The Bioscan 7(4): 705-709, 2012
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

EFFECT OF METHANOLIC EXTRACTS FROM THE LEAVES OF TULSI (*OCIMUM SANCTUM*) ON THE OVARY OF *GONOCEPHALUM BRACHYELYTRA* (KASZAB), (COLEOPTERA: TENEBRIONIDAE)

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KEY WORDS
Extract
Gonocephalum
Ovary
Tulsi

Received on :
13.07.2012

Accepted on :
04.10.2012

ABSTRACT
In *Gonocephalum brachyelytra* (Kaszab), a pair of about 3.64mm long and white ovary is found. Each ovary is composed of twenty eight to thirty eight numbers of ovarioles. The ovarioles are clearly differentiated into terminal filament, germarium and vitellarium. The germarium possesses four to five developing oocytes and trpohocytes which provide nourishment to the developing oocytes. The vitellarium possesses developing oocyte, maturing oocyte and large yolky globules *i.e.* resorptive oocyte. In the control ovary each ovariole shows normal release of oogonial cells after maturation. Whereas, the insect's ovariole treated with the methanolic tulsi extract releases less amount of oogonial cells. Increase in intercellular spaces observed between the trophocytes. The trophocytes shows their contracting nature. The yolky materials of oocyte show distortion. Clumping of mature oocyte also shows sign of decrease in oogenesis. The intercellular spaces provide pathway for protein to enter into the cytoplasmic area, but not into the nuclear region. It checks the active protein deposition and decrease the process of mitosis and meiosis. As a result prevention in the phenomenon of oviposition is observed. In the present study the extract of tulsi shows antioogenetic activity.

INTRODUCTION

The ovaries have two functions- oogenesis and steroidogenesis that ultimately leads to ovulation. Histologically the ovariole and oogenesis have been studied by several researchers (Gross, 1901; Kohler, 1907; Bonhag and Wick, 1953; Davis, 1956; Natalie *et al.,* 2006; Jeffrey *et al.,* 2011). A detailed study of the oogenesis is called for the determination of germarium *i.e.* actually the trophocytes or the oogonial cells (Bonhag, 1958). Martoza (1964) described short type of germarium and vitellarium. Coleoptera possess telotrophic ovaries in which nurse cells are aggregated terminally in the germarium. Each oocyte is connected to the germarium by a cytoplasmic extension, the trophic cord. The germarium contains trophocytes that provide nourishment to the developing oocyte, maturing oocyte and large yolky globules or resorptive oocyte.

The hormonal regulation of spermatogenesis and oogenesis is also interfered by the environmental toxic chemicals. So many histological studies are present in mammals but not in invertebrate species although for the control of insects numerous leaf extracts are applied. The present study is the first one undertaken to look the structure of ovariole of *Gonocephalum brachyelytra* contributing female high reproductive potential adapted to a system of reproduction and to see the methanolic effect of tulsi on it.

MATERIALS AND METHODS

G. brachyelytra (Kaszab) adult specimens were collected from the local infesting food storages, rice mills and flour mills at Varanasi, India. Cultures were established and maintained as stored food products at 30 ± 2ºC inside the glass jar covered with pieces of muslin cloth fixed with rubber bands. All the insects were cultured under low density condition to ensure proper development and equal size of resultant adults. The insects were reared for two generations on a 50:50 mixture of wheat flour and rice grain. Larvae, pupae and adults were measured and examined carefully. When the larvae pupated, pupae were harvested and examined daily till the formation of adults. For experimental work three groups of same age insects were taken from the culture in three separate glass jar. Two glass jar insects were sprayed with fresh methanolic extract of Tulsi to see their effect on the ovaries of insects. The controlled insects were kept without spraying the extract.

The reproductive organ of insects were dissected out in Insect Ringer and fixed in a variety of fixatives like aquous Bouin's, Allen-Bouin's, Zenker etc. Borax carmine preparation of the entire reproductive system and of the individual component was made after fixation. Paraffin blocks were prepared for histological and histochemical studies. Sections were cut at the thickness of four to seven micron. For the study of histological preparation Mayer's haematoxylin-eosin, Iron haematoxylin-eosin and Brome phenol blue has been employed and studied under light microscopy.

Plant material: Fresh leaves of *Ocimum santum* (Tulsi) were collected from local area of Varanasi city. Specimen sample were deposited in the laboratory. Collected leaves were oven dried at 40º Cover night and then crushed in a crucible and

weighted separately.

Methanolic extract: For methanolic extracts samples of the crushed leaves (5g of Tulsi) were soaked separately in 100ml of methanol in a sealed container at 4ºC for two days. The sample was then filtered and centrifuged for the aqueous extract.

RESULTS

The female reproductive system of *G. brachyelytra* Kaszab (Fig. 1) consists of paired ovaries, a pair of lateral oviducts, a common median oviduct, spermathica, spermathecal accessory gland and an ovipositor. Other accessory glands and bursa copulatrix is absent.

Both the ovaries are connected with a pair of lateral oviducts. The lateral oviduct opens into the common median oviduct which runs posteriorly to open into a genital chamber. The genital chamber forms a tube like structure, the vagina (Fig. 1). It works as aedeagus receiving organ. Spermatheca (the storage organ of sperm) is found on the other sides of vagina. A spermathecal duct originates from the spermatheca.

A pair of long and white ovary found, one on either side of the middle line in the abdominal cavity from the sixth abdominal ganglion up to the middle of third abdominal segment. Each ovary is composed of twenty eight to thirty eight ovarioles. The type of ovarioles is acrotrophic, which are arranged on the dorso-median side of the common duct (Fig. 1).

The space found in between the ovarioles are filled with adipose tissue in which tracheal ramification also penetrates. It is an elongated thread like organ, broad proximally and tapering to a point at the free end (Fig. 1). It contains a chain of developing ova (Fig. 2). The ovarioles are enclosed in outer modified fatty tissue and inner layer of tunica propria. The ovariole is clearly differentiated into terminal filament, germarium, and vitellerium.

Each ovariole is extended anteriorly into fine filamentous structure known as terminal filament. The terminal filament is long, delicate thread like anterior prolongation of the wall of the ovariole, which serves to hold the gonad in its position (Fig. 1). Each ovariole possesses apically placed long germarium (Fig. 3) and four to five developing oocytes (Fig. 2), where one or two developing oocytes lie at the base of germarium (Fig. 3). The germarium also contains trophocytes (Fig. 5). The function of the trophocytes is to provide nourishment to the developing oocyte.

The germarium in its turn is recognizable into two distinct zones. Zone-I has a thin layer of cytoplasm. At the zone-I oogonial cells are not found (Fig. 4). In the zone-II trophocytes are found (Fig. 4). It is to some extent bigger in size. Trophocytes are compact and sometimes assume polygonal outline (Fig. 5). The nuclei are very large and spherical with coarsely granular chromatin and a large number of basophilic nucleoli. In cytoplasm of germarium, a number of nuclear extrusions are present; these are darkly stained with haematoxyline (Fig. 4). The zone II shows amitotic divisions of the trophocytes nuclei, sometimes the trophocytes appear to be binucleate (Fig. 5). The two nuclei are enclosed within distinct nuclear membrane but both have common cell membrane. The lower portion of germarium shows developing oocytes (Fig. 3). At the lower end oocytes are surrounded by many follicular cells which later on reduce to one layer of columnar cells. Some of the oocytes show mitotic activity. The oocyte of zone II shows a large nucleus with somewhat clumped chromatin arranged in the centre of the nucleus (Fig. 4).

Each developing oocyte show ooplasm with yolky material and excentrically placed germinal vesicle. Oocytes can be

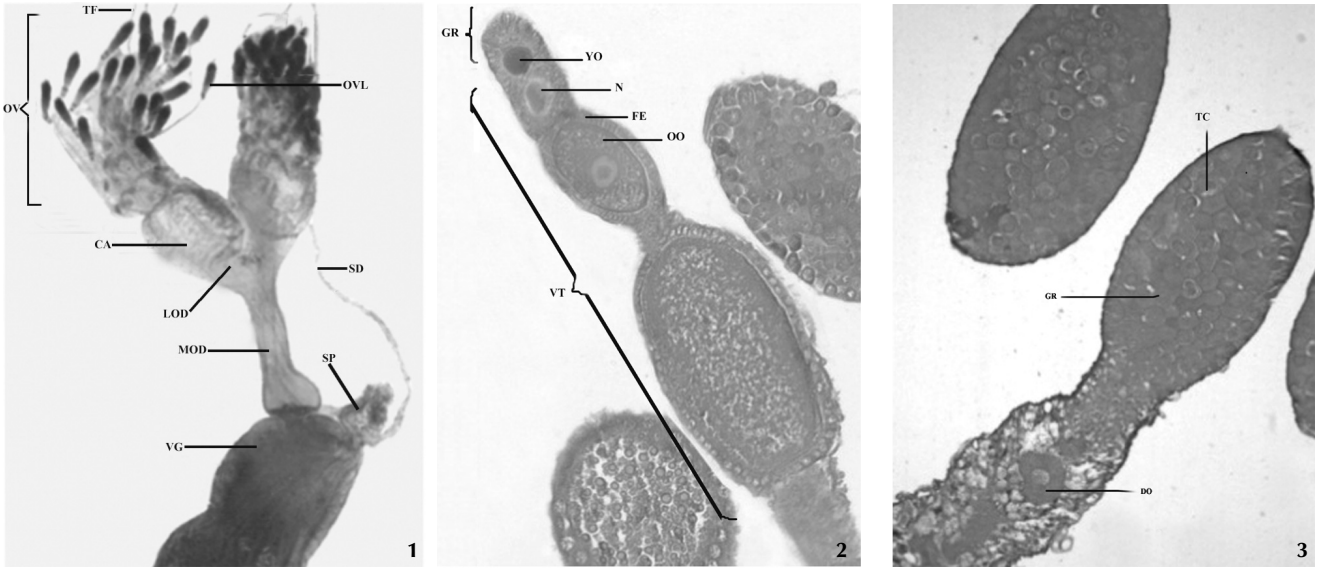

Figure 1 to 3: (1) W.M. of Female reproductive system of *G. brachyelytra* (Kaszab). Terminal filament (TF), Ovariole (OVL), Ovary(OV), Calyx (CA), Lateral Oviduct (LOD), Median Oviduct (MOD), Spermathecal duct (SD), Spermatheca (SP), Vagina (VG). (Aq. Bouin's/Borax carmin); (2) L.S. of ovary *G. brachyelytra* (Kaszab). Germarium (GR), Vitellarium (VT), Youngest oocyte (YO), Nucleus (N), Follicular epithelium (FE), Oocyte (OO).(Aq. Bouin's/HE-eosin X 40); (3) L.S. of ovariole showing germarium and developing oocyte. Germarium (GR), Trophocyte (TC), Developing oocyte (DO). (Aq. Bouin's/HE-eosin X 110)

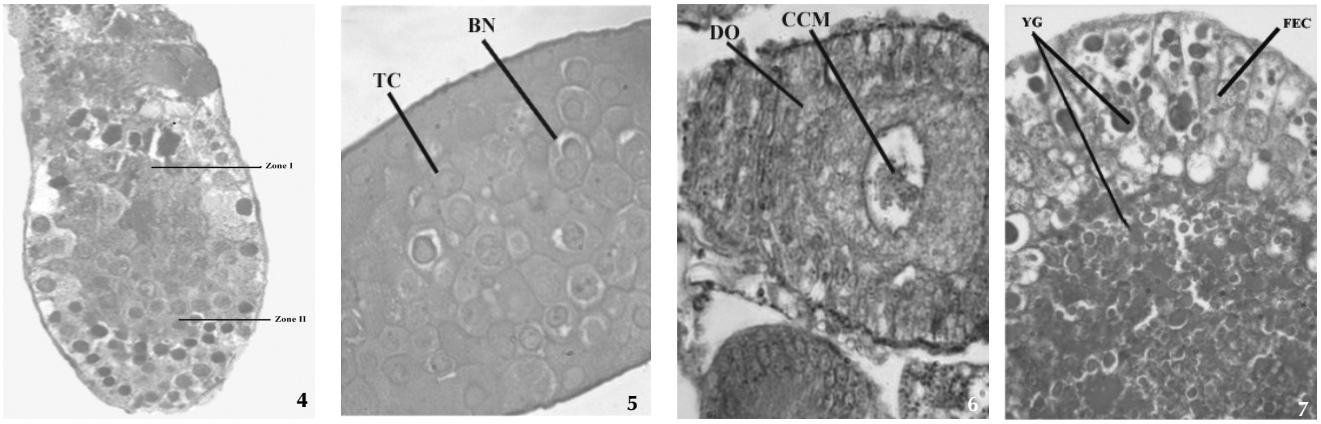

Figure 4 to 8: (4) L.S. of germarium at immature stage showing two distinct zone. Zone I without oogonial cell and Zone II shows trophocytes. (Aq. Bouin's/HE-eosin X 110); (5) L.S. of germarium at mature stage showing Trophocyte (TC) with polygonal outline and binucleat condition (BN).(Aq. Bouin's/HE-eosin X 110); (6) L.S. of developing oocyte (DO) showing clumped chromatin material (CCM) inside the germinal vesicle. (Aq. Bouin's/Brome phenol blue X 110); (7) L.S. of oocyte showing large number of yolk globules (YG) and cuboid follicular epithelial cells (CEC).(Aq. Bouin's/HE-eosin X 110); (8) Resorptive oocyte showing yolk globules (YG) in the follicular epithelial cells (FEC). (Aq. Bouin's/ HEeosin X 400)

differentiated into three main categories, i.e. developing oocyte, maturing oocyte having large yolky material, and atretic or resorptive oocyte filled with large yolky globules (Fig. 8). The follicular epithelium of developing oocyte is cuboidal in shape (Fig. 6), where each cell has single nucleus. The matured yolky oocyte have yolk material inside the follicular cells itself (Fig. 7). The atretic oocyte shows wide space in between the follicular cells and the yolk material which are slowly resorbed in the oocyte body (Fig. 8).

After twenty four hours, the treated ovary shows fewer amounts of oogonial cells than control one. The nuclear membrane becomes darkly stained with haematoxylin eosin stain, contraction of granular chromatin and less number of basophilic nuclei was observed. In the cells of zone-II trophocytes show intercellular space between them (Fig. 10). The germarium possesses trophocytes showing their contracting nature. This shows that with Tulsi treated extract ribonucleoprotein particles are released quickly to the developing oocyte as indicated by hematoxyline (Fig. 10). These cells are comparatively larger than of the control ones. The haemolymph protein passes through inter follicular space in between follicular cells in the developing oocyte (Fig. 9).

The germinal vesicle contains large nucleolus, but it shows liberation of very small nucleoli. These nucleoli pass from the nucleoplasm to the outer ooplasm. The developing oocyte surrounded by the follicular epithelial cells show disintegration. The young oocyte does not have developed space surrounding ooplasmic zone after twenty four hours of treatment with Tulsi extract. The yolky materials are found in lesser amount inside it. The mature oocyte possesses yolky material while atretic oocyte shows little space in between the follicular cells. In the Tulsi treated ovary germinal vesicle of

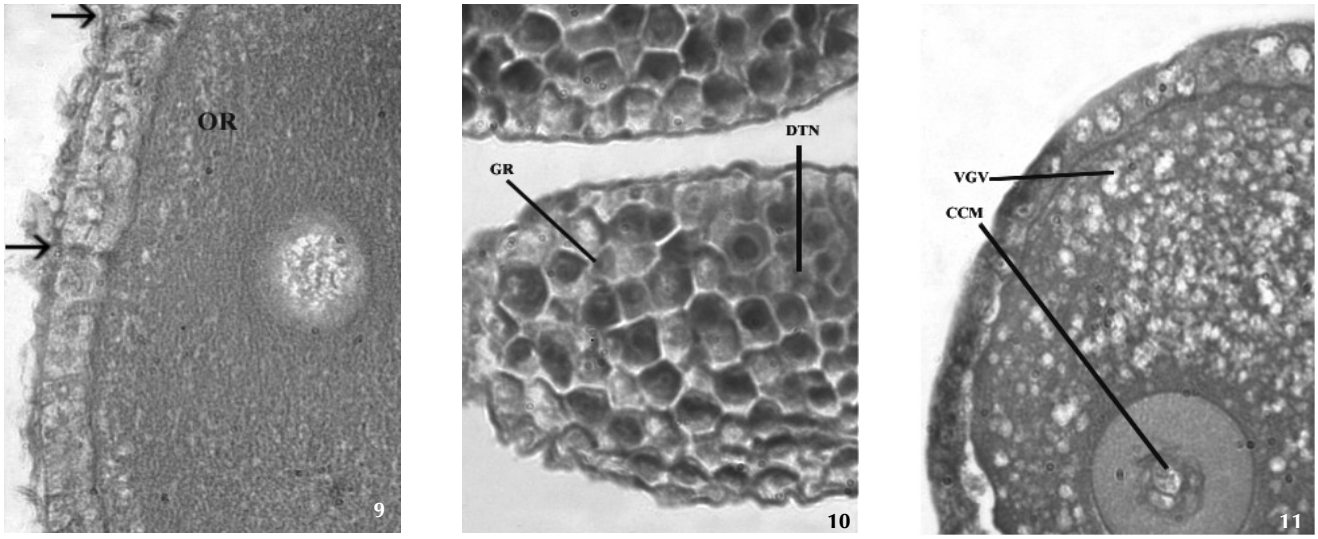

Figure 9 to 11: (9)L.S. of 24 h. Tulsi treated mature oocyte showing heamolyph protein (→) entering in to ooplasmic region (OR) via intercellular spaces to the ooplasm. (Aq. Bouin's/HE-eosin X 110); (10) L.S. of 48 h. Tulsi treated germarium (GR) showing large intercellular spaces and distortion of trophocyte nuclei (DTN).(Aq. Bouin's/HE-eosin X 110); (11) L.S. of 48 h. Tulsi treated maturing oocyte showing clumping in the chromatin material (CCM) and vacuolated germinal vesicle (VGV).(Aq. Bouin's/HE-eosin X 110)

atretic oocyte show large nucleoli which are vacuolated in nature (Fig. 11). Quick resorption of yolk material is found in the matured oocyte. The ooplasm possesses distortive nature of yolky material. The yolky materials possess distortive nature in the oocyte body. After forty eight hours of new treatment, matured oocyte shows clumping in the nuclear material (Fig. 11). Between the follicular epithelial cells, intercellular spaces were observed.

DISCUSSION

Tillyard (1917) and Matsuda (1976) stated that the ovarioles are held closely together by a thick sheath. But such thick sheath was not observed in the present study. The trophocytes may be uninucleated or they may be binucleated as observed earlier in *Hydrophilus olivacious* by Gundevia and Ramamurty (1972). Trophocytes present in germarium of *G. brachyelytra* (Kaszab) are of varying size seen throughout the germarium.

The trophocyte nuclei of zone II increase in number and size as compared to those of zone I. In the zone II, chromatin materials are also increased in amount. In hemipterous telotrophic ovarioles greatly branched appearance of nuclei were observed in *Panorpa* by Ramamurty (1963). The shape of nuclei has its own importance because it attributes its endomitotic polyploid growth. In the zone II of germarium amitotic divisions were observed. Such divisions give rise binucleate conditions. This observation was similar to that of *Hydrophilus olivaceous* described by Gundevia and Ramamurty (1972). But such condition does not seem to have been reported for trophocyte nuclei, although such a phenomenon was known to occur in the follicle cells of the milk weed bug, *Oncopellus* (Bonhag and Wick, 1953) and also in the panoistic ovarioles of grasshoppers. The zone II shows increase in its size as compared to those of zone I and zone II. This seems that they are involved in some cyclical activities, although their total disruption could not be noticed. All this indicates the growth of oocytes.

In the vitellarium younger oocytes are placed close to the germerium as seen earliar by De wilde and De loof, (1973); Inamdar and Joshi, (1984); De wet, (1989), meaning there by the trophocytes are rich in RNA and protein which supply these materials to the developing oocyte through trophic cores during vitellogenesis i.e. the time of yolk deposition. Follicular cells become cuboidal, intercellular spaces develop in between them. Such results were also seen by Burnett (1971), and Ramamurty (1969). Ramamurty found changes in follicular epithelial cells in his ultrastructural studies of *Panorpa* during vitellogenesis. The main yolk protein enters in to the follicular epithelial cell through interfollicular spaces in the developing oocyte.

The *Ocimum santum* treated ovary shows damage of trophocytes. Similar finding had been observed by Sahayaraj *et al*. (2012) in the ovary of *Dysdercus cingulatus* treated with *Acalypha indica* and *Tephrosia purpurea*. The treated vitellogenic follicles of ovary showed increase in intrafollicular spaces. In between the oocyte and follicular cells, spaces are also observed. The change took place in the follicular epithelium and oocytes. It affects the activity of yolk protein. The yolk protein vitellogeny synthesize at fat bodies, was described by Pan *et al*. (1969) in *Periplaneta Americana* and by Gelti Douke *et al*. (1974) in *Drosophila melanogaster*. The yolky materials reflect distortion after treatment with Tulsi extract. Shekhari *et al*. (2008) showed that the treatment of *Xanthogaleruca luteola* with *Artemisia annua* decreases amount of protein in the oocytes. Clumping of mature oocyte shows sign of decrease in oogenesis. Similar thing is earlier observed by Schmutterer (1990). The intercellular spaces provide pathway to protein for entering into the cytoplasmic area but not into the nuclear region. It checks the active protein deposition and decreases the process of mitosis and meiosis. Sreelatha *et al*. (2010) also reported that oocyte maturation and vitellogenesis were disrupted in *Oryctes rhinoceros* when treated from extract of *Eupatorium odoratum* leaves. It prevents the phenomenon of oviposition. In this study it appears that extract of tulsi possesses antioogenic activity. Nagarjun *et al*. (1989) have reported that the extract of leaves of *Ocimum santum* (Linn.) shows antifertility activity in the female rats. Antifertility effect of *Ocimum santum* leaves has also been reported earlier by Kashinathan *et al*. (1972) when given in high amount. The disturbance in the number of ovary had been also described by Madhvi *et al*. (2012) in the ovary of *Corcyra celphalonica* when treated *with Vitex negundo*.

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