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RESEARCH ARTICLE

STUDIES ON THE HISTOLOGICAL CHANGES OF FAT BODY AND SILK GLAND OF BACTERIAL INFECTED SILKWORM *BOMBYX MORI* (L.) (LEPIDOPTERA: BOMBYCIDAE) FED WITH ANTIBIOTIC DRUG (DICLOXACILLIN) TREATED MULBERRY LEAVES

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ABSTRACT

Mulberry silkworm, *Bombyx mori* is the preferred species for the production of silk cocoons. The quality of this species is generally affected by bacterial, viral, fungal and protozoan pathogens. Bacterial pathogens alone are responsible for causing cocoon loss to the extent of almost 70%. Antibiotics are widely and effectively used to control these pathogens affecting particularly *Bombyx mori*. The present study is aimed to find out the histological changes in the fat body, and silk gland of bacterial infected V instar larvae of silkworm fed with antibiotic drug (Dicloxacillin) treated V1 mulberry leaves. Silkworm fat body consisted of nucleus, cytoplasmic vacuole and granular substances. In bacterial infected silkworm's silk gland showed necrosed columnor epithelium with disintegrated nucleus, vacuoles and the lumen contained low secretory substances, whereas in antibiotic drug (Dicloxacillin) treated V1 mulberry leaves fed V instar silkworm's fat body and silk gland showed spectacular changes such as swollen nucleus, with more vacuoles in the fat body, whereas, the silk gland showed shrunken nucleus, vacuole in the epithelium, lumen contained more secretory substances suggested that the mobilization of to secretory substance from posterior silk gland to middle silk gland which enhance and to store more amount of secretory substance for spinning.

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INTRODUCTION

The bacterial diseases of *B. mori* have been the subject of intensive research because of their commercial importance for silk producing countries (Kumar *et al.,* 1997). It is prone to several diseases caused by fungi, bacteria, protozoans and viruses, leading to cocoon crop loss and affecting the entire sericulture industry (Kumar *et al.,* 1999). One of the major constraints in silk production is the diseases in silkworm rearing. Silkworm *Bombyx mori* is domesticated for silk production and are reared in colonial forms. A code of conduct for rearing of silkworm is practiced to ensure survival of silkworm and cocooning. Bacterial flacherie disease caused by *Bacillus thuringiensis* was first isolated and named by Berliner, (1915), while in Japan Ishiwata, (1901) had previously isolated a bacterial strain which he called *"Bacillus sotto"*. However, this is not a serious pathogen. On the other hand, bacterial diseases caused by *Streptococcus, Serratia* and *Proteus* are serious, especially when an artificial diet is used (Kodama and Nakasuji, 1968; Iizuka, 1972). Silk is obtained by sericulture, a technique of farming of silkworms. Silk is a natural animal based textile fiber associated with luxury and class due to its sheer luster and elegance. Mulberry silkworm, *Bombyx mori* – L., is the preferred species for the production of silk cocoons. The quality of this species is generally affected by different viral, fungal and protozoan pathogens. Bacterial pathogens alone are responsible for causing cocoon loss to the extent of almost 70 %. Antibiotics are widely and effectively used to control these pathogens affecting particularly *Bombyx mori* (Manimegalai and Chandramohan 2005). Dicloxacillin is a narrow-spectrum β-lactam antibiotic of the penicillin class. It is used to treat infections caused by susceptible (non-resistant) Gram-positive bacteria. It is active against beta-lactamase-producing organisms such as *Staphylococcus aureus*, which would otherwise be resistant to most penicillins. Dicloxacillin is available under a variety of trade names including Diclocil (BMS) (Miranda-Novales *et al.,* 2006). Dicloxacillin is used to treat mild-to-moderate staphylococcal infections. To decrease the development of resistance, dicloxacillin is recommended to treat infections that are suspected or proven to be caused by beta-lactamase-

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producing bacteria. Dicloxacillin is similar in pharmacokinetics, antibacterial activity, and indications to flucloxacillin, and the two agents are considered interchangeable. It is believed to have lower incidence of severe hepatic adverse effects than flucloxacillin, but a higher incidence of renal adverse effects (Rossi, 2006). Dicloxacillin is used for the treatment of infections caused by susceptible bacteria (Rossi, 2006).

In many insect species, the oenocytes closely associated with the fat body. In the Colorado potato beetle, *Leptinotarsa decemlineata,* the fat body lobes are present in two separate areas. At the end of the trachea, the internal fat body is present. Some fat bodies are situated close to the body wall, the peripheral fat bodies (De Loof and Lagasse, 1970). Hill, (1965) has revealed that the incorporation of glycine in the protein of fat body of the desert locust during ovarian development. In the *Gastrophilus* larvae, the fat body contains some very large cells measuring 350 to 400µ in diameter. They are filled with haemoglobin which is obtained from the blood of their host, the horse. These cells are richly supplied with the tracheoles and thus, it may help in respiration. The arrangement of all these cells within a fat body lobe is illustrated in the cockroach, *Periplaneta americana* (Bodenstein, 1953). The fat body cells may have one or more nuclei with a large vacuole. The size of the nucleus has been reduced following prolonged starvation (Wigglesworth, 1967). According to Wyatt, (1980), haemolymph proteins are synthesized in insect fat body. In *Bombyx mori,* the blood proteins are synthesized by fat body (Shigematsu, 1960). The histological features of fat body have been investigated for few species of insects such as *Drosophila* (Gaudecker, 1963); *Diploptera punctata* (Barbara and clark, 1971); *Aedes aegypti* (Behan and Hegedorn, 1978) and *Gryllotalpa afncana* (Kalavathy, 1988) during larval period. The silk gland is divided into anterior, middle and posterior parts of these the middle part is the largest and is twisted in the shape of letter "S". The glands are well differentiated in the fourth and fifth instar larvae. They are so large in the fully grown final instar larva that they occupy almost the entire body cavity and account for 50 % of the total weight of the larva (Kurabar, 2000). They are important organs which produce liquid silk as the sources of cocoon fibre, which is used for spinning the cocoon. The cocoon protects the pupa. The pupa metamorphoses into adult within ten to fifteen days. These are situated on the ventrolateral sides of mid intestine. The posterior ends are blind. The paired ducts unite anteriorly and open into spinneret, the median projection in the labium. The wall of the silk gland is composed of three layers, the outer tunica propria of uniform thickness throughout the gland, the middle glandular layer and inner tunica intima enclosing the lumen of the gland. The tunica intima of anterior portions has thick chitinous substance, which is renewed at each ecdysis or moult, but the tunica intima of middle and posterior region is very thin and is not shed at the time of moulting (Kurabar, 2000).

Shimura *et al.* (1976) have reported that fibroin is composed of atleast three proteins ranging in size about 25 KD to 100 KD with almost the same amino acid composition. During the fifth larval instar, fibroin synthesis occurs in extremely large amounts (0.25 mg/cell) in the posterior silk gland. The fibroin gets translocated into the middle silk gland where, it is stored. To meet the demands of such high rate of fibroin synthesis, a functional adaptation of the posterior silk gland takes place (Suzuki 1976; Shimura, 1978; Goldsmith and Kafatos, 1984). The spinneret draws out the silk fluid in the form of silk filament. The threads of two sides are called brins which are stuck together by sericin layer of both and form a single filament (Akai, 1984). As the larvae develop, the cells of silk glands become larger and the secretory function also becomes active. In this phase, there is gradual branching of the nuclei. In late fifth instar stage, there is intense branching of nuclei occupying most of intracellular space (Kurabar, 2000). 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). Arthopod silk gland differs widely in both structure and anatomical location. The histology of trichopteran silk glands has been studied by several workers (Lucas, 1893; Gilson, 1894; Marshall and Vorhies, 1906; Glascow, 1936; Brickenstein, 1955; Barth, 1962 and Allegret and Denis, 1963). Silk gland is also a kind of the dermal glands. It derives from the invagination of the labial ecotoderm. The posterior silk gland cells secrete fibroin and the middle silk gland secretes two types of sericin (Oba, 1958). Silk fibroin synthesized in the posterior silk gland cells are secreted and stored in the gland lumen, and finally it's spun through the spinneret as a cocoon filament (Akai, 1976, 1984). Nambiar *et al.* (1991) have elaborated on the ultrastructure of the eri silkworm spinneret which was circular with an opening in the center. The silk glands in the last instars of silk producing lepidopteran larvae are known to pass through four consecutive phases i.e., growth, secretary, regression and degenerative phases and are revealed in *Bombyx mori* (Tashiro *et al.,* 1976) and *Galleria mellonella* (Sehnal *et al,* 1983; Sehnal and Akai, 1983).

MATERIALS AND METHODS

Collection of silkworm *Bombyx mori*

The first day of V instar of popular Indian bivoltine hybrid ($CSR_2 \times CSR_4$) silkworm *Bombyx mori* (Local Bivoltine) race were collected from Silkworm Culture Centre at 2nd Agraharam, Salem in Tamilnadu, and they were maintained up to cocoon.

Silkworm Rearing Method

The larvae were reared simultaneously both in control and experimental groups separately on mulberry leaves dipped in antibiotic (Dicloxacillin) solution in the laboratory. Proper environmental conditions provided to the silkworms with photoperiod of 12:12 h light and darkness as recommended by Krishnaswamy *et al.* (1973). The first day of V instar larvae were placed at ambient temperature of 25 ± 27ºC and relative humidity of 70 to 80%. The larvae were reared in card board boxes measuring 22×15×5 cms covered with nylon net and placed in an iron stand with ant wells (Govindan, *et al.,* 1981). The antibiotic drug (Dicloxacillin) was diluted 0.5% concentrations. Fresh V1 mulberry leaves were soaked with 15 minutes for Dicloxacillin solution and then were dried in air for 10 minutes. The treated leaves were used for feeding the V instars larvae of silkworm *Bombyx mori* (Suleman, 1999).

Preparation of tissue samples for histological study

Bacterial infected and antibiotics treated V1 mulberry leaves fed V instars *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

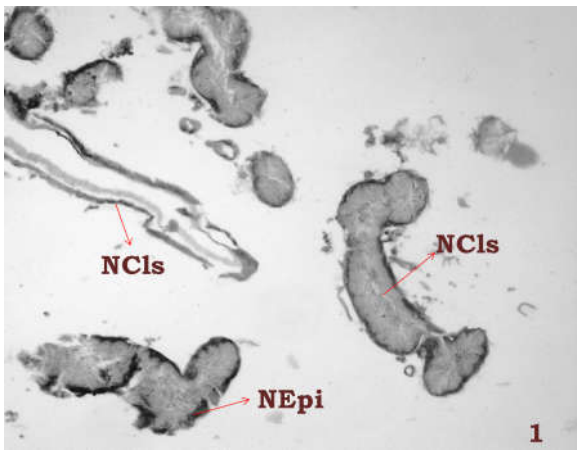
body and silk gland were fixed by immersion in Bouin's solution or 0.5% formalin in separated sterilized sample bottles or vials.

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 °C). Sections were cut at 6µ thickness were deparaffinized using ascending grades of alcohol and stained with haematoxylin and counter stained with aqueous eosin for microscopical observations and microphotographs were taken (Gurr, 1958).

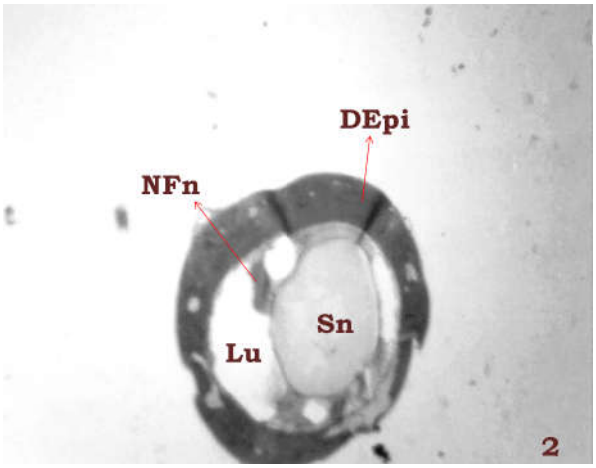
RESULTS

The fat body of diseased V instar of *B. mori* larvae showed the presence of shrunken pycnotic and necrotic epithelial cells indicates less synthetic and secretory activity (Fig. 1). Probably these substances not mobilized from the fat body via haemolymph to the silk gland. The anterior silk gland (ASG) of diseased *B. mori* larvae showed certain remarkable changes in the histological architecture, such as the presence of an outer thick, pynotic and disintegrated epithelium. Below this region contained necroted intima which was disintegrated and the lumen was partially filled with sericine and necroted fibroin substances (Fig. 2). The middle silk gland (MSG) of diseased silkworm exhibited certain histological architecture. The presence of outer thick pynotic epithelium surrounds the lumen. The lumen was filled with condensed sericine and fibroin substances than the control (Fig. 3). The PSG of diseased silkworm showed the occurrence of an outer pycnotic and disintegrated necrotic epithelial cells with shrunken nucleus. The epithelial layer was shrunken with less vacuoles indicates, less synthetic and secretory activity than the control silkworm (Fig. 4). The lumen was partially filled with fewer amounts of secretory substances; suggest that fibrillar protein is less synthesized from this gland. The trophocytes of the fat body are have prominent nucleus of antibiotic drug (Dicloxacillin) (0.5%) treated V instar larvae contained secretory nucleus with more vacuoles, indicates the synthesized and secretory substances mobilized from the fat body via haemolymph into the silk gland. The fat body histological architecture was similar to normal silkworms indicates the antibiotic drug (Dicloxacillin) cure the disease and to stimulate the fat body to synthesize and secretory activity (Fig. 5). The histological architecture of the ASG of diseased *B. mori* fed with antibiotic drug (Dicloxacillin) (0.5%) treated mulberry leaves exhibited certain remarkable changes such as an occurrence of intact epithelium similar to healthy silkworms. The intima also intact below the epithelium, the lumen was filled with clear sericin and fibroin secretory substances than the diseased silkworms (Fig. 6). The histological architecture of the dicloxacillin treated silkworm's MSG showed the presence of a degenerated epithelium with broad central lumen. The lumen was fully filled with clear sericin and fibroin secretory substances similar to healthy silkworms than the diseased worms (Fig. 7). The histological architecture of the PSG of *B. mori* fed with antibiotic drug (Dicloxacillin) (0.5%) treated mulberry leaves showed certain changes than the diseased worms such as the presence of an intact outer epithelium with more vacuoles and shrunken nuclei. The lumen was filled with more amounts of sericin around the fibroin secretory substances like homogenous and fibrillar than the diseased worms comparatively similar to healthy silkworms (Fig. 8).



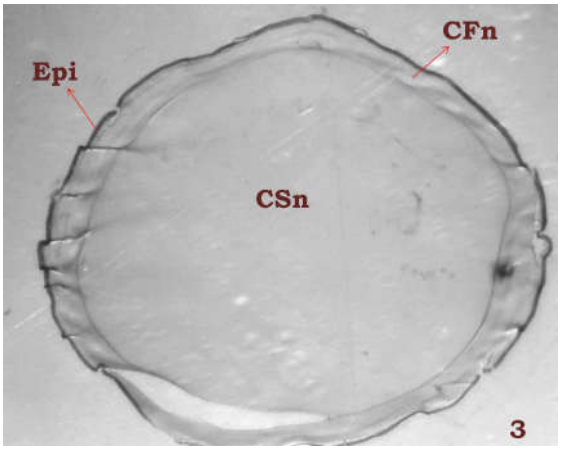
NCls - Necroted Cells
NEpi - Necroted Epithelium

Fig. 1. Histology of bacterial infected V instar silkworm larvae Fat body (X Ca 100)



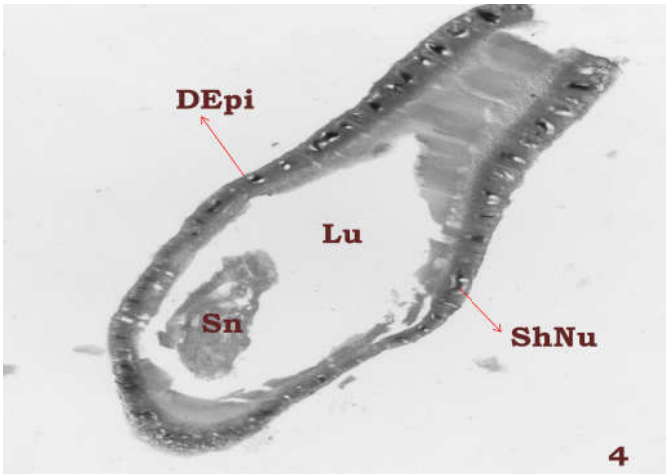
DEpi - Disintegrated Epithelium
Lu - Lumen
Sn - Sericine
NFn - Necroted Fibroin

Fig. 2. Histology of bacterial infected V instar silkworm larvae ASG (X Ca 100)



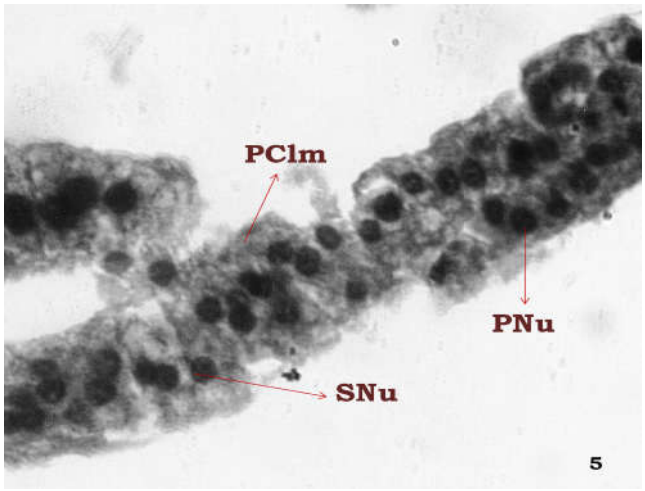
Epi-Epithelium
CSn-Condensed Sericine
CFn-Condensed Fibroine

Fig. 3. Histology of bacterial infected V instar silkworm larvae MSG (X Ca 100)



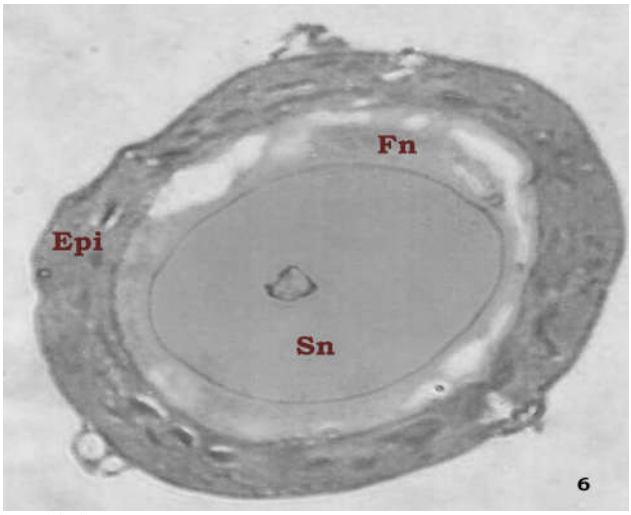
ShNu - Shrunken Nucleus
Lu - Lumen
DEpi - Disintegrated Epithelium
Sn – Sericine

Fig. 4. Histology of bacterial infected V instar silkworm larvae PSG (X Ca 100)



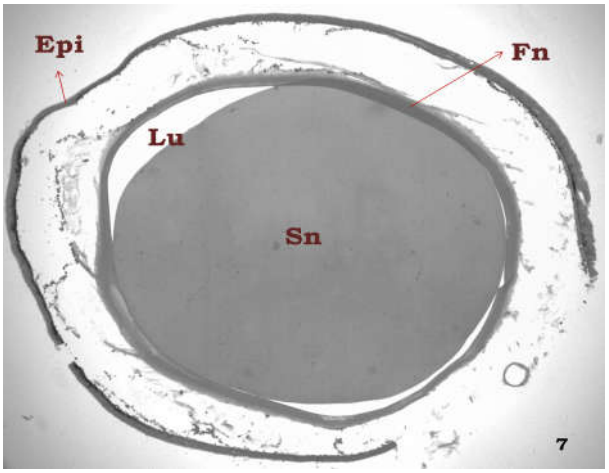
PClm - Prominent Cell mass
SNu - Secretory Nucleus
PNu - Prominent Nucleus

Fig. 5. Histology of Dicloxacillin treated V instar silkworm larvae Fat body (X Ca 100)



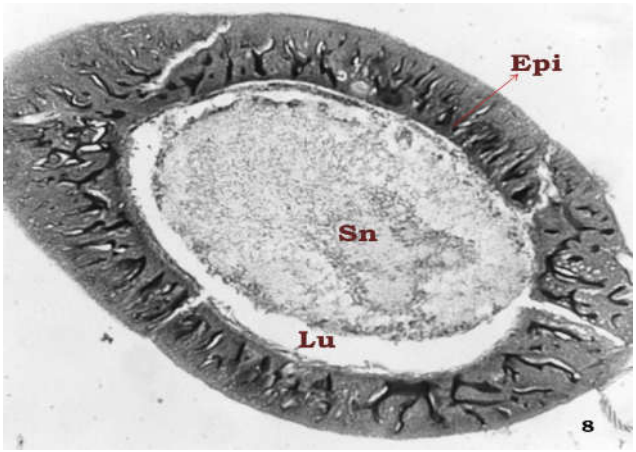
Epi - Epithelium
Sn - Sericin
Fn - Fibroin

Fig. 6. Histology of Dicloxacillin treated V instar silkworm larvae ASG (X Ca 100)



Lu - Lumen
Fn - Fibroin
Epi – Epithelium
Sn - Sericin

Fig. 7. Histology of Dicloxacillin treated V instar silkworm larvae MSG (X Ca 100)



Lu - Lumen
Epi - Epithelium
Sn – Sericin

Fig. 8. Histology of Dicloxacillin treated V instar silkworm larvae PSG (X Ca 100)

DISCUSSION

The present observation on *Bombyx mori* has revealed that the lobe and ribbon shaped fat body of this insect appears to be identical to that of other hemipteran and heteropteran insects (Buys, 1924). 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. These may be attributed due to utilization of these substances by the silk gland for the act of spinning. It is interesting to note that the gradual decreased secretory activity and mobilization of more nutrient substances from IV to V instar larvae during the period of spinning have been antagonistic with an increased secretory activity of the silk gland in *Bombyx mori* from IV instar larvae to V instar larvae, suggested that the existence of a close relationship between these two structures in relation to spinning. Wigglesworth, (1967) has reported the occurrence of cytoplasmic vacuoles and food reserves in the fat body of *Rhodinus prolixus* that had been found following prolonged starvation. According to him, the food reserves such as protein and lipids in the fat body are

likely to have been utilized during starved period resulting in the formation of cytoplasmic vacuoles. These observations indicate that the appearance of cytoplasmic vacuoles together with the accumulation of nutrient substances seem to be related to the synthetic activity of the cells of fat body during particular physiological state of the animal. From I instar to the V instar larvae, the silk glands of *Bombyx mori* have grown an increase in length and diameter externally and an enlargement of cells and nuclei internally. The maximum growth of silk gland occurs in the last larval instars similar to that reported in other silkworms (Sehnal *et al.,* 1983; Barsagade and Tembhare, 2000). It has been shown for *Bombyx mori* that it's fat body undergoes marked histological changes during the period from IV instar larvae to V instar larvae before spinning. A situation similar to this exists in female insects. The observations of Luscher, (1968); Scheurer, (1969); Wujest, (1978); Rajasekarapandian, (1995) and Ravichandran, (1996) have shown that the fat body exhibits a higher synthetic activity at the time of accumulation of yolk spheres in the immature and maturing oocytes of *Leucophae maderae, Oryctes rhinocerous, Nanphoe cinerea, Odontopus varicarnis, Pherosophos hilaris* and *Laccotrephes ruber,* respectively.

The present study on *Bombyx mori* also revealed that the differentiation of silk gland into three regions similar to that found in *Antheraea mylitta* (Barsagade and Tembhare, 2000). These results are in parallel with the works of Tashiro *et al.* (1976) who have reported for *Bombyx mori* during the development. Further, it has been revealed in the present study that the lumen contains more amount of secretory substances in all the instars of silkworm fed with antibiotics treated mulberry leaves than the silkworm fed with V1 leaf variety. The metamorphosis of silk gland of *Bombyx mori* of IV and V instar larvae have shown spectacular changes in the endomitotic development and the cells contain polyploid and multilobulated nuclei and vacuolated cytoplasm were comparatively more in number when the silkworm fed with antibiotics treated mulberry leaves than V1 fed *Bombyx mori.* 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). Besides rapid synthesis and accumulation of the chromatin materials in nuclei and cytoplasmic inclusion in the cell bodies, the growth phase is also characterized by the continuous growth and pigmentation of cuticular intima similar to that found in other species of silkworms (Sehnal and Akai, 1990). The silk glands in *Bombyx mori* are well evident as the fine thread like structures since I instar larvae to II instar larvae, they are however, differentiated into anterior and posterior regions, while in the IV and V instar have three regions, i.e. anterior, middle and posterior, become distinct. 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 (Prudhomme *et al.,* 1985; Sehnal and Akai, 1990; Barsagade and Tembhare, 2000).

The silk glands in the last instar of silk producing lepidopteran larvae are known to pass through four consecutive phases, i.e., growth, secretory, regression and degenerative phases are revealed in other silkworms (Sehnal *et al.,* 1983; Sehnal and Akai, 1983). Similar phases have also been observed in the present study that the silk glands in the last instar larvae became regressed and degenerated both in the worms fed with V1 and antibiotics treated mulberry leaves. These changes may be due to structural changes in the epithelial layer of MSG and PSG of silkworm larvae of *B. mori* during the process of spinning. The concomitant increase in the secretory phase of the silk gland and the accumulation of more amounts of secretory materials in the lumen of the IV and V instar larvae of ASG, MSG and PSG of silkworms when fed with antibiotics treated mulberry leaves than V1 leaf variety. These changes may be attributed due to the feeding of ntibiotics treated mulberry leaves which perhaps due to the presence of more nutritive substances than the V1 leaf variety. It may be suggested from the present study that the V1 leaf variety treated with antibiotics is an indicator for the stimulation of secretory phase in the larvae of silkworms than the same age groups fed with V1 leaf alone. From these results, it may be inferred that the V1 leaf variety treated with antibiotics is comparatively superior to the V1 leaf variety for sericulture industry to enhance the synthesis of more amount-of secretory materials by the silk glands of *B. mori* and also influences the larval and economic characters such as silk production and the nature of silk trait. This V1 leaf variety treated with antibiotics must be recommended to the farmers to get more performance both in economic characters, silk gland secretion and silk production than V1 leaf variety. From the present study, it is inferred that antibiotic drug treated V1 leaves fed *B. mori* larvae is superior then control V1 leaves fed *B. mori* larvae.

Conclusion

In the present study, the antibiotic drug Dicloxacillin to inhibit the bacterial growth on fat body, silk gland of silkworm larvae tissue and also increase the secretory activity of fat body and silk gland. The fat body involved in the regulation of secretory activity of the silk gland which inturn appears to be essential for the utilization of these nutrients for the act of spinning process. The concomitant increase in the secretory phase of the silk gland and the accumulation of more amount of secretory substance such as sericin and fibroin in the lumen of the V instar silkworm larvae of ASG, MSG and PSG of silkworms when fed with antibiotic drug Dicloxacillin (0.5%) treated mulberry leaves than the fat body and silk gland of bacterial infected silkworms.

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