Afaf et al. Annals of Veterinary and Animal Science 2018
http://naturepub.org/navas
[Received: 20 Jun 18, Accepted: 13 Oct 18, Published: 30 Oct 18]

eISSN: 2313-5514
pISSN: 2312-9123

AVAS
Annals of Veterinary and Animal Science

RESEARCH ARTICLE V:5(5) OPEN ACCESS

Long term feeding effects of antiestrogen (tamoxifen) on growth and reproduction of Nile tilapia (*Oreochromis niloticus)*

Abd El Megid Afaf [1], Baiomy Mahmoud [1] and El Asely Amel [*2]

ABSTRACT

The aim of this work was to determine the anabolic and reproductive effects of antiestrogen (tamoxifen) in dietary supplemented Nile tilapia, *Oreochromis niloticus* of an average weight 5.30±0.2 g were fed four experimental diets containing (0, 25, 50 and 100 mg) tamoxifen/ kg food represented by; control, G1, G2 and G3 respectively for 90 days. Growth, serum growth hormone, plasma ions concentrations, serum steroids and gonadal histo-pathology were measured at 30, 60 and 90 days. The highest body weight and length were recorded at G1 which fed 25 mg tamoxifen for 90 days. While 30 days feeding resulted in significant decrease in growth hormone levels at G1 and G3 with continued decrease in its levels at 60 and 90 days. Sodium ion concentration significantly decreased at 30 and 60 days feeding periods at all treatment groups, but significant increase was noticed in serum potassium at 90 days for all tamoxifen fed groups. A significant increase in calcium and phosphorus serum levels were recorded 60 days post feeding tamoxifen incorporated diets. Tamoxifen exhibited time dependent significant increase in serum estradiol concentration of females fed 25 mg tamoxifen. Testosterone concentration was significantly higher 60 days post feeding at G1 and G2 then reduced significantly at 90 days after feeding. Feeding for 30 days didn't alter the histological picture of both males and females, but other feeding periods showed pathological changes in both male and female gonads. These results suggest the use of tamoxifen as growth promoter for tilapia at concentration of 25 mg/kg food for 30 days.

Key words: Tamoxifen, tilapia, growth, reproduction.

INTRODUCTION

Aquatic organism represents a first source of wide variety of micronutrients and protein to the most of the world's poorest population (Kawarazuka, 2010). Benefits of high consumption of fish has been associated with decrease risk of developing coronary heart disease, high blood pressure, some cancers, rheumatoid arthritis and other inflammatory diseases (FAO, 2010). Tilapias are of high importance in world fisheries. They are easy to culture and reproduce, with rapid sexual maturation and become marketable at 6-7 months from hatching. Nile tilapia is also an excellent laboratory animal that deserves to be studied (Maclean et al., 2002).

Tamoxifen is a an antiestrogen which has been widely used around the world for the treatment of hormone responsive breast cancer for more than 43 years (Osborne, 1998). It is an anti-cancer drug that blocks the stimulation of tumor cell growth by estrogens (Lazzeroni et al., 2012). In addition to its antitumor activity, it exerts beneficial effects on osteoporosis as shown by clinical trials of long-term treatment of postmenopausal women (Love et al., 1992). The first use of tamoxifen in the field of aquaculture was conducted by Hines and Watts (1995) for production of all male tilapia fry. Depending on its ability in shifting sex ratios toward males through binding to estrogen (Lichtenfels et al., 2017), leading to increase in the androgen levels. Other trials for production of all male tilapia using tamoxifen were achieved by El Asely et al., (2007) and Singh et al. (2012). The main objective of this study was to evaluate the effect of treating *O. niolticus* juvenile with tamoxifen in different concentrations and different durations on both growth and reproductive associated parameters.

MATERIALS AND METHODS

Fish

About 400 healthy Nile tilapia (*Oreochromis niloticus*) with an average weight 5.30±0.2 g were placed in well prepared fiberglass tank

* Corresponding author: amel.alaasly@fvtm.bu.edu.eg
[1]Department of Biochemistry, Faculty of Veterinary Medicine, Benha University, Egypt.[2]Department of Aquatic animals Diseases and Management, Faculty of Veterinary Medicine, Benha University, Egypt

(500 L) tanks filled with de-chlorinated water for at least two hours till the temperature inside the bags equal to that of outside then the bags were slightly opened and the contents evacuated into the tanks. The water temperature was adjusted to 26±2 ºC; the oxygen level was maintained at optimal level using aerators. Fish were fed with basal diet at a rate of 5% from the body weight three times daily for two weeks till fish become acclimated to the lab conditions.

Drug

Tamoxifen 20 mg tablets "Amryia Pharma Ind., Alexandria, Egypt".

Preparation of tamoxifen treated food

Treatment of food with tamoxifen (tam) was done according to Hines and Watts (1995). To prepare diet containing 25 mg, 50 mg and 100 mg tam per each kilogram, dissolution of 25 mg, 50 mg and 100 mg of tam each in one liter 95% ethanol. Each prepared alcoholic solutions were added to one kilogram of the basal diet (Al Qahira Company, Egypt) and mixed well then left to dry. After dryness of the treated food, it were packed and stored at -4ºC until use.

The Experimental design:

Fish were divided into four groups in duplicate; one control and three treated groups. The control group fed on basal diet and the three treated groups were fed with tam treated food [Cont. (0 mg), G1 (25 mg), G2 (50 mg), G3 (100 mg)]. The treated and control groups received experimental diets for period of three months at a rate 5 % of BW at the first 30 and 60 days and then 4% from BW at the last 30 days from the experiment. Food was offered three times daily. Number of dead fish was recorded daily in all groups during the entire experiment. At the end of 30, 60 and 90 days fish were weighed and the total length were recorded. Serum samples were taken from 20 fish/ tank for determination of growth, estradiol and testosterone hormones levels. Electrolytes (calcium, phosphorus, sodium and potassium) concentrations were also measured in serum. Gonads were excised and fixed in

Table (1): Mean ± (SE) body weight (g) of *Oreochromisniloticus* fed tamoxifen at conc. of 0 (control), 25 (G1), 50 (G2) and 100 (G3) mg/kg food for 30, 60 and 90 days.

Groups	Feeding periods (days)		
	30	60	90
Control	6.42 ± 0.46^{cC}	15.52 ± 1.45^{bB}	19.38 ± 1.15^{cA}
G1	15.66 ± 1.46^{aC}	19.81 ± 1.01^{aB}	31.95 ± 2.41^{aA}
G2	11.38 ± 1.18^{bC}	18.90 ± 1.19^{aA}	19.54 ± 0.93^{cA}
G3	15.24 ± 1.00^{aC}	20.58 ± 1.62^{aB}	23.11 ± 1.55^{bA}

a, b & c: There is a significant difference (P>0.05) between any two means, within the same column have a different superscript letter.
A, B & C: There is a significant difference (P>0.05) between any two means for the same attribute, within the same row have a different superscript letter.

Table (2): Mean ± (SE) growth hormone conc. (ng/ml) of *Oreochromisniloticus* fed tamoxifen at conc. of 0 (control), 25 (G1), 50 (G2) and 100 (G3) mg/kg food for 30, 60 and 90 days.

Groups	Feeding periods (days)		
	30	60	90
Control	0.03 ± 0.01^{bC}	0.07 ± 0.01^{aA}	0.06 ± 0.01^{aB}
G1	0.01 ± 0.00^{cC}	0.01 ± 0.00^{cB}	0.02 ± 0.01^{bA}
G2	0.05 ± 0.01^{aA}	0.03 ± 0.01^{bB}	0.01 ± 0.00^{cC}
G3	0.02 ± 0.01^{bB}	0.01 ± 0.00^{cC}	0.03 ± 0.01^{bA}

a, b & c: There is a significant difference (P>0.05) between any two means, within the same column have a different superscript letter.
A, B & C: There is a significant difference (P>0.05) between any two means for the same attribute, within the same row have a different

neutral formalin buffer for histopathological examination.

Determination of serum growth hormone

Serum growth hormone was determined using commercially available ELISA kits (Immunospec, USA) according to the manufacturer instructions.

Table (3): Mean ±(SE) serum ion conc. ((*m Eq*/l)) of *Oreochromisniloticus* fed tamoxifen at conc. of 0 (control), 25 (G1), 50 (G2) and 100 (G3) mg/kg food for 30, 60 and 90 days. conc. of 25, 50 and 100 mg/kg food for 30, 60 and 90 days.

Groups	Feeding periods (days)		
	30	60	90
	Na^+		
Control	187 ± 1.15^{aB}	199 ± 2.31^{aA}	169 ± 1.15^{cC}
G1	178 ± 1.73^{bB}	188 ± 1.73^{bA}	188 ± 2.31^{aA}
G2	163 ± 1.15^{cC}	195 ± 2.31^{aA}	187 ± 1.15^{aB}
G3	188 ± 1.73^{aA}	178 ± 1.73^{cB}	177 ± 9.24^{bB}
	Ka^+		
Control	7.8 ± 0.17^{aA}	7.8 ± 0.17^{aA}	5.9 ± 0.06^{dB}
G1	6.9 ± 0.17^{cB}	6.3 ± 0.17^{bC}	7.9 ± 0.17^{aA}
G2	6.8 ± 0.12^{cB}	7.7 ± 0.12^{aA}	6.3 ± 0.12^{cC}
G3	7.2 ± 0.06^{bA}	6.4 ± 0.12^{bC}	6.8 ± 0.23^{bB}
	Ca++		
Control	8.2 ± 0.12^{bcA}	7.5 ± 0.17^{dB}	8.2 ± 0.06^{bA}
G1	8.1 ± 0.12^{cC}	11.0 ± 0.58^{aA}	9.6 ± 0.17^{aB}
G2	9.0 ± 0.12^{aB}	8.3 ± 0.12^{cC}	9.6 ± 0.29^{aA}
G3	8.5 ± 0.12^{bB}	9.9 ± 0.17^{bA}	9.6 ± 0.06^{aA}
	P^{++}		
Control	6.3 ± 0.12^{aB}	6.9 ± 0.23^{bA}	5.3 ± 0.12^{dC}
G1	5.9 ± 0.17^{bC}	8.5 ± 0.23^{aA}	7.8 ± 0.17^{aB}
G2	6.3 ± 0.12^{aA}	5.1 ± 0.06^{dB}	6.1 ± 0.06^{cA}
G3	6.1 ± 0.06^{abB}	6.1 ± 0.06^{cB}	6.5 ± 0.23^{bA}

a, b & c: There is a significant difference (P>0.05) between any two means, within the same column have a different superscript letter.
A, B & C: There is a significant difference (P>0.05) between any two means for the same attribute, within the same row have a different superscript letter.

Determination of serum Estradiol II (E2) and testosterone:

Serum estradiol and testosterone were determined by specific ELISA kits (Immunospec, USA) following the manufacturer instructions.

Determination of serum electrolytes

Serum sodium, Potassium, Calcium and inorganic phosphorus were determined calorimetrically.

Histopathological examination:

Fixed testes and ovaries from different were subjected to serial dilutions of alcohol (methyl, ethyl and absolute ethyl), cleared in xylene and embedded in paraffin at 56 degree in hot air oven for 24 hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by sledge microtome. The obtained tissue sections were collected on glass slides, de-paraffinized, and stained by hematoxylin &eosin stain for examination through the light microscope (Bancroft et al; 1996).

Statistical analysis

The Statistical analysis was carried out using ANOVA with two factors under significance level of 0.05 for the whole results using SPSS ı (ver. 19). Data were treated as complete randomization design according to Steel et al. (1997). Multiple comparisons were carried out applying LSD.

Table (4 Mean ±(SE) Estradiol hormone conc. (ng/ml) of *Oreochromisniloticus* fed tamoxifen at conc. of 0 (control), 25 (G1), 50 (G2) and 100 (G3) mg/kg food for 30, 60 and 90 days.

Groups	Feeding period (days)		
	30	60	90
Control	699.5 ± 3.93^{bC}	711.4 ± 1.10^{dB}	1254.1 ± 7.85^{cA}
G1	707.8 ± 4.62^{bC}	2844.7 ± 5.31^{aB}	2994 ± 6.93^{aA}
G2	782.3 ± 3.00^{aB}	1681.4 ± 4.04^{bA}	750.7 ± 3.18^{dC}
G3	287.4 ± 4.16^{cC}	815.3 ± 3.00^{cB}	2058 ± 11.55^{bA}

a, b & c: There is a significant difference (P>0.05) between any two means, within the same column have a different superscript letter.
A, B & C: There is a significant difference (P>0.05) between any two means for the same attribute, within the same row have a different superscript letter.

Table (5): Mean ±(SE) testosterone hormone conc. (ng/ml) of *Oreochromisniloticus* fed tamoxifen at conc. of 0 (control), 25 (G1), 50 (G2) and 100 (G3) mg/kg food for 30, 60 and 90 days.

Groups	Feeding periods (days)		
	30	60	90
Control	3.47 ± 0.02^{bC}	5.96 ± 0.03^{cB}	6.55 ± 0.22^{bA}
G1	3.98 ± 0.08^{aC}	14.95 ± 0.17^{aA}	5.82 ± 0.06^{cB}
G2	3.60 ± 0.03^{bB}	11.4 ± 0.17^{bA}	2.17 ± 0.01^{dC}
G3	2.91 ± 0.03^{cC}	4.15 ± 0.02^{dB}	7.24 ± 0.25^{aA}

a, b & c: There is a significant difference (P>0.05) between any two means, within the same column have a different superscript letter.
A, B & C: There is a significant difference (P>0.05) between any two means for the same attribute, within the same row have a different superscript letter

RESULTS

Effect of tamoxifen on body weight

As shown in (Table: 1),a significant increase in body weight was observed at all tamoxifen fed groups 30 days post feeding. It was also noticed that G1 and G3 recorded the highest significant values (P>0.05) along the whole periods of experiment compared to the control group.

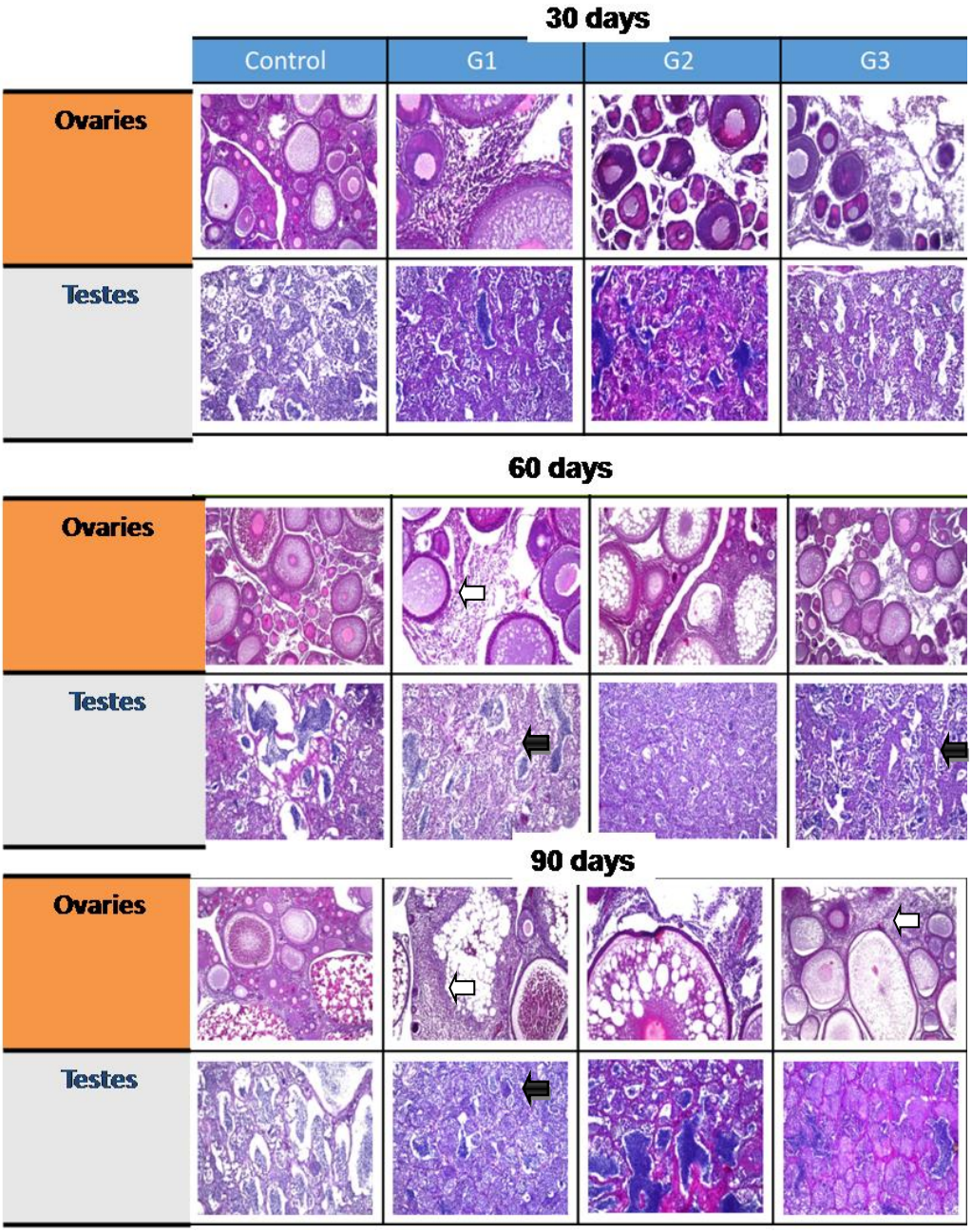

Fig1:Histopathology of O. *niloticus* gonads fed tamoxifen at conc. of 0 (control), 25 (G1), 50 (G2) and 100 (G3) mg/kg food for 30, 60 and 90 days. White arrow (⇦) Focal inflammatory cells infiltration was detected in between the follicles and oocytes. Black arrow (⬅) few spermatozoa in the lumen of few tubules with thickening and fibrosis in between the somniferous tubules with atrophy

Effect of tamoxifen on growth hormone levels

Growth hormone concentration in the serum of control group 60 days post feeding showed the highest significant values compared to other feeding periods and tamoxifen fed groups (Table: 2). But great variations and fluctuation in the results of the hormone concentrations within treated groups along the different feeding periods were noticed (Table: 2). Where, in G2 the growth hormone levels showed the highest values at 30 days feeding then it declined by 60 and 90 days treatment. While, G1 and G3 growth hormone levels showed significant decrease compared to the control and its levels continued in decrease at 60 and 90 days.

Effect of tamoxifen on serum electrolytes concentrations

As shown in (Table: 3) significant reduction at sodium concentration was recorded at G1 and G2 the control and G3, but this reduction was modulated at the 60 and 90 days feeding periods. On the other hand G3 exhibited significant reduction at sodium concentration by the 90th day from start feeding. Potassium ion concentration in the serum was also affected by tamoxifen treatment; leading to significant reduction in its levels 30 days post feeding at all groups compared to the control, but by the day 90 potassium ion concentration exhibited significant increase at the three treated groups compared to the control(Table: 3). Calcium and phosphorus ions concentrations didn't show fluent results, but the results were waved between increase and decease within the different groups, even the control groups especially at 90 days post feeding, but significant increase in calcium and phosphorus serum levels were recorded 60 days post feeding tamoxifen incorporated diets at all groups(Table: 3)

Effect of tamoxifen on serum estradiol and testosterone concentrations

Estradiol (E2) hormone in G1 showed significant gradual increase associate with the time, with the highest significant values ($p<0.05$) at 90 days feeding period (Table: 4). Gradual progressive increase was observed in E2 values in G3 with increasing to the exposure time, with the highest significant increase among other treated groups was noticed at 90 days (Table: 4). Testosterone hormone in tilapia fingerlings fed with 25 and 50 mg tamoxifen incorporated diets exhibited the highest significant ($p<0.05$) values at 30 and 60 days feeding periods compared with control groups and other concentration (Table, 5). At 90 days feeding, slight reduction in testosterone concentrations at G1, but severe drop in G2 was noticed (Table: 5), on the other side gradual and significant ($p<0.05$) increase in testosterone concentration was clear in G3 at 90 days feeding period (Table: 5)

Effect of tamoxifen on gonadal histopathology

Fig (1) showed that, 30 days feeding didn't alter the histological picture of both males and females, but other feeding periods showed pathological changes in both male and female gonads. At 60 days feeding progressive changes in the histological structures of both males and females gonads, spermatozoa was detected in the lumen of few tubules with thickening and fibrosis were detected in between the somniferous tubules with atrophy. Focal inflammatory cells infiltration was detected in between the follicles and oocytes. These alterations were obviously recorded with increasing to the doses and duration of exposure Fig (1).

DISCUSSION

To the best of our knowledge, few studies were done regarding the effect of tamoxifen on fish as growth promoter, and most of studies discuss the use of tamoxifen to produce mono sex populations. Tamoxifen action on sex reversal was hypothesized to depend on the binding action of tamoxifen to the estrogen receptors, leading to increase in the androgen levels so the androgen: estrogen ratio is increased causing testicular development. (Hines and Watts, 1995 and

El Asely et al., 2007). In the current study a significant increase in the body weight was observed at all tamoxifen fed groups 30 days post feeding. But in the other feeding periods; only 25 mg tamoxifen incorporated diet recorded the highest significant values. This was in agreement with the results of El Asely et al. (2007) who reported that a significant increase in the growth rates were found in tamoxifen treated fish at concentrations 50 and 100 mg for 30 days. An increase in tilapia weight was also obtained by Singh et al. (2012) who found that immature *Oreochromis niloticus* treated by 200 µg of tamoxifen for 60 days produced fish with significant growth as compared to the control ones. In concurrent with our results, long duration didn't significantly increased the weight Mandiki et al. (2005) recorded no significant changes between tamoxifen (50 mg/kg food) fed Eurasian perch juveniles and control groups after 90 days.

The recorded results showed low levels of growth hormone in groups of tilapia treated by tamoxifen compared to control group. Rozenboim et al. (1993) who found that TAM-treated birds displayed significantly lower values in plasma growth hormone concentrations than those of control birds at 20 and 69 d of age. This could be attributed to that tamoxifen inhibits both basal and GRF-stimulated GH release from pituitary cells (Malaab et al., 1992).

Sodium and potassium ions concentrations of the current results showed almost semi-static results of different tamoxifen concentrations along the time of experiment. This was in partial agreement with the results obtained by (Grey et al., 1997) who found that there were no differences between the groups in the levels of serum sodium and potassium during a double-blind comparison of tamoxifen 20mg per day compared to placebo, over two years on forty-six healthy late-postmenopausal women.

Regarding to the effect of tamoxifen on serum calcium and phosphorus levels, the present study revealed that almost tamoxifen supplemented groups show slight increase or nearly stable in results except of phosphorus in groups fed (50 and 100 mg tamoxifen supplemented group for 60 days) which showed slight decrease in its levels. This was in partial agreement with the results of (Williams et al., 1991) who examined the effect of tamoxifen on the estrogen-induced formation of medullary bone in male Japanese quail model. In their experiment, this compound did not elicit changes in serum calcium and phosphorus levels. But our results are opposite with those obtained by (Kailajärvi 2000) who observed that there was statistically significant decreases occurred in serum calcium in seven postmenopausal women with breast cancer during 3 months after initiating the therapy of tamoxifen, and with (Filipović et al., 2015) who studied the effects of tamoxifen at a dose of 0.3mg kg-1 bw. daily for 3 weeks on biochemical markers of bone metabolism by using middle-aged orchidectomized (Orx) rats and found that blood serum levels of calcium (Ca2+) and phosphorus (P) were significantly decreased.E2 increased gradually by the increase of tamoxifen concentration and by the time. This was in agreement with the results of Lazier et al. (1996) who found that treatment of mature female tilapia (*Oreochromis niloticus*) with tamoxifen resulted in increased serum E2 levels. Concerning the effect of tamoxifen on testosterone levels, 60 days treatment showed the highest compared with other treatment periods specially G1 and G2 and this was in agreement with the results of (Rozenboim et al., 1993) who studied the effect of tamoxifen on testosterone level in WL male chicks and they found an early rise of plasma testosterone content.

Concerning the results obtained from histological examination to gonads, significant damage to the either testes or ovaries were noticed by increasing both concentrations and periods of administrations. Nearly the same results

were recorded by (El Asely et al., 2007) who found destruction and vacuolation in the cells of the somniferous tubules of tamoxifen treated tilapia. Similarly, Raslan (2012) found that *Oreochromis niloticus* tamoxifen treated groups with 50 and 100 mg for 15 and 30 days, testis showed mild degeneration while the ovary showed normal histological structure. On the other hand, no differences was found in the testes histology between control and tamoxifen treated gilt head sea bream after 25 days of treatment with tamoxifen at dose of 100μg/g feed, but also an increased in the sperm concentration and higher motility index were recorded García-Hernández et al. (2016).

CONCLUSION

In conclusion, the results proved that administration of tamoxifen for long period in Nile tilapia produce negative effect either on fish growth or reproduction. Therefore, if necessary it is recommended to be applied with dose 25 mg/kg food for 30 days for increasing the growth at such critical period of fish growth curve, without hazardous effects on fish physiology.

REFERENCES

1. Bancroft J, Stevens A, Turner D (1996). Theory and Practice of Histological Techniques 4th Ed Churchill Living Stone, New York Edinburgh. Madrid, Sanfrancisco.
2. El Asely AM, Abbass AA, Shaheen AA (2007). Effect of dietary administration of steroidal and non steroidal compounds on sex ratio of *Oreochromis niloticus*. Benha Veterinary Medical Journal, 18 (2):37-50.
3. FAO (2010). The state of world fisheries and Aquaculture (p.197).Rome.
4. Filipović B, Šošić-Jurjević B, Ajdžanović V, Živanović J, Isenović E, Popovska-Perčinić F, Milošević V (2015). Tamoxifen Stimulates CalcitoninProducing Thyroid C-Cells and Prevents Trabecular Bone Loss in a Rat Model of Androgen Deficiency. Journal of Anatomy, 226: 489-496.
5. García-Hernández M, Rodenas M, Cabas I, Garcia-Alcazar A, Chaves-Pozo E, Garcia-Ayala A (2016). Tamoxifen Disrupts the Reproductive Process in Gilthead Seabream Males and Modulates the Effects Promoted by 17α-Ethynylestradiol. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 179: 94-106.
6. Grey A, Evans M, Kyle C, Reid I (1997). The Anti Oestrogen Tamoxifen Produces Haemodilution in Normal Postmenopausal Women. Journal of Internal medicine, 242: 383-388.
7. Hines GA, Watts SA (1995). NonSteroidal Chemical Sex Manipulation of Tilapia. Journal of the World Aquaculture Society, 26(1): 98-102.
8. Kailajärvi MAO, Virtanen A, Salminen E, Irjala K (2000). Early Effects of Adjuvant Tamoxifen Therapy on Serum Hormones, Proteins and Lipids. Anticancer Research, 20(2B):1323-1327.
9. Kawarazuka N (2010). The Contribution of Fish Intake, Aquaculture, and Small-Scale Fisheries to Improving Nutrition: A Literature Review. The WorldFish Center Working Papers.
10. Lazier C, Langley S, Ramsey N, Wright JM (1996). Androgen Inhibition of Vitellogenin Gene Expression in Tilapia (*Oreochromis niloticus*). General and Comparative endocrinology, 104: 321-329.
11. Lazzeroni M, Serrano D, Dunn BK, Heckman Stoddard BM, Lee O, et al. (2012) Oral low dose and topical tamoxifen for breast cancer prevention:modern approaches for an

old drug. Breast Cancer Research 14(5): 214.
12. Lichtenfels M, Dornelles A, Petry F, et al (2017.) The anticancer estrogen receptor antagonist tamoxifen impairs consolidation of inhibitory avoidance memory through estrogen receptor alpha. Journal of Neural Transmission, 124(11): 1331-1339.
13. Love RR, Mazess RB, Barden HS, Epstein S, Newcomb P A, Jordan VC, Carbone PP, DeMets DL (1992). Effects of Tamoxifen on Bone Mineral Density in Postmenopausal Women with Breast Cancer. New England Journal of Medicine, 326: 852-856. DOI: 10.1056/NEJM199203263261302
14. Maclean N, Rahman M, Sohm F, Hwang G, Iyengar A, Ayad H, Smith A, Farahmand H (2002). Transgenic Tilapia and the Tilapia Genome. Gene, 295: 265-277.
15. Malaab S A, Pollak MN, Goodyer CG (1992). Direct effects of tamoxifen on growth hormone secretion by pituitary cells in vitro. European Journal of Cancer, 28(4-5): 788-793.
16. Mandiki SN, Babiak IJ, Bopopi M, Leprieur F, Kestemont P (2005). Effects of sex steroids and their inhibitors on endocrine parameters and gender growth differences in Eurasian perch (Perca fluviatilis) juveniles. Steroids, 70(2): 85-94.
17. Osborne CK (1998). Tamoxifen in the Treatment of Breast Cancer. New England Journal of Medicine, 339: 1609-1618.
18. Raslan WM (2012). Physiological Studies on Momosex Nile Tilapia.Master dissertation, Faculty of vetrinary medicine, Benha university.
19. Rozenboim I, Snapir N, Arnon E, Aryeh RB, Burke W, Sharp P, Koch Y, Robinzon, B (1993). Precocious Puberty in TamoxifenTreated Cockerels: Hypothalamic Gonadotrophin Releasing Hormone and Plasma Luteinising Hormone, Prolactin, Growth Hormone and Testosterone. British poultry science, 34: 533-542.
20. Singh R, Singh A, Tripathi M (2012). Effect of a Non Steroidal Tamoxifen on the Gonad and Sex Differentiation in Nile Tilapia, *Oreochromis niloticus*. Journal of environmental biology, 33: 799.
21. Steel R (1997). Analysis of Variance I: The One-Way Classification. Principles and Procedures of Statistics A Biometrical Approach: 139-203.
22. Williams DC, Paul DC, Herring JR (1991). Effects of Antiestrogenic Compounds on Avian Medullary Bone Formation. Journal of Bone and Mineral Research, 6: 1249-1256.