



DIMORPHISM IN NUCLEAR POLYHEDROSIS VIRUS (*BmNPV*) (FAMILY: BACULOVIRIDAE) CAUSING 'GRASSERIE' DISEASE IN SILKWORM (*Bombyx mori* L.): LIGHT AND ELECTRON MICROSCOPY AND PROTEIN PROFILE

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ABSTRACT

The present study was aimed to microscopically assess dimorphism in nuclear polyhedrosis virus (*BmNPV*) responsible for 'Grasserie' disease in silkworm, *Bombyx mori* L. Tissue sections of infected larvae integument were found ruptured, had abundant lump cells and released milky fluid containing occluded bodies (OBs). Nuclei of columnar epithelial cells of integument were almost covered with *BmNPV* polyhedra. Scanning electron micrograph (SEM) revealed full lesions in infected integuments with very fragile hypodermis. Transmission electron micrograph (TEM) showed that infected different parts of peripheral membrane contain lipid globules started to diffuse continuously to form a large structure to occupy maximum OBs. In advanced stage of infection, two viral phenotypes were noticed. 1st form occluded within polyhedra was responsible for primary infection in mid-gut cells. The 2nd form 'budded virus (BV)' never became occluded and was released into haemolymph and spread infection by cell to cell contact within insect body. The fully transformed lipid globules discharged the 1st form whereas BV was observed in integument, mid gut and Malpighian tissues. Interestingly, the infected mid gut cells were dilated in presence of OBs and gradually increased in number to form chain-like structure which broke up no sooner the number of OBs reached highest carrying capacity, releasing polyhedra in gut juice. Inner layer of infected Malpighian tissues were loose and fragmented with OBs in between the striations of Malpighian cells. BVs, the new finding from *B. mori*, are responsible for virulence of *BmNPV* in silkworm. 29 and 17 kDa proteins in multivoltine (Nistari) and bivoltine (NB₄D₂), respectively, were absent in the haemolymph of *BmNPV* infected counterpart. The total quantity of haemolymph protein was more in multivoltine samples than in bivoltine samples.

Keywords: *Bombyx mori*, budded virus, Grasserie, polyhedra, occluded bodies, innate immune system

INTRODUCTION

The silk industry faces severe setbacks due to frequent disease outbreaks since most of the commercially reared silkworm species, including *Bombyx mori*, are highly susceptible to the diseases like pebrine, flacherie, grasserie and muscardine. In India approximately 40% crop loss is attributed to these diseases (Sheeba Rajakumari *et al*, 2007). Grasserie, incited by nuclear polyhedrosis virus (NPV), is second major disease in silkworm next to pebrine in tropical areas including India. Usually high temperature (30-45°C) and humidity (90-100%), prevalent in tropical regions, are conducive to the proliferation of polyhedrosis diseases. Generally all larval instars, especially the 4th and 5th ones, are

infected by the disease in India causing 20-50% cocoon crop loss (Nataraju *et al.*, 1998). The low productivity of silk is mainly due to the frequent disease outbreaks and limited genetic diversity among the commercially exploited silkworms. It is, therefore, necessary to identify the nature of damage caused by these pathogens so that effective preventive remedial measures are adopted. Silkworms are most common target for *Bombyx mori* nuclear polyhedrosis virus (*BmNPV*) belongs to Baculoviridae, a family of enveloped double stranded DNA. In *BmNPV*-infected larva, the skin becomes thin and fragile and body turns milky white to yellowish in colour with inter-segmental swellings. Infected larvae become restless and crawl aimlessly along the edges of rearing tray. The viral disease in *Bombyx mori* comprises mainly the inclusion of polyhedra caused by *BmNPV*. The appearance of *BmNPV* occlusion bodies (OBs) in the haemolymph of infected silkworm has first been described by Cornalia (1856). Recent reports on the presence of anti-viral proteins against some viruses strongly suggest the involvement of functional anti-viral immune system in silkworm. Information on haemolymph protein of the silkworm responsible innate immunity system from diseases including viral infections is reported (Chakrabarty *et al.*, 2011). Many attempts have been made by the different workers to control this silkworm disease (Patil, 1995; Rabindra, 1994, Nataraju *et al.*, 2000; Kawakami, 2001). However, the losses due to *BmNPV* are escalating at an alarming rate due to the emergence of new resistant strains of pathogen (Sen *et al.*, 1999). In the present communication detailed histological and electron micrographical and protein profile studies on the nature of damage in silkworm by *BmNPV* are reported so as to formulate suitable disease management technology for the sustenance of sericulture industry.

MATERIALS AND METHODS

Isolation and purification of virus

Infected pupae, obtained from silk growers of Bandhkhala village, Birbhum district, West Bengal, India were used in the present study. *BmNPV* isolates were propagated in *Bombyx mori* silkworm larvae and purified from live moth using Percoll Cushions (PVP coated Silica particle, Sigma Chemicals, USA). The polyhedral particles from the haemolymph of *BmNPV* infected silkworm larvae were centrifuged in endotoxin free water (E.F. water) and suspension filtered twice through cotton followed by three washings in E.F. water. The polyhedra were isolated from the infected pupae and purified by centrifugation at 3000 rpm for 10 min. The suspension of polyhedra particles was layered over percoll cushions and centrifuged at 18000 rpm for 30 min. in RC5C high speed refrigerated centrifuge (Sorvall) with swing out rotor (SH-MT12) to obtain purified occlusion bodies (Bhattacharya *et al.*, 1993). After centrifugation, the polyhedra were stored at 4°C for further processing.

Inoculum concentration for experiment

Fresh ecdysed 5th instar larvae were inoculated at '0' h with *BmNPV* @ 1.5×10^8 OBs ml⁻¹ using conventional diet contamination method. Further, leaf dishes (28.27 cm²) were dipped in 1 ml polyhedral suspension, dried and then fed to larvae for a period of 6 h. Larvae were used for light and electron microscopic study.

Light microscopic study

Both healthy and infected integuments of 5th instar larvae were collected. The tissues were fixed in 10% aqueous Bouin's fluid for 16-18 h. The tissues were then dehydrated through graded alcohols, cleared with xylene and embedded in paraffin wax. Sections of 5 µm thickness were cut. The deparaffinized sections were brought to water, stained with Delafield's haematoxylin and counterstained with alcoholic eosin for histological observation under compound light microscope (×1000) (Leitz Diaplan Phase Contrast Microscope). The procedure was followed as per Steinhaus (1949) and Bergold (1958).

Electron microscopy study

Tissues of integument, gut and Malpighian tubule of healthy and infected silkworms (Nistari and NB₄D₂) taken at different larval stages were dissected and cut into small pieces of 5 x 7 mm size for SEM and 2

× 2 mm for TEM. The samples were washed thrice in buffer for 1 h at 4°C and kept in a thermos-flask using cool pack for further processing. For SEM, the samples were centrifuged at 1000 rpm, pellets suspended in 0.1 M phosphate buffer (pH, 7.4), again centrifuged at 1000 rpm for 10 min. and fixative solution (2% ρ -formaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer) added in 3:7 ratio. The pellets were again suspended and fixed in fixative solution for 2-3 h at 4°C. The samples were further centrifuged for 10 min. at 4°C, supernatant discarded and buffer added to the pellets. Gold coating was done by IB2 Ion Coater (Eiko Engineering, Japan). The samples were observed under SEM (S-530; Hitachi, Japan.) and photo-micrographed at 300-1500 x.

For TEM, the fixation was done by perfusing the tissue with 0.9% phosphate buffer saline (pH 7.2) for 10 min. and subsequently by 2% ρ -formaldehyde for 20 min. The samples were again fixed in fixative solution for 8-12 h at 4°C. The samples were post-fixed in 1% osmium tetroxide (OsO_4) for 1 h at 4°C. The samples were dehydrated in ascending grades of acetone, infiltrated and embedded in araldite CY 212 (TAAB, UK). Thick sections of 1 μm size were cut with an ultra-microtome, mounted onto glass slides and stained with aqueous toluidine blue. For electron microscopic examination, thin sections of gray silver colour interference (70-80 nm) were cut and mounted in 300 mesh-copper grids. The sections were stained with alcoholic uranyl acetate and alkaline lead citrate, then gently washed with distilled water, observed under Morgagni 268D TEM (FEI Company, The Netherlands) and photo-micrographed at 1000-8000 x at an operating voltage 80 KV. Images were digitally acquired by using a CCD camera (Megaview III, Fei Company) attached to the microscope. The procedure was followed as per Undeen (1997).

Protein profile study

Haemolymph samples were collected from 3-day old 5th instar larvae at different time intervals of post injection into pre-cooled (below 4°C) eppendorf tubes containing few crystals of phenyl-thiourea to prevent oxidation of haemolymph and centrifuged subsequently at 6,000 rpm for 5 min. in Sorvall RC5C centrifuge to remove haemocytes and other tissue debris (pellet). The supernatant was stored at -20°C until use. Haemolymph collected from uninfected samples was used as control. Each sample was collected from approximate 15 silkworms, centrifuged for 10 min. at 12,000 rpm at 4°C and stored in a lysis solution of 8 M urea, 4% (w/v) CHAPS, 1% (w/v) dithiothreitol (DTT), and 1% (w/v) protease inhibitors cocktail (Sigma P2714). Total protein content in the supernatant was determined as per Bradford (1976) and absorbance measured at 750 nm in a spectrophotometer (Shimadzu). SDS-PAGE was carried out on 4% stacking gel and 12% polyacrylamide gel (separating gel) with 0.1% SDS and stained with Coomassie brilliant blue. Each protein sample (15 μg) was loaded onto gels after dissolving in loading buffer followed by 95°C treatment for 5 min. SDS-PAGE was carried out at a constant electric current of 10 mA for stacking gel and 20 mA for separating gel until the bromophenol reached the end of the gel. SDS-PAGE was carried out for 2 h until bromophenol reached the end of the gel (Laemmli, 1970).

RESULTS AND DISCUSSION

The *BmNPV* infected larvae had thin and fragile skin and their bodies became milky white to yellowish in colour with inter-segmental swellings (Fig. 1a) as compared to the healthy larvae (Fig 1b). The infected larvae were restless and crawled aimlessly along the edges of rearing trays. ‘Grasserie’ disease is observed in all larval instars especially in 4th and 5th instars during all seasons causing 20-50% recurring crop losses in India (Nataraju *et al.*, 1998). Integument of *BmNPV* infected silkworm was observed full of lesions and was clearly manifested as compared to the normal ones. *BmNPV* infected larval skin appeared to be covered with amorphous material which surrounded the surface of viral envelopes. The material formed hollow structure where nucleocapsids of *BmNPV* had lost their density. The skin of infected larvae in advanced stage showed the formation of pores. It is probable that the amorphous materials of infection lead to the elimination of pus, and stored below the integument

surrounding viral envelopes are the host proteins which might have reacted with NPV. The envelopes of *BmNPV* seemed to be more stable against host protein attack than nucleocapsids.

Light microscopy

The tissue sections of integument of infected larvae were ruptured and deformed, and formed many lump cells as compared to the tissue sections of integument of healthy larvae (Fig. 2a-c). Hypertrophies of nucleus of columnar epithelial cells (CEC) were material formed hollow structure where nucleocapsids of *BmNPV* had lost their density. The skin of infected larvae in advanced stage showed the formation of pores. It is probable that the amorphous materials of infection lead to the elimination of pus, and stored below the integument surrounding viral envelopes are the host proteins which might have reacted with *BmNPV*. These were noticed 2 h post-inoculation (hpi) indicating the initiation of *BmNPV* infection in integument (Khurad *et al.*, 2004). CEC, goblet cell (GC) and basal metabolic cell (BMC), with a few occluded bodies (OBs), were observed abnormal at early stage of infection at 96 hpi (Fig. 2b). All the nuclei of integument cells were covered with *BmNPV* at 120 hpi (Fig. 2c). Enlarged nuclear region supports the histological observation of Steinhaus (1949) and Bergold (1958). Benz (1963) reported that non-occluded virions of *BmNPV* usually initiate infection cycles in tissues and organs like haemocytes, fat body, hypodermis, tracheal matrix, muscle, nerve ganglia and gonadal tissues in *B. mori* at varying degrees at later stages of infection. The hypodermis was extremely fragile and body wall ruptured releasing the milky fluid. OBs were found in maximum number in mid gut for virus multiplication and spreads of infection. Maximum number of large size budded virus (BV) were found in Malpighian tissue for spreading rapid infection spontaneously. This is the new finding in the present study.

The healthy larval mid gut had layers of different tissues (Fig. 2d) whereas infected mid guts were dilated with a series of ODs in soft tissue which gradually increased in number and later released polyhedra in gut juice (Fig. 2e). Deformed columnar epithelial tissues were filled with OBs, and BVs were exposed from mid gut epithelial layer in advanced stage of infection, whose mid gut wall appeared emerged (Fig. 2f). The Malpighian tubule of healthy silkworm larvae showed the presence of inner soft Malpighian tissue guarded by continuous peripheral membrane (Fig. 2g). However, the infected Malpighian tissues were loose with OBs in between the striations of Malpighian cells. Basic round structure of outer membrane was lost and these became fragile. BVs were mostly in groups and larger in size than OBs (Fig. 2h-i). During *BmNPV* infection process, two viral phenotypes were encountered: one form was OBs within polyhedra and is responsible for primary infection in mid gut cells. The second form of virus, BV, never became occluded and was released into haemolymph. BV spreads infection from cell to cell within insect body (Haas-Stapleton *et al.*, 2005). The initiation of replication of BV is believed to occur in cells by endocytosis (Herniou *et al.*, 2003). However, the silk glands and majority of insect tissues have a fibrous extracellular matrix, the basal lamina (BL). Therefore, BV needs to penetrate BL for systemic infection. Reports reveal that BL organization and thickness influences the passage of macromolecules into certain tissues where it acts as a selective filter (Rahman and Gopinathan, 2004).

Reddy and Locke (1990) using colloidal gold particles as tracer observed the movement of macromolecules through BL in *Calpodex ethlius* (Lepidoptera: Hesperidae) larvae because the charge and size of tracer was in the same range as those of large haemolymph proteins. The tissues involved in import-export of haemolymph proteins (fat body, epidermis and pericardial cells) possess BL that allow passage of gold particles (of ≤ 15 nm size). Once the particles crossed BL they entered into endocytic pathway without further hindrance. However, silk glands, Malpighian tubules, heart and muscle tissues are not known to be involved in the synthesis of large haemolymph proteins due to bilayered laminae with additional less permeable layer inside the outer permeable one. The outer layer adjacent to haemolymph resembled the complete filtering laminae of fat body, epidermis and pericardial cells. The inner layer apposed to cell membrane was impermeable to the smallest tracer particles (6 nm), as the midgut connective tissue sheath prevented the particles of > 6 nm from entering the compartment

between it and midgut basal lamina, hence particles >6 nm were retained in silk gland, Malpighian tubule and muscle BL (Torquato *et al.*, 2006). *BmNPV* virions have approximate mean diameter of 95 nm and length of 315 nm, making the passage of BV through silk gland BL difficult (Brancalhão *et al.*, 2009). Therefore, BV is retained in fat globules, Malpighian tubules and silk glands. This is an interesting finding in the present study.



Fig. 1: Silkworm, *Bombyx mori* a) Grasserie infected fifth stage silkworm b) Healthy silkworm

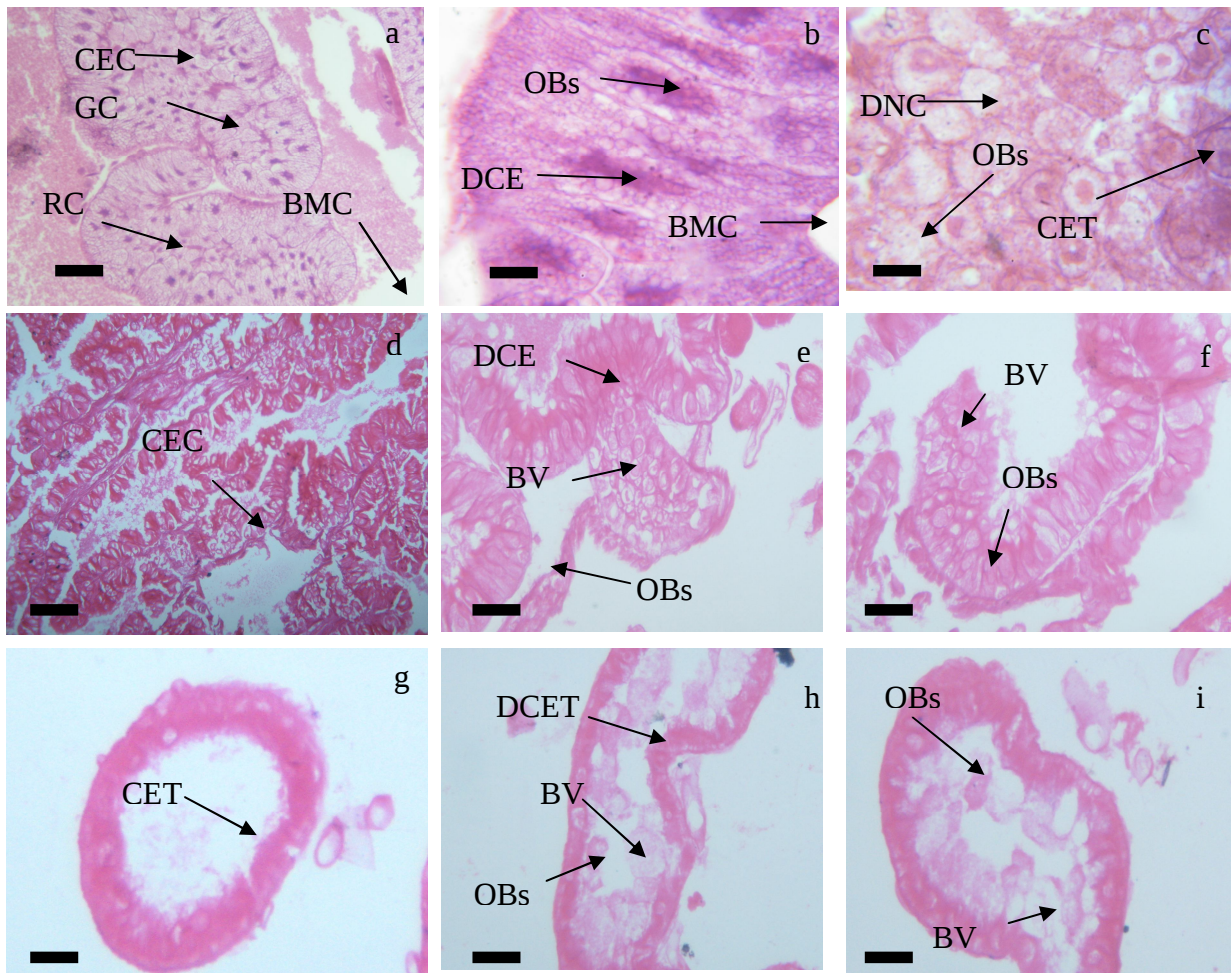


Fig. 2: Histology a) Normal larval integument. Columnar epithelial cells (CEC), Goblet cell (GC), regenerative cell (RC), Basal metabolic cell (BMC); b) Infected larval integument (preliminary stage at 96 hpi). Deformed CEC cells (DCE), GC, BMC and OBs; c) Infected larval integument (advanced stage, at 120 hpi). Denucleated cell (DNC) and OBs; d) Normal larval mid gut. CEC; e) Infected larval midgut. DCE and OBs, budded virus (BV); f) Infected larval midgut in advance stage. OBs and BV. g) Normal larval Malpighian tissue. Columnar epithelial tissue (CET). h) Infected larval Malpighian tissue. Deformed CET tissue (DCET), OBs and BV; i) Infected larval Malpighian tissue. CET, OBs and BV. In all case above Bar = 20 μ m and magnification $\times 1000$.

Scanning electron microscopy

BmNPV formed a large number of hexagonal, pentagonal or tetragonal polyhedra of 1-5 μm in dia. in cell nuclei (Fig. 3a). The viral particles contained nucleic acid and protein and were rod shaped measuring $330 \times 80 \text{ nm}$ in size (Fig. 3b). Integument of healthy larva was packed of bristles but was absent in *BmNPV* infected integument. The integuments of infected silkworm had lesions which was distinguishable from normal integuments. The skin of *BmNPV* infected larva was covered with an amorphous material. The material formed hollow structure whereas the nucleocapsids of *BmNPV* had lost their density. The skin of infected larva indicated the formation of pores at the advanced stage of infection leading to the elimination of pus and stored below the integument (Fig. 4a-f).

Fig. 3: a) SEM of *Bombyx mori* nuclear polyhedrosis virus ($\times 85000$). Bar = $1 \mu\text{m}$; b) Ultra-microscopic view of nucleocapsids of *B. mori* NPV ($280 - 330 \times 40 - 85 \text{ nm}$)

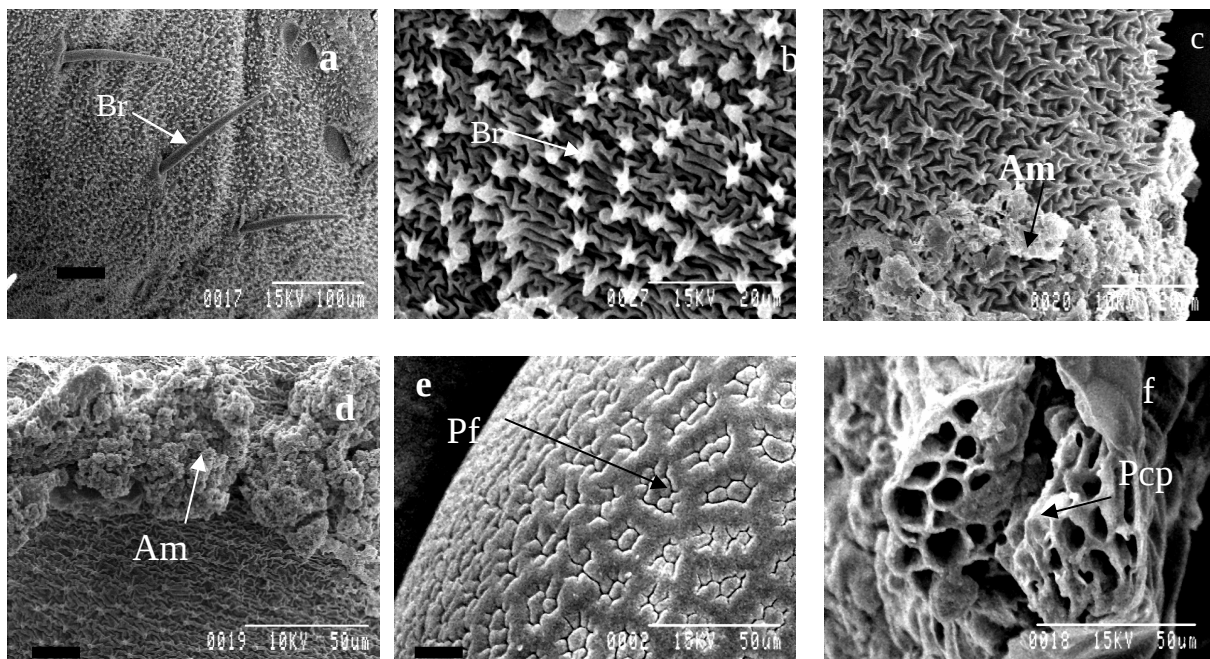
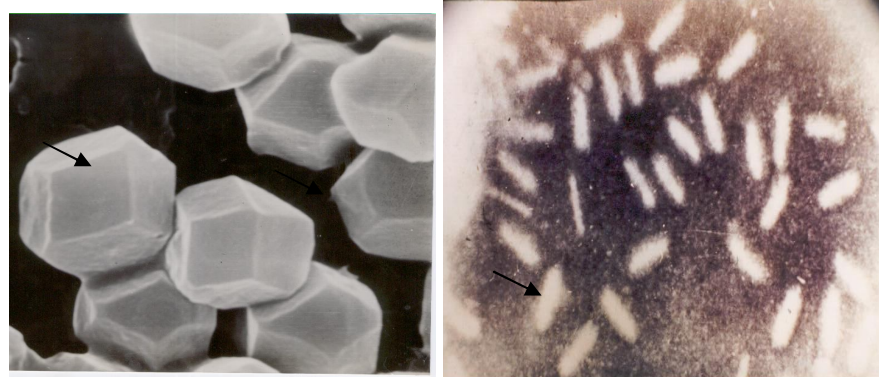


Fig. 4: Scanning electron micrograph a) Normal larval integument ($\times 300$) bristles (Br); b) Normal larval integument ($\times 800$) Br; c) Infected larval integument (preliminary stage $\times 1000$), amorphous material (Am); d) Infected larval integument (advanced stage $\times 1000$), Am; e) Infected larval integument (late advanced stage, $\times 1200$) pus formation (Pf); f) Infected larval integument near to death ($\times 1500$), pus oozing out from pore (Pcp)

Transmission electron microscopy

The transmission electron microscopy of different parts of *BmNPV* infected *B. mori* revealed globular lipid structures, present in healthy larval integument (Fig. 5a), were transformed into elongated structures at later stage of infection (Fig. 5b) which clearly indicated that the peripheral membrane of lipid globules diffused to form a continuous larger structure to incorporate maximum number of OBs (Fig. 5c). The fully transformed lipid globules gradually discharged OBs in adjacent matrix (Fig. 5d) and released polyhedral structures. The 2nd form, the BV, never became occluded (Fig. 5e). Healthy mid gut possessed several layers of different tissues (Fig. 5f) while infected mid guts were dilated due to the presence of OBs in soft tissue. The number of OBs gradually increased in chain with disease progression. The chain broke up once the number of OBs reached highest carrying capacity thereby releasing polyhedra in gut juice (Fig. 5g). The Malpighian tubule of healthy larvae showed the presence of inner soft tissues guarded by continuous peripheral membrane (Fig. 5h). However, in infected larva the inner Malpighian tissue was loose and fragmented with OBs in between the striations of Malpighian cells. BV spread infection from cell to cell (Fig. 5i-j). TEM examination of various parts of *BmNPV* infected *B. mori*, compared to the normal uninfected counterpart, clearly revealed that the peripheral membrane of lipid globules began to diffuse to form a continuous larger structure to incorporate maximum number of OBs. The fully transformed lipid globules gradually discharged OBs in adjacent matrix and the polyhedral structures were released.

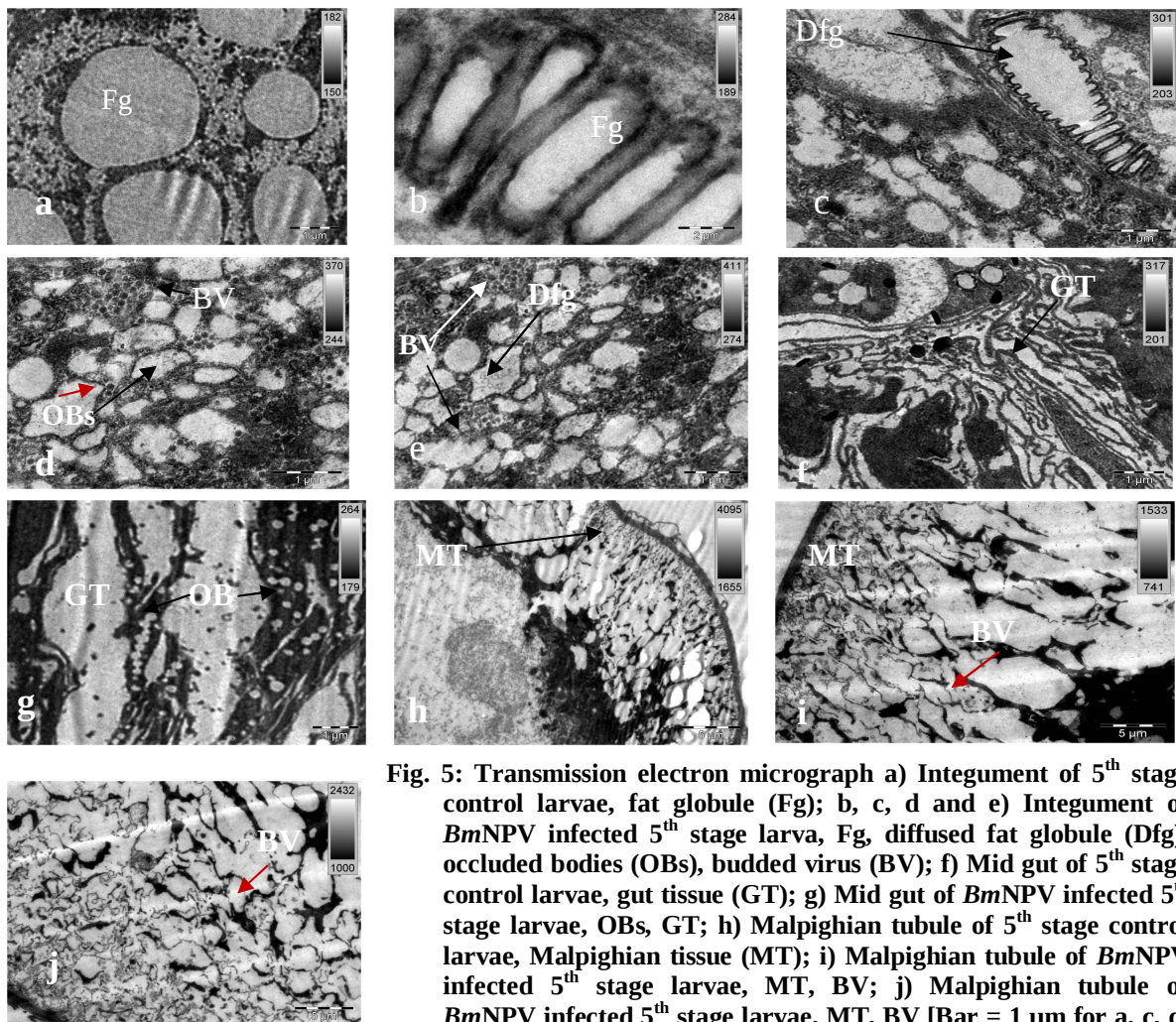


Fig. 5: Transmission electron micrograph a) Integument of 5th stage control larvae, fat globule (Fg); b, c, d and e) Integument of *BmNPV* infected 5th stage larva, Fg, diffused fat globule (Dfg), occluded bodies (OBs), budded virus (BV); f) Mid gut of 5th stage control larvae, gut tissue (GT); g) Mid gut of *BmNPV* infected 5th stage larvae, OBs, GT; h) Malpighian tubule of 5th stage control larvae, Malpighian tissue (MT); i) Malpighian tubule of *BmNPV* infected 5th stage larvae, MT, BV; j) Malpighian tubule of *BmNPV* infected 5th stage larvae, MT, BV [Bar = 1 μ m for a, c, d, e, f and g; 2 μ m for b and 5 μ m for h, i and j].

Protein profile study

The proteins profile study revealed that banding pattern of infected larval haemolymph protein was altered in comparison to normal haemolymph (Fig. 6a-d). SDS-PAGE analyses revealed that the four fractions (Nistari: Control and Infected as well as Bivoltine: Control and Infected) contained protein in between 15-240 kDa common as a major or even the sole component. However, a total of 21 protein bands were present in the haemolymph of both the control batches of multivoltine (Nistari) and bivoltine (NB₄D₂) but only 20 protein bands were present in the haemolymph of both *BmNPV* infected batches of multivoltine (Nistari) and bivoltine (NB₄D₂). Protein fraction number 16th (Refractive index: 0.32; 29 kDa) and 19th (Refractive index: 0.043; 17 kDa) which were already present (innate) in the haemolymph of larva (control) in multivoltine (Nistari) and bivoltine (NB₄D₂), respectively, were either completely absent or weakly present in the haemolymph of *BmNPV* infected larva. These protein fractions have definitely a role in protection against infection through innate immune system. The total protein content was much more in multivoltine than that of bivoltine samples that apparently appear from the graphical representation.

As the primary/major route of infection is through food, there must be some antiviral mechanism/substances existing in gut juice of larvae. The presence of such antiviral substances was observed by earlier workers (Funkoshi and Aizawa, 1989). Owing to domesticated and mass reared for several centuries, the mulberry silkworm presumably has developed a weakened immune system which has made the insect highly susceptible to viral infection (Yao *et al.*, 2006). A number of antibacterial and antiviral substances have been identified and isolated from the haemolymph and intestinal fluid of silkworm and a virus-inactivating protein (27-28 kDa) was isolated from digestive juice of the *B. mori* by Uchida *e. al.*(1984). Increase in total protein content in intermediate stage of infection and decrease in protein content in later stage is in agreement with the observation of Watanabe and Kobayashi (1969). Similarly, the protein content in haemolymph did not increase which may be attributed to the fact that the protein synthesized in fat bodies and mid gut after infection may have been utilized for virus multiplication but haemolymph protein was unutilized by virus which is in agreement with the findings of Gururaj *et al.* (1999a,b). This strongly suggested that 15 kDa and 27-28 kDa, low molecular weight proteins could be the factor responsible for promoting the activity or immunity in all the races.

Kovacs-Simon (2011) reported that bacterial lipoproteins are a set of membrane proteins with much broad ranging functionality, cellular physiology through cell division and virulence. It has been shown to play key roles in adhesion to host cells, modulation of inflammatory processes and translocation of virulence factors into host cells. As such, a number of lipoproteins have been shown to be potential vaccines. Insect haemolymph contains a single major class of low to high density lipoproteins, called 'lipophorins' because of their role in lipid transport. In mammals, the importance of the innate immune system is now appreciated even more than before because recent findings have revealed that it is essential to make the adaptive immune responses active (Gamo, 1978). A group of structure-related proteins with a molecular mass of approximately 30 kDa accumulate in haemolymph of *B. mori* belong to the lipoprotein family (Gamo, 1978). 30 kDa proteins were found to be stored in the larval haemolymph of silkworms in a stage-dependent fashion in larval haemolymph of *B. mori*. In silkworm, the 30 kDa proteins are major plasma proteins which are detected in later stage of 4th instar larvae and it becomes a major haemolymph proteins in the early pupal stage because of progressive increase in expression after 3rd/4th day of 5th instar larvae, increased gradually as the silkworm grew, and became a major component of haemolymph in pupation (Gamo, 1978; Izumi *et al.*, 1984). At late pupa stage, proteins around 30 kDa began to decrease, almost disappearing during emergence from the cocoon. Except for sequence similarity between 30 kDa proteins and the micro-vitellogenin from *Manduca sexta* (Wang *et al.*, 1989), so far no 30 kDa proteins have been reported in any other lepidopterans, suggesting that they may be unique to specific insect species. Since some 30 kDa proteins (6G1 lipoprotein) of silkworm have been reported to have a role in defence against fungal infection (Ujita *et al.*, 2002, 2005), it is possible that the 30 kDa proteins may also participate in self-

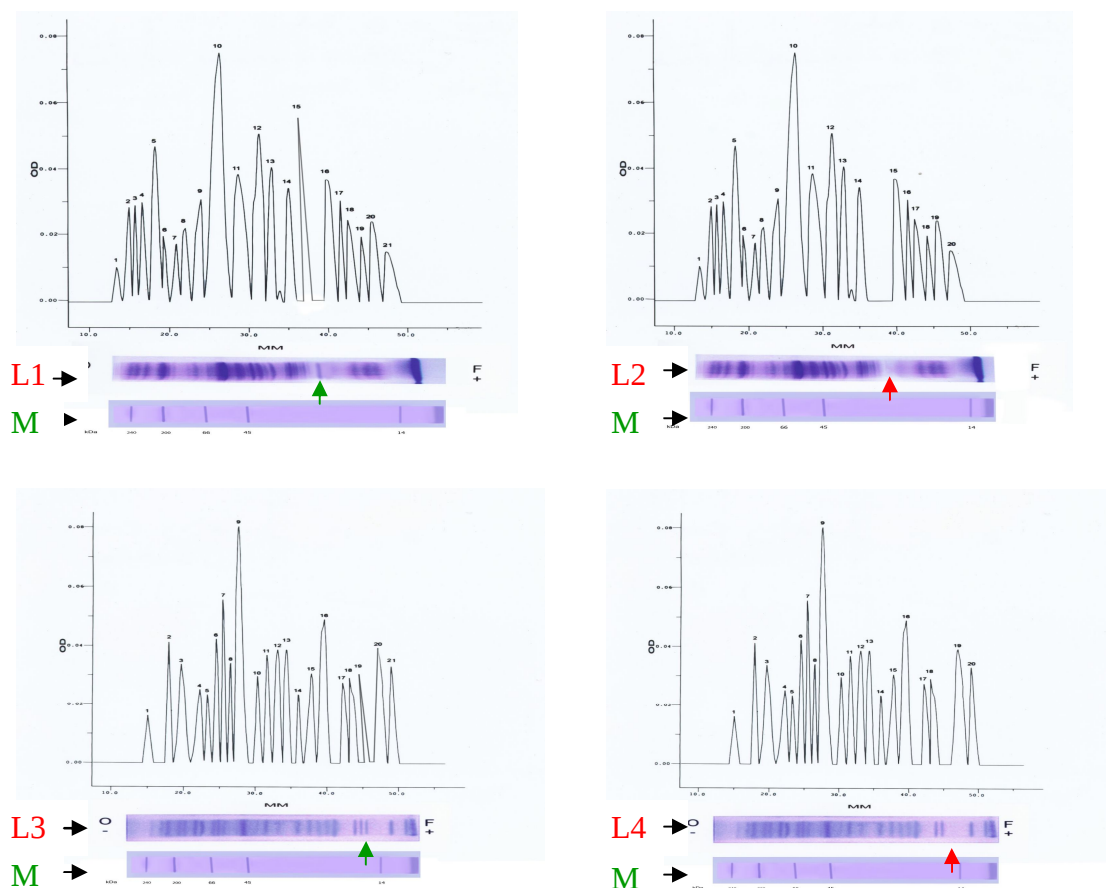


Fig. 6. SDS PAGE of Haemolymph protein a) Nistari control (Lane 1: 29 kDa protein is present indicated by arrow); b) Nistari *BmNPV* infected (Lane 2, (Lane 2: 29 kDa protein is absent indicated by arrow); c) NB4D2 control (Lane 3: 17 kDa protein is present indicated by arrow); d) NB4D2 *BmNPV* infected (Lane 4: 17 kDa protein is absent indicated by arrow). Marker indicated by M.

defence response. The general trend for them is that their expression started at 5th instar larval stage, increased steadily to maximum and declined gradually thereafter. The new finding of ~29kDa protein from insect haemolymph attracts for the exploration of above novel function. However, characterization of protein is under process.

It may be summarized that some of the existing antiviral proteins/lipoproteins (~30 kDa) present in the haemolymph of healthy silkworm protects the insect through innate immune system, which is destroyed due to *BmNPV* infection. The possibility of exploring such proteins in silkworm may help to resist viral infection during rearing to reduce huge economic loss. Further study on amino acid and gene sequence analysis are under way so as to identify the novel protein lost due to the *BmNPV* infection. Study on the proteins responsible for innate immune system is our goal towards control the 'Grasserie' disease in silkworm, as insect has no long time effect of adaptive immunity. Besides, innate immunity is the basis for adaptive immunity of higher vertebrates also.

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