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Deltamethrin induced histolopathogy in male reproductive organ of *Chrotogonus Trachypterus* Blanchard (Orthoptera; Acrididae)

Shashi Meena[1], Vinod Kumari[2]
[1-2] Department of Zoology, University of Rajasthan, Jaipur, India

Abstract
Histopathological changes in the male reproductive organ of *Chrotogonus trachypterus* Blanchard were examined when topically treated sub lethal dose (9.93 ppm) of deltamethrin. Testes were removed out, dehydrated, stained and observed under light microscope. Degeneration and vacuolization were started ino germ cells 12 h after treatment. Considerable changes were observed 24 h after treatment where testicular wall protruded out along its periphery. Disintegration of cytoplasm and formation of vacuoles were observed in the spermatogonia and spermatocytes in maturation region. Disruption in reduction division, pycnotic nucleus and karyorrhexis in the spermatocyte widely observed. Pronounced damaged of cytoplasmic and nuclear material was observed 48 h after treatment. These findings represented sublethal effects of deltamethrin at cellular level and supported the indication of less survival abilities of *C. trachypterus*.

Keywords: *Chrotogonus Trachypterus* blanchard, deltamethrin, histopathological changes

1. Introduction
The *Chrotogonus trachypterus* Blanchard is a acridid grasshopper and commonly called as surface grasshopper. It damage wide variety of crops and vegetables in their seedling stage. The control strategy for damaging stages of the pest mainly relies upon synthetic chemical insecticides that have been using since long period. Synthetic pyrethroids are new group of insecticides and safer, due to its lower toxicity to mammals and higher biodegradability in nature. They have extremely higher insecticidal activity at lower doses. These are synthetic analogue of naturally occurring substance pyrethrin, which derived from *Chrysanthemum cinerariaefolium* flowers. They are mainly nerve poison and lipophilic in nature. (Dong, 2007 [3]; Soderlund, 2012 [25]. and Silver *et al*., 2014) [21]. Synthetic pyrethroids are extensively used against agricultural, domestic and public health pests and their potential role in management strategy (Rozilawati *et al*, 2005 [18]; Tommy *et al*, 2013). Deltamethrin is one of the important pyrethroid having one cis-isomer which considerably increases its efficacy. It kills pests by contact directly and disturbs functioning of nervous system. They induce ions imbalance in voltage gates of sodium channels which leads to constant flow of action potential and further blocks nerves activity (Gupta, 1999 [7]; Shrivastava *et al*., 2011). Deltamethrin mediated histopathological changes were earlier reported in midgut of *Periplanata americana* and *C. trachypterus* (Singh, 1990 [22]; Majumdar *et al*., 2016) [12].
This experiment was carried out to determine the histopathological changes occurred in the testis follicles of male *C. trachypterus* from the treatment with sub lethal concentration (LC_{50}) of deltamethrin.

2. Materials and methods
Nymphs and adults of *C. trachypterus* were collected from field and the stock culture was maintained in the laboratory at room temperature (32±2˚C and 60±5% R.H). Adult males were topically treated with sub lethal concentration (LC_{50}-9.93 ppm) of deltamethrin at base of the wing by using a micro syringe (Hemilton- Bonadoz, Schwez). Testes follicles were removed/excised from abdominal segment. Histopathology carried out on both controlled and treated test insects. Tissues were fixed in Bouins fluid for 20 hrs and proceed for dehydration in alcohol series of ascending order 30%, 50%, 70%, 90% for 30 minutes while in 100% for 1 hr. Tissues further kept in alcohol and xylene (1:1) solution for 1 hr and then embedded in paraffin wax for 1 hr with 3 changes. Sections were further cut (5 micron) followed by double staining in haematotoxyline and alcoholic eosin and mounted in DPX. Observations were taken under light compound microscope and photographs were taken using Cannon compound microscope with proper magnification. Comparison was made with normal histology of male grasshopper testis.

3. Results
a. Structure of normal testis
Male reproductive organs of *C. trachypterus* Blanchard typically consist of a pair of testes connected with paired seminal vesicles and a median ejaculatory duct. The testes lie above the gut in the abdomen and are often close to the midline. Each testis contains many testis tubes or follicles. Each testis follicle is full of a mass of developing germ cells arranged in a proper sequence that shows the process of spermatogenesis from the apical to base end. At the distal end of each testis follicle is germarium in which germ cells divide to produce spermatogonia. The wall of each follicle is a thin epithelium supported on a basal lamina. The follicles are bound together by a peritoneal sheath. There are three region of development of germ cells just beneath the germarium. The testis follicles are grouped in large sacs and the adjacent sacs are separated by epithelial septa (Es). On an average there are 16.5±0.40 testis follicles in a pair of testis. Size of testis follicles ranged from 0.1 to 0.2 mm with an average 0.12±0.01 mm.
Zone of growth consists primary spermatogonia enclosed in

cysts, divided and increase in size to form spermatocytes. In growth region spermatogonia produce spermatocytes by mitotic division. (Fig.1,2). The spermatogonia obtain nutrient from a large apical cell with which they have cytoplasmic connections. Around the primary spermatogonia are connective tissue cells of irregular elongated shape, smaller in size and having a deeply staining nucleus. These cells are concerned with the formation of cyst wall which surrounds various stages of the germ cells in separate compartment except the spermatozoa. The primary spermatogonia give rise to groups of secondary spermatogonia which are enveloped by cyst, each group of spermatogonia forming a spermatocyte by mitotical division. Secondary spermatogonia are small than primary spermatogonia in size, stained deeply and not finely analyzed in light micrographs. All the cells of a cyst are generally at the same stage of development. Interzonal bodies connected spermatogonia each other with their cytoplasmic ends. Later spermatogonia loose this rosette like arrangement and seen in irregular manner. In normal testis follicles showed synchronous cell division spermatogonia to spermatocytes. Spermatocytes undergo their division and development normally and differentiate in spermatids.

Zone of maturation, in this region spermatids are produced by two meiotic divisions in the spermatocyte. During resting period the chromatin is arranged in the form of granules in nucleus of the early spermatocytes. The spermatocytes are followed by a zone of spermatids. Various stages of maturation division can be observed in this region (Fig. 3).

Zone of transformation, in which the spermatids develop into spermatozoa, a process known as spermieogenesis. This zone contains spermatozoa at different levels of development. Each spermatozoa consists of an elongated nucleus as head and long tail attached to it. In surface grasshopper sperms are bound together in sperm bundle. The number of sperms ultimately produced by a cyst depends on the number of spermatogonial divisions and this is fairly constant for a species. About 16 to 21 sperms are present in a sperm bundle in orthoptera (Chapman, 1973) [2].

b. Histopathology (light microscopy)

After 12 h of treatment no marked effect was observed in morphology of testis while, slight pathological changes have been occurred in germ cells. Some follicles were noted with loosening of germ cells, degeneration and formation of vacuoles occurred in the spermatogonia as well as spermatocytes (Fig. 5, 6). Arrangement of the chromosomes at meiosis was slightly disrupted at growth zone. Some spermatocytes showed karyorrhexis and pycnosis of chromatin material (Fig. 7, 8). Degeneration of cytoplasm in spermatids was associated with karyorrhexis while, loss of nucleus was also observed in transformation region (Fig. 7, 8). Degeneration of cytoplasm and formation of vacuoles were observed in transformation region as starting disruption of normal structure of testis. Spermatozoa were morphologically normal but present in scattered form. Most of the follicles remained unaffected (Fig. 6, 8).

Marked changes were observed 24 h after treatment as testicular epithelium protruded out along its periphery (Fig. 9). Few spermatogonia were present and the number of spermatocytes also decreased. Degeneration of cytoplasm was observed in spermatogonial region which was more pronounced. In growth zone arrangement of the chromosomes at meiosis was severely disrupted. There was further increase in the number of affected spermatocytes with pycnotic nucleus and karyorrhexis (Fig. 10). The chromatin material in many spermatocytes has shrunken from most of the nucleus forming a cresent shaped structure on one side. Some nuclei of spermatids also showed the process of pycnosis and dissolution (karyorrhexis and chromatolysis) in transformation region. Some of the spermatids exhibited loss of nucleus (Fig. 11, 12). Spermatozoa retained their normal texture.

Damage persisted and became more pronounced in insects 48 h after treatment as compared to control. The cyst wall was seen to be dissolved in several follicles (Fig. 13). It was common to observe cysts with degenerating spermatocytes and others showing wide empty spaces, probably as a result of germ cell degeneration and reabsorption (Fig. 13, 14). Shrinkage resulted due to disintegration of cytoplasm and increased spaces between cells which lead into formation of vacuoles were observed (Fig. 15, 16). Growth zone of testis were filled with pycnotic spermatogonia and spermatocytes. The destruction is more obvious that almost all the spermatogonia in most of the follicles showed pycnosis and decrease in number (Fig. 14, 15, 16). Spermatogonia, spermatocytes and spermatozoa were severely damaged beyond recognition. The most affected were spermatocytes which showed clumping of chromatin material in general, loosening of germ cells was prominent. Many pycnotic cells were seen in this region and hypertrophid cells were also observed which were eosinophilic and diad compact cells. In the transformation zone cells totally damaged and tissue lost its consistency due to formation of vacuoles. Number of spermatids and spermatozoa decreased and degeneration of cytoplasm, pycnosis and karyorrhexis occurred in them (Fig. 15, 16).

Almost the whole cytoplasmic and nuclear material was damaged. Spermatids became thickened and became elongated cells. These elongated cells suggest a physiological inactivation of spermatids. Consequently the testicular debris increased. Sperms lost their arrangement due to breakage of cyst wall which were observed near the periphery in bunch but no definite pattern was observed (Fig. 16).

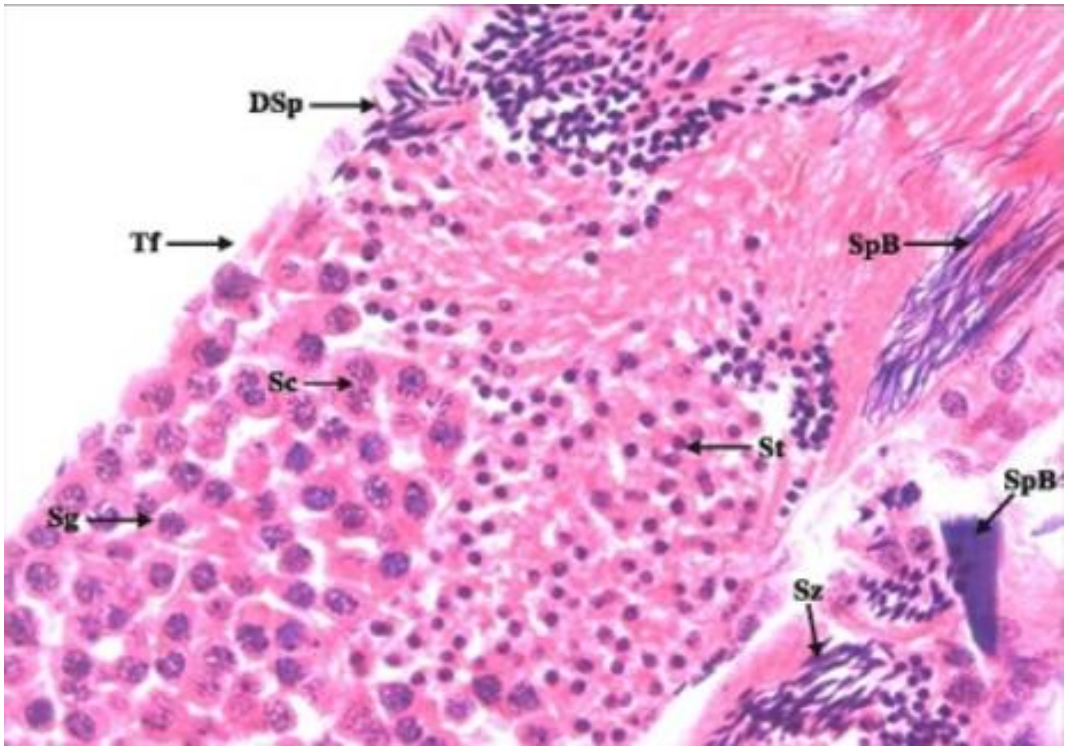

Fig 1: light micrograph of normal testis of C. trachypterus showing testicular follicle (tf) with successive zone of spermatogonia (sg), spermatocytes (sc) sipermatids developing sperms (dsp) and sperm bundles (spb). Haematoxylin-Eosin, 200X

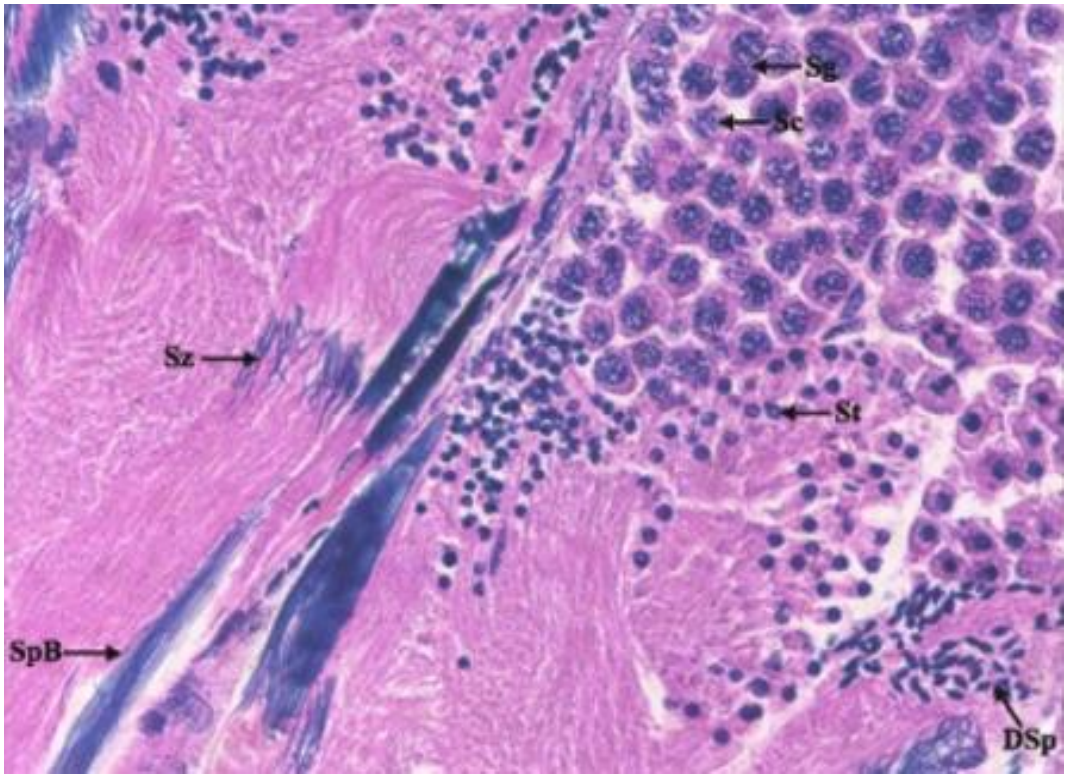

Fig 2: light micrograph of normal testis of C. trachypterus showing testicular follicle with pro minent sperm bundles (spb). With developing sperms (dsp) and spermatozoa Haematoxylin-Eosin, 200X

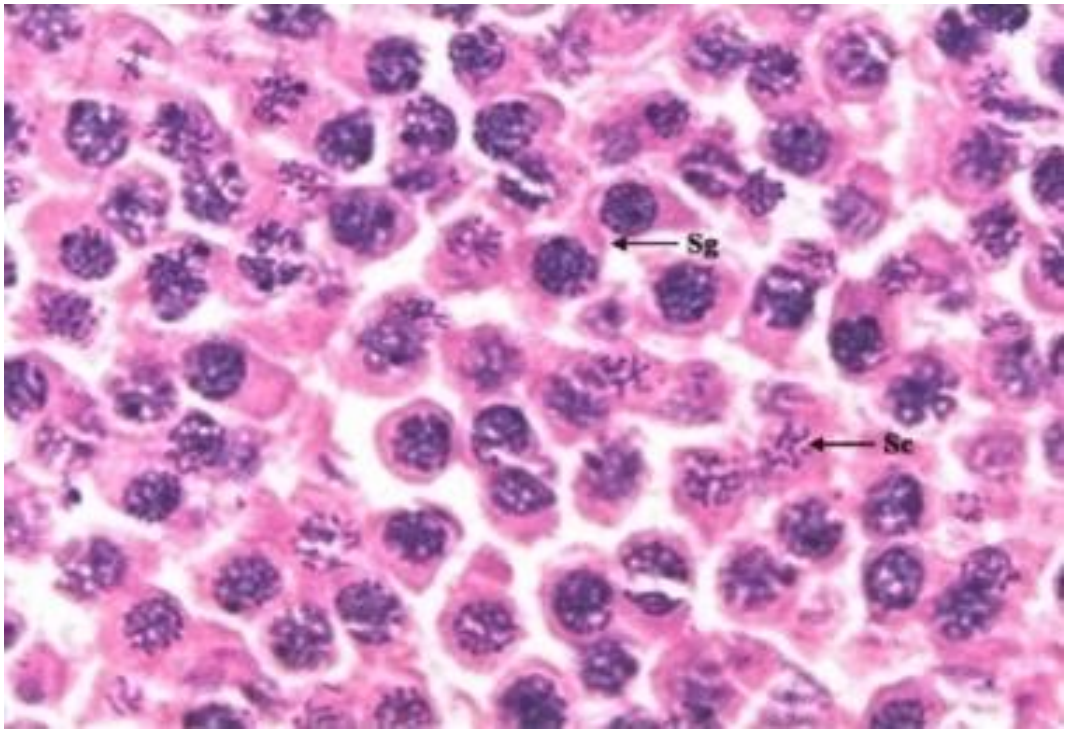

Fig 3: light micrograph of normal testis of C. trachypterus showing spermatogonia (sg) and spermatocytes (sc) at different satges of cell division. Haematoxylin-Eosin, 400X

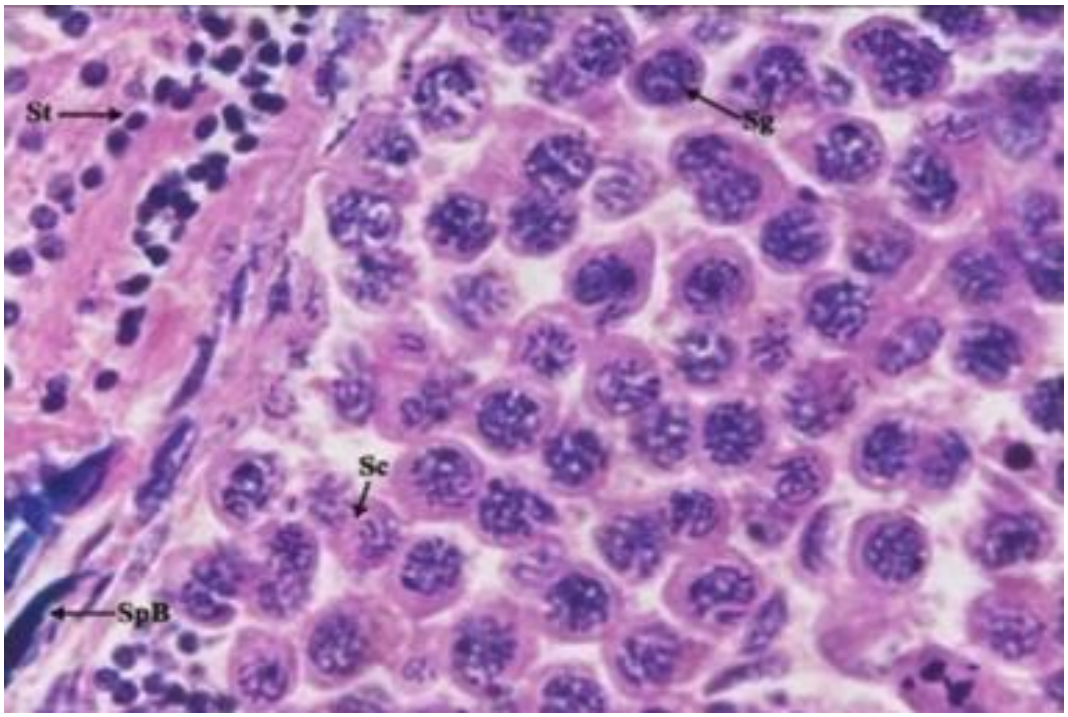

Fig 4: light micrograph of normal testis of C. trachypterus showing spermatocytes (sc) at different satges of cell division, spermatids (st) and sperm bundle (spb), Haematoxylin-Eosin, 400X

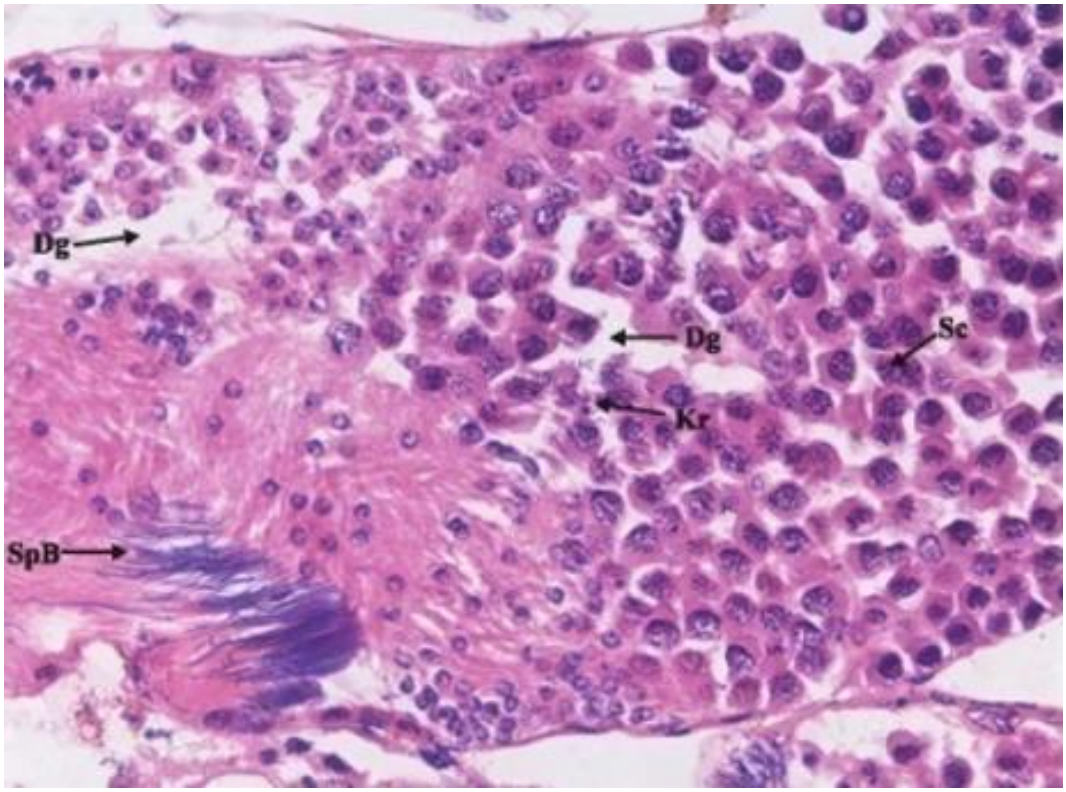

Fig 5: light micrograph of 12 h treated testis of C. trachypterus exhibiting slight degrneration (dg), karyorrhexis (kr) and loosening of germ cell. Haematoxylin-Eosin, 200X

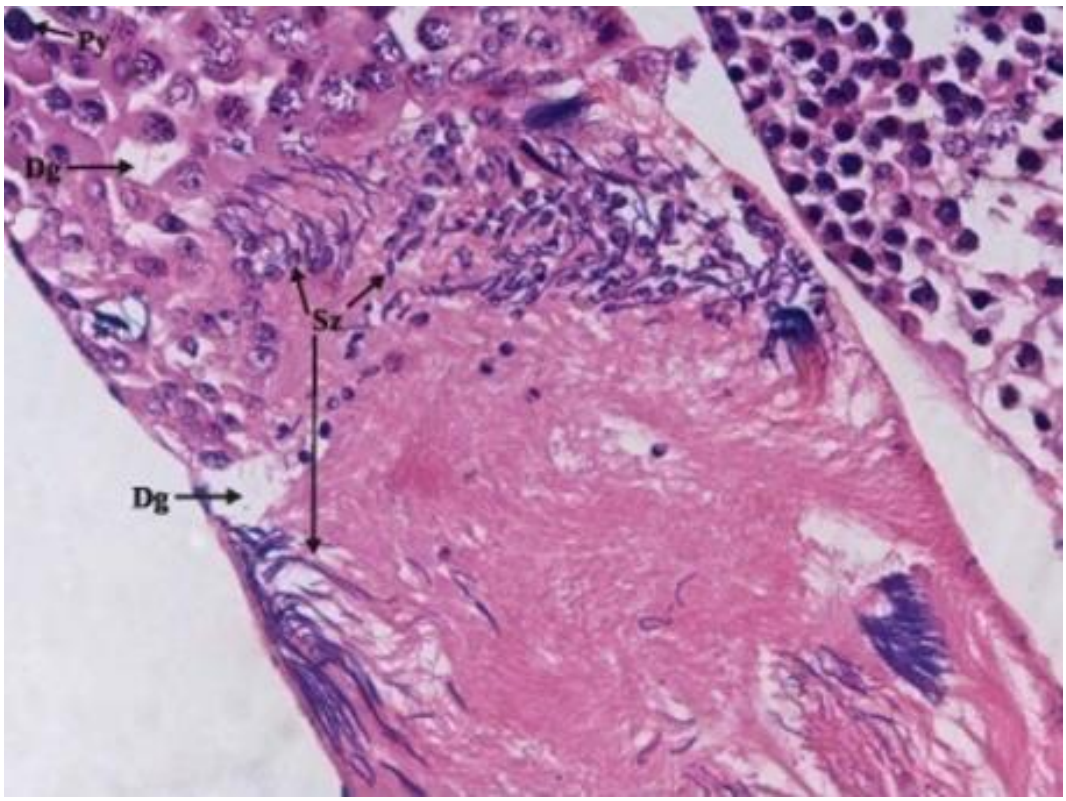

Fig 6: light micrograph of 12 h treated testis of C. trachypterus exhibiting degrneration (dg), pycnosis (py) and scattered spermatozoa (sz). Haematoxylin-Eosin, 200X

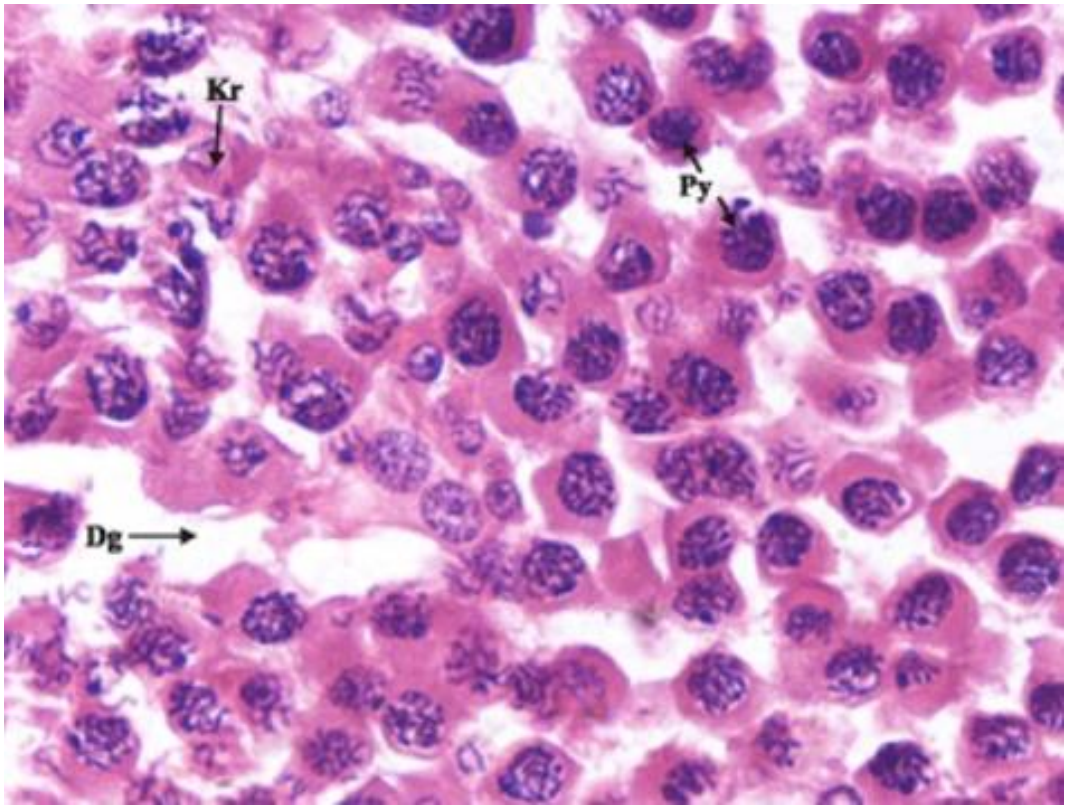

Fig 7: higher magnification of 12 h treated testis of C. trachypterus exhibiting degrneration (dg) of the spermatocytes with karyorrhexis (kr) and pycnosis (py). Haematoxylin-Eosin, 400X

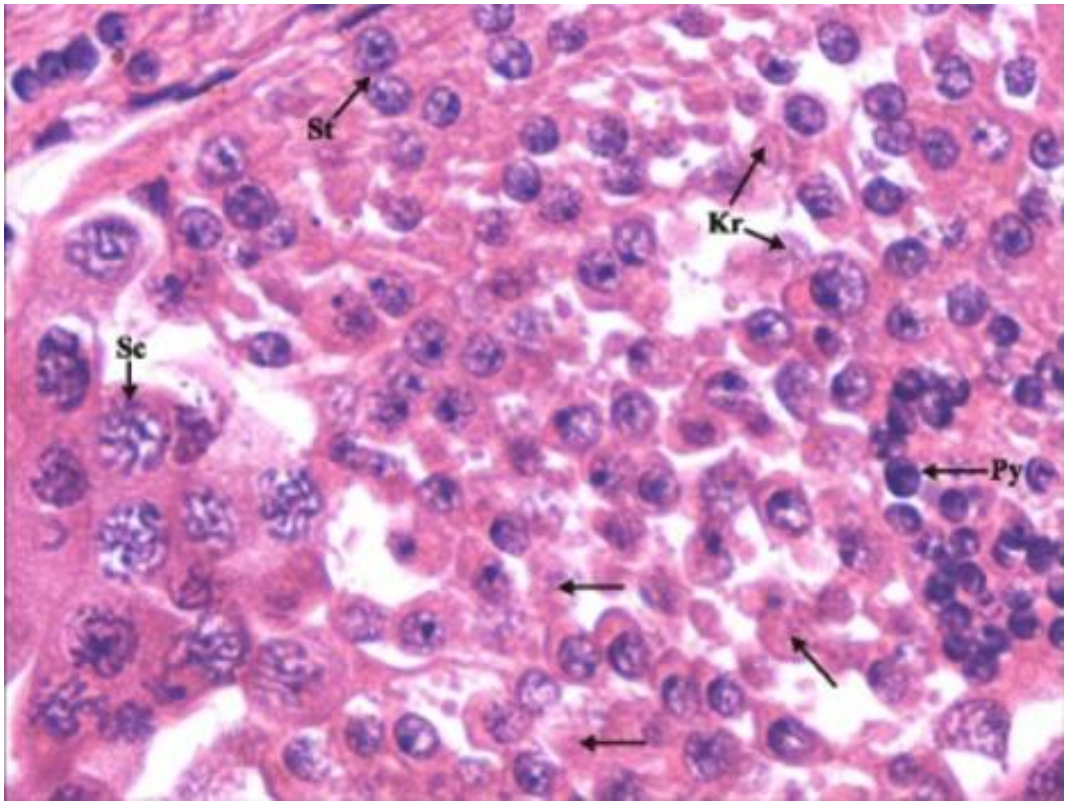

Fig 8: higher magnification of 12 h treated testis of C. trachypterus showing loss of nucleus (↟) karyorrhexis (kr) in spermatids (st). Haematoxylin-Eosin, 400X

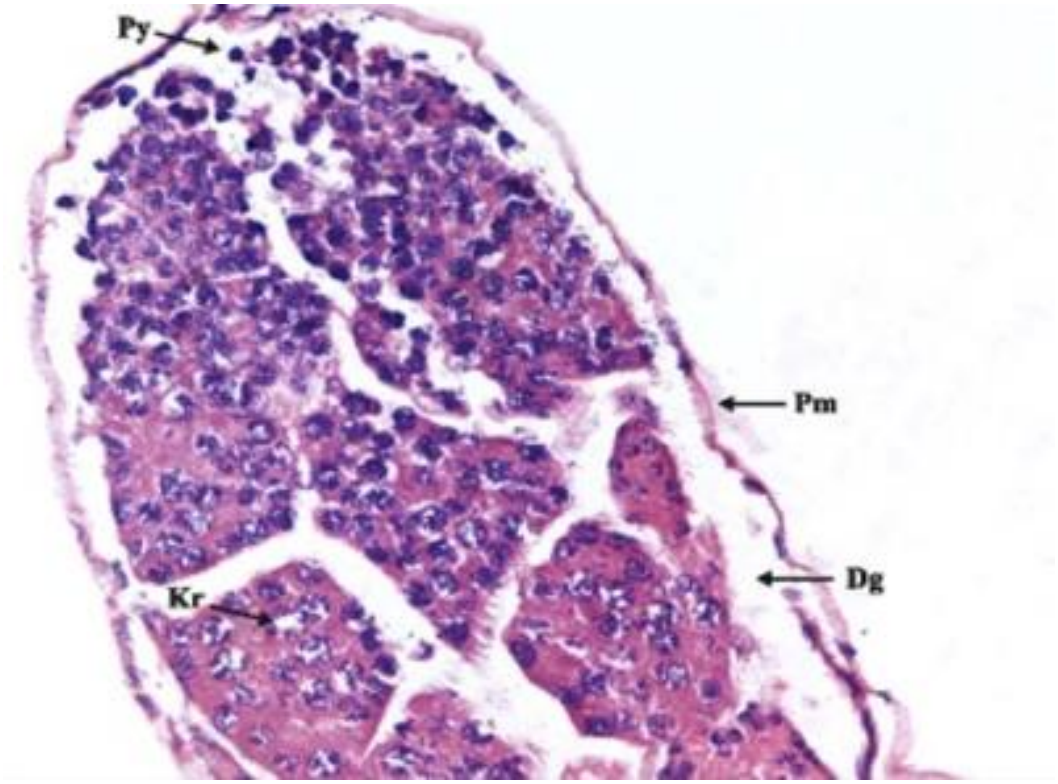

Fig 9: light micrograph of 24 h treated testis of C. trachypterus exhibiting karyorrhexis (kr) and protruding peritoneal membrance (pm). Haematoxylin-Eosin, 200X

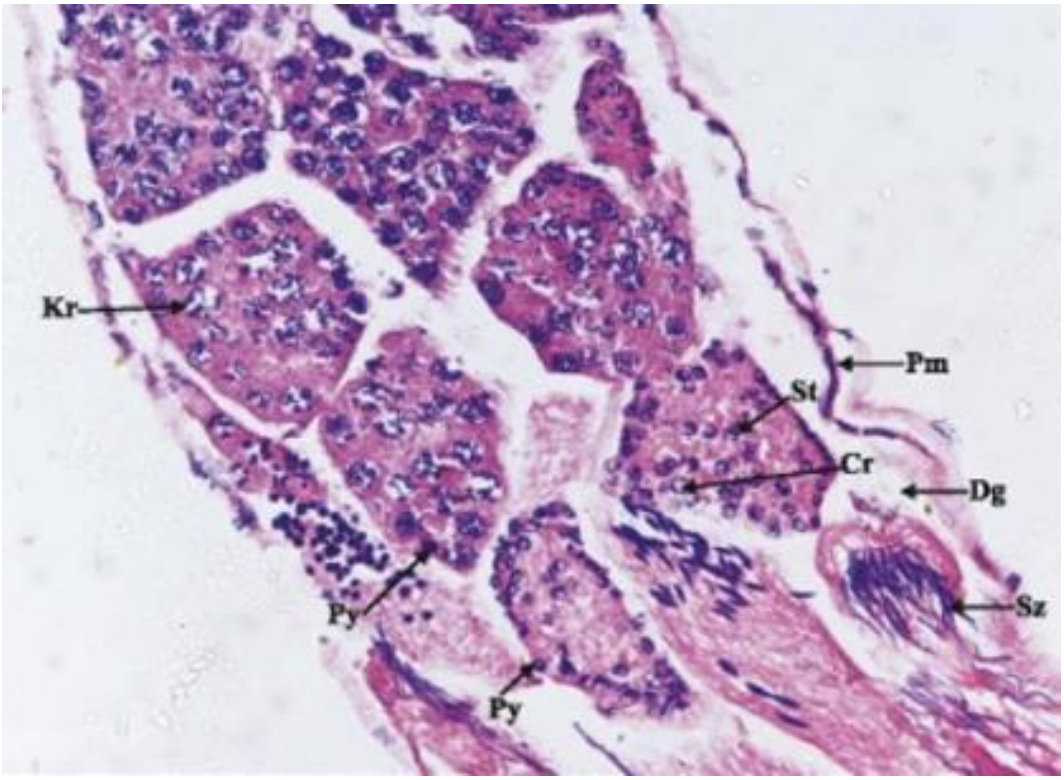

Fig 10: light micrograph of 24 h treated testis of C. trachypterus exhibiting degrneration (dg) of connective tissue chromatolysis (Cr) and pycnosis (py) in spermatids (st). Haematoxylin-Eosin, 200X

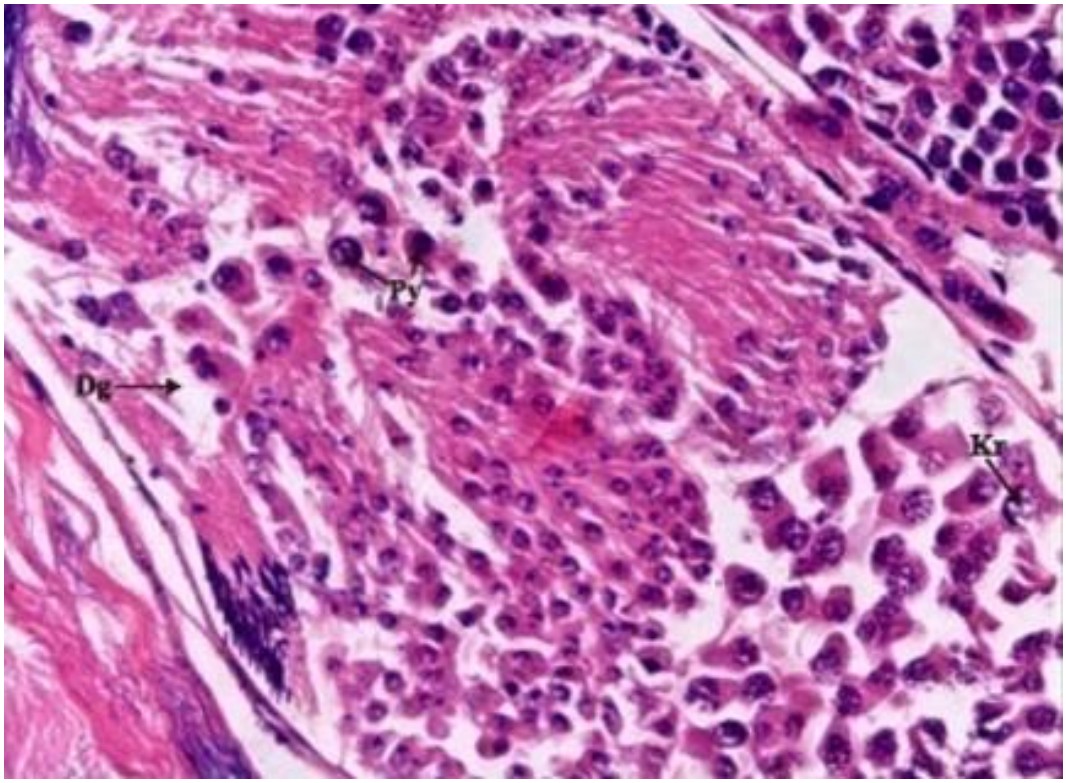

Fig 11: light micrograph of 24 h treated testis of C. trachypterus exhibiting karyorrhexis (kr) and pyscnosis (py) in germs cells. Haematoxylin-Eosin, 400X

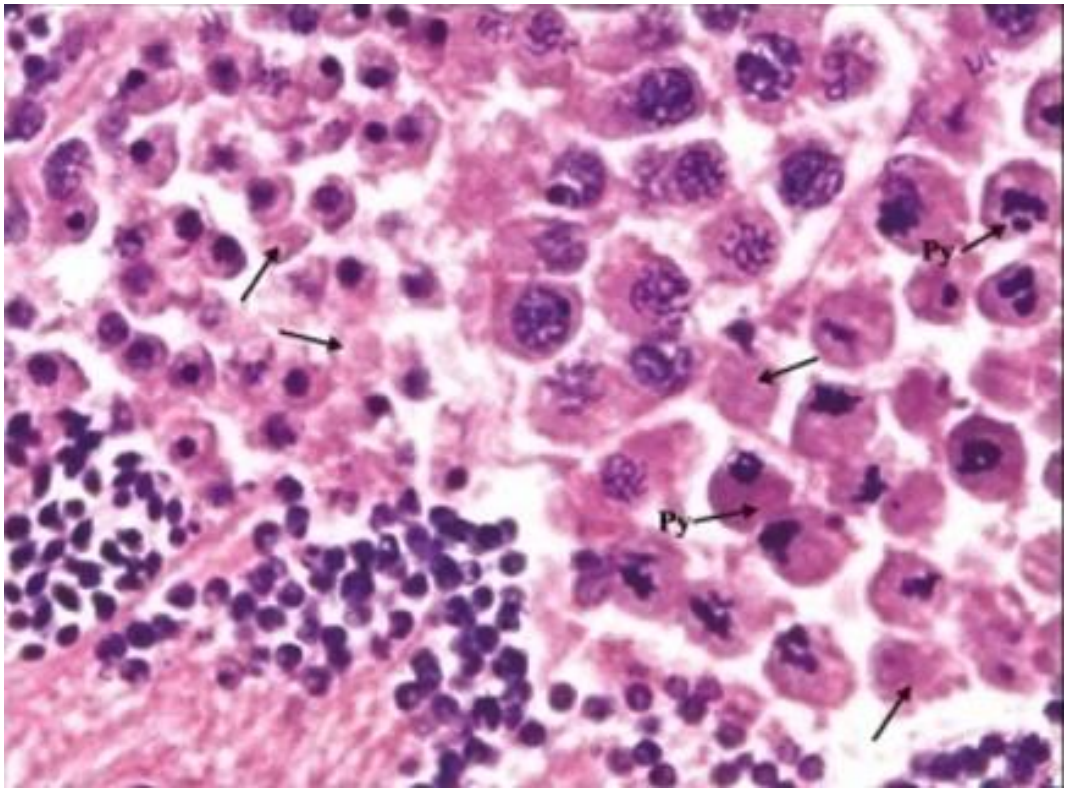

Fig 12: higher magnification of 24 h treated testis of C. trachypterus exhibiting pycnosis (py), loss of nucleus (↑) and degeneration in spermatids (st). Haematoxylin-Eosin, 400X

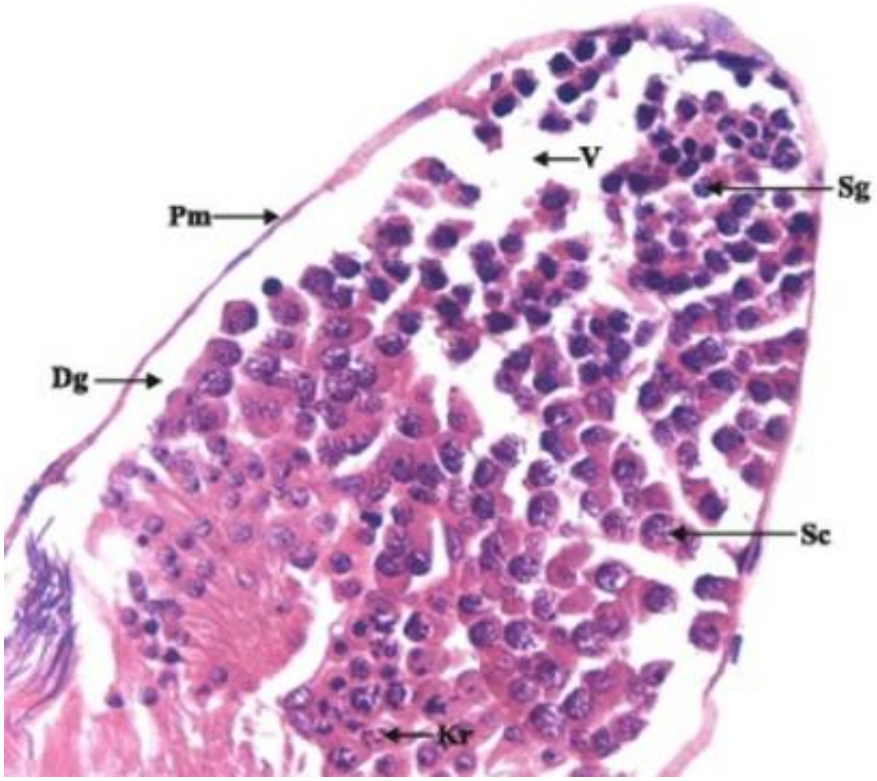

Fig 13: light micrograph of 48 h treated testis of C. trachypterus exhibiting degeneration (dg) karyorrhexis (kr) spermatogina (sg) and spermatocytes (sc) and vacuolization (v). Haematoxylin-Eosin, 200X

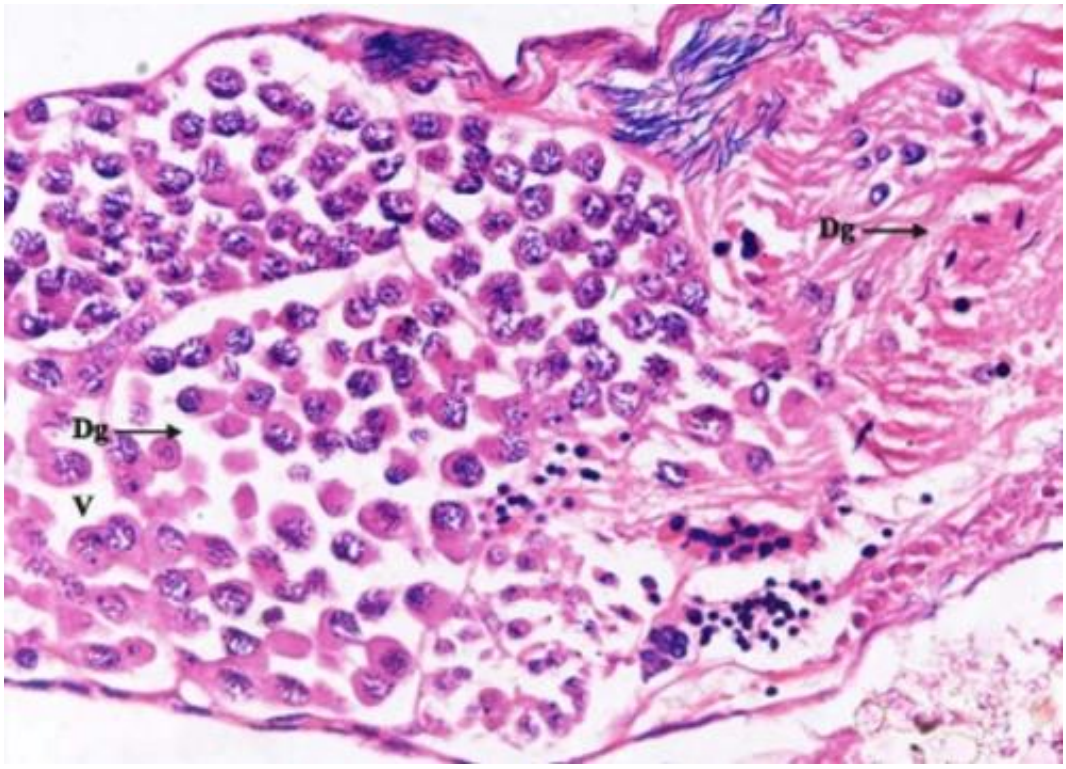

Fig 14: light micrograph of 48 h treated testis of C. trachypterus exhibiting vacuolization (v). and degeneration (dg) of connective tissue and germ cells. Haematoxylin-Eosin, 200X

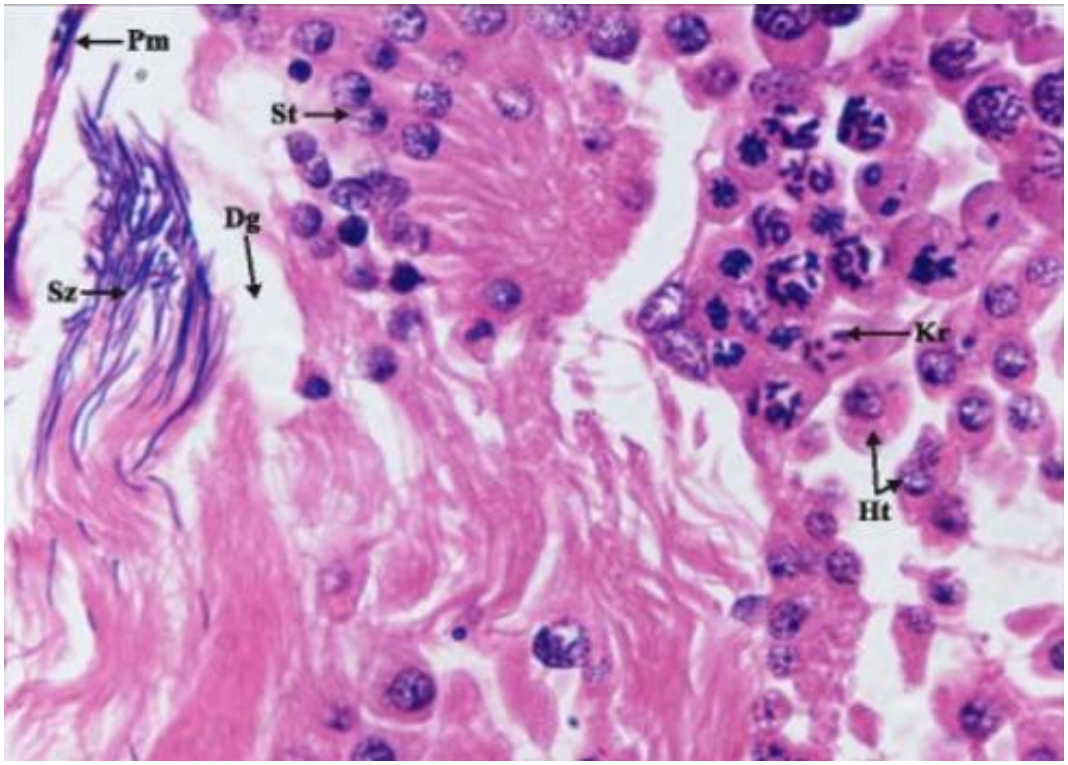

Fig 15: higher magnification of 48 h treated testis of C. trachypterus exhibiting karyorrhexis (kr) hypertrophid cells (Ht) and less number of spermatids (St) and spermaoza (Sz) and degeneration of connective tissue (dg). Haematoxylin-Eosin, 400X

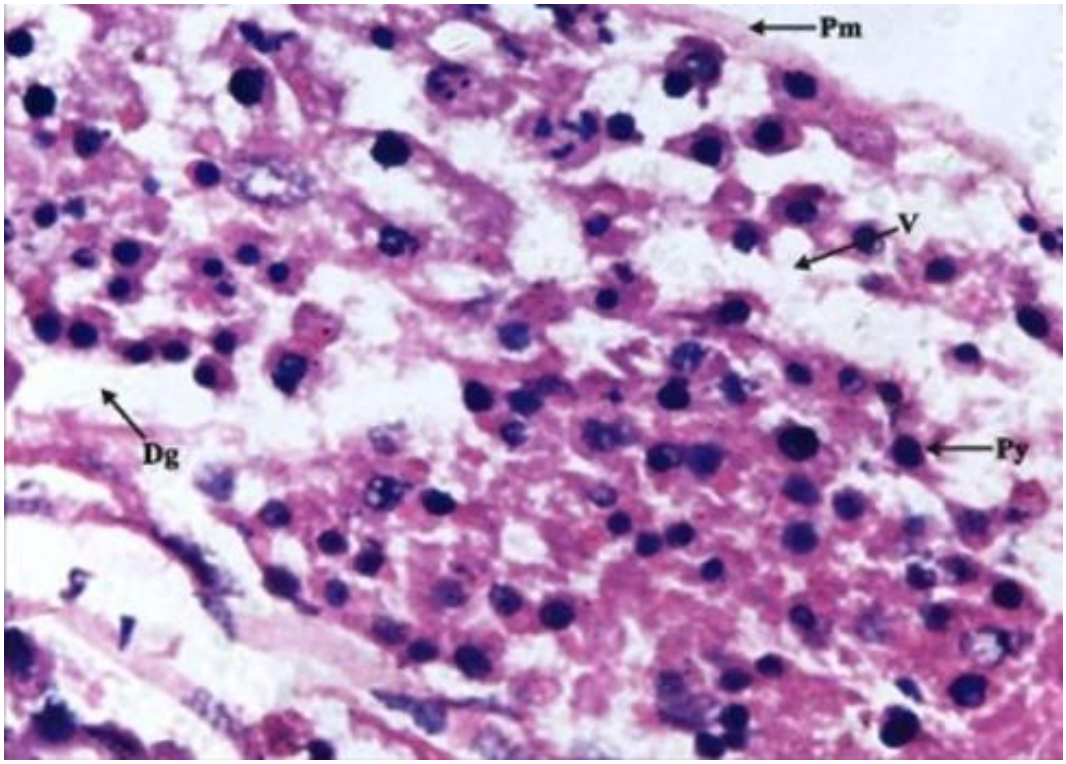

Fig 16: light micrograph of 48 h treated testis of C. trachypterus exhibiting pycnotic nuclei (py) and vacuolization (v). degeneration (dg) of matrix and peritoneal membrane (pm). Haematoxylin-Eosin, 200X

4. Discussion

A histological study of *C. trachyperus* revealed that testes are compact structure consisting successive order of testicular follicles with germ cells located in different zones of development. Similar patterns were observed in *Locusta migratoria* (Sheikher and Mittal, 1986) [19], *Atractomorpha crenulata* (Muse, 2000 & 2002) [14, 15], *Heteracris littoralis* Ramb. (Ghajawi *et al*., 2007), *Schistocerca gregaria* and *Poecilocerus pictus* (Martoja, 1964; Gupta & Rathore; 2006 [9]; Hussien *et al*., 2008 and Reda *et al*., 2010) [16]. Each of testicular follicles contains germ cells at various stages of development. Spermatogenesis is a sequential order of cell division through which spermatogonia produced spermatocytes which further give rise to spermatids. The spermatid transformed into sperm involving distinguished morphological reorganizations in the cells.

Pathological changes appeared in degrading patterns of testes follicles after treatment with deltamethrin. Present observations are in agreement with Ahi (1988) [1]. who revealed that in males of *P. pictus*, differentiating germ cells showed pycnosis, which caused prominent damage in germ cells 16 days after treatment with sub lethal concentration of aldrin. Pycnotic spermatogonia and spermatocytes were also observed with endosulfan treatment (Janak, 1992) [11]. However, Singh (2002 & 2003) [23, 24]. noted that there were no apparent morphological changes in testis of *Earias fabia* Stoll but testicular tissue showed remarkable degeneration in spermatid cysts and sperm bundles when treated with thiotepa. *H. littoralis* (Orthoptera) nymphs resulted with degenerated testis follicular cells, deformed spermatids and sperm bundles when treated with azadirachtin (Ghazawi *et al*., 2007) [6]. Testis follicular epithelium of *S. gregaria* totally disintegrated with treatment with sub lethal dose of anti-chitin compound (cascade), plant extract (*Oriza sativa*, bran extract) and synthetic pyrethroids (Karate). Cyst formation inhibited around the spermatogonia at higher doses (Hussein *et al*., 2008) [10]. 5th male nymphal instars of *S. gregaria* showed severe abnormalities in germ cells and seized spermatogenesis at various stages of growth after 1 day treatment with Lufox LC_{50} (Reda *et al*., 2010) [16]. Deltamethrin induced cytological abnormalities in the male reproductive system of *Chrysomya megacephala* causing detachment and disruption of the epithelium of the testicular tissue. Number of spermatocytes, spermatogonia and spermatozoa were also reduced. Vacuolization in the various zones, necrosis and pycnosis in nucleus is also reported (Gupta and Amir, 2018) [17]. However, it should be pointed out that factors that regulate spermatogenesis are not well understood (Dumser, 1980) [4]. There is no strong evidence to indicate that hormones are generally involved (Engelmann, 1970) [5], but in some moths, the moulting hormone could facilitate the process by increasing the permeability of the wall of the testis to some macromolecular factors (Wild and Loof, 1973) [27]. It seems that deltamethrin prevent the spermatogonia from cyst formation and therefore the spermatocytes failed to make normal division to complete spermiogenesis and showed aberration and degeneration.

6. Conclusion

Deltamethrin induced toxic affect on the male reproductive organ of *C. trachypterus* at sublethal dose (9.93 ppm). It significantly caused deformation and degeneration of germ cells at various stages of growth and further blocked the normal process of spermieogenesis. Thus, deltamethrin at low concentration can be used to control population of *C. trachypterus* as effective tool in insect pest management strategy.

7. Acknowledgement

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