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IMPACT OF NEEM GOLD ON ULTRA STRUCTURAL CHANGES IN THE ADULT MALE ACCESSORY REPRODUCTIVE GLANDS OF *ODONTOPUS VARICORNIS* (DIST.) (HEMIPTERA: PYRRHOCORIDAE).

Thankappan R. Kumar*, Sekar Sethuraman, Kanagaraj Anandaraj, Vaithiyanathan Iyyappan
Department of Zoology, Annamalai University, Annamalai Nagar, Tamilnadu, India.

A B S T R A C T

The male accessory reproductive glands of insect, in most cases the gland exhibits a single layered glandular epithelium surrounded by the basement membrane and muscular wall. They may be ectodermal in origin, when they known as ectodenia, and in this case they open into the ejaculatory duct, ectadenia, occur in coleopteran and possibly other groups. Glands of mesodermal origin mesadenia are found in Orthoptera. The primary function of these secretions is to facilitate sperm transfer; they may also act as barrier to further insemination. Electron microscopic studies on the adult male accessory reproductive glands of *Odontopus varicornis* insects have revealed presence of a single layer of columnar epithelium, characterized by the multi shaped endoplasmic reticulum, polymorphic golgi bodies and vesicles. The male accessory reproductive gland in control insects indicates its apocrine mode of secretion and its relative quantity of secretion appears to be higher than that of the glands of treated insects. A significance changes in the structure of the glands have been observed after the treatment of neem gold (25ppm median lethal concentration). It indicates the presence of numerous rough endoplasmic reticulum with cisternae which are considerably distended and induce fine granular products. The lumen of this gland reveals the presence of less granular secretary materials identical to the rough endoplasmic inclusions and less electron dense substance which are identical to golgi vesicles. The gland, further exhibits the mitochondria with numerous microvilli, swollen nucleus and secretary vesicles with multi vesicular bodies. The gland of the insects after treatment reveals the occurrence of the degenerative epithelium, shrunken nucleus and large number of smooth endoplasmic reticulum. It shows the absence of multi vesicular bodies indicating its less secretary activity. Based on the observations, it may be inferred that this gland involves itself in apocrine mode of secretion during reproductive cycle and this secretion seems to facilitate the transport of spermatozoa into female through seminal fluid and may contribute rich materials for the maturity and physiological activity of spermatozoa.

Keywords: Basement membrane, polymorphic, microvilli, cisternae, ectadenia, columnar epithelium, neem gold.

INTRODUCTION

Reproduction in insects is an essential physiological process from the view point of propagation of the insect species. The male and female reproductive system generally consists of paired gonads connected to a median duct leading to the gonophore. Accessory glands are often present with in the male are usually concerned with spermatophere formation and sperm maintenance and in the female provide glue sticking the eggs to the substratum or provide the substance for a complex egg case Gillott, (2003). In insects, the male accessory reproductive gland varies in number, size, shape and location Leopold, (1976) and Ranganathan, (1982). In *Odontopus varicornis* the male accessory reproductive gland lies at the postero median end of the abdominal ganglion and below the rectum. The morphology of accessory gland in adult reproductive system of *Odontopus varicornis* appears as pale white colour, transparent, short pear shaped body, supplied with tracheal tubes. It is made up of delicate, transparent tubules in separate coil around the seminal vesicle of the basal end of the vas deferentia and opens in to an ejaculatory duct Davey, (1985). The male accessory

* Corresponding Author:
Email: sethuraman65@gmail.com

reproductive glands of insects, in most cases, the gland exhibits a single layered glandular epithelium surrounded by basement membrane and muscular wall Cruz-Landim, (2008). They may be ectodermal in origin are known as ectadenia and in this case they open into the ejaculatory duct, occur in Coleopteran and possibly other groups. Glands of mesodermal origin, mesadenia are found in Orthroptera and in some cases Tenbrio for instance both ectadenia and mesadenia are present Chapman, (1972). In *Odontopus varicornis* both the apocrine and merocrine mode of secretion is seen in mated males and only apocrine is reported in virgin males. (Leopold, 1976 and Simmons, 2001). The primary function of this secretion is to facilitate sperm transfer, but they may also act as barrier to further insemination, either physically (or) by altering the behaviour of the female. In some cases the secretion may have some nutritional value for the females (or) they may accelerate oocyte maturation Leopold, (1976) and Simmons (2001). The secretion of the male accessory reproductive gland contains protein components which have a wide variety of functions Hinton, (1974); Leopold, (1976); Chen, (1984); and Happ, (1984). One of the major functions is in the assembly of spermatophore, a structure which serves as the vehicle for the transfer of sperms from male to female. The development and synthetic activity of the glands associates with the reproductive system of the male are regulated hormonally in several insect species Radhakrishnan and Taylor, (2007), (2008); Szopa *et al.,* (1985). The ultra-structural studies of MARGs are scanty in insects especially in Hemiptera. Hence this study has aimed to find out the ultra-structural changes in the MARGs of *Odontopus varicornis.*

MATERIAL AND METHODS

The male accessory reproductive gland (control and treated) was dissected and separated. The tissue was fixed in 3% glutaraldehyde, 0.1M cacodylate and 0.025% calcium chloride buffer (pH 7.4) for 3h at 4^0C. After washing the tissue thoroughly in the same buffer, it was post fixed in 1% aqueous osmium tetra oxide (OSO_4) for 2-3h. The tissue was washed again thoroughly in the same buffer and was dehydrated through a graded series and embedded in flat silicone mould for one hour. Thin sections were cut in LKB_U-Ultra microtome. Thin sections were double stained with uranyl acetate ads lead citrate for 5minutes. They were viewed under Philip's (Zoo) Holland transmission microscope (TEM) and electron photomicrographs were taken (Dallai *et al.,* 1999).

RESULTS AND DISCUSSION

The MARGs of insects after eclosion appear white and transparent. As it gain more secretary materials and receives sperm from the testis via vas deference, the gland appear full, grayish and opaque. The wall of mesadenes consists of long columnar epithelium with thin basement membrane and delicate muscle bands around the gland. An intricate network of intercellular spaces which can be considerably distended surrounds the basal parts of the cells. During maturation, it becomes more and more pronounced and extends further towards the apical to the blister like dilations extended septate desmosomes furnish the mechanical coherence of the epithelium (fig. 1). The nuclei are basally located, the epithelial cells are large, globular and swollen (fig. 2 &3). The most spectacular changes concern the rough endoplasmic reticulum (fig.4). The rough endoplasmic reticulum increase drastically at the days of maturation and occupies most of the cytoplasmic materials (fig. 5 and 6). The cisternae of rough endoplasmic reticulum are distended and enclosed a fine granular material.

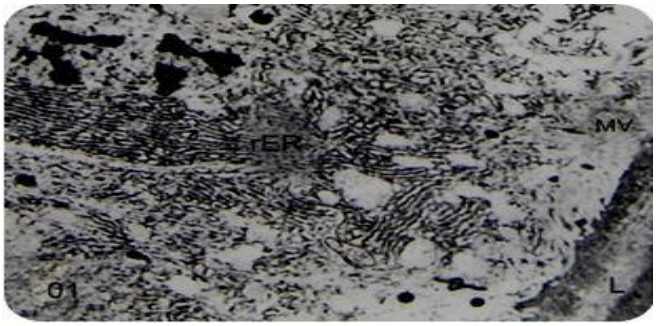

Figure 1. Uitrastructure of MARGs – of control insect (MV) – Microvilli (L) – lumen (rER) – rough endoplasmic reticulam.

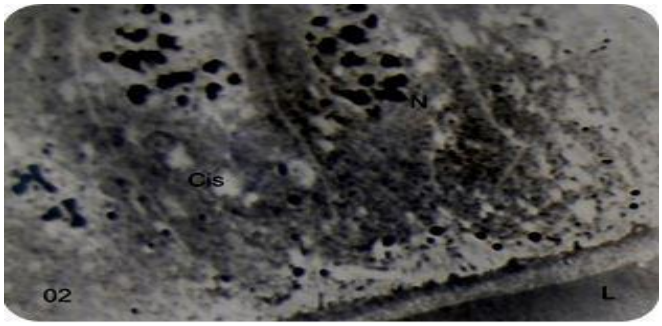

Figure 2. Ultrastructure of MARGs – of cotrol insect (Cis) – Cisternae (N) – nucleus (L) – lumen.

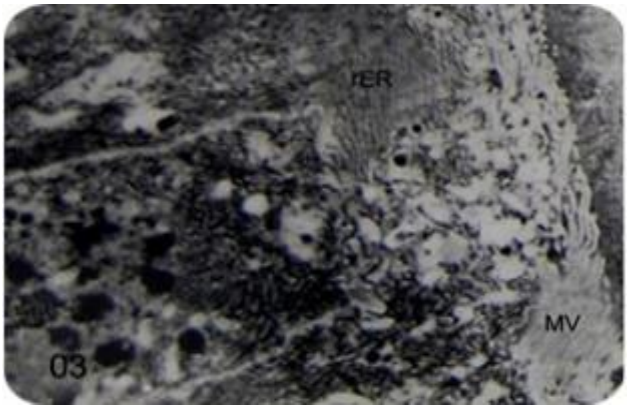

Figure 3. Ultrastructure of MARGs – of control insect (MV) – Microvilli (rER) – rough endoplasmic reticulam.

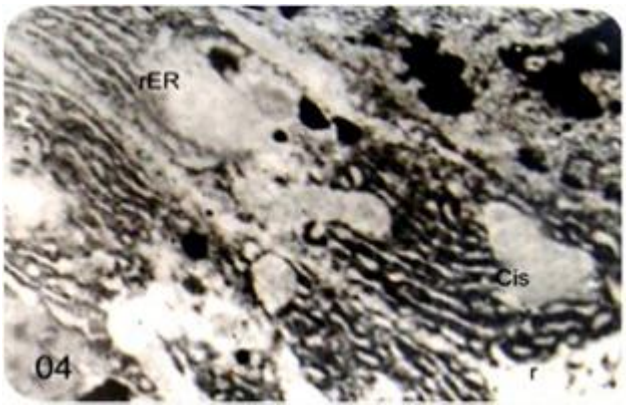

Figure 4. Ultrastructure of MARGs – of control insect (rER) – rough endoplasmic reticulam (Cis) – cisternae.

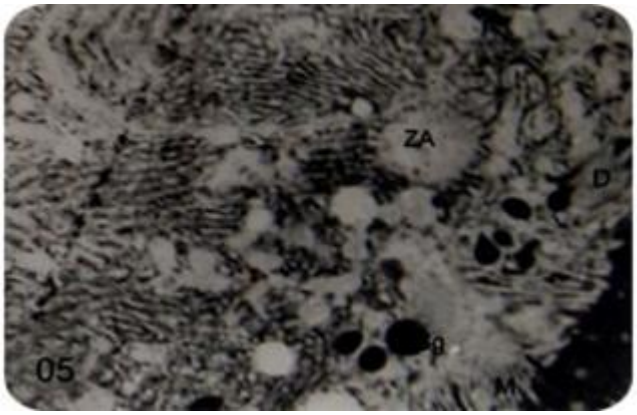

Figure 5. Ultrastructure of MARGs – of control insect (D) – desmosomes (M) – merocrine secrection (ZA) – Zonulae of adherence (SG) – Secretory globule.

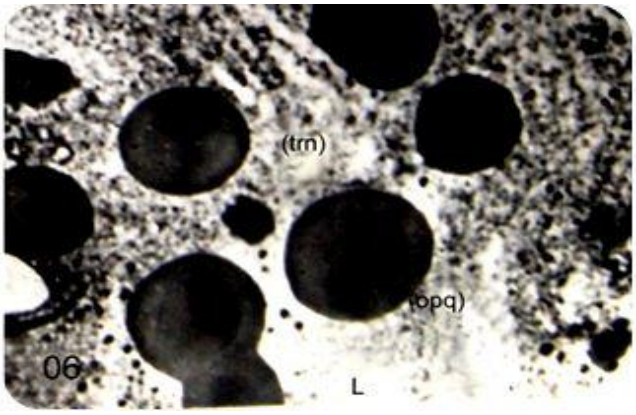

Figure 6. Ultrastructure of MARGs – of control insect (L) – Lumen (opq) – opaque (trn) – tranparent.

The MARGs contains lumen which has transparent and opaque secretion (fig. 7 & 8) which synthesis the secretary granules having an electron dense in the center and electron less core in the periphery (fig.8). The protein synthesized at the rER as represented by the swollen cisternae was directly transported to the adjacent golgi vesicles where in the golgi vesicles themselves transformed into membrane limited granules. Tongue *et al.,* (1972); and Craig, (1967) observed in the glands of the male *Culex pipens*, three types of granules and four types of secretary cells. In this insect the mode of secretion was mesocrine and apocrine. Apocrine was the most frequent mode of secretion various form of rER polymorphic golgi apparatus, many lysosomes, cored vesicles, numerous dense granules, microtubules were all in abundance in the process of secretion. De Marzo *et al.,* (1976); Marchini and Del Bene, (2003); Freitas *et al.,* (2007). Pinocytotic vesicles and phagosomes are numerous endocytosis of phagosomes and exocytosis of pinocytic vesicles are evident. Riemann and Thorson, (1976a). All these indicated the high secretary activity in the mesadene of control male insect. Further, the occurrence of disintegration of the epithelial cell of the gland concomitant with high secretary activity showing the flow of granules and clear globules towards the free cell surface in *Odontopus varicornis*, strongly suggest that the mesadenia is highly secretary in nature releasing their contents through apocrine and mesocrine mode of secretary activity. The protein content of the gland decreases significantly in neem gold treatment insects it appears that the secretion of the gland is involved in the transference of sperms to the female and perhaps in initiating the oviposition behavior. The secretion could play a role in sperm concentration and / or nourishing Simmons, (2001). The mitochondria are fewer in number (fig. 9 & 10). The number and length of the microvilli are less showing that the secretary activity of the cells is

evidently less. Golgi complex are few in number with less vesicles (fig. 9).The drastic changes seen in accessory gland is the occurrence of greatly reduced columnar cells due to loss of cytoplasm. The lumen is quite large and contains less secretary materials and cytoplasmic vacuoles (fig.9 & 10).

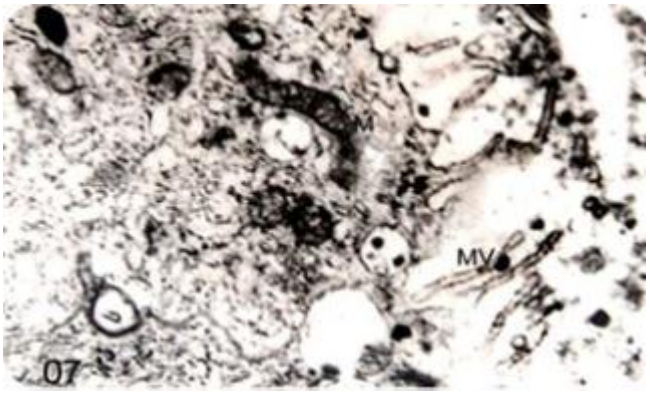

Figure 7. Ultrastructure of MARGs – of treated insect (MV) – Microvilli (M) – Mitochondria.

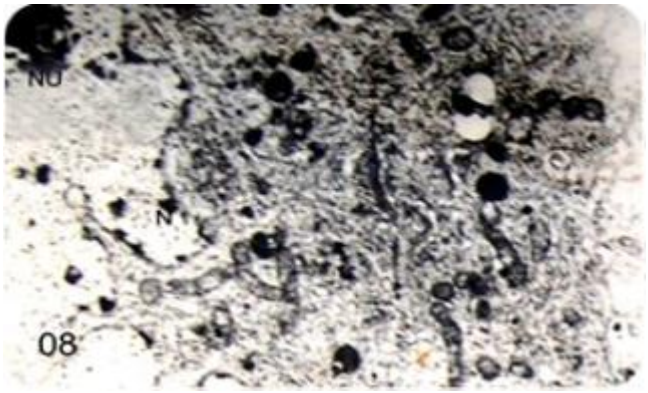

Figure 8. Ultrastructure of MARGs – of treated insect (N) - Nucleus (NU)- Nucleolus.

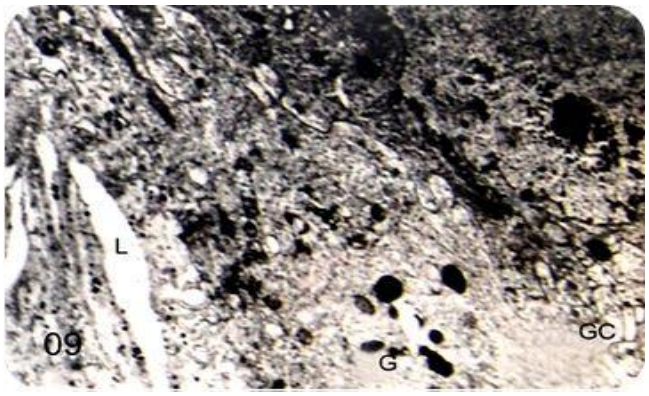

Figure 9. Ultrastructure of MARGs – of treated insect (GC) - Golgi complex (L) - Lumen.

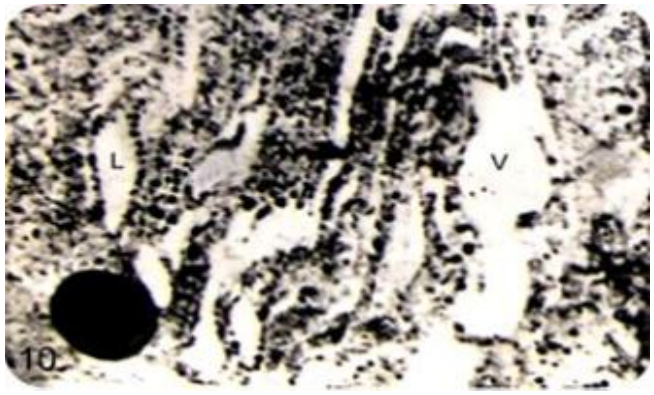

Figure 10. Ultrastructure of MARGs – of treated insect (V) - Vacuole (A) - Apocrine secrection (L) - Lumen.

It is known that the secretary substance of accessory reproductive glands seems to vary widely in physiological properties, biochemical composition and physiological function. According to Leopold (1976), the secretary substance of MARGs has certain components which may promote sperm maturation and provide nourishment to the stored sperms. The activation of sperms is one of the major functions of accessory reproductive gland Alberts *et al.,* (2004); Wedell and Cook, (1999). From these ultra-structural findings, it may be inferred that the lumen contains more vacuoles with less secretary materials of rER and golgi complex. Further, the lumen of the gland consists of less electron dense and electron lucent materials. Probably, the electron dense material consists of an outer transparent core and an inner dense core may be utilized during the stress condition. The transparent secretary material filled in the lumen perhaps rich in control insects than the treated one. These observations reveals that the neem gold prevents the secretary nature of the MARGs therefore, no nourishment to the stored sperms. It is inferred from the above findings that the neem gold seems to exert its action directly on the male accessory reproductive glands and reducing drastically the reproductive potential of the insect *Odontopus varicornis.*

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