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Histological alterations in some organs of monosex tilapia (*Oreochromis niloticus*, Linnaeus, 1758) produced using methyltestosterone

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ABSTRACT

17α-methyltestosterone is the most efficient and least expensive method in monosex production in Egypt. This study was carried out to investigate the effect of this hormone on farmed Nile tilapia (*Oreochromis niloticus*) using some histological parameters. Fish samples were collected from four localities (Assiut; as a control and Beheira, Alexandria, Kafr El-Sheikh; as monosex populations). Histological sections in liver, kidney, and testis were prepared, stained, and examined according to histology protocol. Microscopic examination has been done for random samples showing some alterations, such as damages central vein (CV), nicrotic area (N), and hyperplasia in the hepatocytes (H) and Kuffer cells (Kc) in fish collected from Kafr El-Shiek farms. Sections of liver of tilapia from Alexandria showed hydropic degeneration (HD) in the hepatocytes (H), hyperplasia in the Kupffer cells (Kc) and blood congestion (BC) in the central vein. Moreover, sections of liver of tilapia from Beheira showed the hepatocytes (H) and the central vein (CV) with presence of melanomacrophages (M) and hyperplasia in the hepatocytes and Kupffer cells (Kc). Kidney of tilapia from monosex farms showed enlarged glomeruli (EG) and damaged renal tubules, detached epithelial cells and damaged renal tubules. Testis showed degeneration of the interstitial tissues and disorganization in the seminiferous tubules. It has been concluded that, the overdose of methyltestosterone (MT) induction affected some oranges in monosex fish.

INTRODUCTION

The Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) is likely to be the most important farmed fish in the 21 century (Ridha, 2006). Tilapia tolerates stress induced by handling and grows and reproduces in a wide range of environmental conditions (Tsadik and Bart, 2007).

As the goal of achieving more productivity in growing *O. niloticus*, at the unit time, monosex production constituting totally of males is important to culture (Mair and Little, 1991). Male monosex cultures are preferred to females because of the differential growth in favour of males due to the metabolic energy is channeled towards growth (Tran-Duy *et al.*, 2008; Angienda *et al.*, 2010). Another problem with females; there is a greater reallocation of metabolic energy towards reproduction and their early maturation and ability to breed every month resulting in overpopulation of stocked tilapia ponds and the stunting of growth (Fashina-Bombata and Megbowon, 2012). On the other hand, varying from small to large due to the faster growth male in a mixed sex of tilapia makes it more difficult to establish uniformity of product. For producers wanting high yields of large-sized fish, all male fry are preferred.

Although, manually production of all male tilapia can be accomplished by such techniques as separating the males and females, hybridization, chromosomal manipulation, and hormonal sex reversal, oral administration of *O. niloticus* has been reported to be the most used method in commercial uses (Green and Teichert-Coddington, 2000; Wahbi and Shalaby, 2010; Celik *et al.*, 2011). The most efficient and least expensive method is monosex production is using of 17α-methyltestosterone. 17α-Methyltestosterone (MT) is a synthetic steroid derivative of a male specific hormone used to masculinize tilapia juveniles (Abucay and Mair, 1997; Green *et al.*, 1997; Gale *et al.*, 1999). The effect of MT is dependent on various factors such as dose, timing, duration of treatment, and mode of administration (Mirza and Shelton, 1988). There is however a global concern of the effect of this steroid on the fish flesh, consumers (man) and the environment (water bodies into which effluent is released) which forms the basis of this study (Sayed and Moneeb, 2015; Sayed *et al.*, 2016).

Recently, the success of tilapia production in Egypt stemmed from the drive to ensure monosex male production through sex reversal using 17α-methyl testosterone. The uptake and depletion of MT have been reported in several species of teleost fish, including cichlids (Fagerlund and Dye, 1979; Goudie *et al.*, 1986; Curtis et al., 1991; Cravedi *et al.*, 1993; Rinchard *et al.*, 1999). As MT administrated orally is readily metabolized, research on the fate of MT and on its metabolites need to be addressed for human and environmental safety issues. Another problem associated with the use of MT is that, at high doses or prolonged treatment, MT induces gonadal intersex-uality and paradoxical feminization (van den Hurk *et al.*, 1982; Goudie *et al.*, 1983; Solar *et al.*, 1984; Blazquez *et al.*, 1995; Rinchard *et al.*, 1999; Papoulias *et al.*, 2000). Piferrer and Donaldson, (1989) suggested that paradoxical feminization might be due more to aromatization than to inhibition of in vivo synthesis of androgens. Also, high doses of MT administration caused changes in the hematology and biochemical parameters of farmed tilapia (Sayed and Moneeeb, 2015). Erythrocytes alterations, nuclear abnormalities, and apoptosis as biomarkers were sued indicating the cellular damage as side effects of the high dose of MT in farmed tilapia at Egypt (Sayed *et al.*, 2016). The present study investigated the hsitopatholpgical changes in some body tissues of farmed tilapia produced by overdose MT administration in monosex production.

MATERIALS AND METHODS

Sample collection

Healthy male's fishes of the Nile tilapia, *Oreochromis niloticus* were collected from Assiut farms as control (no hormones treatment, contains males and females but males only used in this study) and monosex fishes from three farms of Beheira, Alexandria, and Kafr el- Sheikh as monosex farms in Egypt (Sayed *et al.*, 2016). The data of those farms and fishes were reported in (Sayed *et al.*, 2016).

Water quality assessment

Water-quality criteria [pH, dissolved oxygen, water temperature, conductivity, salinity, turbidity, phenols, chloridate, fluridate, sulphate, nitrate, cyanide, and ammonia] of the chosen sites were monitored during the fish collection. Total Fe, Cd, Pb, Zn, Cr, and Hg were measured using graphite furnace AA (GFAA) spectroscopy. Sampling and assessment of water quality were done according to the traditional manual methods (APHA, 2005). Data of the selected farms reported in (Sayed *et al.,* 2016).

Histological changes

Fish of each group were removed and sacrificed. Small pieces of the tissues (liver, goands, and kidney) were taken and immediately fixed in Davsion's solution. Fixed tissues were processed routinely for paraffin embedding technique. Embedded tissues were sectioned at 5-7µ in thickness and then stained with Harris's hematoxylin and eosin stain (H & E) according to (Bancroft and Stevens, 1982). Sections were examined and studied using BX50F4 OLYMPUS microscope (Olympus optical Co. LTP. Japan). All procedures of the current work have been approved by the Committee of the Faculty of Science of Assiut University, Egypt.

RESULTS

Physico-chemical water parameters

The measured physico-chemical parameters of the water samples collected from Assiut farms as control and three farms of Beheira, Alexandria, and Kafr el-Sheikh as monosex farms were reported in Sayed *et al.* (2016). Most of these parameters showed the highest values in the water of the monosex farms in comparison to Assiut farms

Histopathological studies

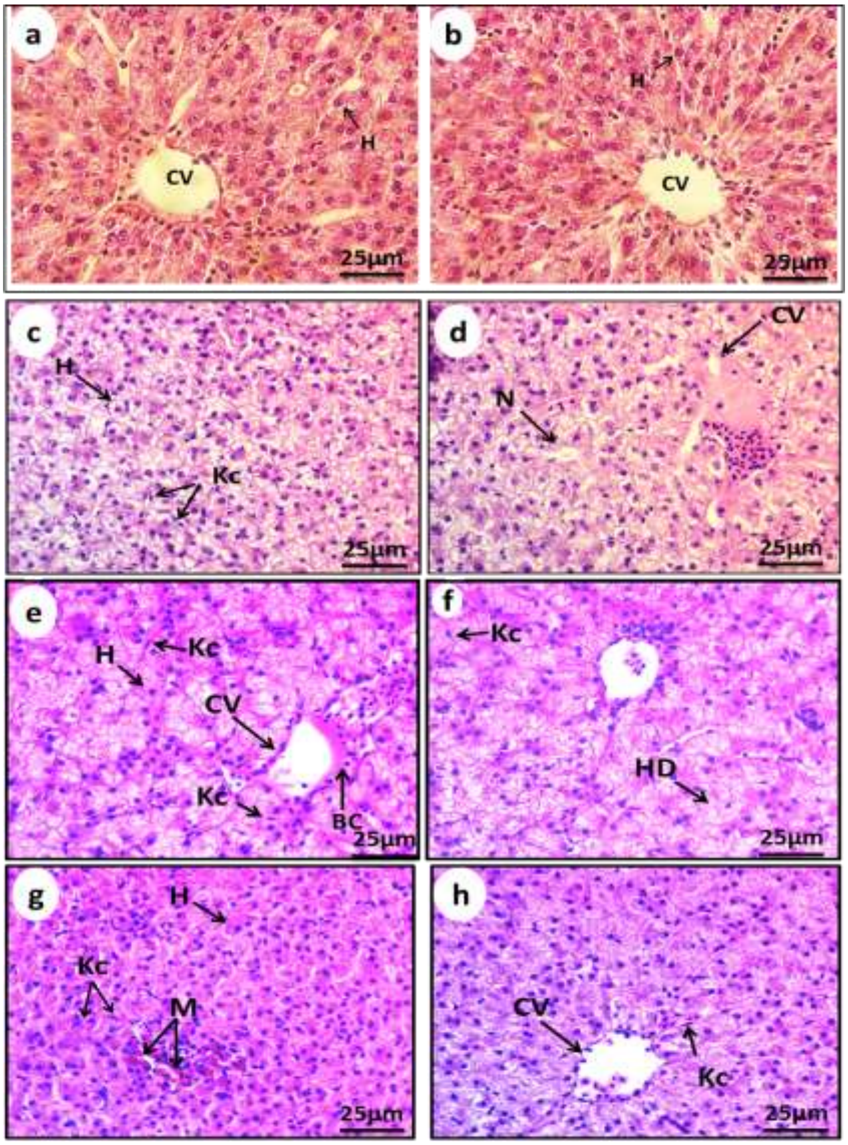
Liver

No alterations were observed in the control liver tissues of Tilapia from Assiut farms. The structural details of the control liver are shown in (Figs.1: a & b). In control liver of Tilapia the cords of hepatocytes tend to have a regular radial pattern enclosing the sinusoidal network for a short distance into the perivenular areas. These areas become less regular outside the perivenular zone. The individual hepatocyte is polygonal in shape and has a single spherical nucleus. The nuclei are mostly centrally located within the hepatocytes with some nuclei tending to occur closer to the cell periphery bordering the sinusoids. The endothelial cells that line these sinusoids as well as their nuclei are flattened and elongated (Figs.1: a & b).

Variable alterations had indicated in the liver of tilapia from Kafr El-Shiek farms. These alterations were mainly represented by liver nonhomogenous architecture (Figs.1: c & d). Sinusoidal lumen was collapsed and hydropic degenerations with the rupture of hepatocyte membranes were observed. Proliferation of the hepatic cells with a decrease in cell size and hyperplasia in the Küpffer cells was observed. Accordingly, the hepatocytes lost their normal polygonal shape and boundary between cells became invisible. Areas of hepatic necrosis started to appear in some regions and rupture of the wall of the central vein were noticed (Fig.1: d).

Variable alterations had indicated in the liver of tilapia from Alexandria. These alterations show the hepatocytes were characterized by the absence of nuclei while the others were having pyknotic nuclei and cytoplasmic vaculation. Also, infiltration of the inflammatory cells around the central vein and between the hepatocytes, proliferation of hepatocytes (crowded of nuclei) was observed. The number of Küpffer cells increased some cells suffering from hydropic degeneration in some areas and blood congestion inside the central vein (Figs.1: e & f).

Variable alterations had indicated in the liver of tilapia from Beheira. These alterations show rupture of the wall of the central vein were noticed. The hepatocytes are delimited by ruptured cell membranes and dispersion of cell contents and loss of stainability. An aggregation of melanomacrophage between the hepatocytes as observed. Proliferation of hepatocytes (crowded of nuclei) with the number of Küpffer cells increased (Figs.1: g & h).



Figs. 1: (a & b) Sections of control liver of Tilapia from Assiut farms showing the normal structure of hepatocytes (H) and central vein (CV). (c&d) sections of liver of tilapia from Kafr El-Shiek farms showing the damages central vein (CV), necrotic area (N), and hyperplasia in the hepatocytes (H) and Kupffer cells (Kc). (e&f) Sections of liver of Tilapia from Alexandria showing hydropic degeneration (HD) in the hepatocytes (H), hyperplasia in the Kupffer cells (Kc) and blood congestion (BC) in the centeral vein. (g&h) sections of liver of Tilapia from Beheira showing the hepatocytes (H) and the central vein (CV) with presence of melanomacrophages (M) and hyperplasia in the hepatocytes and Kupffer cells (Kc) (H &E; X400).

Kidney

No alterations were observed in the control kidney tissue of Tilapia from Assiut farms. The structural details of the control kidney are shown in (Figs.2: a & b). The kidney of Tilapia consists of the renal corpuscle which is roughly spherical consisting a double membrane capsule (Bowman's capsule) enclosing a tuft of blood capillaries (glomerulus). There is a space in between the glomerulus and the capsule, which called the Bowman's space. The renal tubules are numerous and their cross sections exhibit a round or oval outline with a narrow lumen. The collecting tubules are lined with cubical or low columnar epithelial cells with basally located nuclei (Figs. 2: a & b).

Examination of kidney sections of Tilapia from Kafr El-Shiek farms showed hypertrophies of glomeruli, rupture of the renal corpuscles with disorganization in the glomerular tuft. Indistinct lumen, focal tubular necrosis, and degeneration of the epithelial cells lining the renal tubules were observed (Figs. 2: c & d).

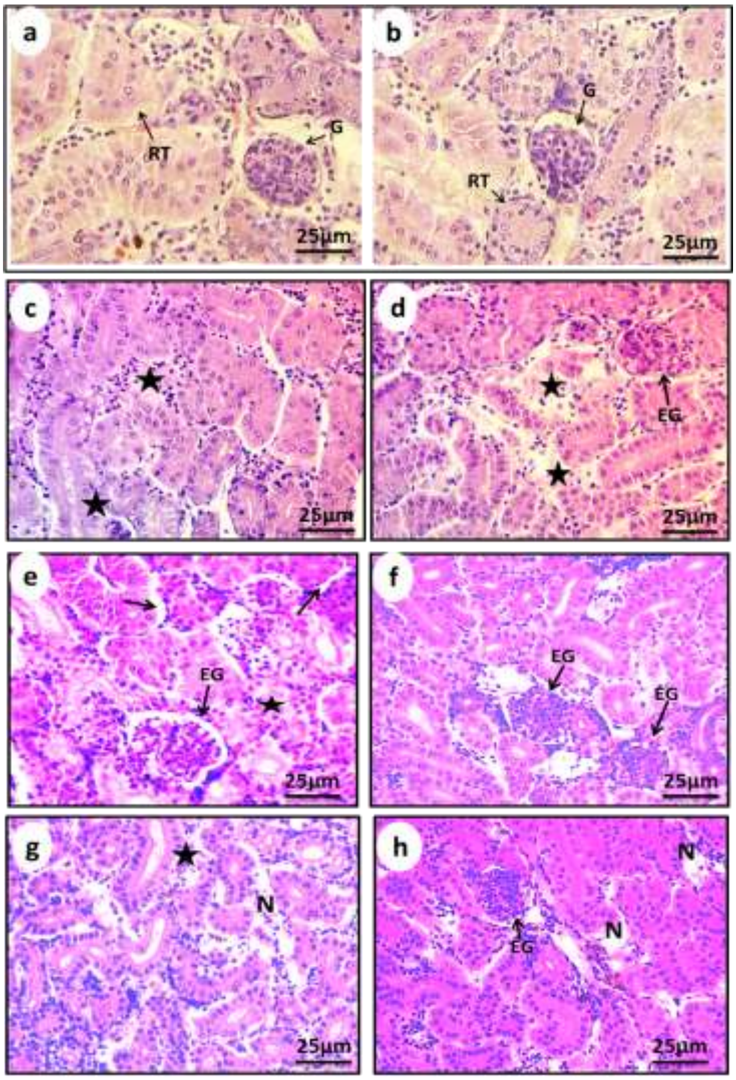


Fig. 2 (a & b) Sections of control kidney of Tilapia from Assiut farms showing the normal structure of glomerulus (G) and renal tubules (RT). (c&d) sections of kidney of Tilapia from fromKafr El-Shiek farms showing enlarged glomeruli (EG) and damaged renal tubules (stars). (e&f) Sections of kidney of Tilapia from Alexandria farms showing enlarged glomeruli (EG), damaged renal tubules (star) and detached epirthelial cells (arrows) and damaged renal tubules (star). (g &h) sections of kidney of Tilapia from Beheira showing enlarged glomeruli (EG), necrotic areas (N) and damaged renal tubules (star) (H &E; X400).

Examination of kidney sections of Tilapia from Alexandria showed rupture of Bowman's capsule and the glomerular tuft, hypertrophies of glomeruli, degeneration of the epithelial cells lining the renal tubules and degeneration of the parietal cell of renal corpuscles. Proliferation of renal tubules was observed (Figs. 2: e & f).

Examination of kidney sections of Tilapia from Beheira showed indistinct lumen. Focal tubular necrosis was also noticed. Rupture of Bowman's capsule and the glomerular tuft, hypertrophies of glomeruli, degeneration of the epithelial cells lining the renal tubules and degeneration of the parietal cell of renal corpuscles. Proliferation of renal tubules and necrotic was observed (Figs. 2; g & h).

Testis

Examination of testis sections of Tilapia from Assiut farms showed the normal structure of seminiferous tubules and interstitial tissue (Figs.3; a & b). Variable alterations had indicated in the testis of tilapia from Kafr El-Shiek farms. These alterations were degeneration of the interstaitial tissue and in the seminiferous tubules (Figs.3; c & d). Examination of testis sections of Tilapia from Alexandria showed the degeneration in the interstitial tissue and disorganization in the seminiferous tubules (Figs.3: e & f). Examination of testis sections of Tilapia from Beheira showed the structure of seminefrous tubules and interstial tissue with presence of complete degeneration (Figs.3; g & h).

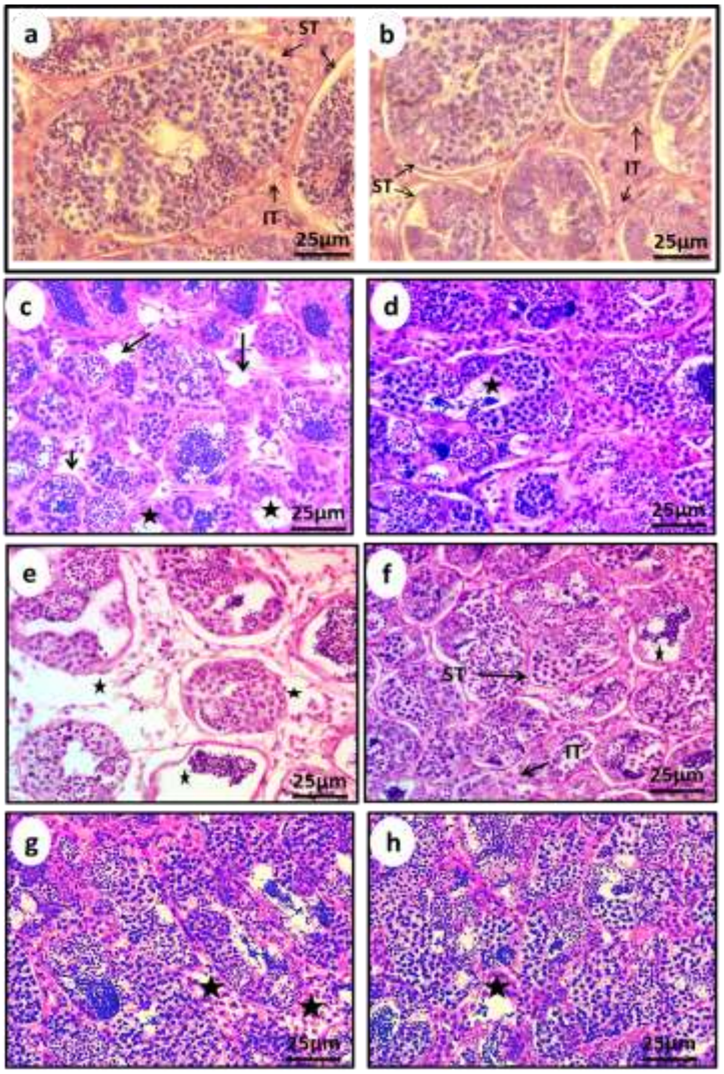


Fig. 3 (a & b) Sections of control testis of Tilapia from Assiut farms showing the normal structure of seminiferous tubules (ST) and interstitial tissue (IT). (c&d) sections of testis of Tilapia from Kfer el-shiek showing degeneration of the interstaitial tissue (arrow) and in the seminiferous tubules (stars). (e&f) sections of testis of Tilapia from Alexanderia showing the degeneration in the interstitial tissue (star) and disorganization in the seminiferous tubules. (g&h) sections of testis of Tilapia from Beheira showing the structure of seminefrous tubules (ST) and interstial tissue (IT) with presence of complete degeneration (stars) (H &E; X400).

DISCUSSION

Feeding small amounts of male hormone 17α-methyltestosterone (MT) to tilapia fry before and during sexual differentiation in fish hatcheries can induce male monosex production. This form of sex control made the male tilapias generally grow faster than females, with larger and uniform in size than mixed sex tilapias (Smith and Phelps, 1997; Hussain *et al.*, 2005). Many studies have been reported the effects of MT on some parameters and tissues of tilapia (Khater, 1998; Hasheesh *et al.*, 2011; El-Greisy and El-Gamal, 2012;; Sayed and Moneeb, 2015; Sayed and Abou Khalil 2016; Sayed *et al.*, 2016) experimentally and in field but to our knowledge this is the first investigation which deals with the study of the effects of use of methyltestosterone in tilapia sex reversal at Egypt in the field using histological biomarkers.

Fish liver is a very interesting organ for the study of interactions between environmental factors and hepatic structures and functions. Thus research on liver is important, especially in the field of problems induced by aquaculture conditions and aquatic pollution. In the present study, different histopathological alterations were recorded in fish liver collected from monosex farms produced by MT administration. These alterations are similar to that recorded by Khater (1998) who studied the effect of different doses of 17α-methyltestosterone on the liver where they reported that, liver tissue treated with 60 mg MT for 14 days, showed diffuse hydropic degeneration and the central vein was congested. He also reported that hemorrhage was seen in the hepatic parenchyma. Moreover, Hasheesh *et al.*(2011) studied the effect of 17α-methyltestosterone hormone on the liver of Nile Tilapia and reported that the treated fish showed diffuse vacuolar degeneration followed by mild and severe hepatocytic vacuolations. On contrast, other studies after exposure to MT did not show any pathological differences among the treatments (Kefi *et al.*, 2013). Deborah (1990) studied the effect of the synthetic steroid 17α-methyltestosterone on the growth and organ morphology of channel catfish (*Ictalurus punctatus*) and found that there is no deviation from the normal morphology in livers taken from both treated and control specimens.

The kidney is a vital organ for the body and proper kidney function is to maintain the homeostasis. It is not only involved in the removal of wastes from the blood but it also responsible for selective reabsorption which help in maintaining volume and pH of blood and body fluid erythropoiesis (Iqbal *et al.*, 1998). In the present study, different histopathological alterations were recorded in fish kidney. These alterations are similar to that caused by pesticides and heavy metals (Mohamed, 2009; Mekkawy *et al.*, 2013). Gannam and Lovell (1991) studied the effects of feeding 17α-methyltestosterone and 11-ketotestosterone, to channel catfish *Ictalurus punctatus*, and found that there was slight vacuolation of epithelial cells of the renal tubules in the trunk kidneys of the MT- and KT-fed fish.

The effect of 17α-methyltestosterone on the gonads appears to be complex (Ahmad *et al.*, 2002). Macintosh (1988) showed the higher level of 17α-methyltestosterone (60 mg/Kg of feed) produced some testicular degeneration which lowered the GSI value. Ahmad *et al.* (2002) found that male and female GSI was significantly decreased at high MT doses (5, 10, 20 and 40 mg MT/kg feed), while non-significant change were observed at low MT doses (0.5, 1.0 and 2.5 mg MT/kg feed). In the present study, different histopathological alterations were recorded in fish testis. These alterations are similar to that recorded by Shen *et al.* (2015) who studied the effects of 17 α-methyltestosterone on sex reversal gonadal structure, and growth in yellow catfish *Pelteobagrus fulvidraco* and recorded that, high dose caused large amounts of vacuolated seminiferous lobules was observed which may be due to the degeneration of testes. Also, in *Tilapia mossambica*, treated with MT (50 µg/g of diet), some germ cells in the gonads of possible genetic females underwent oogenesis even under the influence of androgen hormone, but were found to be degenerated eventually (Nakamura *et al.,* 1974). High doses and long-term administration of 17α-MT produced sterile males with malformation in both Grouper and milk fish (Lee *et al.*,1986; Tan-Fermin *et al.*,1994). Our results were in agreement with Yamazaki (1972) in pink and chum salmon and with Hirose and Hibiya (1968a, 1968b) in goldfish and rainbow trout, where they reported that, MT administration of 2.5 mg/kg induced the degenerative changes in the ovaries and testes. Furthermore, Higgs *et al.* (1977) found clear signs of gonads degeneration in coho salmon affected by MT causing fish sterility, which might be considered advantageous in fish culture.

CONCLUSION

It could be concluded that, high dose and misuse of MT in aquaculture can cause histopathological changes in some fish organs, accordingly affecting all liver, kidney, and testis functions. Therefore, alternative methods more safe to fish and environment to produce monosex populations should be considered.

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