Ultrastructural Changes in Midgut of CPV infected Tropical Tasar Silkworm, *Antheraea mylitta* (D) (Lepidoptera : Saturniidae)

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The tropical tasar silkworms, *Antheraea mylitta* (D) produce famous silk 'Kosa' in central part of India. Due to outdoor rearing it became susceptible to viral infection including cytoplasmic polyhedrosis virus (CPV). The common mode of entry of cytoplasmic polyhedrosis virus is per os and cause grasserie disease to the larvae. Histopathological studies elucidated the insect CPV virus produces infective polyhedral inclusion bodies (PIBs) in the midgut cell cytoplasm of virus infected fifth instar larvae. The PIBs multiply enormously in the cytoplasm without invading the nucleus. Ultrastructural studies confirmed the pathological effects of CPV on midgut cell cytoplasm. The multiplication of polyhedral inclusion bodies took place into the vacuoles and form virogenic stromata in the cytoplasm of cells. However, the encapsulations of polyhedral inclusion bodies into the polyhedrin protein occurred and polyhedra were released into the lumen. At the late stage of infection, cells showed the regressed cytoplasmic organelles with large vacuoles and elongated mitochondria. Hence, the horizontal transmission of CPV causing the midgut cells disintegration in the tasar silkworm, *Antheraea mylitta* (D) confirmed during infection.

Key words: *Antheraea mylitta* (D), Cytoplasmic polyhedrosis virus, Midgut, Polyhedral inclusion bodies

Introduction

Among the diseases, virosis (Grasserie) caused by cytoplasmic polyhedrosis virus (CPV) is very common which accounts for the loss of 25–30% of total cocoon crop of tasar silk industry (Sahay *et al*., 2000; Singh *et al*., 2006) and thus pose a serious problem in tasar sericulture. The appearance of polyhedral bodies of CPV was first time observed in the midgut cells of diseased silkworm larvae by Ishimori (1934) and Aruga (1971). Since then, cytoplasmic polyhedrosis has been recognized as one of the most important disease of silk-worm infecting substantial economic losses to the sericulture industry in silk producing countries (Aruga, 1971). Although the CPV have very wide range, they are affecting mainly the Lepidopteran insects (Martignoni and Iwai, 1991). The CPV remain in a stable infectious state in the environment even after death of infected host. A large number of progeny of CPV particles released due to liquid oozing from the body and became available as a source for transmission to other susceptible individuals (Richards *et al*., 1999). The released CPV remain viable to accomplish in part by the polyhedrin protein matrix that surrounds the infective units of the virions and provides some degree of protection against environmental degradation (Rohrmann, 1986a).

*A. mylitta* eco-races showed distinct insusceptibility to the preoral infection of a CPV to subdermal infections and the host resistance to preoral infections depending on inhibitory mechanism in the gut against the invasion of the virus into the midgut cells (Watanabe, 1971).

It has been reported that CPV may persist with its host in occult or latent form of infection through various natural stressors, crowding and temperature conditions (Steinhaus, 1958a, b; Tanada *et al*., 1964). Payne (1981) reviewed the mechanism and frequency of occurrence of such infection. An effective mechanism would ensure virus maintenance in low density population in order to avoid the vertical transmission of diseases. In the lepidopteran insects, vertical transmission was observed in armyworm, *Mythimna separata*, gypsy moth, *Lymantria dispar* (Shaprio and Robertson, 1987) and silkworm *Bombyx mori* (Khurad *et al*., 2004). The vertical transmission of TnCPV in *Trichoplusiani* population were observed during
rearing (Fuxa et al., 1992, 2002). The virus transmitted in the progeny either may kill the host larvae at an early stage or would latent form and express at late stage with favorable fluctuation in the environmental conditions.

Material and Methods

Tasar silkworm, *Antheraea mylitta* (D) is the principle non-mulberry silk producing insect and is cultivated in the tropical forest of India including the region of Bhandara, Chandrapur and Gadchiroli District of Vidarbha in the Maharashtra state. The non dipasuring cocoons were brought to the laboratory and rearing was carried out at the insectaries of the Department of Zoology, RTM Nagpur University, Nagpur.

Isolation and purification of cytoplasmic polyhedrosis virus (CPV)

The grasserie infected fifth instar larvae were collected from the rearing field and were triturated individually. The larvae were crushed in the pestle and mortar and the homogenate was filtered through muslin cloth. The filtrate was observed under the microscope for presence of PIBs in it. The homogenate was contaminated with other microbes and it was purified by repeated centrifugation until clear layer of PIBs was obtained. The isolated PIBs were stored in refrigerator until their use. The serial ten fold dilutions in distilled water were prepared initially. The 60 larvae starved for six hours and distributed in four trays. First two trays containing equal number of larvae were inoculated individually by providing 1 sq. cm piece of *Terminalia arjuna* leaves coated with 20 µl suspension (about 2000 PIBs) 1 x 10^5 PIBs /ml. The larvae of third and forth tray were also fed with 1 sq. cm piece of leaves dipped in distilled water and thereafter they were provided with fresh leaves. The larvae in the third and forth tray were treated as control and were placed for away from the inoculated ones. Both the inoculated and control larvae were scarified at varying intervals of 24 h, 48 h and 74 h and termed them as early, mid and late stage of infection.

Transmission Electron Microscopy (TEM)

The transmission electron microscopy was carried out at All India Institute of Medical Sciences, New Delhi. The midgut dissected gently and fixed in cold Karnovsky's fixative for 48 h. The tissues were washed repeatedly in cold phosphate buffer (pH-7.2, 0.1 M) and post fixed for one hour in Osmium tetroxide. The tissues were stained enbloc with 0.5% uranyl acetate during dehydration in graded alcohol and embedded in Araldite or Styrene- Methacrylate. The ultrathin sections were collected on pioloform coated grid after cutting with glass knife on LKB-4800A Ultramicrotome. At various stages 0.5 µm thick alternate sections were cut and observed at desirable magnification under Morgagni-200 transmission electron microscope (TEM).

Results

Isolation and purification of polyhedral inclusion bodies (PIBs)

The purified PIBs were observed under the light microscope. The PIBs were mostly hexahedral, tetragonal, spherical,icosahedral, colourless and highly retractile bodies measuring about 1.5 to 2.5 µm in diameter (Figs. 1a, b).

Histopathological effects of CPV virus on the midgut

In the fifth instar larvae of tasar silkworm, *Antheraea mylitta* (D), the midgut epithelium was greatly folded and rests upon a basement membrane. The cells of midgut epithelium were differentiated into three types, the columnar cells(CEC), the goblet cells(GC) and the regenerative cells(RC). The columnar cells were tall and closely associated with each other so that their boundaries were indistinct. They were interspersed apically with goblet and basally with regenerative cells. The columnar cells contain granular cytoplasm and fine brush border facing towards the lumen. The nuclei were large, spherical or elliptical and situated in the middle or apical half of the cells. The chromatin material well distinct and scattered.