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Original Article

Effect of Male Hormone (17α-Methyl Testosterone) on the Histological Changes of Male Dwarf Gourami *Trichogaster Lalius* (Hamilton, 1822)

Anupa Biwsas*, Samarnedra Behera, Sanjeev Kumar****

Abstract

The present study was conducted to know about the effect of different concentrations of synthetic androgen 17α- Methyl testosterone (MT) on histological changes in gonads of male Dwarf Gourami, *Trichogaster lalius* (Hamilton, 1822). Fishes were fed with homogenous mixture of the hormone in ethyl alcohol in its feed for 90 days. On the basis of histological study, the hormonal actions on the testicular changes were distinguished on the basis of the spermatocytes present i.e. primary spermatocytes, secondary spermatocytes and spermatozoa. In lower doses (5 mg/Kg & 10 mg/Kg of feed), the concentrations of the gonadal materials were so closely placed that the identification of different stages was found very difficult up to 45 days. In the higher dose (15 mg/Kg of feed), the gonadal materials were found less concentrated which leads a negative impact on the gonads after 60 days of treatment.

Keywords: Histological Changes; 17α- Methyl Testosterone; Synthetic Androgen; *Trichogaster Lalius.*

Introduction

West Bengal is one of the states of India having a rich wealth of freshwater resources and fish germplasm diversity. It is also a pioneer state in ornamental fish production and export. Due to congenial climatic conditions, Kolkata and its surrounding districts have emerged as promising breeding centers for ornamental fish and a considerable number of small fish farmers and amateurs are engaged in this trade. It is found that 288 exotic varieties of ornamental fish populations are in West Bengal (Bhaskar *et al*., 1989) and 52 native ornamental fishes are available here (Ghosh *et. al*., 2003).

The Dwarf Gourami *(Trichogaster lalius)* is a peaceful freshwater fish, also known as the "Dwarf Gourami". Gourami is the name used for a big variety of perciform fish characterized by flat body and two elongated rays of pelvic fins used as sense of touch. Since they reach only 2 inches, they can be housed in small tanks and are a good fish' for beginners because of their low aggressiveness, easy care and nice look. Males can be easily distinguished from females for their colors. The male is a bit bigger than the female and has turquoise and orange-red iridescent vertical bands on the entire body and on fins; its color mutations with total orange-red body and turquoise dorsal fin, or total turquoise body with just some red at the edges of the fins. The dwarf gourami female is totally silver with pale turquoise vertical stripes (Patro *et al.,* 2015).

Nakorn and Sangsri (1995) reported that the testes of control Tawes (*Puntius gonionotus*) were relatively round with a single stalk attached to peritoneum. They comprised numbers of lobules in which various stages of spermatogenic cells, spermatocytes, spermatids and spermatozoa were found. In the groups of fish being treated for 30 days three types of gonads; testes, regressed ovaries and undifferentiated gonads were observed. The testes of genetic males represented the control fish.

Manosroi *et al*., (2004) reported that the presence of spermatogonia and ovocells in the gonads were used as an indicator of males and females respectively. Intersex gonads contained both oogenic and spermatogenic tissue. Sterile gonads contained large amounts of connective tissue with numerous vessels.

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The present study was conducted to investigate the effect of MT on histological changes of *T. lalius*, i.e., primary spermatocytes, secondary spermatocytes and spermatozoa.

Materials and Methods

The study was conducted during April 2009 to July 2009 in the laboratory of Department of Fisheries Resources Management, Faculty of Fishery Sciences, Chakgaria, Kolkata to understand the effect of different concentrations of dietary administration synthetic androgen 17α-Methyl testosterone on histological changes of male Dwarf Gourami, *Trichogaster lalius* (Hamilton, 1822). The samples were collected from Gullif Street, near to Syambazar, Kolkata, and West Bengal and acclimatized to the laboratory condition by feeding with the commercially available aquarium feed (i.e. Tokyo, Japan). The fishes were acclimatized for 15 days before starting of the experiment. During the experiment fishes were fed with the hormone incorporated feed upto 3 months.

Preparation of Hormone Incorporated Feed

An androgenic steroid hormone as 17α-methyltestosterone (MT) was used in the present study. It was obtained from the (Sigma chemicals Ltd). Three different kinds of feed were prepared by adding three doses of MT as 5 mg of MT per Kg of feed, 10 mg of MT / Kg of feed and 15 mg / Kg of feed. A hormone treated feed will be prepared as described by (Killian and Kohler, 1991).

Histological Study

For observing histological change fish were sacrificed in each 15 days and microscopic slides were prepared by the following procedure of Agarwal (1996). The development stages of germ cells in the testes were studies by the following methods.

Collection and Fixation of Tissue

For histological study, the middle parts of the gonadal tissues (testes) of *T. lalius* were selected. The tissues were put into Bouin's fluid for 24-48 hours as per size of tissue (Testes).

Post Fixation Treatment & Washing

The tissues (testis) were removed from the fixatives and subjected to overnight washing with flowing clear tap water till the noxious formaldehyde odour was not remain.

Dehydration

Then tissue was treated with graded alcohols (i.e. 30, 50, 70, 90 and 100 %) to dehydrate it.

Dealcoholization

Two changes of Xylene (1 hr each) were made to clean the tissues from alcohol. For better impregnation of wax into the tissue, the xylene penetration into the tissue is very important. After xylene treatment the tissue must be transparent and should come up to float on the top.

Infiltration

Paraffin wax (melting point 50-60^{0}C) was used for infiltration of tissue. Three changes of wax (45 min each) were made to make tissue xylene free.

Embedding and Block Preparation

For the preparation of blocks, pure paraffin wax melted in water bath in between 58-60^{0}C Metal 'L' moulds was adjusted according to the size of blocking materials. The melted paraffin was taken from water bath and the blocking disc was filled. After permitting a layer of wax to be solidifying on the bottom of the disc, the completely infiltrated tissue was carefully taken from the paraffin wax and put inside the blocking disc according to the size. Care must be taken, so that the wax on the top of the disc should not be solidified during keeping the material in the blocking disc. For this reason, a heated needle or forceps was put inside the wax of the disc. After the proper positioning of the tissues, the wax inside the disc was allowed to solidify. After few minutes, the 'L' moulds were removed from the wax block. The prepared blocks were kept separately inside the labeled polythene packets.

Trimming and Sectioning

The paraffin blocks were trimmed carefully to 6 to 7 mm^2 by sharp blades. The trimmed blocks were fixed to the wooden holder with the material facing away from it. Molted wax was poured on the holder and the block was kept on it. The block was padded with more wax at the base to make it strong. After being confirm, the blocks were firmly fixed with holder, the sectioning was done by using microtome (Spencer 820 Type). Each section was cut into 5µ

thickness. The ribbons containing tissue were collected on clear glass side with the help of fine brush.

Spreading and Fixing

Glass slides were cleaned properly by concentrated sulphuric acid, soap and finally with tap water. After cleaning, the slides were air dried and a thin layer of glycerin, egg albumin was rinsed over it. Then the ribbons with materials (about 10 to 12 sections depending on the size) were spread over the clean glass slides. The tissue were made wrinkle free allowed to fix on slides by keeping them on hot plates (30^0C) for 2-5 minutes.

Dewaxing and Staining of the Tissues

Tissues fixed on slides were dewaxed with descending order of graded alcohols (100%, 90%, 70%, 50% and 30%) and stained with Haematoxylin and Eosin by using standard techniques (Agarwal, 1996). After staining the slides were air dried.

Mounting

One or two drops of mountant (D.P.X) were put on the dried slide. Then a cover slip was put over it. During putting cover slip the slide was slowly lower when the mountant would flow ahead of the descending glass without trapping air bubble between the cover slip and slide. The excess of mountant on the slides was allowed for drying.

Labeling and Storing

Labeling was done on the slide by glass marking pens to avoid future confusion. The slides were stored inside box to protect them from dust and dirt.

Microscopic Observation

The prepared slides were thoroughly observed under Advanced Trinocular Research Microscope (Olympus Model 8x51) at different magnification. The development stages of germ cells inside the seminiferous tubules of the testis were noticed carefully. Coloured microphotographs of selected histological sections were taken as and when required.

Results and Discussion

In the present study from the GnSI value it is understood that the gonadal development in the treated fishes are found better than the control. Among the treated fishes the GnSI of 10 mg / Kg was

Table 1: Histological changes in testis of *Trichogaster laliaus* during May to July 2009

Months	Control	5 mg/Kg	10 mg/Kg	15 mg/Kg
Initial	ILS was more, LW was more prominent, and PS, SS and SZ were present.			
15thday	ILS was less, LW was less, PS, SS, and SZ was present. SZ was larger in shape.	ILS was more, ILW was packed with SZ. SZ was larger in shap e.	ILS was less, LW was less prominent. PS, SS and SZ were present and smaller in size.	LW was thin, ILS was packed with PS,SS and Sertoli cells were present
30thday	ILM was more, ILS was less, L W was more prominent, PS, SS, SZ was present. SZ was larger in shape.	ILS was more, ILW was thin, PS, SS were present and smaller in size.	ILM was packed with spermatogenic cells. SZ were larger in size, PS, SS was less and smaller in size.	ILS were more, LW were thin, PS, SS were pre sent and small in size.
45thday	LW was more prominent, ILS was more, more SZ, PS and SS was present.	ILW was thin, and ILW was packed with PS, SS and less number of SZ and small in size.	ILS was more, LW was thin, PS, SS was more, and SZ was smaller in size.	LW was prominent, ILS were more, PS, SS, SZ were present and small in size.
60thday	ILS was packed with SZ, ILS was less, and LW was prominent.	ILS was less, ILM were packed with PS, SS and SZ, spermatogenic cells were smaller in size.	ILS was less, LW was very thin, and LM was packed with SZ.	ILS was less, LW was thin, LM was packed with PS, SS and SZ.SZ was larger in size.
75thday	ILS were more, LW were thin, PS, SS were more and SZ present.	ILS were less, PS, SS and SZ were smaller in size.	ILS were more, PS, SS, SZ were sm aller in size.	LW were prominent but cells were not prominent tend to sterile conditions.
90thday	ILS was more, ILM contained more PS and SS, less SZ.	ILS were less, PS,SS, SZ were smaller in size,	ILS was more spermatogenic cells were more.	LW were pro minent, ILS were more, Sterile areas were more

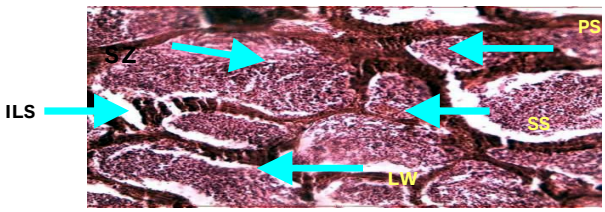


Plate-1

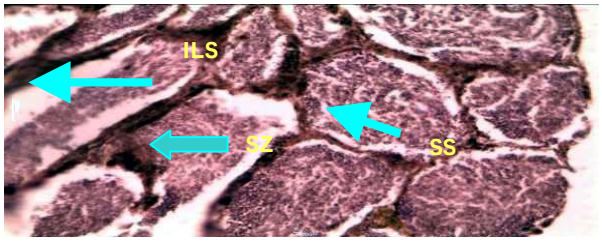
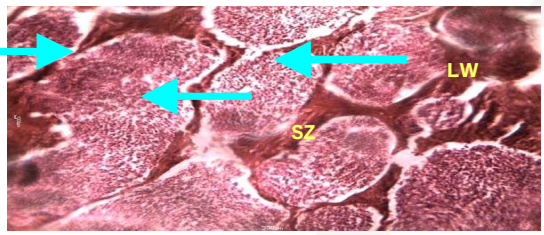


Plate -2



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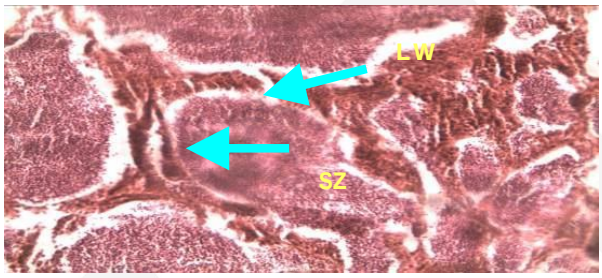

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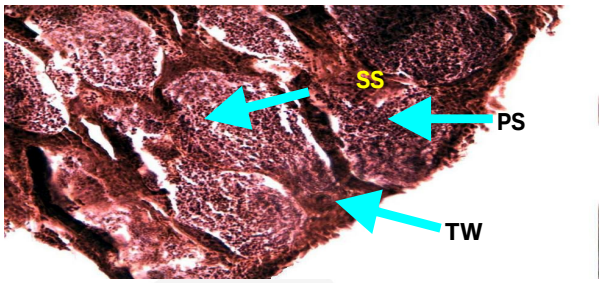

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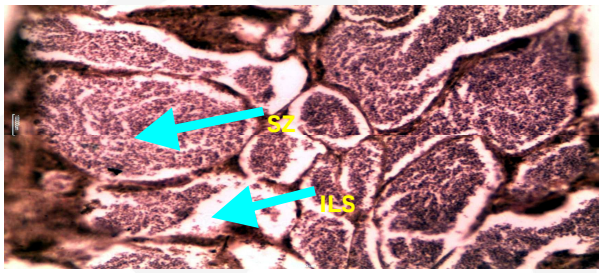

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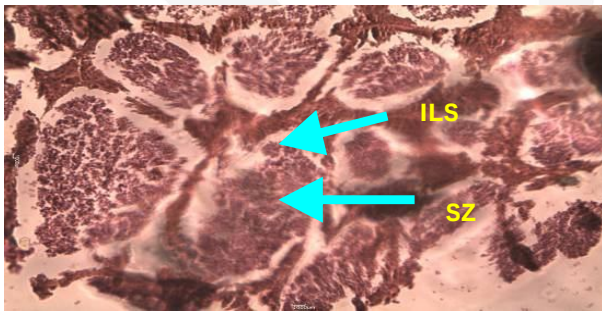


Plate-7

Photomicrograph of the parts of transverse section of testis of *Trichogaster lalius* showing

Plate 1: Testis of control fish at initial period

Plate 2: Testis of control fish at 15th day)

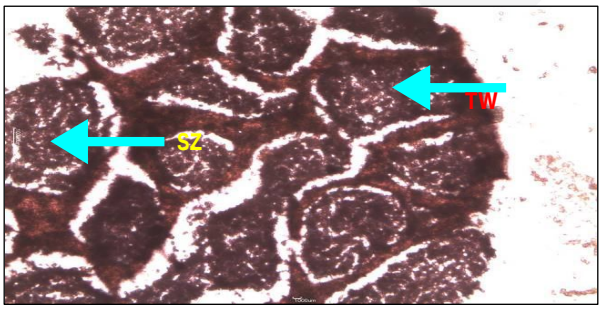
Plate 3: Testis of control fish at 30th day

Plate 4 : Testis of control fish at 45th day

Plate 5: Testis of control fish at 60th day

Plate 6: Testis of control fish at 75th day

Plate 7: Testis of control fish at 90th day



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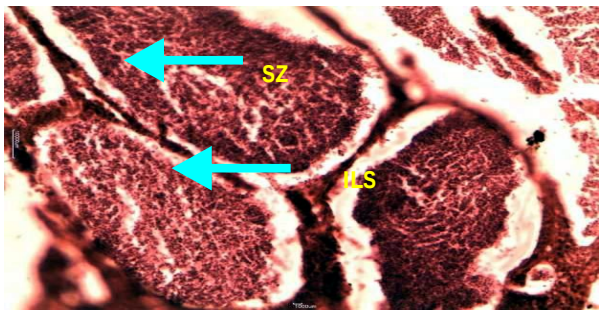

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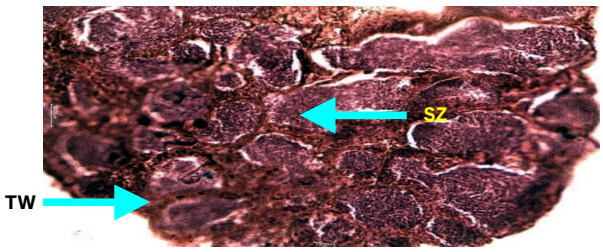

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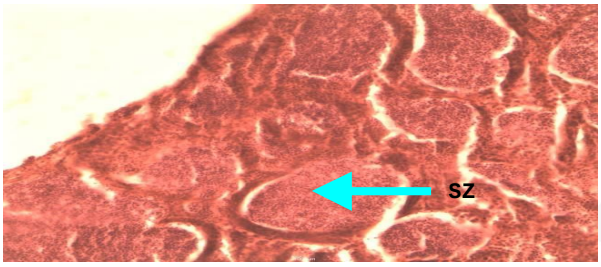

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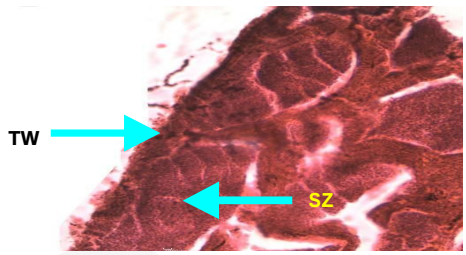

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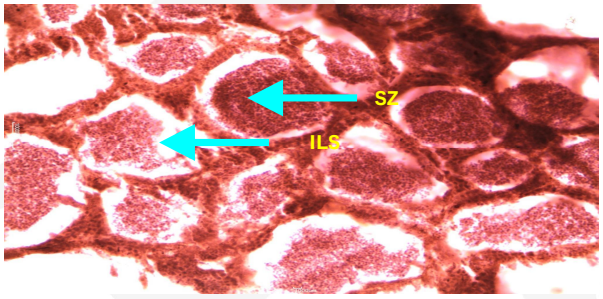
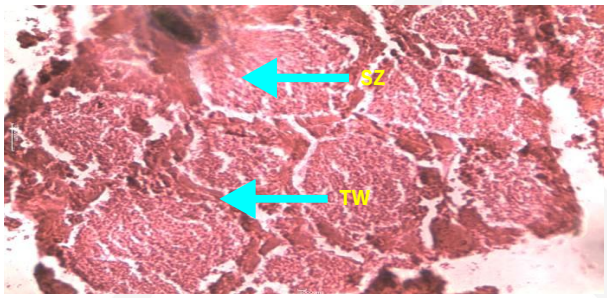


Plate-13

Photomicrograph of the parts of transverse section of testis of *Trichogaster lalius* showing
Plate 8: Testis of 5 mg/Kg hormone treated fish at 15th day. **Plate 9:** Testis of 5 mg/Kg hormone treated fish at 30th day
Plate 10: Testis of 5 mg/Kg hormone treated fish at 45th day. **Plate 11:** Testis of 5 mg/Kg hormone treated fish at 60th day
Plate 12: Testis of 5 mg/Kg hormone treated fish at 75th day. **Plate 13:** Testis of 5 mg/Kg hormone treated fish at 90th day



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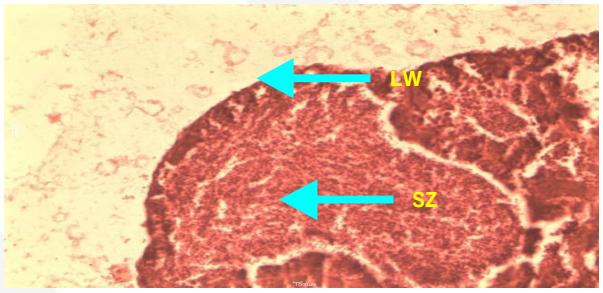

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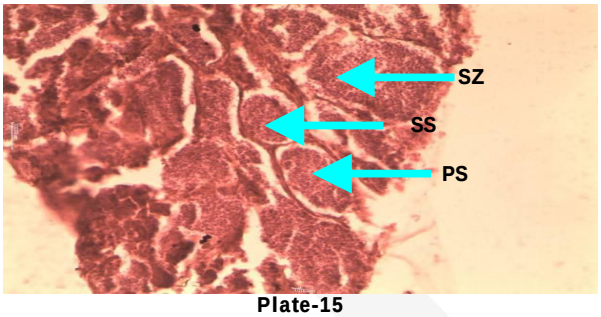

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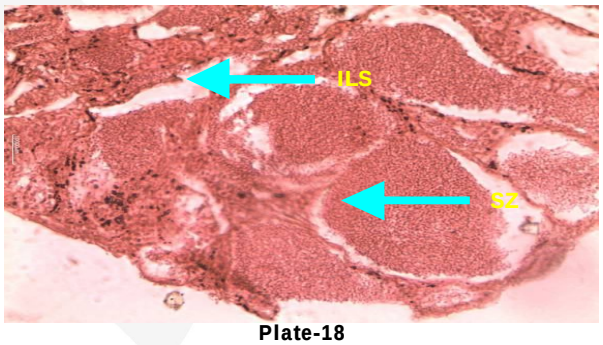

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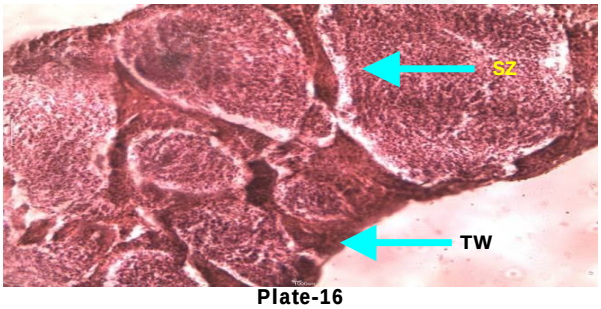

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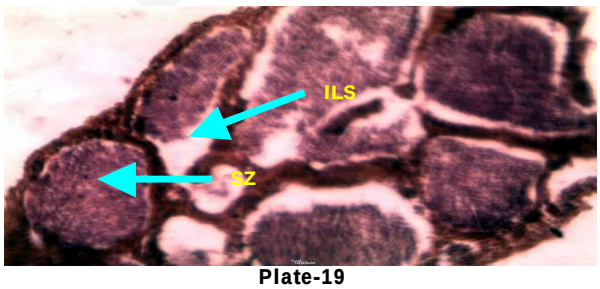
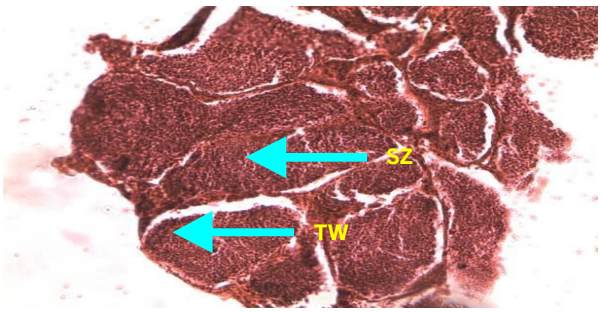


Plate-19

Photomicrograph of the parts of transverse section of testis of *Trichogaster lalius* showing
Plate 14: Testis of 10 mg/Kg hormone treated fish at 15th day. **Plate 15:** Testis of 10 mg/Kg hormone treated fish at 30th day
Plate 16: Testis of 10 mg/Kg hormone treated fish at 45th day. **Plate 17:** Testis of 10 mg/Kg hormone treated fish at 60th day
Plate 18: Testis of 10 mg/Kg hormone treated fish at 75th day. **Plate 19:** Testis of 10 mg/Kg hormone treated fish at 90th day



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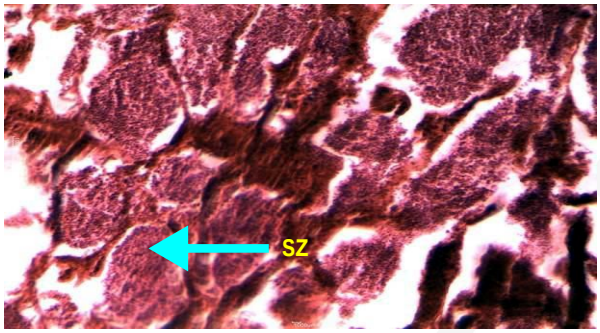

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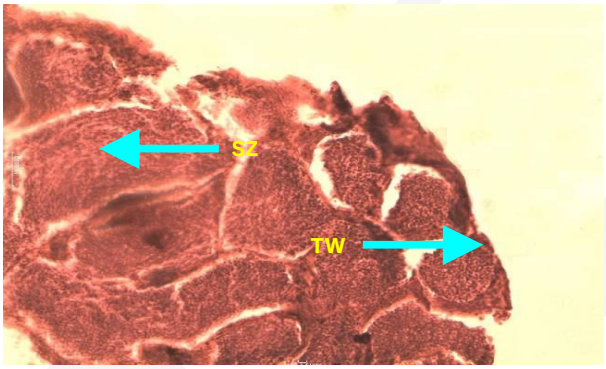

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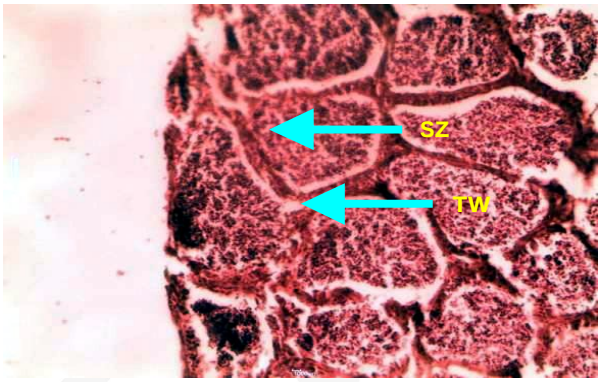

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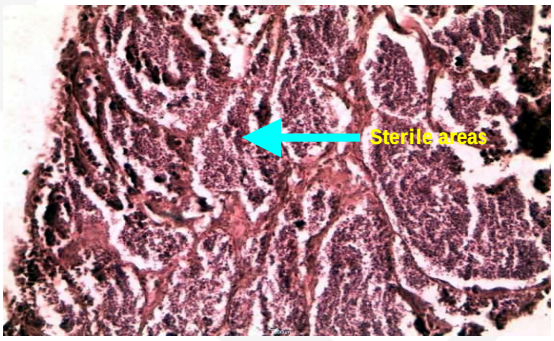

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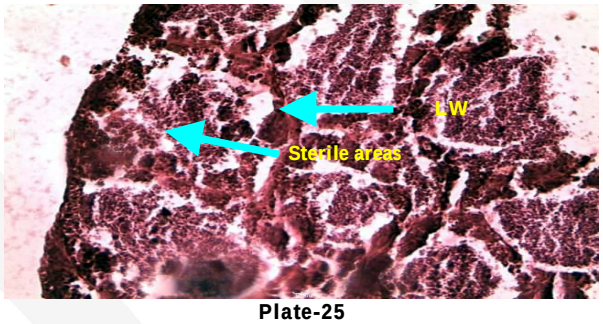

Plate-25

Photomicrograph of the parts of transverse section of testis of *Trichogaster lalius* showing
Plate 20: Testis of 15 mg/Kg hormone treated fish at 15th day
Plate 21: Testis of 15 mg/Kg hormone treated fish at 30th day
Plate 22: Testis of 15 mg/Kg hormone treated fish at 45th day
Plate 23: Testis of 15 mg/Kg hormone treated fish at 60th day
Plate 24: Testis of 15 mg/Kg hormone treated fish at 75th day
Plate 25: Testis of 15 mg/Kg hormone treated fish at 90th day

found better than other two (5 and 15 mg/kg). The development of gonadal materials (Primary spermatogonia, secondary spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa i.e. sperms) insides the follicles of the male gonads was also seen accordingly in the different treatments. In the testis of fish, when undergoing reproductive activity (spermatogenesis), about six spermatogenic elements have been identified and described by Guraya (1986). The elements of spermatogenesis are produced from sperm mother cell of germinal epithelium and passes through different maturation stages as primary spermatogonia, secondary spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa (sperms). On the basis of histological study, the testicular change of *T. lalius* due to hormone administration through diet was distinguished into primary spermatocytes, secondary spermatocytes and spermatozoa. This is because the concentrations of the gonadal material was so closely placed that the identification of different stages was found very difficult. In the present study sterile areas were also found in high dose of 15 mg/Kg hormone treated fish at the end of 60th day of treatment which is in agrrement of findings of the earlier workers (Simpson, 1976; Johnstone *et al*., 1978; Hurk and

Slof, 1981; Billard, 1992; Boris *et al*., 1994; Pandian and Sheela 1998).

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