IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 6, April 2022
Impact Factor: 6.252

Impact of Botanical Extracts on Histopathology of Midgut of CSR2 Race of Mulberry Silkworm (Bombyx Mori L.) Inoculated by Staphylococcus Aureus

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Abstract:*In the present study changes in midgut epithelium of 5th day of 5th instar silkworm race CSR2 infected with a gram-positive bacteria Styphalococcus aureus and plant extract treated group and control group were studied. Large numbers of newly developed cells appeared in the bacteria infected part of the midgut epithelium. After inoculation, and along with their development, the bacteria old columnar cells were discharged into the midgut lumen during development. On the other hand, in the uninfected portion of the midgut only a few cells developed, and no columnar cells were discharged. Similarly, the marked replacement of midgut epithelial cells during larval development were also observed in larvae treated by plant extract. In the larvae infected with S.aureus, the columnar cells lost their regenerative ability, and because of the exfoliation of infected columnar cells, the midgut epithelium consisted mainly of uninfected goblet cells at a late stage of infection. The degree of epithelial regeneration varied with the silkworm strain and the dosage of the bacteria.*

Keywords:Plant Extract, Staphylococcus aureus, Midgut, Goblet Cell, etc.

I. INTRODUCTION

The lepidopteran midgut is formed by a folded epithelial cell separated from underlying muscles and trachea by a thin basal membrane and composed of three main cell types Goblet cells, columnar cells and regenerative cells. (Ciaffi 1979; Baldwin and Hakim 1991) The columnar (cylinderical) cells of the mid-gut are active functional cells, whose inner brush border projecting into the lumen promotes secretion and absorption. Goblet cells (calyciform) are small secretory cells interspersed among columnar cells. The goblet cells have reduced cytoplasm and their striated border has deep invagination to form cavity. Among the active epithelial cells there are small basal, embryonic or replacement cells called regeneration cells.

II. MATERIAL AND METHODS

Experimental Animal:

The disease free layings (dfls) of popular Bivoltine race CSR_2 was obtained from the directorate, District Sericulture Office, Shahupuri, Kolhapur for laboratory rearing. The silkworm larvae of Bivoltine race CSR_2were reared in the laboratory on mulberry leaves as per the method of Krishnaswami *et al.,* (1978).

Microorganisms:

The microbial culture of Gram-positive bacterium *Staphylococus aureus* was made available from Department of microbiology, Shivaji University Kolhapur, culture was maintained by monthly transfer in the new broth medium.

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III. Medicinal Plants Used for Study

1. *Syzygium cumini*(Seed powder)

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2. *Euphorbia geniculata*(leaf powder)

***Terminalia arjuna (*Bark powder)**

3. *Mormordicta charantia* (leaf powder)

***Momordica charantia* (leaf powder)**

4. *Terminalia arjuna (*Bark powder)

***Euphorbia geniculata* (leaf powder)**

Histological Methods:

For histopathological studies the midgut of silkworm larvae of CRS2 race from each group (i.e normal, inoculated and plant extract treated) were dissected under stereoscopic binocular microscope by using chilled ringer solution. Midgut was removed from the healthy, inoculated as well as the plant extract treated groups silkworms. The tissues were then fixed in freshly prepared Bouin's fixative for histological studies. Thereafter the tissue was dehydrated, cleared inn xylene and embedded in paraffin wax at 58 to 60^0c, Blocks were sectioned at 6 µm thickness and stained with Eherlich Haematoxylene-Eosin (HE) staining technique (Humason 1962).

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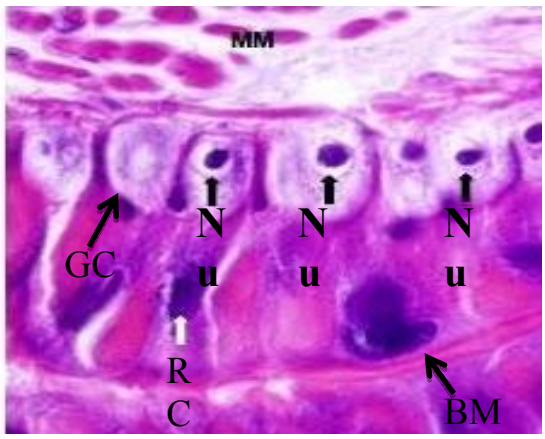

Figure 1: Cross section of mid gut of healthy fifth instar larva of mulberry silkworm B.mori L showing columnar epithelial cell and compact darkly stained nucleus, goblet cell, muscle layer and regenerative cell (Bar = 4.2 µm).

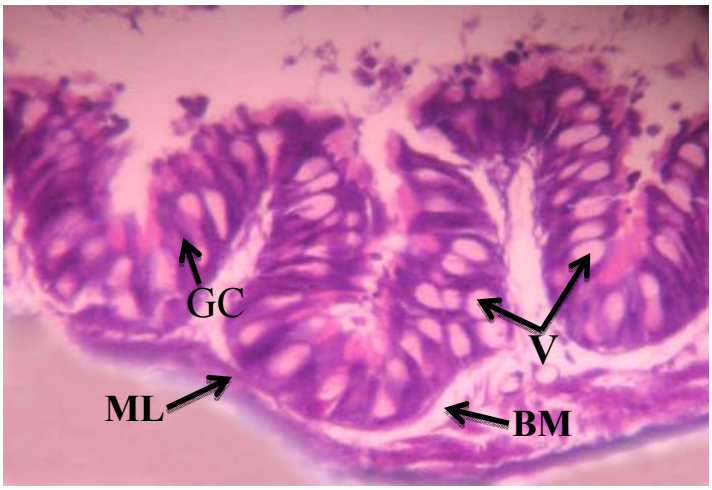

Figure 2: Cross section of mid gut after early bacterial infection showing vacuoles in columnar epithelial cell, goblet cell and regenerative cell, brush border membrane towards lumen and vacuoles in cytoplasm (Bar = 4.2 µm).

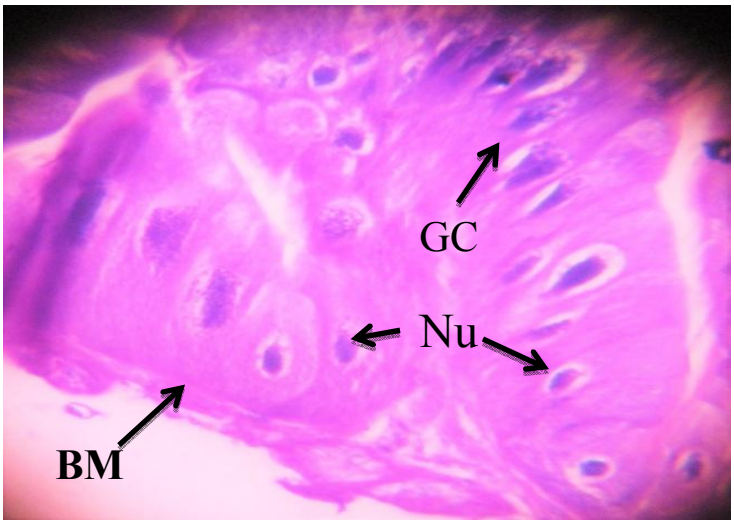

Figure 3: Cross section of mid gut after Treatment of plant extract in inoculated group showing vacuoles in columnar epithelial cell, goblet cell and regenerative cell, brush border membrane.

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IV. RESULT

Histological Observations

Normal Midgut

The mid-gut in the larvae is the main organ involved in digestion and absorption. It is a straight and long tube occupying the major part of the alimentary tract. Histologically, a stratum of enteric epithelium, the outer ends of whose cells rest upon a basement membrane, lines the mid-gut. The latter is followed by an inner layer of circular muscles and an outer layer of longitudinal muscles.

The outermost coat of the mid-gut is a thin peritoneal membrane. Both muscles are composed of striated fibres and their positions are the reverse to what obtains in the fore-gut. The enteric epithelium of mid-gut is devoid of cuticle, but have a delicate detached sheath called peritrophic membrane. It is produced by the delamination of thin sheets from the surface of the cells throughout the length of the mid-gut. The mid-gut cells are chiefly of two types, columnar cells and secretory or goblet cells. The columnar (cylinderical) cells of the mid-gut are active functional cells, whose inner brush border projecting into the lumen promotes secretion and absorption. Goblet cells (calyciform) are small secretory cells interspersed among columnar cells. The goblet cells have reduced cytoplasm and their striated border has deep invagination to form cavity. The regenerative cells are scattered singly along the gut.

Midgut of Infected Silkworm

In the mid-gut of diseased worms' digestion was abortive. Digestive action was reduced. Absorption was also poor. The digestive enzyme secretory cells have enzymes but the enzymatic materials unreleased. Large pieces of ingested mulberry leaves occuluded the digestive passage. The epithelial layer of the mid-gut wall had lost its continuity. Pentrophic membrane had lost its integrity and convoluted. It got detached from the epithelial layer. Bacterial conglomerates were seen corroding the epithelial layer. Intracellular parasitation of bacterial cells was seen as dark mass inside. The lumen was enriched with bacterial population. Goblet cells were partially vacuolated. Regenerative cells were pyknotic. Vacuolations are also common in cytoplasm. The goblet cells are necrotic and loaded with secretions but the secretions remained unreleased due to the lack of cellular dysfunction. In the regenerative cells nuclei are prominent but the nuclei have polymorphic nature.

V. DISCUSSION

The silkworm larvae are voracious feeder and the efficiency of feeding, digestion, absorption and feed type decides the economics of sericulture. Minimising the feed intake and break down in feed conversion interrogates the energy budget and ends in poor quality cocoon formation. Hence the digestive system needs much attention in silkworm larvae. As oral entry of pathogenic microbes are quite common (Lu Yup-lian and Liu Fu-an, 1991) the alimentary system must be endowed with special defensive mechanism to inhibit the action of pathogens to have a good digestive function. This is ensured by the presence of a cuticular epithelium or propria intima, and peritrophic membrane bordering the gut lumen. This defensive fort has been further fortified with epithelial cells and musculature.

In the present investigation cytopathological changes were reported in columnar, goblet and regenerative cells in the mid-gut epithelium. Columnar cell architecture had been changed in several places. Columnar cells became hypertrophic and sloughed off into the lumen. bacterial toxins affect the microvillated apical membrane of the columnar epithelial cells and forms pores in the epithelial cells. columnar cells Cell off into the lumen and the cellular degeneration was limited to the anterior part of the mid-gut. in the present investigation goblet cells were much affected. Necrosis of goblet cells leads to secretary dysfunction. The regenerative cells seen on the basal membrane near columnar cell replaces the dead or wornout cells. Due to infection, they are also damaged.

ACKNOWLEDGEMENT

I am thankful to Former Guide Dr. G. P. Bhawane and Guide Dr. S. S. Patil for their guidance. Thanks to Department of Zoology, Shivaji University Kolhapur for providing the facilities for research work.

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