Normal histology of *Dysdercus similis*

The ovaries of adult *Dysdercus similis* are quite big and well developed. The total length of the ovary is 12mm. Each ovary is composed of several ovarioles. The apical filaments of each ovary unite anteriorly to form a long suspensory ligament which remains attached to prosternal apophysis of the thorax. A ovariole consists of the terminal filament, germarium and vitellarium. Comprise the egg tube which contains the developing germ cells and accessory tissues. The pedicel remain attached to the ovariole by the lateral oviduct.

Each ovariole is covered by an outer epithelial sheath. At the anterior end of the ovariole the outer epithelium sheath is multilayered. This epithelial sheath becomes thinner in the posterior region near the pedicel. The nuclei of the outer epithelial sheath are elliptical containing coarse chromatin particle.

The terminal filament is long and synchiutium contains irregularly arranged oval nuclei.

The germarium is the anterior most chamber of the ovariole which contains trophocytes, oogonia, young oocytes and prefollicular tissue. A large part of the germarium remains occupied by the trophocytes. Four zones can be easily recognized in the germarium of *Dysdercus*.

Zone-I: The undifferentiated cells are abundant and they show all the stage of mitotic activity.

Zone-II: is the transitional region of trophocyte differentiation.

Zone–III: is characteristic due to fully differentiated trophic tissue.

Zone- IV: the trophic nuclei of this zone remain arranged in the form of small aggregates.

At the posterior end of the trophic core a number of cytoplasmic projections are found which ultimately form the nutritive cords. These cords remains attached to
the developing oocytes. They are mainly responsible for the supply of nutritive substances of the trophocytes to the developing oocytes.

The young oocytes remain embedded in a mass of nucleated cytoplasm which occupies the posterior part of the germarium and extends up to the vitellarium. The young oocytes can be differentiated from the oogonia by the help of their size and attachments with the nutritive cords.

The vitellarium follows the germarium and contains a series of oocytes arranged in a single row. Due to the enlargement of oocytes the vitellarium is distended which forms a series of follicles which become progressively larger towards the posterior end. In the eight days old adult *Dysdercus* ovary there are usually 11-12 oocytes. There is a marked increase in size of the oocyte from the anterior to the posterior region of the ovariole.

**Normal Histology of the Adipose Tissue of Adult *Dysdercus similis* (Freeman):**

The adipose tissue or the fat body of insects is a definite organ (Wigglesworth, 1965). In insect it is found from the very early stages. Two layers of the fat body can be distinguished - peripheral zone is close to the hypodermis and the second, a more central position around the visceral organs. The body tissue is covered by the adipose tissue. The adipose tissue of the deeper and superficial layer is identical; the size of the cell is about 19.4μ to 20.8μ. While the nucleus is about 8.3 μ to 9.3 μ in size. The outline of the nuclear membrane is wavy.

The central globules are conspicuous and are numerous. These globules are protein positive. The peripheral globules are of uniform size and are distributed throughout the cytoplasm. These are protein positive in nature. The area adjacent to the nucleus is shown protein positive reaction. Water vacuoles are absent.

**Control Series:** The control insects of the present investigation do not show any deviation from the normal histology in terms of the ovary and adipose tissue.
Histological Observation (Haematoxylin-Eosin) in the Adipose Tissue of *Dysdercus similis*

**Control Group: Ovary of the female *Dysdercus similis***

**Ovary of 2 days old female:** At two days in the ovary, the germarium were showing trophocytes, oocytes and an active mitotic cell division. At this stage oocytes were already shifted in the vitellarium where they were surrounded by follicular epithelium and interfollicular tissue. Follicular epithelial cells were mostly columnar. In the pedicel interfollicular tissue was very prominent. The migration of oocytes into the vitellarium were observed at this early stage. No yolk synthesis was noticed at this stage. The nucleus was towards the periphery. The Tunica propria was attached to Follicular epithelium (Fig. 1).

**Ovary of 4 days old female:** In the Germarium nutritive chords were clearly visible. More oocytes were come out into the vitellarium. Ovary was increased in length. Ten to eleven oocytes were visible at this stage. Follicular epithelium was cubical. The yolk synthesis was started near the periphery of the oocyte. More deposition of granules was started in the terminal oocytes. The nucleus was towards the periphery. Chorion layer still not formed but vacuolization was noticed (Fig.2).

**Ovary of 6 days old female:** The follicular epithelial cells were cubical with large nucleus. The yolk synthesis was in progress. Yolk granules were deposited in all oocytes. Chorion formation was started at this stage, vacuoles were also observed (Fig.3).

**Ovary of 8 days old female:** In oocytes the yolk synthesis and chorion formation was completed. The follicular epithelial cells were flattened. Some cells were noticed with double nuclei. The interfollicular bridges were completely broken (Fig.4).
Histological observation:

**Treated Group: Effect of 0.01µg/µl JHA on the ovary of female Dysdercus similis**

**Ovary of 2 days old female:** After two days of treatment with 0.01µg/µl JHA the germarium was found to be degenerated cells causing appearance of vacuoles. Detachment of follicular epithelium was also begun. The follicular epithelial cells were columnar. The vitellarium contained developing oocytes. The ovary was smaller in size as compared to those in the control group of same age. The ooplasm was cleaned, yolk granules were not observed and the nucleus was present towards the periphery. The vacuoles were observed in the ooplasm. The tunica propria was attached with follicular epithelium cells (Fig. 5).

**Ovary of 4 days old female:** After four days of treatment, the cells of germarium were not properly formed into trophocyte, young oogonia and prefollicular tissue, the vacuolization was also increased. The nutritive chords were clearly visible. The follicular epithelium was thick and its cells were pycnotic. The ooplasm of developing oocyte were with large vacuoles. The germinal vesicle was small in size. Here the ooplasm was contracted and shrunk, but it was granular due to which free spaces were formed in between follicular epithelium and ooplasm (Fig.6).

**Ovary of 6 days old female:** The germarium was found to be more vacuolar and large numbers of loosely arranged trophocytes were noted. The follicular epithelium was thin (columnar cells) and broken at few places. The vitellarium contained largely number of oocytes. Due to shrinkage of ooplasm free spaces were formed between the follicular epithelium and ooplasm. The chorion formation was started but it gets ruptured at some places due to shrinkage of ooplasm. The terminal oocytes were showing oosorption (Fig. 7).
Ovary of 8 days old female: After 8 days of treatment with 0.01µg/µlJHA loosening of the cells of germarium was noted and the germarium was found to be shrunk. The follicular epithelium was fragmented and the oocytes were not intact with the ovariole. Here, the oocytes were observed with much vacuolization and little deposition of yolk was found as compared to corresponding control group of the same age. The Yolk was accumulated to the centre of the oocyte (Fig. 8).
Histological observation:

**Treated Group: Effect of 0.03µg/µl JHA on the ovary of female Dysdercus similis**

**Ovary of 2 days old female:** After two days of treatment with 0.03µg/µl JHA, the germarium appeared spongy due to loosening of cells. These cells were not clearly differentiated. Vacuoles were also seen in germarium. The cells were with degenerated nuclei, the cytoplasm was also vacuolated.

The vitellarium possessed undifferentiated oocytes, these oocytes were with vacuolated and shrunk ooplasm. Follicular epithelium was thick and its detachment from oocyte was also noted. Thus creating spaces in between follicular epithelium and oocyte. The interfollicular epithelial bridges were distorted and broken in some part of ovariole (Fig. 9).

**Ovary of 4 days old female:** The germarium was not showing much difference as compared to the previous dose of same age days but in the oocyte the small spherical vacuoles were observed which less in quantity as compared to the previous dose. The oocyte was shrunk and contracted due to that the space between the follicular epithelium and oocyte was increased than the previous day of treatment. (Fig. 10)

**Ovary of 6 days old female:** In the germarium loosening of cells were observed due to which large vacuoles were formed. In the oocyte yolk deposition was observed but the chorion formation was not observed and shrinkage of ooplasm was also noted. The terminal oocytes were shown oosorption. Follicular epithelium was shown folds around to the ooplasm as a result of these larger vacuoles were formed in the centre of the oocytes (Fig. 11).

**Ovary of 8 days old female:** After eight days of treatment, the yolk deposition was very less and the follicular epithelium was shown folds. The terminal two to three oocytes were showing oosorption still the chorion was not formed (Fig. 12).
Histological observation:

Treated Group: Effect of 0.002µg/µl ecdysone on the ovary of female *Dysdercus similis*

**Ovary of 2 days old female:** After two days of treatment with 0.002µg/µl ecdysone, the germarium was showing the loosening of cells and the germarium looked more spongy. The follicular epithelium was thick. In the developing oocyte the germinal vesicle was present towards the periphery and the nutritive cords were clearly visible. The vitellarium was larger in size with 8 to 9 developing oocytes as like to control ovary of same age (Fig. 13).

**Ovary of 4 days old female:** The germarium contained large number of trophocytes, oogonia and nuclei. The yolk synthesis and the follicular epithelium were in progress. The follicular epithelium was thick cubical and the chorion formation was started at this stage (Fig. 14).

**Ovary of 6 days old female:** The germarium was not affected and the vitellarium was shown mature oocytes which was fully deposited by yolk granules and it was surrounded by chorion (Fig. 15).

**Ovary of 8 days old female:** After eight days of treatment egg laying was started.
Histological observation:

**Treated Group: Effect of 0.03µg/µl ecdysone on the ovary of female *Dysdercus similis***

**Ovary of 2 days old female:** After two days of treatment with 0.03µg/µl ecdysone the size of the ovary and oocytes was smaller as compared to those of control group of same age. The germarium was showing vacuolization. The nutritive chords were broken at various places. The shrinkage of oocyte was observed at the periphery of the terminal oocyte. Follicular epithelium was thick with columnar cells. The nucleus was present in the centre of the oocyte (Fig. 16).

**Ovary of 4 days old female:** After four days of treatment, the germarium was showing loosely arranged cells due to which vacuoles were seen more than the previous day of treatment. The trophocytes were with more irregular cell boundaries due to fragmented nuclei and cytoplasm. In vitellarium the terminal oocyte was shown oosorption while the fourth and fifth oocytes were shown shrinkage towards the periphery. The shrinkage was more than the previous day. The follicular epithelium was thick and follicular cells were columnar still the yolk was not deposited into the oocyte, the nucleus was at the centre of the oocyte. The tunica propria was detached from the oocyte (Fig. 17).

**Ovary of 6 days old female:** The germarium was found to be more spongy due to loosening of trophocytes and it caused vacuolization. The shrinkage of the oocytes was increased than previous dose. The yolk deposition was very less as compared to the control group of same age (Fig. 18).

**Ovary of 8 days old female:** After eight days of treatment the germarium as well as vitellarium both was damaged by this dose. The oocyte was found to be more damaged because the ooplasm was shown shrinkage due to which the yolk was accumulated in the centre. The follicular epithelium was broken at various sites still the chorion was not formed. The free spaces were increased in between follicular epithelium as compared previous dose and the vacuoles were also increased (Fig. 19).
Histological observation:

Treated Group: Effect of 0.75µg/µl ecdysone on the ovary of female *Dysdercus similis*

**Ovary of 2 days old female:** After two days of treatment with 0.75µg/µl ecdysone the gerarium was showing loosely arranged trophocyte, young oocytes, and prefollicular tissue. The vacuolization was also noticed. The developing oocytes were smaller than those seen in the corresponding controls. The follicular epithelium was thick (Fig. 20).

**Ovary of 4 days old female:** After four days of treatment, the gerarium was showing loosely arranged cells due to which vacuoles were seen more than the previous days of treatment. In the oocyte the ooplasm was shrunk, vacuolated and fragmented. The follicular epithelium was thick and fragmented at some places (Fig. 21).

**Ovary of 6 days old female:** After six days of treatment the degeneration of trophocytes were observed due to which the gerarium was more vacuolar. The developing oocyte was showing fragmentation of the ooplasm. Still the yolk was not deposited in the oocyte. The follicular epithelium was damaged very much and the follicular cells were converted into syncitium like structure (Fig. 22).

**Ovary of 8 days old female:** After eight days of treatment with 0.75µg/µl ecdysone the deposition of yolk was very less and it accumulated in the centre of the oocyte. The structure of the oocyte became irregular which was not oval or spherical because the follicular epithelium was showing folds at various places. At the folded region the follicular epithelium was thick or multilayered and the cells were cubical but at the regular size it was thin single layered and the cells were flattened (Fig. 23).
Histological observation:

Treated Group: Effect of 1.5µg/µl ecdysone on the ovary of female *Dysdercus similis*

**Ovary of 2 days old female:** After treatment with 1.5µg/µl ecdysone the size of the ovary was reduced as compared to the control group. The germarium was shown degeneration of cells due to which vacuolization was increased. The nutritive chords were broken but the ooplasm was cleaned and shown oblique bands (Fig.24).

**Ovary of 4 days old female:** The germarium and vitellarium both were affected and shown shrinkage which was very much same as the six days of the corresponding previous dose. Fragmentation of ooplasm was also observed in the oocyte. The follicular epithelium was ruptured at various places. Free spaces were formed in between ooplasm and follicular epithelium. The shapes of the oocyte get disturbed. The germarium was degenerated and reduced at greater extent (Fig. 25).

**Ovary of 6 days old female:** The oocytes were found to be more fragmented than the previous day of treatment with same dose. Still the yolk deposition was not observed. The follicular epithelium was flattened and broken down at various places. The ooplasm was accumulated at the centre of the oocyte (Fig. 26).

**Ovary of 8 days old female:** The ovary was same as like the previous day of treatment with same dose, but the ooplasm reduced at greater extent. The follicular epithelium was found to be thicker (Fig. 27).
Histochemical Observation for detection of protein (Mercuric Bromophenol blue technique) in ovarian tissue of *Dysdercus similis*

**Control group: Ovary of female *Dysdercus similis***

**Ovary of 2 days old female:** The germarium of the two days control ovary had shown strong reaction with mercuric bromophenol blue stain and the vitellarium was showing moderate reaction. The oocytes were cleaned (Fig. 28).

**Ovary of 4 days old female:** At four days the germarium was shown with trophic core and nutritive cords which were moderately protein positive. Here the protein yolk deposition was started and it was strongly protein positive, the vacuolization was also observed it was protein negative. The follicular epithelium was thick and moderately protein positive. The germinal vesicle was toward the periphery (Fig. 29).

**Ovary of 6 days old female:** The germarium was moderately protein positive while the trophic nuclei were strongly protein positive. In vitellarium the yolk deposition was in progress and the protein positive yolk bodies were deposited all over the oocytes. The yolk spherules were of various sizes, $G_1, G_2, G_3$. The vacuoles were also observed these were protein negative. The follicular epithelium was cubical with prominent nuclei and it was shown moderate reaction. Here the chorion formation was started (Fig. 30).

**Ovary of 8 days old female:** At eight days the yolk deposition was almost completed and the yolk was strongly protein positive. The chorion formation was also completed and it was strongly protein positive (Fig. 31).
Histochemical observation for protein

Treated Group: Effect of 0.01µg/µl JHA on the ovary of female *Dysdercus similis*

**Ovary of 2 days old female:** After two days of treatment with 0.01 µg/µl JHA the germarium was shown moderate reaction with mercuric bromophenol blue. In the vitellarium the oocytes were cleaned and it was also moderately protein positive (Fig. 32).

**Ovary of 4 days old female:** After four days of treatment with 0.01 µg/µl JHA in the germarium, the nucleus of trophocytes and cell boundaries were strongly protein positive, while the nutritive chords and trophic core were moderately protein positive and the vacuolization occurred in the ooplasm was protein negative. The follicular epithelium was thick and was shown pycnotic nuclei, which was strongly protein positive. The protein positive yolk granules were still not deposited in the ooplasm (Fig. 33).

**Ovary of 6 days old female:** After six days of treatment with 0.01µg/µl fenoxycarb the germarium was moderately protein positive, but the vacuoles were protein negative. Here in the vitellarium the strongly protein positive yolk granules were deposited which were less in number as compared to the control group of the same age. The follicular epithelium was thin with flattened cells and it was strongly protein positive, while the ooplasm of the oocyte was accumulated into the centre of the ooplasm due to shrinkage free spaces were formed in between them which were protein negative. The chorion formation was not started (Fig. 34).

**Ovary of 8 days old female:** After eight days of treatment the yolk was accumulated at the centre of the ooccyte and the follicular epithelium was shown irregular structure around the yolk and the chorion was formed completely. The yolk was deposited very less but it was strongly protein positive as compared to control group of same age (Fig. 35).
Histochemical observation for protein

Treated Group: Effect of 0.03µg/µl JHA on the ovary of female *Dysdercus similis*

**Ovary of 2 days old female:** After two days of treatment with 0.03µg/µl JHA the ovary was found to be moderately protein positive and the ooplasm was clear. The detachment of ooplasm from follicular epithelium was noted (Fig. 36).

**Ovary of 4 days old female:** After four days of treatment the germarium was showing moderate reaction with protein and the space was formed by shrinkage of ooplasm it was protein negative. Here the strongly protein positive yolk granules were started to deposit towards the periphery of the oocyte. The follicular epithelium was cubical and strongly protein positive. (Fig.37)

**Ovary of 6 days old female:** After six days of treatment, the ooplasm was shown strongly protein positive yolk granules but they were deposited less as compared to corresponding control. The vacuoles were increased into the ooplasm and it was protein negative as well as the shrinkage of the ooplasm was also increased. Still the chorion formation was not observed (Fig. 38).

**Ovary of 8 days old female:** After eight days of treatment the yolk deposition was less as compared to corresponding control group and the previous dose of treatment with 0.01 µg/µl JHA with same age the follicular epithelium shown folds and the oosorption was also observed (Fig. 39).
Histochemical Observation for protein

Treated Group: Effect of 0.002µg/µl Ecdysone on the ovary of female *Dysdercus similis*

**Ovary of 2 days old female:** After two days of treatment with 0.002µg/µl ecdysone the germarium was strongly protein positive. The vacuoles and the intercellular spaces in the germarium were protein negative. In the vitellarium the developing oocytes were shown vacuolization which was protein negative and the germinal vesicle was towards the periphery, it was moderately protein positive. The follicular epithelium was thick and strongly protein positive (Fig. 40).

**Ovary of 4 days old female:** The germarium was strongly protein positive. The proteinous yolk granules were deposited towards the periphery which was strongly protein positive. The vacuolization was occurred and it was protein negative. The follicular epithelium was cubical in nature and was strongly protein positive. (Fig. 41).

**Ovary of 6 days old female:** After six days of treatment with 0.002µg/µl ecdysone the vitellarium was shown mature oocytes which were completely filled with protein yolk bodies and were strongly protein positive. The chorion formation was completed. The follicular epithelium was single layered and strongly protein positive (Fig. 42).

**Ovary of 8 days old female:** After eight days of treatment with 0.002µg/µl ecdysone egg laying was started.
Histochemical Observation for protein

**Treated Group: Effect of 0.03µg/µl Ecdysone on the ovary of female *Dysdercus similis***

**Ovary of 2 days old female:** After two days of treatment with 0.03µg/µl ecdysone, the size of the ovary was become smaller as compared to the previous dose with 0.002µg/µl ecdysone. The trophocytes were completely packed and strongly protein positive. The ooplasm of developing oocytes were cleaned and moderately protein positive. But shrinkage of ooplasm was also observed (Fig. 43).

**Ovary of 4 days old female:** After four days of treatment with 0.03µg/µl ecdysone in the germarium the loosening of cells were observed and vacuolization was occurred, but the germarium was strongly protein positive while the vacuoles were protein negative. The germinal vesicle was towards the periphery and rich in chromatin material and it was strongly protein positive. The deposition of strongly protein positive yolk bodies were more toward the periphery and nucleus of the oocyte. Here the shrinkage of the ooplasm was increased due to which free space was formed in between the ooplasm and follicular epithelium which was thin and moderately protein positive (Fig. 44).

**Ovary of 6 days old female:** After six days of treatment the germarium was found to be more spongy but it was strongly protein positive. While the vacuoles were protein negative. The shrinkage of ooplasm was increased than the previous day. The follicular epithelium was broken at various places but it was strongly protein positive. Here the yolk deposition was same as that of previous day (Fig. 45).

**Ovary of 8 days old female:** After eight days of treatment due to shrinking the yolk was accumulated at the centre of the oocyte. The yolk deposited was very less as compared to the control group.
Histochemical Observation for protein

Treated Group: Effect of 0.75µg/µl Ecdysone on the ovary of female Dysdercus similis

**Ovary of 2 days old female:** After two days of treatment with 0.75µg/µl ecdysone, the germarium was strongly protein positive, the germarium was also shown vacuoles due to loosening of trophocytes which was protein negative. The developing oocyte was smaller in size as compared to the corresponding controls. The oocyte was clear the follicular epithelium was thick and moderately protein positive (Fig. 46).

**Ovary of 4 days old female:** After four days of treatment, the germarium was shown degeneration of cells but it was moderately protein positive. The oocyte was shown protein positive granules at the periphery. Here the ooplasm was shrunk and the yolk deposition was less as compared to control as well as the previous dose of same days.

**Ovary of 6 days old female:** After six days of treatment, the degeneration of trophocytes was increased therefore they stain moderately. The proteinous yolk was deposited less than the previous day of treatment and it was moderately protein positive. The shrinkage was also increased than the previous day. The follicular epithelium was broken down at various places and it was thick and weakly protein positive (Fig. 47).

**Ovary of 8 days old female:** After eight days of treatment with 0.75µg/µl ecdysone, the ovary was moderately protein positive and the oocyte was become irregular in structure due to over shrinkage of ooplasm. The yolk contain was very less. Still the chorion formation was not observed the yolk was weakly protein positive (Fig.48).
Histochemical Observation for protein

Treated Group: Effect of 1.5µg/µl Ecdysone on the ovary of female

*Dysdercus similis*

**Ovary of 2 days old female:** After two days of treatment with 1.5µg/µl ecdysone, the germarium was shown degeneration of trophocytes due to which very few trophocytes were present and hence vacuolization was increased. But it was shown very strong reaction with protein. The ooplasm was cleaned at the centre and was strongly protein positive. The follicular epithelium was thick and moderately protein positive (Fig. 49).

**Ovary of 4 days old female:** After four days of treatment, the germarium as well as vitellarium both were affected by this dose at this day of treatment. The shrinkage of ooplasm was increased tremendously and due to which the ooplasm get fragmented. The protein positive yolk was still not deposited (Fig.50).

**Ovary of 6 days old female:** After six days of treatment the shrinkage of the ooplasm was increased very much and the yolk deposition was very less it was moderately protein positive (Fig. 51).

**Ovary of 8 days old female:** After eight days of treatment with this highest dose. The germarium was shrunk and the vacuolization was increased at greater extent and it was weakly protein positive. The oocytes were shown shrinkage of ooplasm and the yolk was accumulated at the centre, the yolk was moderately protein positive. Due to shrinkage of ooplasm the free spaces were formed and it was protein positive (Fig. 52).
Histochemical observation for detection of lipid (Sudan black-B) in ovarian tissue of *Dysdercus similis*

**Control group: Ovary of female *Dysdercus similis***

**Ovary of 2 days old female:** At two days in the germarium the trophocyte, oocytes and a lot of mitotic cells were observed which was shown weak reaction with sudan black B. In the vitellarium the developing oocytes were cleaned and sudan positive, but it was shown weak reaction. The follicular epithelium was multilayered and was shown weak reaction. The germinal vesicle was shown weak reaction with sudan black B (Fig. 53).

**Ovary of 4 days old female:** In four days ovary the germarium was sudan black B positive and was shown moderate reaction while the trophic tissue and the pycnotic nuclei were also was shown moderate reaction. The vitellarium was found to be bigger in size because number of oocytes transferred into it, the yolk deposition was started at the periphery which was sudan black B positive and it was shown strong reaction. The ooplasm was shown vacuolization which was sudan black B negative. The follicular epithelium was thick and the cells were cubical and it was shown moderate reaction. The germinal vesicle was towards the periphery and it was shown weak reaction (Fig. 54).

**Ovary of 6 days old female:** In the oocyte the vacuoles were observed it were sudanophobic while the yolk deposited lipid bodies were sudan positive and it was shown strong reaction. The lipid yolk spherules were with various sized i.e. L1, L2, L3 was noted. The follicular epithelium was cubical and binucleated which was shown weak reaction. The chorion formation was started and it was shown strong reaction with sudan black B (Fig. 55).

**Ovary of 8 days old female:** On eight days in oocyte the yolk synthesis was almost completed and the chorion was formed and was shown strong reaction with sudan black. The lipid yolk droplet was larger in size and was shown moderate reaction, while the chorion was shown strong reaction with sudan black (Fig. 56).
**Histochemical observation for lipid:**

**Treated Group: Effect of 0.01µg/µl JHA on the ovary of female *Dysdercus similis***

**Ovary of 2 days old female:** After two days of treatment with 0.01µg/µl JHA, the germarium was shown weak reaction with sudan black B. The cell boundaries of trophocytes, trophic core, and nutritive chords were shown weak reaction. But the vacuoles were formed due to degeneration of trophocyte it was sudan negative. The germinal vesicle was at the center of the oocyte and it was shown weak reaction with sudan black B. The follicular epithelium was shown moderate reaction. The ooplasm was clean and was shown weak reaction with sudan black B (Fig. 57).

**Ovary of 4 days old female:** After four days of treatment the germarium was shown weak reaction. In the vitellarium yolk deposition was started in the terminal oocytes. The ooplasm was granular and it was shown moderate reaction, while some of them were shown strong reaction. Due to the shrinkage of ooplasm the space was formed in between follicular epithelium and ooplasm it was shown negative reaction. The nucleus was shown weak reaction. The follicular epithelium was thick and the cells were cubical in nature. Still the chorion formation was not started (Fig. 58).

**Ovary of 6 days old female:** The germarium was found to be more vacuolar and larger numbers of loosely arranged trophocytes were noted. The trophocytes were shown moderate reaction with sudan black B while the vacuoles were sudan negative. In the vitellarium the growing oocytes were observed and the deposition of yolk granules were started, some of them was shown moderate reaction while few of them were strongly sudan positive. The follicular epithelium was broken down at various places and had flattened cells. The free spaces were formed in between the ooplasm and follicular epithelium due to shrinkage of ooplasm and it was sudan negative. Due
to shrinkage the ooplasm was accumulated in the centre of the oocyte. The chorion formation was started. The ooplasm was vacuolated and the vacuoles were sudan negative (Fig. 59).

**Ovary of 8 days old female:** After 8 days of treatment with 0.01µg/µIJHA, the oocytes were shrunken and due to shrinkage the shape of the oocytes became irregular and the yolk was accumulated in the centre. The lipid yolk bodies were shown moderate reaction but very few of them were shown strong reaction. The oocytes were observed with different L₁, L₂ lipid bodies because the larger sized lipid spherules were broken down into L₁ and L₂. The yolk deposition was very less as compared to control group of same age. The follicular epithelium was shown strong reaction with sudan black B (Fig. 60).
Histochemical observation for lipid

Treated Group: Effect of 0.03µg/µl JHA on the ovary of female *Dysdercus similis*

**Ovary of 2 days old female:** After two days of treatment with 0.03µg/µl JHA, the germarium appeared spongy due to loosening of cells and it was shown weak reaction with sudan black. The vacuoles were also seen in the germarium which were sudan black B negative.

The vitellarium was possessed undifferentiated oocytes. The oocytes were with vacuolated and shrunk ooplasm. The vacuoles were sudan negative while the shrunk ooplasm was moderately sudan black positive. The follicular epithelium was shown weak reaction it was thick and detached from oocyte. Thus spaces in between follicular epithelium and oocyte were sudan black B negative (Fig. 61).

**Ovary of 4 days old female:** The germarium did not show any change and it was sudan positive but was shown weak reaction and it was found to be more vacuolar. The small spherical vacuoles were observed in oocyte which were less in quantity as compared to the previous dose and it was sudan negative. The oocytes were shrunken and contracted due to that the space between the follicular epithelium and oocyte was increased than the previous days of treatment and the space was sudan negative (Fig. 62).

**Ovary of 6 days old female:** In the germarium loosening of cells were observed due to which large vacuoles were formed. The germarium was shown weak reaction and the vacuoles were sudan negative. In the oocyte little yolk deposition was observed towards the periphery. The yolk was moderately sudan black B positive, the shrinkage of ooplasm was seen at various places. The terminal oocytes were shown oosorption. Still the chorion formation was not observed. The follicular epithelium was strongly sudan positive and shown folds (Fig. 63).

**Ovary of 8 days old female:** The yolk deposition was very less. The follicular epithelium was shown folds and oosorption. It was shown moderate reaction with sudan Balck B the free spaces were formed in between Follicular epithelium and ooplasm was sudan negative (Fig. 64).
Histochemical observation for lipid

Treated Group: Effect of 0.002µg/µl Ecdysone on the ovary of female *Dysdercus similis*

**Ovary of 2 days old female:** After two days of treatment with 0.002µg/µl ecdysone. The gerarium was shown loosening of cells due to that it looked more spongy and was shown weak reaction with sudan black B. Large number of vacuoles were observed in the oocyte which were sudan negative. The nutritive chords were seen clearly visible and was shown weak reaction. The germinal vesicle was towards the periphery and was shown moderate reaction (Fig. 65).

**Ovary of 4 days old female:** The gerarium contained large number of trophocyte, oogonia and nuclei which were sudan black B positive nature and was shown moderate reaction. The oocyte had small as well as large vacuoles and the yolk deposited all over the oocyte which was shown moderate reaction. The follicular epithelium was thick and was shown moderate reaction and the chorion formation started at this stage (Fig. 66).

**Ovary of 6 days old female:** The gerarium did not show any difference and was shown weak reaction. The vitellarium was shown mature oocytes which were fully deposited by yolk granules and was shown moderate reaction with sudan black B. Chorion formation was almost completed and it was strongly sudan positive (Fig. 67).

**Ovary of 8 days old female:** Egg laying was started at this stage or days of treatment.
Histochemical observation for lipid

Treated Group: Effect of 0.03µg/µl ecdysone on the ovary of female Dysdercus similis

Ovary of 2 days old female: After two days of treatment with 0.03µg/µl ecdysone the size of the ovary was smaller as compared to those in the control group of the same age and it was shown weak reaction with sudan black. But some or few nuclei in the germarium were shown moderate reaction with sudan black. The terminal oocyte was shown shrinkage towards the periphery (Fig. 68).

Ovary of 4 days old female: After four days of treatment, the germarium was shown weak reaction while the vacuoles were formed was shown negative reaction with sudan black. The pycnotic nuclei were shown moderate reaction in the germarium. The nutritive chords was also was shown weak reaction. The terminal oocyte was shown oosoption while the fourth and fifth oocyte was shown shrinkage toward the periphery. The shrinkage was more than the previous days of treatment. The nucleus was towards the periphery. The nuclear membrane was not so distinct and the chromatin material was sudan positive and was shown weak reaction. The follicular epithelium was thin with flattened cells and show weak reaction with sudan black. The space formed in between follicular epithelium and ooplasm was sudan B negative (Fig. 69).

Ovary of 6 days old female: The germarium as well as vitellarium both were damaged by this dose. The germarium was shown weak reaction. While the band like structure formed into the oocytes due to shrinkage and accumulation of ooplasm and it was shown weak reaction with sudan black. The follicular epithelium was broken down at various places and was shown weak reaction (Fig. 71).
Histochemical observation for lipid:

Treated Group: Effect of 0.75µg/µl ecdysone on the ovary of female *Dysdercus similis*

**Ovary of 2 days old female:** After two days of treatment with 0.75µg/µl ecdysone the germarium was shown loosely arranged trophocytes, young oocytes and prefollicular tissue. Here some trophocytes were strongly sudan positive while some of them were moderately positive. The pychnotic nuclei were strongly sudan positive and rest of the reason was shown weak reaction. The vacuoles were sudan negative. The developing oocytes were smaller in size than those seen in the corresponding controls. The oocyte was shown weak reaction and the follicular epithelium was thick and it was shown moderate reaction. Free space was formed in between ooplasm and follicular epithelium caused by shrinkage of ooplasm which was sudan negative (Fig. 72).

**Ovary of 4 days old female:** The whole germarium was shown weak reaction with sudan black B. The vacuolization were increased and it was sudan negative. In the vitellarium the oocytes were was shown shrinkage due to which the ooplasm get contracted and converted into a oblique band like fragment and was shown weak reaction. The follicular epithelium was thick but broken down at various places and was shown weak reaction (Fig. 73).

**Ovary of 6 days old female:** The degeneration of trophocyte was observed due to that the germarium was more vacuolar hence was shown weak reaction while the vacuoles were sudan negative. The shrinkage of the oocyte was increased than the previous days of treatment. There was no any significant change with sudan. Still the lipid positive yolk deposition was not started in the oocyte. The follicular epithelium was damaged and it also was shown weak reaction with sudan black B (Fig. 74).

**Ovary of 8 days old female:** After eight days of treatment with 0.75µg/µl ecdysone the ovary was shown weak reaction with sudan black B. the yolk deposition in the oocyte was very less and it was weakly sudan negative (Fig. 75).
Histochemical observation for lipid:

**Treated Group: Effect of 1.5µg/µl ecdysone on the ovary of female Dysdercus similis**

**Ovary of 2 days old female:** After two days of treatment with 1.5µg/µl ecdysone the size of the ovary became smaller as compared to control group. The germarium as well as vitellarium both was shown weak reaction with sudan black B (Fig. 76).

**Ovary of 4 days old female:** After four days of treatment with 1.5µg/µl ecdysone the germarium and vitellarium both was affected and was shown weak reaction but only follicular epithelium was shown moderate reaction. Nucleus was shown strong reaction (Fig. 77).

**Ovary of 6 days old female:** In the developing oocytes still sudan positive yolk deposition was not observed and therefore it was shown weak reaction. The ooplasm was shown shrinkage and due to which oblique bands of ooplasm was formed. The follicular epithelium was also broken at some places. The vacuoles were increased and it was sudan B negative, the interfollicular bridges were also broken (Fig. 78).

**Ovary of 8 days old female:** After eight days of treatment with 1.5µg/µl ecdysone the germarium was shown degeneration of cells and it was shown weak reaction with sudan balck B. The oocytes were shown accumulation of ooplasm at the centre. Still the yolk was not deposited hence the ooplasm was weakly sudan negative. The follicular epithelium was thick and was shown strong reaction. The nucleus was sudan negative. Still the chorion formation was not observed, and the free spaces were formed in between follicular epithelium and ooplasm, it was sudan negative (Fig. 79).
Histochemical observation for detection of carbohydrates in the ovarian tissue of Dysdercus similis:

Control group: Ovary of female Dysdercus similis

Ovary of 2 days old female: The two days control ovary was shown, trophocytes, oocytes and a lot of mitotic cells in the germarium which was PAS positive and was shown weak reaction with PAS. In the vitellarium the developing oocytes were clear and were shown weak reaction. The follicular epithelium was thick and shows strong reaction with PAS (Fig. 80).

Ovary of 4 days old female: In the fourth days of control ovary the germarium had large number of pycnotic nuclei which was moderately PAS positive. The trophocyte, trophic core, nutritive chords were clearly visible but it was weakly PAS positive. The prefollicular tissue was surrounded to the young oocytes and it was also weakly PAS positive.

In the primary oocyte the follicular epithelium was multi layered and the ooplasm was cleaned and was weakly PAS positive. But In the remaining oocytes the PAS positive yolk deposition was started towards the periphery and it was shown strong reaction with PAS. The follicular epithelium was cubical with prominent nuclei. The vacuoles were appeared at this stage and it was PAS negative (Fig. 81).

Ovary of 6 days old female: In the six days ovary the yolk deposition was in progress. The PAS positive yolk granules were with various sizes like G₁, G₂, G₃ all were was shown strong reaction with PAS. The vacuoles were also observed which PAS negative were. The follicular epithelium was cubical and binucleated the vacuoles were observed into the follicular cells which were PAS negative. Here the chorion formation was started (Fig. 82).

Ovary of 8 days old female: In the oocytes the deposition of strongly PAS positive yolk bodies were completed and the chorion formation was also completed it was weakly PAS positive (Fig. 83).
Histochemical observation for Carbohydrate:

Treated Group: Effect of 0.01\(\mu g/\mu l\) JHA on the ovary of female

*Dysdercus similis*

**Ovary of 2 days old female** - After two days of treatment with 0.01\(\mu g/\mu l\) JHA, the germarium was shown moderate reaction with PAS. Few pycnotic nuclei were seen in germarium and were shown moderate reaction with PAS. The developing oocytes were shown clear ooplasm with weak reaction. The nucleus was towards the periphery of the oocyte. The follicular epithelium was columnar with prominent nuclei and moderately PAS positive nature (Fig. 84).

**Ovary of 4 days old female**: After four days of treatment, the germarium was shown weak reaction with PAS, while the pycnotic nuclei were shown moderate reaction. The vacuoles appeared in the germarium were PAS negative. In the vitellarium strongly PAS positive yolk granules started to deposit in the oocyte. The shrinkage of ooplasm was observed into some oocytes due to which free spaces were formed in between follicular epithelium and ooplasm and it was PAS negative. The follicular epithelium was thin with cubical cells and was shown weak reaction. But the deposition of strongly PAS positive material was less as compared to control group of same age (Fig. 85).

**Ovary of 6 days old female**: The germarium was shown weak reaction with PAS due to degeneration of cells. The vacuoles were observed into the germarium and it was PAS negative. In the vitellarium deposition of strongly PAS positive material was less and the vacuolization was increased as compared to the control. The follicular epithelium was broken at some places with flattened cells. In the oocyte the ooplasm get accumulated in the centre. Here the chorion formation was stared (Fig. 86).

**Ovary of 8 days old female**: After eight days of treatment with 0.01\(\mu g/\mu l\) JHA the oocytes were shrunken and due to which the shape of the oocytes were disturbed and it became irregular. Here the PAS positive material was present in less quantity than the control group of same age. The follicular epithelium was shown folds at some places, thin and due to which the oocyte became irregular in shape (Fig. 87).
Histochemical observation for Carbohydrate:

Treated Group: Effect of 0.03µg/µl JHA on the ovary of female *Dysdercus similis*

**Ovary of 2 days old female:** After two days of treatment with 0.01µg/µl JHA the ovary was appeared spongy due to loosening of cells and it was shown weak reaction with PAS. The vacuoles were observed in it which was PAS negative. In the vitellarium the oocyte were shrunken and it was shown weak reaction with PAS (Fig. 88).

**Ovary of 4 days old female:** The germarium was shown weak reaction with PAS. In the vitellarium the oocytes were shown shrinkage due to which the ooplasm detached from follicular epithelium and the space was formed between the follicular epithelium and ooplasm which was PAS negative. The PAS positive granules were started to deposit towards the periphery of the oocytes. The germinal vesicle was shown weak reaction with PAS and follicular epithelium was thick and was shown moderate reaction with PAS (Fig. 89).

**Ovary of 6 days old female:** After six days of treatment in the germarium loosening of cells were observed due to which larger vacuoles were formed and it was PAS negative. In the oocyte deposition of strongly PAS positive yolk bodies were increased as compared to previous days of treatment. The follicular epithelium was thin. The shrinkage of ooplasm was seen at various sites of the oocyte. Still the chorion formation was not observed (Fig. 90).

**Ovary of 8 days old female:** After eight days of treatment with 0.03µg/µl JHA the yolk deposition was negligible as compared to control group of same age. The follicular epithelium was shown folds and oosorption. The follicular epithelium was moderately PAS positive still the chorion formation was not observed (Fig. 91).
Histochemical observation for Carbohydrate:

**Treated Group: Effect of 0.002µg/µl ecdysone on the ovary of female *Dysdercus similis***

**Ovary of 2 days old female:** After two days of treatment with 0.002µg/µl ecdysone the germarium was shown loosening of cells which was weakly PAS positive and large numbers of vacuoles were also observed and it was PAS negative. The nutritive chords were clearly visible and were shown weak reaction with PAS. In the vitellarium the developing oocytes were shown vacuolization and the germinal vesicle was towards the periphery and it was shown weak reaction. The PAS positive granules were started to deposit in the oocyte (Fig. 92).

**Ovary of 4 days old female:** After four days of treatment in ovary the germarium was shown large number of trophocytes, oogonia and nuclei and it was shown weak reaction with PAS. The vacuoles were PAS negative. The vitellarium also was shown vacuolization and PAS positive yolk bodies. The follicular epithelium was cubical and it was shown weak reaction with PAS. The chorion formation was started at this stage (Fig. 93).

**Ovary of 6 days old female:** The germarium was not was shown any change it was same as like previous days of treatment. In the vitellarium the oocytes were fully deposited by strongly PAS positive material and surrounded by chorion. The chorion was weakly PAS positive (Fig. 94).

**Ovary of 8 days old female:** Egg laying was started after eight days of treatment.
Histochemical observation for Carbohydrate:

Treated Group: Effect of 0.03µg/µl ecdysone on the ovary of female *Dysdercus similis*

**Ovary of 2 days old female:** After two days of treatment with 0.03µg/µl ecdysone the size of the ovary became smaller as compared to those in the control group of the same age and the germarium was shown weak reaction with PAS. But the pycnotic nuclei were shown moderate reaction with PAS. The oocyte was shown shrinkage towards the periphery and the ooplasm was cleaned and it was weakly PAS positive (Fig. 9.5).

**Ovary of 4 days old female:** After four days of treatment with 0.03µg/µl ecdysone the germarium was shown weak reaction with PAS while the vacuoles were shown negative reaction. The pycnotic nuclei were shown strong reaction with PAS. In the vitellarium terminal oocyte was shown shrinkage towards the periphery. Here the shrinkage of oocytes was increased as compared to previous day. The germinal vesicle was present toward the periphery of the oocyte and it was shown weak reaction. The follicular epithelium was thin and it was shown weak reaction. The PAS positive yolk deposition was started. The vacuoles were increased and it was PAS negative (Fig. 9.6).

**Ovary of 6 days old female:** After six days of treatment with 0.03µg/µl ecdysone the germarium was spongier and it was shown weak reaction with PAS. Due to spongyness the vacuoles were formed and it was PAS negative. In the vitellarium very less PAS positive yolk granules were deposited into the oocyte. The follicular epithelium was shown folds and the yolk was accumulated at the centre (Fig. 9.7).

**Ovary of 8 days old female:** The germarium as well as vitellarium both were damaged by this dose. The germarium was shown weak reaction with PAS. In the vitellarium the oocytes were damaged because the ooplasm was accumulated at centre of the oocyte due to over shrinkage of ooplasm. The PAS positive material was very less as compared to the other dosage and the control group and it was moderately PAS positive (Fig. 9.8).
Histochemical observation for Carbohydrate:

Treated Group: Effect of 0.75µg/µl ecdysone on the ovary of female *Dysdercus similis*

**Ovary of 2 days old female:** After two days of treatment with 0.75µg/µl ecdysone the germarium was shown weak reaction with PAS but the pycnotic nuclei were shown moderate reaction with PAS. The vacuoles were also noted into the germarium which was PAS negative. The developing oocytes were smaller than the corresponding control. The PAS positive granules were observed in the ooplasm. The follicular epithelium was thick and was shown weak reaction with PAS. The cells were cubical (Fig. 99).

**Ovary of 4 days old female:** the germarium was shown weak reaction with PAS. The vacuolization was increased as compared to previous days of treatment with same dose and it was PAS negative. The vitellarium was shown shrinkage and the ooplasm was shown weak reaction. The yolk was deposited towards the periphery and it was strongly PAS positive. The follicular epithelium was shown weak reaction it was thick and broken down at various places (Fig. 100).

**Ovary of 6 days old female:** Here the degeneration of trophocytes was observed due to that the germarium was more vacuolar and it was shown weak reaction with PAS. The shrinkage of the oocyte was increased than the previous days of treatment still the yolk deposition was less in the oocytes. The follicular epithelium was damaged and was shown weak reaction with PAS.

**Ovary of 8 days old female:** After eight days of treatment the ovary was shown weak reaction with PAS. The oocytes became irregular in structure due to shrinkage. Still the chorion was not formed. The yolk was deposited less and it was weakly PAS positive. The free spaces were formed in between ooplasm and follicular epithelium was PAS negative (Fig. 101).
Histochemical observation for Carbohydrate:

**Treated Group: Effect of 1.5µg/µl ecdysone on the ovary of female Dysdercus similis**

**Ovary of 2 days old female:** After two days of treatment with 1.5µg/µl ecdysone, the size of the ovary became small as compared to the control group. The germarium was show weak reaction with PAS. In the oocyte ooplasm was cleaned and it was weakly PAS positive. The follicular epithelium was thick and was shown weak reaction (Fig. 102).

**Ovary of 4 days old female:** After four days of treatment with 1.5µg/µl ecdysone the as compared to previous day of treatment germarium as well as vitellarium both were affected by this highest dose. The PAS positive granules were still not deposited in the oocyte and hence it was weakly PAS positive (Fig. 103).

**Ovary of 6 days old female:** After four days of treatment the shrinkage of ooplasm was increased than previous days of the same treatment. The deposition of PAS positive material was very less and the free space was formed due to shrinkage was PAS – negative still the chorion formation was not observed (Fig. 104).

**Ovary of 8 days old female** after eight days of treatment with 1.5µg/µl ecdysone the ovary was same as like that of previous day of treatment, but the shrinkage of ooplasm was increased and it was accumulated into the centre of the oocyte. Very few PAS positive yolk was present into it. The chorion formation still was not observed and the follicular epithelium was broken at various places it was weakly PAS positive. The free spaces were PAS negative (Fig. 105).
Histological observation (Haematoxylin-Eosin) in the Adipose Tissue of *Dysdercus similis*

**Control Group: Adipose tissue of the female *Dysdercus similis***

**Adipose tissue of 2 days old female:** At two days nucleus was big, clearly visible and prominent. Cytoplasm showed central and peripheral globule and the cells were quite active (Fig.106).

**Adipose tissue of 4 days old female:** At four days the nucleus was prominent the size of the nucleus was increased as compared to previous day and the vacuolization was observed in the cytoplasm. At this stage protein began to accumulate and cytoplasm was elaborated. The cells were still active containing sharp nucleoli (Fig.107).

**Adipose tissue of 6 days old female:** At six days cell boundaries were distinct. Nucleus was found to be bigger than the 4 days control fat cells. Central and peripheral globules were prominent. Cytoplasm was vacuolated. The size of the cells was decreased (Fig.108).

**Adipose tissue of 8 days old female:** Adipose cells were contained clean cytoplasm. Nucleus was moderate in size. The size of the fat cells was same as like previous day. The central and peripheral globules were also less in number (Fig.109).
Histological observation

**Treated Group:** Effect of 0.01µg/µl JHA on the adipose tissue of the female *Dysdercus similis*

**Adipose tissue of 2 days old female:** After two days of treatment with 0.01µg/µl JHA fat cells were large but vacuolization was observed and the nucleus was moderate sized. The cells were active and containing sharp nuclei (Fig.110).

**Adipose tissue of 4 days old female:** After four days of treatment with 0.01µg/µl JHA the fat cells were large but contained lesser quantity of cytoplasm and many vacuoles were present in it. The nucleus was small (Fig.111).

**Adipose tissue of 6 days old female:** After six days of treatment the fat cells were decreased in size contained vacuolated cytoplasm. However, cytoplasm was moderate and the nucleus was sharp. The cell boundaries were clearly visible. The central globules were clearly observed. But the peripheral globules were not so distinct (Fig.112).

**Adipose tissue of 8 days old female:** The fat cells were decreased in size as compared to control the secretary activity was also less as compared to corresponding control. The nucleus was large in size. The cytoplasm was vacuolated and cleaned (Fig.113).
Histological observation

Treated Group: Effect of 0.03µg/µl JHA on the adipose tissue of the female Dysdercus similis

Adipose tissue of 2 days old female: After two days of treatment with 0.03µg/µl JHA the fat cells were found to be small in size. There was less cytoplasm, with many vacuoles. Nucleus was largest. The central globules were clearly observed (Fig.114).

Adipose tissue of 4 days old female: After four days of treatment with 0.03µg/µl JHA the fat cells were not enlarged. The nucleus was large containing nuclei. The cytoplasm was clear and vacuolated. The vacuoles were increased as compared to control group (Fig.115).

Adipose tissue of 6 days old female: The fat cells were found to be small in size as compared to the corresponding control group of same age and the cytoplasm was cleaned, vacuolization was increased. The peripheral and central globules both were smaller in number. The nucleus was reduced in greater extent (Fig.116).

Adipose tissue of 8 days old female: The fat cells reduced in greater extend as compared to corresponding control of the same age. The depletion of cytoplasmic material was also noticed and the cell boundaries were not clearly visible (Fig.117).
Histological observation

Treated Group: Effect of 0.002µg/µl ecdysone on the adipose tissue of the female Dysdercus similis

Adipose tissue of 2 days old female: After two days of treatment with 0.002µg/µl ecdysone, the fat cell showed vacuolization. The nucleus was moderate sized and the fat cells were quite active. In cytoplasm the peripheral globules were observed but the cell boundaries were not clearly visible (Fig.118).

Adipose tissue of 4 days old female: After four days of treatment with 0.002µg/µl ecdysone, the cells were large in sized, cytoplasm was contained moderate sized nucleus. The cells were very active. The vacuoles were less as compared to the previous days.

Adipose tissue of 6 days old female: After six days of treatment with 0.002µg/µl ecdysone, the fat cells were reduced, the nucleus was moderate sized. The cytoplasm was vacuolated (Fig.119).

Adipose tissue of 8 days old female: After eight days of treatment with 0.002µg/µl ecdysone, the cells were decreased in size containing many vacuoles. Nucleus was large. The peripheral and central globules were very less in number.
Histological observation

**Treated Group:** Effect of 0.03µg/µl ecdysone on the adipose tissue of the female *Dysdercus similis*

**Adipose tissue of 2 days old female:** After two days of treatment with 0.03µg/µl ecdysone, the cells of the adipose tissues were medium sized, containing moderate sized vacuoles. The nucleus was small. The cytoplasm contained less number of peripheral and central globules as compared to control group (Fig.120).

**Adipose tissue of 4 days old female:** After four days of treatment with 0.03µg/µl ecdysone, the adipose cells were vacuolated. Nucleus was small in size and the cytoplasm was moderate. The cells were quite active (Fig.121).

**Adipose tissue of 6 days old female:** After six days of treatment the fat cells were active, few vacuoles were seen in the cytoplasm near the nucleus. The peripheral and central globules were moderate into the cytoplasm (Fig.122).

**Adipose tissue of 8 days old female:** After eight days of treatment the size of the fat cells were decreased and the less cytoplasmic material was observed as compared to the control. Nucleus was also smaller in size and vacuoles were found in large quantity. The cell boundaries were indistinct.
Histological observation

**Treated Group: Effect of 0.75µg/µl ecdysone on the adipose tissue of the female *Dysdercus similis***

**Adipose tissue of 2 days old female:** After two days of treatment with 0.75µg/µl ecdysone the cytoplasm of fat cells was contained few vacuoles. The nucleus was big, the cytoplasm contained less material. The cell boundaries were indistinct (Fig.123).

**Adipose tissue of 4 days old female:** After four days of treatment with 0.75µg/µl ecdysone the cytoplasm of the cells was moderate and contained large number of vacuoles. As compared to the control the nucleus was sharp and big. The central and peripheral globules were also less in number (Fig.124).

**Adipose tissue of 6 days old female:** After six days of treatment, the size of the cells was big, rich in cytoplasm but the cytoplasm did not contain many vacuoles. Nucleus was moderate sized. The depletion of cytoplasmic material was observed (Fig.125).

**Adipose tissue of 8 days old female:** After eight days of treatment with 0.75µg/µl ecdysone the nucleus was reduced in a greater extent. The cytoplasm showed vacuoles. The depletion of cytoplasmic material was increased (Fig.126).
Histological observation

Treated Group: Effect of 1.5µg/µl ecdysone on the adipose tissue of the female *Dysdercus similis*

**Adipose tissue of 2 days old female:** After two days of treatment with 1.5µg/µl ecdysone the fat cells were big active, vacuolated with moderate cytoplasm. The nucleus was small but the intensity of cytoplasmic material was less. The cell boundaries were indistinct (Fig.127).

**Adipose tissue of 4 days old female:** After four days of treatment fat cells were enlarged and active cytoplasm showed large number of vacuoles and the cell boundaries were indistinct. Nucleus of the fat cells was prominent. The cytoplasm was clear (Fig.128).

**Adipose tissue of 6 days old female:** The adipose cells were reduced in size with vacuolated cytoplasm. The nucleus was pycnotic. The cytoplasm was clear.

**Adipose tissue of 8 days old female:** After eight days of treatment with 1.5µg/µl ecdysone the cells were more vacuolated. Nucleus was pycnotic containing number of nuclei. The fat cells were reduced in sized as compared to the control group. The cell cytoplasm contained very few central globules (Fig.129).
Histochemical observation for the detection of Protein in Adipose tissue of *Dysdercus similis*

By staining the section of Cornoy No.2 fixed fat tissue with mercuric/bromophenol blue method of Bonhag (1955), proteins are found to be present around the peripheral globules in the cytoplasm. The chromatin material of the nuclei also gives positive reaction for this staining due to the presence of nucleoprotein in them. The fat protein is seen to be deposited in the form of granules in the peripheral globules and their concentration varies in different stages. These proteins are utilized most at the time of emergence and also at the time of egg laying. This showed that its synthesis was related to the growth and ovarian development as reported in other insects by Permtick and Butz (1970).

**Histochemical Observation for Protein**

**Control Group: Adipose tissue of female *Dysdercus similis***

**Adipose tissue of 2 days old female:** At two days the protein deposition was clearly visible in the peripheral globules of fat cells and it was intense (Fig.130).

**Adipose tissue of 4 days old female:** At four days there was heavier deposition in numerous peripheral globules and a granular deposition in the peripheral parts of the fat cells was observed. The vacuoles were very less (Fig.131).

**Adipose tissue of 6 days old female:** At six days in fat body the protein deposition was less than the previous day. The cytoplasmic strands between the central globules and nuclei also react positively with mercuric Bromophenol blue (Fig.132).

**Adipose tissue of 8 days old female:** At eight days treatment fat bodies showed depletion of protein and the vacuoles were also appeared (Fig.133).
Histochemical Observation for Protein

Treated Group : Effect of 0.01µg/µl JHA on adipose tissue of female

*Dysdercus similis*

Adipose tissue of 2 days old female: After two days of treatment with 0.01µg/µl JHA, the protein deposition was somewhat less than the control group fat cell. The nucleus was prominent and showed strong reaction with protein. The central globules were weakly protein positive (Fig.134).

Adipose tissue of 4 days old female: After four days of treatment the protein deposition was less than the corresponding control. The cytoplasm was cleaned and vacuoles were increased. The fat cells were weakly PAS positive (Fig.135).

Adipose tissue of 6 days old female: After six days of treatment the proteins was depleted completely. The nucleus was reduced and moderately PAS positive. The cytoplasm was weakly PAS positive protein (Fig.136).

Adipose tissue of 8 days old female: The depletion of protein was increased. The cytoplasm was clear. Peripheral globules were less in number and the cell boundaries were not clearly visible (Fig.137).
Chapter-4

Observations

Histochemical Observation for Protein

Treated Group: Effect of 0.03µg/µl JHA on adipose tissue of female Dysdercus similis

Adipose tissue of 2 days old female: After two days of treatment with 0.03µg/µl JHA the size of the cells was small and the deposition of protein in the peripheral globules was less than the previous dose of same age. The vacuolization was also increased. The cytoplasmic string was clearly visible (Fig.138).

Adipose tissue of 4 days old female: The nuclei of the fat cells were pycnotic and the peripheral globules were present throughout the cytoplasm but the numbers of peripheral globules were less than the corresponding control. The nucleus was not so distinct (Fig.139).

Adipose tissue of 6 days old female: The depletion was almost completed. The nucleus was prominent and strongly protein positive. The cytoplasm was cleaned and weakly protein positive and vacuolated (Fig.140).

Adipose tissue of 8 days old female: The vacuolization was observed throughout the cytoplasm. The depletion of protein was almost completed. The nucleus was weakly protein positive. The cytoplasmic strings and cell boundaries were also disturbed (Fig.141).
Histochemical Observation for Protein

Treated Group: Effect of 0.002µg/µl ecdysone on adipose tissue of female *Dysdercus similis*

**Adipose tissue of 2 days old female:** After two days of treatment with 0.002µg/µl ecdysone the protein granules were heavily deposited into the cytoplasm of the fat cells. The nucleus was moderately protein positive and the cytoplasm was strongly protein positive (Fig.142).

**Adipose tissue of 4 days old female:** The peripheral globules were observed throughout the cytoplasm and the cells were strongly protein positive. The nucleus was large in sized and strongly protein positive. The vacuoles were very less.

**Adipose tissue of 6 days old female:** The vacuoles were appeared into the cytoplasm and depletion of protein was started. The nucleus was strongly protein positive and big. The cytoplasm was weakly protein positive (Fig.143).

**Adipose tissue of 8 days old female:** The vacuolization was increased. The depletion was clearly visible. The nucleus was strongly protein positive.
Histochemical Observation for Protein

Treated Group: Effect of 0.03µg/µl ecdysone on adipose tissue of female *Dysdercus similis*

**Adipose tissue of 2 days old female:** After two days of treatment of 0.02µg/µl ecdysone the deposition of protein granules was less than the previous dose of same age treated insect and the cells were weakly protein positive (Fig. 144).

**Adipose tissue of 4 days old female:** After four days of treatment the protein deposition was increased but it was less than the previous days of with same treatment (Fig. 145).

**Adipose tissue of 6 days old female:** After six days of treatment the depletion of protein was observed, the size of the fat cells was also reduced (Fig. 146).

**Adipose tissue of 8 days old female:** After eight days of treatment, depletion of protein was increased and the vacuolization was also increased and the fat cells were shown weak reaction with protein.
Histochemical Observation for Protein

Treated Group: Effect of 0.75μg/μl ecdysone on adipose tissue of female *Dysdercus similis*

**Adipose tissue of 2 days old female:** After two days of treatment with 0.75μg/μl ecdysone, no change was observed into the fat cells. It was same as like the treatment of previous dose of same age (Fig.147).

**Adipose tissue of 4 days old female:** The protein deposition was slightly decreased into the four days after treatment with 0.75μg/μl ecdysone. Very few peripheral globules were observed and it was weakly protein positive (Fig.148).

**Adipose tissue of 6 days old female:** After two days of treatment with 0.75μg/μl ecdysone, the depletion of protein was observed and the cells were weakly protein positive (Fig.149).

**Adipose tissue of 8 days old female:** After eight days of treatment the cells were vacuolated and depletion of protein was completed and the fat cells were shown weak reaction with protein (Fig.150).
Histochemical Observation for Protein

Treated Group: Effect of 1.5µg/µl ecdysone on adipose tissue of female *Dysdercus similis*

**Adipose tissue of 2 days old female:** After two days of treatment with 0.75µg/µl ecdysone, no change was observed into the fat cells. It was same as like the treatment of previous dose of same age (Fig.151).

**Adipose tissue of 4 days old female:** The protein deposition was slightly decreased into the four days after treatment with 0.75µg/µl ecdysone. Very few peripheral globules were observed and it was weakly protein positive (Fig.152).

**Adipose tissue of 6 days old female:** After two days of treatment with 0.75µg/µl ecdysone, the depletion of protein was observed and the cells were weakly protein positive (Fig.153).

**Adipose tissue of 8 days old female:** After eight days of treatment the cells were vacuolated and depletion of protein was completed and the fat cells were shown weak reaction with protein (Fig.154).
Histochemical observation for detection of lipid in adipose tissue of *Dysdercus similis*

By staining the section of Formal-calcium fixed fat tissue with McManus Sudan Black B Method (1946).

The cell boundaries of fat cells were clearly visible in which the nucleus is surrounded by central and peripheral globules. The saturated and unsaturated lipids are found in the central globules while only unsaturated lipids were found in peripheral globules.

**Histochemical Observation of Adipose Tissue:**

**Control group: Adipose tissue of female *Dysdercus similis***

**Adipose tissue of 2 days old female:** At two days there was an intense deposition of lipid both in the central and peripheral globules. The vacuoles were also observed into the cytoplasm of the adipose cells (Fig.155).

**Adipose tissue of 4 days old female:** At four days the central and peripheral globules were increased in number and the deposition of the lipid was increased as compared to previous day and it was strongly sudan positive. The cells were compactly filled with lipid. The vacuoles were less as compared to previous day and it was sudan negative (Fig.156).

**Adipose tissue of 6 days old female:** At six days the depletion of lipid was started (Fig.157).

**Adipose tissue of 8 days old female:** At eight days, there was depletion of lipids and large number of vacuoles were observed into the cytoplasm and the cells were weakly sudan positive (Fig.158).
Histochemical Observation for Lipid

Treated Group: Effect of 0.01µg/µl JHA on adipose tissue of female *Dysdercus similis*

**Adipose tissue of 2 days old female:** After two days of treatment with 0.01µg/µl JHA there was less deposition of lipid as compared to control. The nucleus was strongly sudan positive. While the cytoplasm of the cells were weakly sudan positive (Fig.159).

**Adipose tissue of 4 days old female:** After four days of treatment the vacuoles were observed into the cytoplasm and the cytoplasm was somewhat clear only few central and peripheral lipid positive globules were observed (Fig.160).

**Adipose tissue of 6 days old female:** After six days of treatment with 0.01µg/µl JHA the depletion of lipid was increased and the cytoplasm was clear. The vacuoles were also increased into the cytoplasm and the cells became smaller in size (Fig.161).

**Adipose tissue of 8 days old female:** After eight days of treatment with 0.01µg/µl JHA sudan. The depletion of lipid was almost completed the lipid positive material was negligible. The nucleus was moderately sudan positive while the cytoplasm was weakly sudan positive. The vacuoles became fused to one another and formed larger vacuole.
Histochemical Observation for Lipid

**Treated Group: Effect of 0.03µg/µl JHA on adipose tissue of female Dysdercus similis**

**Adipose tissue of 2 days old female:** After two days of treatment with 0.03µg/µl JHA the cells were same as compared to previous days of treatment with 0.01µg/µl JHA (Fig.162).

**Adipose tissue of 4 days old female:** After four days of treatment very few lipid positive bodies were seen in the cytoplasm, vacuolization was increased and depletion of lipid was started. The nucleus was moderately Sudan positive and the cytoplasm was also moderately sudan positive and the cytoplasm was also moderately sudan positive (Fig.163).

**Adipose tissue of 6 days old female:** After four days of treatment the depletion of lipid was almost completed. The size of the cells was reduced at greater extent. The nucleus was strongly sudan positive and the peripheral globules was moderately sudan positive (Fig. 164).

**Adipose tissue of 8 days old female:** After eight days of treatment the cells became very small in size and much vacuolization was observed. The cytoplasm was cleared. The nucleus was weakly sudan positive while the cytoplasm were moderately sudan positive (Fig. 165).
Histochemical Observation for Lipid

Treated Group: Effect of 0.002µg/µl ecdysone on adipose tissue of female *Dysdercus similis*

**Adipose tissue of 2 days old female:** At two days there was an intense deposition of lipid both in the central and peripheral globules. The vacuoles were also observed into the cytoplasm of the adipose cells.

**Adipose tissue of 4 days old female:** At four days the central and peripheral globules were increased in number and the deposition of the lipid was increased as compared to previous day and it was strongly sudan positive. The cells were compactly filled with lipid. The vacuoles were less as compared to previous day and it was sudan negative.

**Adipose tissue of 6 days old female:** At six days the depletion of lipid was started.

**Adipose tissue of 8 days old female:** At eight days, there was depletion of lipids and large number of vacuoles were observed into the cytoplasm and the cells were weakly sudan positive.
Histochemical Observation for Lipid

Treated Group: Effect of 0.03µg/µl ecdysone on adipose tissue of female *Dysdercus similis*

**Adipose tissue of 2 days old female:** After two days of treatment with 0.03µg/µl ecdysone the peripheral globules was less in number but the central globules were moderate and moderately sudan positive. The nucleus was weakly sudan positive and the cytoplasm was moderately sudan positive (Fig. 166).

**Adipose tissue of 4 days old female:** After two days of treatment, the deposition of lipid was little bit increased. Both the central and peripheral globules were increased in cytoplasm and it was moderately sudan positive. The nucleus was moderately sudan positive. The vacuoles were also observed and it was sudan negative (Fig. 167).

**Adipose tissue of 6 days old female:** After six days of treatment the depletion of lipid was observed (Fig. 168).

**Adipose tissue of 8 days old female:** After eight days of treatment with 0.03µg/µl ecdysone, the depletion of lipid increased and the cytoplasm was clear, it was weakly sudan positive while the vacuoles were sudan negative.
Histochemical Observation for Lipid

Treated Group: Effect of 0.75µg/µl ecdysone on adipose tissue of female *Dysdercus similis*

**Adipose tissue of 2 days old female:** After two days of treatment with 0.75µg/µl ecdysone the cells were quite active. The deposition of lipid was observed. The nucleus was smaller in size the cytoplasm was moderately sudan positive (Fig.169).

**Adipose tissue of 4 days old female:** After four days of treatment with 0.75µg/µl ecdysone the cells were slightly increased in size. Large number of central and many peripheral globules were observed and it were weakly sudan positive (Fig.170).

**Adipose tissue of 6 days old female:** After six days of treatment, depletion of lipid was started. The size of the cells was reduced. The cytoplasm was weakly sudan positive (Fig.171).

**Adipose tissue of 8 days old female:** After eight days of treatment the depletion of lipid was almost completed. The cell becomes reduced in size and the nucleus was weakly sudan positive, while the vacuoles were observed in large amount which were sudan negative (Fig.172).
Histochemical Observation for Lipid

Treated Group: Effect of 1.5µg/µl ecdysone on adipose tissue of female *Dysdercus similis*

**Adipose tissue of 2 days old female:** After two days of treatment with 0.75µg/µl ecdysone the cells were quite active. The deposition of lipid was observed. The nucleus was smaller in size the cytoplasm was moderately sudan positive (Fig.173).

**Adipose tissue of 4 days old female:** After four days of treatment with 0.75µg/µl ecdysone the cells were slightly increased in size. Large number of central and many peripheral globules were observed and it were weakly sudan positive (Fig.174).

**Adipose tissue of 6 days old female:** After six days of treatment, depletion of lipid was started. The size of the cells was reduced. The cytoplasm was weakly sudan positive (Fig.175).

**Adipose tissue of 8 days old female:** After eight days of treatment the depletion of lipid was almost completed. The cell becomes reduced in size and the nucleus was weakly sudan positive, while the vacuoles were observed in large amount which were sudan negative (Fig.176).
Histochemical observation for the detection of carbohydrate in Adipose tissue of *Dysdercus similis*

By staining the section of chilled Cornoy No.2 fixed fat tissue with periodic Acid Schiff method (PAS) (Mc Manus, 1948). Carbohydrates were present in the cytoplasmic strands and peripheral globules.

**Histochemical Observation of Adipose Tissue:**

**Control group: Adipose tissue of female *Dysdercus similis***

**Adipose tissue of 2 days old female:** At the two days the cytoplasm was strongly PAS positive, the nucleus was sharp and moderately PAS positive. The deposition of glycogen in the cytoplasm was observed (Fig.177).

**Adipose tissue of 4 days old female:** At four days, the peripheral globules packed with glycogen and it was clearly observed. They were much more in number than that in the two days female fat body. The vacuoles were present and it was PAS negative. The cytoplasmic strand were also present and it was PAS positive (Fig.178).

**Adipose tissue of 6 days old female:** At six days in the fat cells the numbers of peripheral globules were less and the vacuolization was noticed in the cytoplasm with less the peripheral globules were less (Fig.179).

**Adipose tissue of 8 days old female:** At eight days, there were very few peripheral globules were present into the cytoplasm which was PAS positive. The depletion of carbohydrate was noticed (Fig.180).
Histochemical Observation for Carbohydrate

Treated Group: Effect of 0.01µg/µl JHA on adipose tissue of female *Dysdercus similis*

**Adipose tissue of 2 days old female:** After two days of treatment with 0.01µg/µl JHA the deposition of glycogen into the cytoplasm was observed but it was somewhat less as compared to control group of the same day fat body. The cytoplasmic strands were clearly visible and it was strongly PAS positive (Fig.181).

**Adipose tissue of 4 days old female:** After four days of treatment with 0.01µg/µl JHA the peripheral globules were moderately PAS positive near to the nucleus. The vacuolization was increased. The cytoplasm was contained moderate cytoplasmic material. But it was weakly PAS positive. The nucleus was large sized (Fig.182).

**Adipose tissue of 6 days old female:** After six days of treatment with 0.01µg/µl JHA the peripheral globules were very less and the depletion of glycogen was almost complete. The cell cytoplasm was vacuolated and it was vacuolated and it was PAS negative (Fig.183).

**Adipose tissue of 8 days old female:** After eight days of treatment with 0.01µg/µl JHA, the vacuolization of the cells were increased and the cell boundaries were clearly observed and the depletion of carbohydrate was completed. The cell cytoplasm was weakly PAS positive because it was cleaned. While the nucleus was moderately PAS positive (Fig.184).
Histochemical Observation for Carbohydrate

Treated Group: Effect of 0.03µg/µl JHA on adipose tissue of female *Dysdercus similis*

**Adipose tissue of 2 days old female:** After two days of treatment with 0.03µg/µl JHA only few peripheral globules were present and the deposition of glycogen was near to the periphery of the cytoplasm. The cells were with large vacuoles (Fig.185).

**Adipose tissue of 4 days old female:** After four days of treatment with 0.03µg/µl JHA the deposition of glycogen was increased. The cytoplasm was vacuolated. The nucleus was weakly PAS positive while the cytoplasm was strongly PAS positive (Fig.186).

**Adipose tissue of 6 days old female:** After six days of treatment the depletion of glycogen was noted. The cytoplasm was with less number of peripheral globules and it was weakly PAS positive. The nucleus was strongly PAS positive and larger in sized (Fig.187).

**Adipose tissue of 8 days old female:** The cytoplasm was found to be more vacuolar and the nucleus was prominent but weakly PAS positive while the cytoplasm was cleaned and weakly PAS positive (Fig.188).
Histochemical Observation for Carbohydrate

Treated Group: Effect of 0.002µg/µl ecdysone on adipose tissue of female *Dysdercus similis*

**Adipose tissue of 2 days old female:** After two days of treatment with 0.002µg/µl ecdysone the fat cells were PAS positive and the deposition of glycogen was heavy. The vacuoles were observed and it was PAS negative. Nucleus was small and moderately PAS positive (Fig.189).

**Adipose tissue of 4 days old female:** After four days of treatment the intensive deposition of the glycogen was observed into the peripheral globules the cytoplasm was completely filled with peripheral globules and it was strongly PAS positive (Fig.190).

**Adipose tissue of 6 days old female:** After six days of treatment the depletion of the glycogen was started, the vacuoles were observed (Fig.191).

**Adipose tissue of 8 days old female:** After eight days of treatment with 0.002µg/µl ecdysone very few glycogen was observed into the cytoplasm and the vacuoles were increase which were PAS negative.
Histochemical Observation for Carbohydrate

Treated Group: Effect of 0.03µg/µl ecdysone on adipose tissue of female Dysdercus similis

Adipose tissue of 2 days old female: After two days of treatment with 0.03µg/µl ecdysone, the deposition of glycogen was observed near the nucleus. The cytoplasm was cleaned and weakly PAS positive. The nucleus was moderately PAS positive (Fig.192).

Adipose tissue of 4 days old female: After four days of treatment with 0.03µg/µl ecdysone the deposition of glycogen was slightly increased.

Adipose tissue of 6 days old female: After six days of treatment the depletion of glycogen was started and the vacuoles were appeared (Fig.193).

Adipose tissue of 8 days old female: After eight days of treatment the vacuolization was increased and the cytoplasm was cleared (Fig.194).
Histochemical Observation for Carbohydrate

Treated Group: Effect of 0.75µg/µl ecdysone on adipose tissue of female *Dysdercus similis*

**Adipose tissue of 2 days old female:** After two days of treatment with 0.75µg/µl ecdysone the little deposition of glycogen was observed. They cytoplasmic strands and the peripheral globules were not so distinct it was weakly PAS positive and nucleus was not so sharp.

**Adipose tissue of 4 days old female:** The deposition of glycogen was rapidly increased and the glycogen was intensively deposited into the cytoplasm. The nucleus was increased in size and it was moderately PAS positive (Fig. 19).5

**Adipose tissue of 6 days old female:** After six days of treatment with 0.75µg/µl ecdysone, no change was observed (Fig.196).

**Adipose tissue of 8 days old female:** After eight days the depletion of glycogen was observed (Fig.197).
Histochemical Observation for Carbohydrate

Treated Group: Effect of 1.5µg/µl ecdysone on adipose tissue of female *Dysdercus similis*

**Adipose tissue of 2 days old female:** After two days of treatment with 1.5µg/µl ecdysone very few deposition of glycogen was observed as compared to corresponding control (Fig.198).

**Adipose tissue of 4 days old female:** After four days of treatment with 1.5µg/µl ecdysone the cytoplasm was fully packed with glycogen. Very few vacuoles were observed and it was PAS positive.

**Adipose tissue of 6 days old female:** After six days of treatment, the depletion of glycogen was started but very few vacuoles were observed. The nucleus was not so distinct. The vacuoles was prominent and PAS negative (Fig.199).

**Adipose tissue of 8 days old female:** After eight days of treatment the depletion of glycogen was almost completed. The nucleus was reduced in size. The cytoplasm was cleaned and weakly PAS positive. The vacuoles were PAS negative. The size of the cell was also reduced as compared to the control group of same age (Fig.200).
BIOCHEMICAL STUDIES

Protein Detection of Ovary of *Dysdercus similis* by SDS Page Electrophoresis:

SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis, is a technique widely used in biochemistry, forensics, genetics and molecular biology to separate proteins according to their electrophoretic mobility (a function of length of polypeptide chain or molecular weight as well as higher order protein folding, post-translational modifications and other factors). The SDS gel electrophoresis of samples having identical charge to mass ratios results in fractionation by size and is probably the world’s most widely used biochemical method.

Fig. 1: Showed the very charged protein fractions were increased in number and intensity in ovaries of adult female insects of control groups of *Dysdercus similis* while depletion in number and intensity of protein fractions was observed in 2 days treated experimental groups due to the effect of JHA and ecdysone.

The molecular mass of the ovaries of control adult female of *Dysdercus similis* ranged from 6.5 to 195 kDa, while ovaries of 2 days JHA and ecdysone treated ovaries of *Dysdercus similis* adult female ranged from 6.5 to 195 kDa as exhibited in Fig. 1.

Thirteen bands in lane 1 of Fig. 1 were observed in the ovaries of control insects. These bands were of 6.5, 14.5, 21, 31, 41.5, 45, 54.5, 66, 80, 97.5, 100, 110 and 195 kDa molecular weight. Five bands of 6.5, 21, 54.5, 66 and 97.5 kDa were of very very high intensity. Three bands of 14.5, 31 and 110 kDa were of very high intensity. Two bands of 45 and 195 kDa were of high intensity. One band of 41 kDa was of low intensity and one band of 80 kDa was of very low intensity. One band of 100 kDa was of very very low intensity.

Thirteen bands in lane 2 of Fig. 1 were observed in ovaries of 2 days 0.01µg/µl JHA treated insects. Out of these five bands of 6.5, 21, 54.5, 66 and 97.5
kDa were of very high intensity. Three bands of 14.5, 31, and 110 kDa were of high intensity. Two bands of 45 and 195 kDa were of low intensity while one band of 41.5 was of very low intensity and two bands of 80 and 100 kDa were of very very low intensity.

Twelve bands in lane 3 of Fig. 1 were observed in ovaries of 2 days 0.3 µg/µl JHA treated insects. Out of these six bands of 6.5, 21.5, 54.5, 66, 80 and 110 kDa were of high intensity. While three bands of 14.5, 31, and 45 kDa were of low intensity. One band of 195 kDa was of very low intensity and remaining two bands of 41.5 and 97.5 kDa were of very very low intensity.

Thirteen bands in lane 4 of Fig.1 were observed in ovaries of 2 days 0.002 µg/µl ecdysone treated insects. The number and intensity of the bands was same as in lane 1 of 2 days ovaries of control female insects.

Eleven bands in lane 5 of Fig. 1 were observed in ovaries of 2 days 0.03 µg/µl ecdysone treated insects. Out of these one band of 21 kDa was of high intensity. Six bands of 6.5, 14.5, 54.5, 66, 80 and 110 kDa were of low intensity. Three bands of 31, 45 and 195 kDa were of very low intensity. One band of 97.5 kDa was of very very low intensity.

Nine bands in lane 6 of Fig. 1 were observed in ovaries of 2 days 0.75 µg/µl ecdysone treated insects. One band of 21 kDa was of high intensity. Five bands of 6.5, 14.5, 54.5, 80 and 110 kDa were of low intensity and three bands of 31, 45 and 195 kDa were of very low intensity.

Seven bands in lane 7 of Fig. 1 were observed in ovaries of 2 days 1.5 µg/µl ecdysone treated insects. One band of 21 kDa was of low intensity. Three bands of 54.5, 80 and 110 kDa were of very low intensity while three bands of 6.5, 31 and 195 kDa were of very very low intensity.
Fig. 2: Showed the very charged protein fractions were increased in number and intensity in ovaries of adult female insects of control groups of *Dysdercus similis* while depletion in number and intensity of protein fractions was observed in 4 days treated experimental groups due to the effect of JHA and ecdysone.

The molecular mass of the ovaries of control 4 days adult female of *Dysdercus similis* ranged from 6.5 to 195 kDa while ovaries of 4 days JHA and ecdysone treated ovaries of *Dysdercus similis* adult females was ranged from 6.5 to 110 kDa as exhibited in Fig. 2.

Thirteen bands in lane 1 of Fig. 2 were observed in the ovaries of control insects. These bands were of 6.5, 14.5, 21, 31, 41.5, 45, 54.5, 66, 80, 97.5, 100, 110 and 195 kDa molecular weight. Seven bands of 6.5, 21, 31, 45, 54.5, 66, and 80 kDa were of very very high intensity. Three bands of 14.5, 110 and 195 kDa were of very high intensity. One band of 41.5 kDa was of high intensity. One band of 100 kDa was of low intensity while band of 41 kDa was of low intensity. One band of 97.5 kDa was of very low intensity.

Twelve bands in lane 2 of Fig. 2 were observed in ovaries of 4 days 0.01µg/µl JHA treated insects. Three bands of 6.5, 21, and 54.5 kDa were of very very high intensity. Six bands of 14.5, 31, 45, 66, 80 and 195 kDa were of very high intensity. Two bands of 41.5 and 110 kDa were of high intensity and one band of 100 kDa was of low intensity.

Five bands in lane 3 of Fig. 2 were observed in ovaries of 4 days 0.03µg/µl JHA treated insects. Two bands of 6.5 and 54.5 kDa were of very high intensity. Two bands of 45 and 80 kDa were of high intensity. One band of 41.5 kDa was of low intensity.
Thirteen bands in lane 4 of Fig. 2 were observed in ovaries of 4 days 0.002 µg/µl ecdysone treated insects. The number and intensity of the bands was same as in lane 1 of 4 days ovaries of control female insects.

Nine bands in lane 5 of Fig. 2 were observed in ovaries of 4 days 0.03 µg/µl ecdysone treated insects. Out of these four bands 6.5, 21, 31 and 54.5 kDa were of very high intensity. One band of 45 kDa was high intensity. Four bands of 41.5, 80, 110 and 195 kDa were of low intensity.

Five bands in lane 6 of Fig. 2 were observed in ovaries of 4 days 0.75 µg/µl ecdysone treated insects. Two bands of 54.5 and 66 kDa were of high intensity. Three bands of 6.5, 45, and 110 kDa were of low intensity.

Three bands in lane 7 of Fig. 2 were observed in ovaries of 4 days 1.5 µg/µl ecdysone treated insects. Two bands were of 54.5 and 110 kDa were of low intensity. One band of 31 kDa was of low intensity while three bands of 6.5, 31, and 195 kDa were of very very low intensity.

Fig. 3: Showed the very charged protein fractions were increased in number and intensity in ovaries of adult female insects of control groups of *Dysdercus similis* while depletion in number and intensity of protein fractions was observed in 6 days treated experimental groups due to the effect of JHA and ecdysone.

The molecular mass of the ovaries of control 6 days adult female of *Dysdercus similis* ranged from 6.5 to 195 kDa while ovaries of 6 days JHA and ecdysone treated ovaries of *Dysdercus similis* adult females was ranged from 6.5 to 66 kDa and 6.5 to 54.5 kDa as exhibited in Fig. 3.

Thirteen bands in lane 1 of Fig. 3 were observed in the ovaries of control insects. These bands were of 6.5, 14.5, 21, 31, 41.5, 45, 54.5, 66, 80, 97.5, 100, 110 and 195 kDa molecular weight. Seven bands of 6.5, 21, 31, 45, 54.5, 66, and 80 kDa
were of very very high intensity. Two bands of 14.5 and 195 kDa were of very high intensity. Two bands of 41.5 and 110 kDa were of high intensity and one band of 100 kDa was of low intensity. One band of 97.5 kDa was of very low intensity.

Twelve bands in lane 2 of Fig. 3 were observed in ovaries of 6 days 0.01 µg/µl JHA treated insects. One band of 195 kDa was of very high intensity. Two bands of 66 and 80 kDa were of high intensity. Five bands of 21, 31, 45, 54.5 and 110 kDa were of low intensity. Four bands of 6.5, 14.5, 41.5 and 100 kDa were of low intensity.

Three bands in lane 3 of Fig. 3 were observed in ovaries of 6 days 0.03 µg/µl JHA treated insects. Two bands of 31 and 66 kDa were of very low intensity while one band of 45 kDa was of very very low intensity.

Thirteen bands in lane 4 of Fig. 3 were observed in ovaries of 6 days 0.002 µg/µl ecdysone treated insects. The intensity of the bands was same as in lane 1 of 6 days ovaries of control female insects.

Nine bands in lane 5 of Fig. 3 were observed in ovaries of 6 days 0.03 µg/µl ecdysone treated insects. One band of 195 kDa was of low intensity. Three bands of 6.5, 45.5 and 80 kDa were of very low intensity while five bands of 31, 41.5, 54.5, 66 and 110 kDa were of very very low intensity.

Four bands in lane 6 of Fig. 3 were observed in ovaries of 6 days 0.75 µg/µl ecdysone treated insects. One band of 6.5 kDa was of very low intensity. Three bands of 31, 66 and 110 kDa were of very very low intensity.

Three bands in lane 7 of Fig. 3 were observed in ovaries of 6 days 1.5 µg/µl ecdysone treated insects. All three bands of 31, 54.5 and 66 kDa were of very very low intensity.
Fig. 4: Showed the very charged protein fractions were increased in number and intensity in ovaries of adult female insects of control groups of *Dysdercus similis* while depletion in number and intensity of protein fractions was observed in 8 days treated experimental groups due to the effect of JHA and ecdysone.

The molecular mass of the ovaries of control 8 days adult female of *Dysdercus similis* ranged from 6.5 to 195 kDa while ovaries of 8 days JHA and ecdysone treated ovaries of *Dysdercus similis* adult females ranged from 6.5 to 66 kDa as exhibited in Fig. 4.

Twelve bands in lane 1 of Fig. 4 were observed in the ovaries of 8 days control insects. These bands were of 6.5, 14.5, 21, 31, 41.5, 45, 54.5, 66, 80, 97.5, 100 and 195 kDa molecular weight. Three bands of 54.5, 66, and 80 kDa were of very high intensity. Three bands of 21, 31 and 45 were of high intensity. Two bands of 6.5 and 14.5 kDa were of very low intensity. One band of 97 kDa was of very very low intensity.

Nine bands in lane 2 of Fig. 4 were observed in ovaries of 8 days 0.01µg/µl JHA treated insects. One band of 66 kDa was of high intensity. Seven bands of 21, 31, 45.5, 54.5, 80, 100 and 195 kDa were of very low intensity. One band of 6.5 kDa was of very very low intensity.

Two bands in lane 3 of Fig. 4 were observed in ovaries of 8 days 0.3µg/µl JHA treated insects. The band of 66 kDa was very low intensity and 31 kDa was of very very low intensity.

Twelve bands in lane 4 of fig.4 were observed in ovaries of 8 days 0.002µg/µl ecdysone treated insects. The intensity of the bands was same as in lane 1 of 8 days ovaries of control female insects.
Six bands in lane 5 of Fig. 4 were observed in ovaries of 8 days 0.03 µg/µl ecdysone treated insects. One band of 66 kDa was of low intensity. Four bands of 6.5, 31, 45 and 100 kDa were of very low intensity and one band of 80 kDa was of very very low intensity.

Three bands in lane 6 of Fig. 4 were observed in ovaries of 8 days 0.75 µg/µl ecdysone treated insects. One band of 66 kDa was of very low intensity. Two bands of 31 and 100 kDa were of very very low intensity.

One band in lane 7 of Fig. 4 was observed in ovaries of 8 days 1.5 µg/µl ecdysone treated insects. The band was of 66 kDa of very very low intensity.
Protein Detection of Adipose tissue of *Dysdercus similis* by SDS Page Electrophoresis:

Fig. 5: Showed the very charged protein fractions were increased in number and intensity in adipose tissues of adult female insects of control groups of *Dysdercus similis* while depletion in number and intensity of protein fractions was observed in 2 days treated experimental groups due to the effect of JHA and ecdysone. The molecular mass of the adipose tissue of control adult female of *Dysdercus similis* ranged from 6.5 to 195 kDa, while adipose tissue of 2 days JHA and ecdysone treated adipose tissue of *Dysdercus similis* adult female ranged from 6.5 to 195 kDa as exhibited in Fig. 5.

Thirteen bands in lane 1 of Fig. 5 were observed in the adipose tissue of control insects. These bands were of 6.5, 14.5, 21, 31, 41.5, 45, 50, 66, 68.5, 97.5, 100, 110 and 195 kDa molecular weight. Five bands of 6.5, 14.5, 97.5, 100 and 100 kDa were of very very high intensity. Three bands of 21, 31 and 110 kDa were of very high intensity. Four bands of 45, 50, 66 and 68.5 kDa were of high intensity. One band of 41.5 kDa was of low intensity.

Thirteen bands in lane 2 of Fig. 5 were observed in adipose tissue of 2 days 0.01 µg/µl JHA treated insects. Out of this one band of 195 kDa was of very high intensity. Two bands of 97.5 and 100 kDa were of very high intensity. Three bands of 6.5, 31 and 110 kDa were of high intensity. Six bands 14.5, 21, 45, 50, 66 and 68.5 kDa were of low intensity and one band of 41.5 kDa was of very low intensity.

Twelve bands in lane 3 of Fig. 5 were observed in adipose tissue of 2 days 0.03 µg/µl JHA treated insects. Out of these two bands of 97.5 and 100 kDa were of high intensity. While three bands of 6.5, 31, and 110 kDa were of low intensity. Six bands of 21, 45, 50, 66, 68.5 and 195 kDa were of very low intensity and remaining one band of 14.5 kDa was of very very low intensity.
Thirteen bands in lane 4 of Fig. 5 were observed in adipose tissue of 2 days 0.002µg/µl ecdysone treated insects. The number and intensity of the bands was same as in lane 1 of 2 days adipose tissue of control female insects.

Thirteen bands in lane 5 of Fig. 5 were observed in adipose tissue of 2 days 0.03µg/µl ecdysone treated insects. Out of these two bands of 97.5 and 195 kDa were of very high intensity. Three bands of 68.5, 100 and 110 kDa were of high intensity. Three bands of 31, 50 and 66 kDa were of low intensity. Two bands of 6.5 and 45 kDa were of very low intensity. One band of 41.5 kDa was of very very low intensity.

Ten bands in lane 6 of Fig. 5 were observed in adipose tissue of 2 days 0.75µg/µl ecdysone treated insects. One band of 97.5 kDa was of high intensity. Four bands of 31, 68.5, 100, and 110 kDa were of low intensity and four bands of 6.5, 50, 60 and 195 kDa were of very low intensity. One band of 45 kDa was of very very low intensity.

Nine bands in lane 7 of Fig. 5 were observed in adipose tissue of 2 days 1.5µg/µl ecdysone treated insects. One band of 97.5 kDa was of low intensity. Five bands of 31, 50, 68.5, 100 and 110 kDa were of very low intensity while three bands of 6.5, 66 and 195 kDa were of very very low intensity.

Fig. 6: Showed the very charged protein fractions were increased in number and intensity in adipose tissue of adult female insects of control groups of *Dysdercus similis* while depletion in number and intensity of protein fractions was observed in 4 days treated experimental groups due to the effect of JHA and ecdysone.

The molecular mass of the adipose tissue of control 4 days adult female of *Dysdercus similis* ranged from 6.5 to 195 kDa, while adipose tissue of 4 days JHA and ecdysone treated adipose tissue of *Dysdercus similis* adult females was ranged from 6.5 to 100 kDa as exhibited in Fig. 6.
Observations

Thirteen bands in lane 1 of Fig. 6 were observed in the adipose tissue of control insects. These bands were of 6.5, 14.5, 21, 31, 41.5, 45, 50, 66, 68.5, 80, 100, 110 and 195 kDa molecular weight. Four bands of 31, 80, 100 and 195 kDa were of very very high intensity. Five bands of 6.5, 14.5, 45, 50 and 68.5 kDa were of high intensity. One band of 41.5 kDa was of low intensity while band of 21 kDa was of very low intensity. Two band of 66 and 110 kDa was of very very low intensity.

Eleven bands in lane 2 of Fig. 6 were observed in adipose tissue of 4 days 0.01µg/µl JHA treated insects. Four bands of 31, 80, 100 and 195 kDa were of very high intensity. Five bands of 6.5, 14.5, 45, 50 and 68.5 kDa were of low intensity. Two bands of 21 and 41.5 kDa were of very very low intensity.

Three bands in lane 3 of Fig. 6 were observed in adipose tissue of 4 days 0.03µg/µl JHA treated insects. Two bands of 80 and 100 kDa were of low intensity. One band of 6.5 kDa was of very very low intensity.

Thirteen bands in lane 4 of Fig. 6 were observed in adipose tissue of 4 days 0.002µg/µl ecdysone treated insects. The number and intensity of the bands was same as in lane 1 of 4 days adipose tissue of control female insects.

Eight bands in lane 5 of Fig. 6 were observed in adipose tissue of 4 days 0.03µg/µl ecdysone treated insects. Out of these two bands of 80 and 195 kDa were of very high intensity. Three bands of 6.5, 31 and 100 kDa were of low intensity and three bands of 45, 50 and 68.5 were of very low intensity.

Five bands in lane 6 of Fig. 6 were observed in adipose tissue of 4 days 0.75µg/µl ecdysone treated insects. One band of 195 kDa was of low intensity. One band of 80 kDa was of very low intensity. Two bands of 6.5 and 100 kDa were of very very low intensity.

Two bands in lane 7 of Fig. 6 were observed in adipose tissue of 4 days 1.5µg/µl ecdysone treated insects. Two bands were of 80 and 100 kDa were of very very low intensity.
Fig.7 : Showed the –vely charged protein fractions were increased in number and intensity in adipose tissue of adult female insects of control groups of *Dysdercus similis* while depletion in number and intensity of protein fractions was observed in 6 days treated experimental groups due to the effect of JHA and ecdysone.

The molecular mass of the adipose tissue of control 6 days adult female of *Dysdercus similis* ranged from 6.5 to 195 kDa while adipose tissue of 6 days JHA and ecdysone treated adipose tissue of *Dysdercus similis* adult females was ranged from 6.5 to 195 kDa and 100 kDa as exhibited in Fig. 7.

Twelve bands in lane 1 of Fig. 7 were observed in the adipose tissue of control insects. These bands were of 6.5, 14.5, 21, 31, 41.5, 45, 50, 68.5, 80, 100, 110 and 195 kDa molecular weight. Two bands of 100 and 195 kDa were of very very high intensity. Five bands of 6.5, 14.5, 41.5, 21 and 50 kDa were of high intensity. Two bands of 41.5 and 195 kDa were of very high intensity. Two bands of 45 and 110 kDa were of very low intensity. One 80 kDa was of very very low intensity.

Ten bands in lane 2 of Fig. 7 were observed in adipose tissue of 6 days 0.01µg/µl JHA treated insects. Two bands of 100 and 195 kDa were of very high intensity. One band of 6.5 kDa was of high intensity. Four bands of 14.5, 21, 31 and 50 kDa were of low intensity. Three bands of 45, 80 and 110 kDa were of very very low intensity.

Four bands in lane 3 of Fig. 7 were observed in adipose tissue of 6 days 0.3µg/µl JHA treated insects. Two bands of 100 and 195 kDa were of high intensity while one band of 6.5 kDa was of low intensity. One band of 50 kDa was of very low intensity.
Twelve bands in lane 4 of Fig. 7 were observed in adipose tissue of 6 days 0.002 µg/µl ecdysone treated insects. The number and intensity of the bands was same as in lane 1 of 6 days adipose tissue of control female insects.

Eight bands in lane 5 of Fig. 7 were observed in adipose tissue of 6 days 0.03 µg/µl ecdysone treated insects. Three bands of 6.5, 100 and 110 kDa were of high intensity. One band of 31 kDa was of low intensity. Three bands of 14.5, 21 and 50 kDa were of very low intensity while one band of 80 kDa was of very very low intensity.

Four bands in lane 6 of Fig. 7 were observed in adipose tissue of 6 days 0.75 µg/µl ecdysone treated insects. Two bands of 6.5 and 195 kDa were of low intensity. Two bands of 31, and 100 kDa were of very low intensity.

One band in lane 7 of Fig. 7 was observed in adipose tissue of 6 days 1.5 µg/µl ecdysone treated insects. The band of 100 kDa was of very very low intensity.

Fig. 8: Showed the very charged protein fractions were increased in number and intensity in adipose tissue of adult female insects of control groups of *Dysdercus similis* while depletion in number and intensity of protein fractions was observed in 8 days treated experimental groups due to the effect of JHA and ecdysone.

The molecular mass of the adipose tissue of control 8 days adult female of *Dysdercus similis* ranged from 6.5 to 195 kDa while adipose tissue of 8 days JHA and ecdysone treated adipose tissue of *Dysdercus similis* adult females ranged from 50 to 100 kDa and 50 kDa as exhibited in Fig. 8.

Seven bands in lane 1 of Fig. 8 were observed in the adipose tissue of control insects. These bands were of 6.5, 21, 31, 50, 100, 110 and 195 kDa molecular weight. Four bands of 6.5, 50, 100 and 195 kDa were of high intensity. Three bands of 21, 31 and 110 were of very very low intensity.
Six bands in lane 2 of Fig. 8 were observed in adipose tissue of 8 days 0.1µg/µl JHA treated insects. Four bands of 6.5, 50, 100 and 195 kDa were of low intensity. Two bands of 21 and 110 kDa were of very very low intensity.

Two bands in lane 3 of Fig. 8 were observed in adipose tissue of 8 days 0.3µg/µl JHA treated insects. The bands of 50 and 100 kDa were very low intensity.

Seven bands in lane 4 of Fig. 8 were observed in adipose tissue of 8 days 0.002µg/µl ecdysone treated insects. The number and intensity of the bands was same as in lane 1 of 8 days adipose tissue of control female insects.

Four bands in lane 5 of Fig. 8 were observed in adipose tissue of 8 days 0.03µg/µl ecdysone treated insects. Two bands of 50 and 195 kDa were of low intensity. Two bands of 6.5 and 100 kDa were of very low intensity.

Two bands in lane 6 of Fig. 8 were observed in adipose tissue of 8 days 0.75µg/µl ecdysone treated insects. One band of 50 kDa was of very low intensity, while one band of 195 kDa was of very very low intensity.

One band in lane 7 of Fig. 8 was observed in adipose tissue of 8 days 1.5µg/µl ecdysone treated insects. The band was of 50 kDa of very very low intensity.