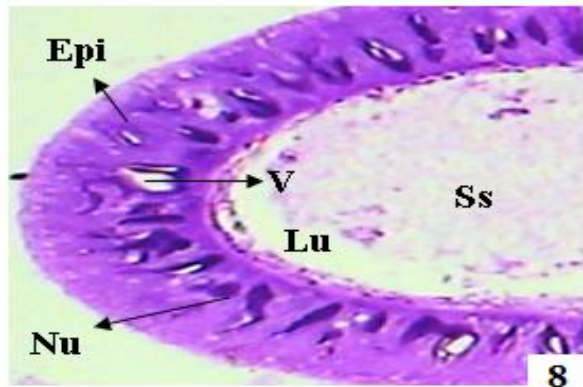


Figure 8: Histological sections of V instar *Bombyx mori* larvae of posterior silk gland (Treated)(× ca 100)
Lu-Lumen
Ss-Secretory substance
Nu-Nucleus
Epi-Epithelium
V-Vacuole

treated mulberry leaves. Further, the granular materials representing the nutrient substances seem to have reduced their concentration in the cytoplasm of fat body cells during the act of spinning. The volume of the nucleus is also found to have reduced significantly. It is evident from the present study that the synthetic and secretory activity of the fat body became concomitantly decreased in accordance with the utilization of these nutrient substances for the act of spinning by this silkworm, *B.mori* from V instar larvae. Further, the secretory activity by the silk glands have been found to have increased concomitantly from V instar larvae of *Bombyx mori* when fed with V₁ than Silver nanoparticles treated mulberry leaves in relation to the act of Spinning.

The structure and secretory activity of the silk gland in *Bombyx mori* fed with V₁ and silver nanoparticles treated mulberry leaves have been thoroughly investigated during the present study. Differentiation of silk glands into three region, anterior (ASG), middle (MSG) and posterior (PSG) as the sericin secretory and fibroin secretory regions, respectively has been noticed in *Bombyx mori* and other silkworm also (Sehnal and Akai, 1990 and Barsagade and Tembhare, 2000 and Centhilnayaki, 2004 and Centhilnayaki *et al.*, 2004). The maximum growth of silk gland occurs in the last larval instars similar to that reported in other silk worms. Further, it has been revealed in the present study that the lumen contains more amount of secretory substances in V instar of silkworm fed with silver nanoparticle treated mulberry leaves than the silkworm fed with V₁, leaf. These changes may be attributed due to an intense secretory activity in the epithelial cells of silk gland rather than V₁, fed silkworms. Similar changes have also been reported earlier by Akai, (1984), Barsagade and Tembhare, (2000), Centhilnayaki, (2004) Centhilnayaki *et al.* (2004). Balasundaram, (2008) and Ganeshprabhu, (2012). From the results, it may be inferred that the V₁, leaf treated with silver nanoparticles is comparatively superior than the V₁, leaf for sericulture industry to enhance the synthesis of more amount of secretory materials by the silk glands of *Bombyx mori* and also influences the larval and economic characters such as silk production and the nature of silk trait. This V₁, leaf treated with silver nanoparticles must be recommended to the farmers to get more performance both in economic characters, silk gland secretion and silk production than V₁, mulberry leaf. From the present study, it is inferred that V₁, mulberry leaf treated with silver nanoparticles is superior to control V₁, mulberry leaf.

The present work is a study on the use of silver nanoparticles for the determination of secretory activity of fat body and silk gland ability of the most commercially important silkworm. Therefore, more future comprehensive works are very much solicited in this line with highest concentrations on this species is required. Studies on histological changes in fat body and silk gland of silkworm *Bombyx mori* may be suggested that the V₁, leaf variety with silver nanoparticles is comparatively superior than the V₁, leaf variety alone for sericulture industry to enhance the synthesis of more amount of secretory materials by the silk glands of *Bombyx mori* and also influences the larval and economic characters such as superior cocoon and silk production. Further, from this present investigation, it is also suggested that an extensive study may be carried out in the

silkworm, *Bombyx mori*, which is a cost effective, economically feasible, ecologically viable and rich silk producing for the individual as well as to the society and also, the nation as a whole by adopting these techniques and produce good quality and higher production of silk.

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6.REFERENCES

- Akai, H. 1984. The ultrastructure and function of the silk gland cells of *Bombyxmori*. In : Insect ultrastructure. R.C.king and H. Akai (eds.) New york, Vol. 2:323-364.
- Barsagade, D.D. and Tembhare, D.B. 2000. Development, Structure and secretory activity of silk gland complex in the Tropical Tasar Silkworm, *Antheraea mylitta*(Drury)(Lepidoptera; Saturniidae). *Sericologia*, 40(2):231-245.
- Balasundaram D, Selvi S and Mathivanan, V. 2008. Studies on comparative feed efficacy of mulberry leaves MR₂ and MR₂ treated with vitamin c on *Bombyxmori* (L.) (Lepidoptera: Bombycidae) in relation to larval parameters. *J curr sci* 12(2),671-68
- Benchamin, K.V. and Jolly M.S. 1986. Principles of silkworm rearing. proceedings of seminar on problems and prospects of sericulture, S. Mahalingam (Ed). Vellore, India, PP.63-108.
- Chenthilnayaki, N., Selvisabhanayakam and Mathivanan, V. 2004. Development of issue on mulberry cultivation in relation to silk on V. mulberry cultivation in relation to silk production. *Indian. J. Environ. & Ecoplan.*, 8(1):111-117.
- Ephurussi, B. and Beadle, G.W. 1936. A technique of transplantation for *Drosophila*. *Am. Natu.*, 70:287-316.
- Etebari, K., Ebadi, R. and Matindoost, L. 2004. Effect of feeding mulberry's enriched leaves with ascorbic acid on some biological, biochemical and economical characteristics of silkworm *Bombyxmori* L. *Int.J. Indust. Entomol.*, 8:81-87.
- Fayaz, A.M.K., Balaji, M., Girilal, R., Yadav, P., Kalaichelvan, T. and Venketesan, R. 2010. Biogenic synthesis of Silver nanoparticles and their synergistic effect with antibiotics. a study against gram- positive and gram-negative bacteria. *Nanomed. Nanotechnol Biol. Med.*, 6:103-109.
- Govindan, R., Devaina, M.C., Tippetwamy, C. and Rangaswamy, H.R. 1981. Ovipositional behaviour of eri silk *samiacynth ricini* under Dharwad Conditions. *Pro. Seri. Sym. and seminar, TNAU.*, pp.35-38.
- Gurr, E. 1958. *Methods of Analytical. Histology and Histochemistry* Leonard Hill (Books Ltd.), London.

- Horie, Y. and Ito, T. 1965. Nutrition of the Silkworm, *Bombyx mori*-x. Vitamin B requirement and the effects of several analogues: *J. Insect Physiol.*, 11:1585-1593.
- Ito, T. 1980. Application of artificial diets in sericulture, silkworm physiology division, Serv, Expt. St., Japan, 14:163-168.
- Yoy, O. 1994. The spinning apparatus of the silkworm. The silk Press. *sericologia*, 34(1):81-85.
- Kalvathy, S. 1988. Studies on histological changes of ovary, fat body and yolk synthesis during ovarian development in *Gryllotalpa africana*-M.Phil.Thesis, Annamalai University.
- Kathiresan, K., Manivannan, S., Nabeel, M.A. and Dhivya, B. 2009. Studies on silvernanoparticles synthesized by a marine fungus, *Penicillium fellutanum* isolated from coastal mangrove sediment. *Colloids Surf. Biointerfaces.*, 71(1):133-137.
- Koul, A. 1986. Growth and silk production in silkworm (*Bombyx mori* L.) fed on four different varieties of mulberry. *Res.Dev. Report.*, 3(2):13-15.
- Krishnaswami, S., Ahsam, M. and Sriharam, T.P. 1970. Studies on the quality of mulberry leaves and Silkworm cocoon crop production II. Quality differences due to leaf maturity. *Indian J. Seric.*, 9(1):11-25.
- Naik, R.P. and Delvi M.R. 1987. Food utilization in different races of silkworm, *Bombyx mori* L. (Lepidoptera : Bombycidae). *Sericologia*, 7(3):391-397.
- Nirwani, R.B. and kaliwal, B.B. 1996. Effect of Folic acid on economic traits and the change of some metabolic substances of bivoltine silkworm, *Bombyx mori* L. *Korean J. Seric. Sci.*, 38: 118-123.
- Sathyavathi, R., Krishna, M.B., Rao, S.V., Saritha, R. and Rao, D.N. 2010. Biosynthesis of silver nanoparticle. using coriandrum sativum leaf extract and their application in nonlinear optics. *Adv. Sci lett.*, 3:1-6.
- Sehna, F. and Akai, H. 1983. Ultrastructure and function of silk glands in *Galleria mellonella*. In, *The Ultrastructure and functioning of insect cells.* (Akai, H., R.S. King and S. Morohoshieds.). SOC. Insect Cell. Japan, Tokyo, PP.135-138.
- Sehna, F. and Akai, H. 1990. Insect silk glands. their type's development and function and effects of environmental factors and morphogenetic hormones on them. *Int. J. Insect Morphol. Embryol.*, 19:79-132.
- Song, J.Y. and Kim, B.S. 2009. Rapid biological synthesis of silver nanoparticles using plant leaf extracts. *Bioprocess Biosyst. Eng.*, 32:79-84.
- Tashiro, Y., Shimaadzu, T. and Matsura, S. 1976. Lysosomes and related structures in the posterior silk gland cells of *Bombyx mori* L. In late larval Stadium. *cell struct. Fund*, 1:205-222.
- Thyagarajan, S.P., Subramanian, S. and Thirunalasundar, T. 1988. Effectes of *Phyllanthus amarus* on the chronic cauriers of hepatitis B. *Virus. Lancet*, 2:764-766.
- Wigglesworth, V.B. 1967. Cytological changes in the fat body of *Rhodinus prolixus* during starvation, feeding and oxygen want. *J. Cell. Sci.*, 2:248-256.