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Role of exogenous thyroxin hormone on eggs, thyroid gland development and growth performance of the monosex Nile tilapia, *Oreochromis niloticus* Larvae

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ABSTRACT

The present study was conducted to study the effect of exogenous thyroxin hormone on thyroid gland development and to determine the concentration of this hormone in the tissues of eggs, which affects the growth performance of tilapia larvae. The eggs and larvae were exposed to different doses of thyroxin solution from 0.025 mg/l to 0.1 mg/L and immersed in that solution for 1 hr. The present results showed that the level of thyroxin (T4) was higher in the newly hatched eggs then gradually decreased to the lowest level 30±0.04 mg /g wet weight (wt) after 96 hrs of treatment. Both treated larvae and the control group were similar in their concentration of total thyroxin at the period ranging from 3–7 days of post-hatching. The level of thyroxin (T4) was gradually increased after 17 days of post-hatching and reached the highest value of 8.38±2.31 mg/g wet wt. This level decreased and reached the lowest value of 1.97±0.79 mg/g wet wt after 27 days of post-hatching. The histological evidence of thyroxin on the thyroid gland showed that the thyroid follicles of the treated fish with thyroxin have more developed than that of the untreated fish (control group).

INTRODUCTION

The Nile tilapia, *Oreochromis niloticus* has a high economic and commercial value among the fish of fresh water. It is highly preferred in Egypt and extensively cultured over a large area of earthen ponds in fresh water fish farms. Semi–natural spawning occurred by controlling the environmental factors and is used without induction of spawning by hormone. Tilapia has a long spawning season, which extends from April to the end of September, thus it is considered partial spawning fish. The larvae of fish resulting from spawning have many problems at the first days of post hatching, such as larval mortalities were increased, particularly during the period of transition from endogenous nutrition to exogenous feeding on life foods (Watanabe and Kiron, 1994). Poor eggs quality associated with deficiency of diverse assortment of important

biological compound, especially low level of thyroid hormones (lam, 1994) and (Nayak *et al*., 2000).

In freshwater fishes, the concentration of total thyroxin (T4) is generally higher than triiodothyroxin (T3) in both eggs and larvae. In contrast, in marine water fishes, the concentration of T3 is higher than T4 in both eggs and fish larvae (Power *et al*., 2001).

The thyroid hormone treatment has been used to increase the growth, embryonic development and survival rate at the different larvae of fish species as reported by (Brown and Nunez, 1994). In this respect, Zairin *et al.*(2000) and Trijuno *et al*. (2002) have been proven that exogenous of T4 treatment could accelerate eye spot formation, swim bladder and pigmentation of marble goby larvae. Involvement of thyroid hormone in regulation and development through the interaction with growth hormone and steroid hormones are known as stated by Leatherland (1982). Immersion of fertilized eggs and larvae in thyroxin of hormone solution for a short period has shown to be an effective and practical means of administering hormones to finish developmental stages of embryos and larvae (Nugegoda and lam, 1994).

Recently, little attention has been paid to the role of total thyroxin (T4) on survival rate of eggs and post hatching larvae in monosex Nile tilapia larvae in the hatcheries. Therefore, the present study was planned to examine the effect of exogenous thyroxin on the structure of thyroid gland in monosex Nile tilapia larvae and its role on survival fish larvae and the eggs which is still need to be further clarified by immunohistochemistry studies.

MATERIALS AND METHODS

Source of larvae:

The experiment started on the first of April 2019, at Station Research of Elserw Fishes Farm that located in the north of Dakhlya governorate, Egypt. The eggs and larvae were collected from the hatchery of the station which produces monosex tilapia fish using 17α - methyl testosterone for a period 28 days of post hatching as described by El- Greisy and Elgamal (2012).

Experimental design:

About 1600 larvae after post hatching and 1500 fertilized eggs were collected and stocked in the two collecting tanks, containing 50L of dechlorinated fresh water which are gently aerated in each of them. For two days later, the larvae and fertilized eggs were transported into two buckets containing 30 L of fresh water.

The dosage of thyroxin hormone was prepared, the thyroxin hormone in experimental study was derived from levothyroxine sodium Tablets/Thyrax Nvorganon OSS, Netherland) each tablet contained 100 mg of thyroxin. Both Larvae and eggs were exposed to exogenous thyroxin solution (T4, 0.1, 0.05 and 0.025 mg /L of water). The

immersion extended for about 1.0 h in each group. In control groups, the larvae were not treated with thyroxine hormone.

Four experimental tanks were used for stocking larvae that were divided into four groups to choose the best and lethal doses level and then repeated.

1- The first group, the larvae and eggs were treated with 0.1 mg of thyroxin hormone /L of water for 1 hr.

2- The second group, the larvae and eggs were treated with 0.05mg of thyroxin hormone /L of water for 1hr.

3- The third group, the larvae and eggs were treated with 0.025 mg of thyroxin hormone /L of water for 1 hr.

4- The fourth group, the larvae and eggs were used without treatment and used as control, the capacity of each tank was 100 L of dechlorinated fresh water and made of fiber glass. The stocking density of larvae in each tank was 150 larvae, the stocking density of eggs was 100 eggs were placed in small pond made of glass and its capacity was 30L of dechlorinated fresh water.

The experimental condition in each tank was adjusted to be pH that ranged from 7–7.8, dissolved oxygen ranged from 6.5–7.5 mg/L and the water temperature ranged from 25 °C to 27.5 °C. The ponds were kept under natural condition of light (16 hrs light and 8 hrs dark) the experiment was terminated after 35 days of post hatching larvae.

Samples collection:

Ten samples were collected before the water change and were taken daily during the first week. In the second and third weeks, the collection of samples ranged from 3- 5 days a week. After that period, the collection samples were taken every 7 days a week. The mortality rate of larvae was daily recorded from each treatment and the dead larvae were counted and removed. The average of lengths was measured in mm and the total weights were recorded in mg in each treatment during the period of 15 days and 35 days of post hatching respectively.

Histological Studies:

In order to study the changes in the structure and development of thyroid gland, the whole head of small larvae were removed and fixed in Bouin's fluid or 10% neutral buffered formalin for a period of 24–36 hrs. After that, the specimens were transported into 70% of ethyl alcohol, the fixation process was followed by dehydration and clearing in pure xylene and then embedded in fresh of melting paraffin wax. The melting point of paraffin wax ranged from 56–58 $^{\circ}$C. Finally, the specimens were cut at 6 microns in thickness by using rotary English microtome and the sections were stained with haematoxylin and eosin according to El-Greisy and El gamal (2012). In order to show a degree of activity in the thyroid follicles, the changes in the height in the thickness of the epithelial cells that surrounded the follicle cells were measured by using eye piece

micrometer under microscope. The number of measured follicle cells was 10 during each section at the period of age from 5 to 27 days of post hatching and then the averages were recorded.

Immunoassay of thyroid hormones:

In concerning for measuring total thyroxin (T4) in the eggs and the larvae, 50 mg of wet wt were pooled and frozen at - 70 °C until analysis. For measuring the total concentration of T4, the eggs and pooled larvae were homogenized in 1.5 mL of ice cold 0.01 phosphate buffered saline (PBS) for 1 minute by using homogenizer as described by (Tanaka *et al.*,1995), the homogenate of an aliquot of 25 mL in each sample was taken. The level total T4 in each sample was determined by using ELISA kit as described by Abol– Munafi *et al.* (2005). Three replicas of the data were applied for measuring the total T4 concentration in larvae and then the averages were taken.

Statistical analysis:

T- test was used to find out the statistical significance between the means of T4 level at the different treatments and the control group, $p<0.05$ considered as a significance difference occurred between control and the different treatments with T4 hormone.

RESULTS

A) Effect of different doses of total thyroxine (T4) on the survival rate of fertilized eggs:

The effects of total thyroxin hormone (T4) on survival rates of newly hatched larvae and eggs were tested with different doses level of thyroxin 0.1 mg/L, 0.05 mg/L and 0.025 mg/L of water. The experimental study was carried out on fertilized eggs for a period of 96 hrs. The best survival rate obtained after 96 hrs on eggs exposed to 0.025 mg/L of water, the survival rate reached to 55 %. However, the fertilized eggs exposed to high dose level 0.1 mg/L at the same period showed that the survival rate decreased to 33% compared to the control group ($P < 0.05$, Table 1).

B- The concentration of total thyroxin (T4) in the tissues of fertilized eggs:

The concentration of total thyroxin (T4) was high in the tissues of fertilized eggs directly before hatching and the highest concentration was 5.25 ng /g wet wt. The lowest value decreased to 2.93 ng / g wet wt. and their average was 4.42±1.06 as shown in Table (2). The concentration decreased gradually after 10 hrs of post hatching to the maximum value 2.25 ng / g wet wt. and 1.53 ng/g wet wt. and their average was 1.98±0.32 ng/ g wet wt. This value decreased gradually and reached the minimum value in an average 0.30±0.04 ng/gwt. after 96 hrs of treatment in comparison to the control group ($P<0.05$).

C- The concentration of total thyroxin (T4) in the tissue of larvae:

The average level of total thyroxin at zero day of post hatching reached to 4.03±1.25 ng/g wet wt. This value reached 3.53±0.90 ng /g wet wt. in their average after 3 days of treatment. This value of thyroxin increased and reached to a peak value (8.38±2.31 ng/g wet wt) after 17 days of post hatching and these values decreased gradually and reached to the minimum value (1.97±0.79 ng/g wet wt) after 27 days of post hatching ($P < 0.05$, Table 3).

D- Effect of total thyroxin 0.025 mg/L of water on growth of larvae after 15 days of post hatching:

The effect of total thyroxin 0.025 mg/L of water on growth and survival rate of larvae after 15 days of post hatching showed that the average length of larvae was 15.5±1.11 mm and the average of weight was 8±0.70 mg. The survival rate reached to 84% in an average. However, in compared with control group, the average of total length was 13.33±1.24 mm and the average weight was 6.33±1.24 mg ($P < 0.05$). The Survival rate in control group was 73.33% after 15 days of post hatching Table (4).

E - Effect of total thyroxin 0.025 mg/l in water on growth of larvae after 35 days of post hatching:

After 35 days of post hatching, the average of total lengths of larvae in treated group with thyroxin was 26.71±179 mm, however in compared with control group, the average of total length of larvae was 22.4±1.94 mm**.** In respect to the growth in weight, the average of total weight of larvae in treated group was more significantly different than that of the control group ($P < 0.05$). The average of survival rate in the treated group was 88.25 compared to the control group (75.4±2.60) that showed a significant difference ($P < 0.05$, Table 5).

Development of thyroid gland in the early larval stages:

The thyroid follicles were not arranged in a discrete gland in fishes, but they were scattered as follicles throughout the connective tissues of pharynx or between the tissues that located between the gill arches which lined with follicular epithelial cells that surrounded the lumen containing colloid matrix.

In small fry fish 3DPH, the thyroid follicles were small in size and the cells appeared without any containing of colloid material Figs.1 (A and B). The thyroid follicles in larvae fish appeared as diffuse follicles lying in the center of pharyngeal region and are slightly larger than that the peripheral follicles. The thyroid follicles appeared encapsulated in shaped and appeared as independent unit without any communication. In small fry fish, the epithelial cells around the follicles are hardly detected. The thyroid follicles became more distinguished in the larvae after 7 DPH by examining sagittal sections under the microscope Fig.1 (C). The follicle cells appeared in round or in tubular-shaped and containing a little colloid in its lumen cavity. The lumens were more distinguished and surrounded with epithelial cells in the form of cubical

shaped. After 10 days of post hatching, the thyroid follicle appeared embedded in connective tissues in the center of pharyngeal region. The lumen of the thyroid follicle seems to be empty from any of colloid material. Some of follicular cells were small in sizes, however, the other cells appeared in large sizes Fig1 (D). The epithelial cells that surrounded the follicle cells is still appeared in the form of cubical shaped. After 17 days of post hatching, the gel- like viscous iodine began to appear in the center of follicles. The colloid material was eosinophilic and brilliant red in color. The layer of the epithelial cells increased in thickness and became more basophilic and stained blue purple in color fig2 (A). After 23 days of post-hatching, the follicles of thyroid gland increased in size and have large lumens containing eosinophilic colloid material. The epithelial cell appeared in more or less in columnar shaped and stained blue purple in color. The blood vessels scattered between the thyroid follicles, containing red blood cells and the pigment cells were scattered between the blood vessels, Figs. 2 (B and C).

The height of thickness in the epithelial cells:

In order to show the activity degree in the thyroid follicles, it is important to measure the change in the heights of thickness of the epithelial cells that surrounded the follicle cells. In small larvae fish, the follicles are small and appeared in inactive stage. In this case, the layer of the epithelial cells was thin in thickness and reached 17.5±0.25 µm after 5 days of post hatching. This layer increased in the thickness to 26.7±0.08 µm and became more distinguished after 15 days of post hatching. The thickness in the epithelial layer increased as the larvae increased in age after 27 days of post hatching and reached to 29.2±0.08 µm (Table 6 and Fig.2 D).

Table (1): The effect of different doses level of the total thyroxin (T4) on survival rate of fertilized egg for a period of 96 hours after fertilization when compared to the control group

Period / hours	Survival rates of eggs at different doses of T4 mg/L of water			Control group
	0.025	0.05	0.1	
5	100	90	70	80
10	90	85	68	70
15	83	82	65	68
20	75	71	61	61
35	70	68	60	60
40	67	65	55	58
55	63	63	49	53
70	59	58	43	51
80	57	49	37	49
96	55	47	33	45

Table (2): Concentration of total thyroxin (T4) in treated eggs from fertilization until hatching of eggs during the period of 96 hrs.

Treatment period (hrs)	Total value of thyroxin (mg/g wt. wet)			Average of control group mg/g wet wt.
	Minimum value	Maximum value	Average ± SD	
0	2.93	5.25	4.42±1.068*	4.13±0.26*
10	1.53	2.25	1.98±0.32*	2.13±0.26*
20	1.12	1.25	1.17±0.05	1.5±0.16
40	0.70	1.10	0.86±0.20	0.96±0.16
82	0.55	1.02	0.69±0.23	0.64±0.08
96	0.25	0.35	0.30±0.04	0.23±0.03

The concentration analysis for thyroxin was repeated.

* T-test, it was significantly difference in compared to the control group ($p < 0.05$)

Table (3): Concentrations of total thyroxin (T4) in treated larvae from hatching until 27 days of post hatching.

Treatment period (days)	Total value of thyroxin (mg/g wt. wet)			Average of control group mg/g wet wt.
	Minimum value	Maximum value	Average±SD	
0	2.30	5.23	4.03±10.25	3.4±1.15
3	2.25	4.25	3.53±0.95	3.25±0.96
7	1.98	3.96	3.14±0.84	2.56±0.75
10	2.98	6.54	5.24±1.60*	3.86±1.73*
17	5.12	10.23	8.38±2.31**	4.83±1.41*
23	3.45	6.23	4.39±1.29	4.50±0.96
27	0.91	2.81	1.97±0.79	1.46±0.53

The concentrations analysis of thyroxin was repeated.

** T-test, it was significantly different when compared to the control group ($p < 0.05$)

Table (4): Effect of total thyroxin (T4) at dose level of 0.025 mg/L of water on growth after 15 days of post hatching.

Hormonal dose and control group	Total length (mm)			Total weight (mg)			Survival rate (%)
	Min.	Max.	Average±SD (mm)	Min.	Max.	Average±SD (mg)	
T4 0.025 mg/L	14.0	17.0	15.5±1.11	7.0	9.0	8±0.70	84.25±2.58*
Control group	12	15	13.33±1.24	5	8	6.33±1.24	73.33±1.24

Number of measured fish fry was 20

*The survival rate was significantly different when compared to the control group ($p< 0.05$)

Table (5): Effect of total thyroxin at dose level of 0.025 mg/L of water on growth after 35days of post hatching.

Hormonal dose and control group	Total length (mm)			Total weight (mg)			Survival rate (%)
	Min.	Max.	Average±SD (mm)	Min.	Max.	Average±SD (mg)	
T4 0.025 mg/l	25	30	26.71±1.79	15	20	17.5+ 1.87	88.25±1.25*
Control group	20	23	22.40±1.94	12	15	13.5 + 1.29	75.4±2.60

Number of measured fish larvae was 15.

*The survival rate was significantly different when compared to the control group ($p < 0.05$)

Table (6): Changes in the thickness of the epithelial cells in thyroid follicles after treated with total thyroxin (T4) at dose level 0.025 mg/l of water for a period of 27 days of post hatching.

Age of larvae (days)	Height of thickness in µm		Average of height of epithelial cells (in µm)
	Minimum value	Maximum value	
5	15	20	17.5±0.25*
7	20	25	21.5±0.21
9	21	24	22.5±0.11
15	26	28	26.7±0.08
27	28	30	29.2±0.08**

- Number of measured follicles in each fry was 10
- *A significance difference was recorded between the thickness of the wall in early follicles and the wall of old follicles ($p<0.05$).

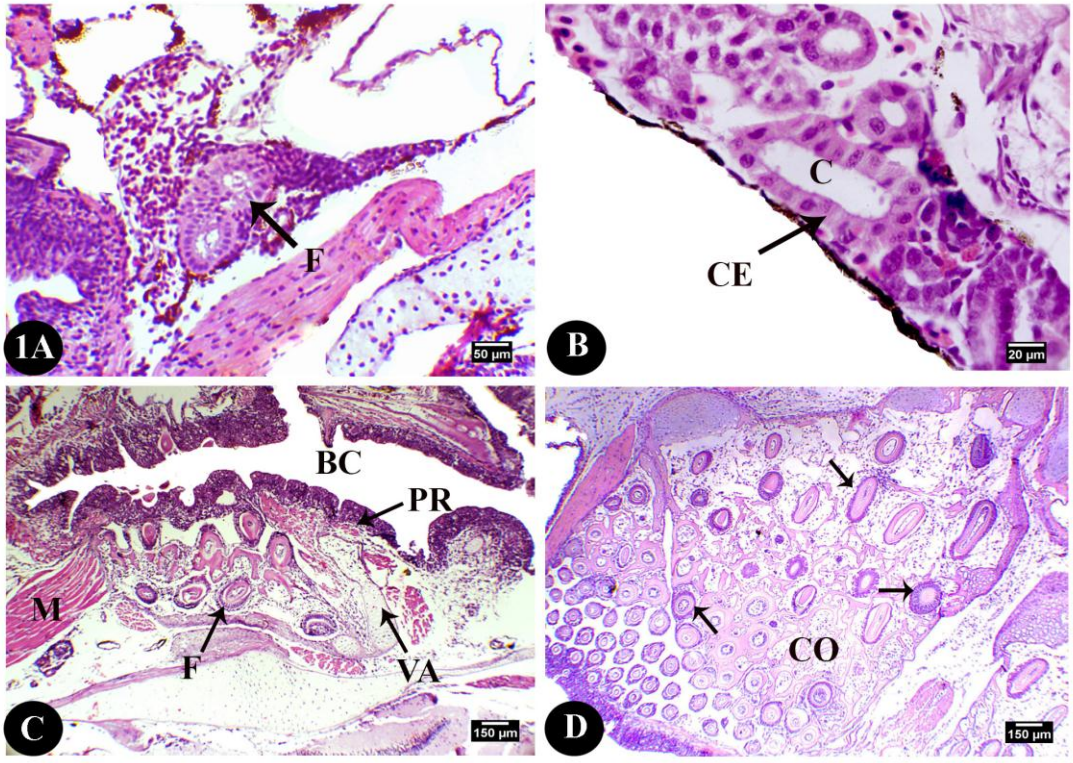

Fig.1 (A&B): The thyroid gland during early developmental stage (3 day), the thyroid follicles (**F**) e granulated nucleus was detected.; m = muscles; Scale bar = 50µm Fig. (C): Sagittal section of the buccal cavity of *Oreochromis niloticus* larvae. The thyroid gland was observed and scattered in encapsulated follicles (**F**) (arrowheads) under the pharyngeal region (**PR**) and located also near to the ventral aorta (**VA**). Scale bar = 150µm.Fig. (D): photomicrograph of thyroid gland during active developmental stage (10 days) showing thyroid follicles were at different developmental stages by (arrowheads), and some follicles have lumen filled with the colloid material (**CO**). Scale bar = 150µm

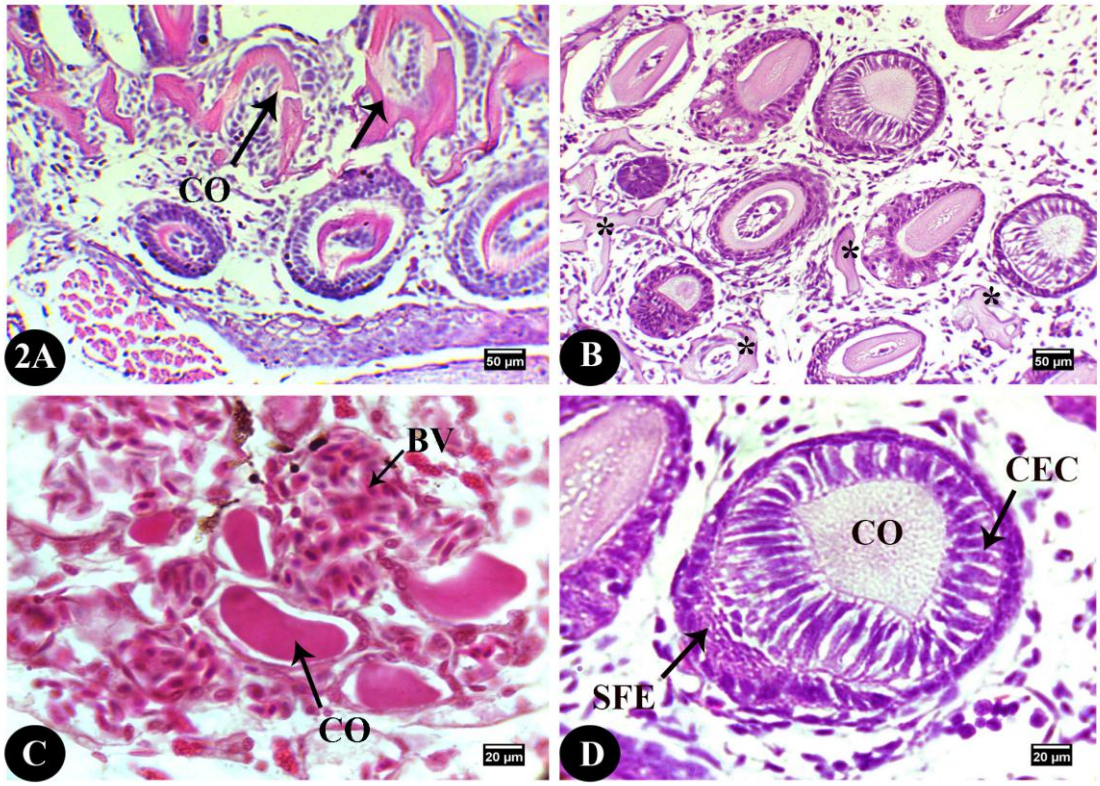

Fig.2 (A): photomicrograph of thyroid gland showing some follicles disintegrated and the colloid (**CO**) depleted during active development. (arrows) Scale bar = 20µm Fig. (B): photomicrograph of thyroid gland showing some follicles completely disintegrated (stars) and reminatant colloidal material (**CO**) (arrows) Scale bar = 50µm, Fig. (C): magnification showing the follicles of thyroid gland surrounded with several blood vessels (**BV**), the lumen filled with colloid (**CO**). (arrows) Scale bar = 20µm.Fig. (D): magnification showing the follicles of thyroid gland displaying well-developed cylindrical epithelial cells (**CEC**) (arrows) hypertrophy (increased cell in height; columnar epithelium), lining lumen stimulated follicles (**SFE**) that to be filled with colloid. Scale bar=20 µm

DISCUSSION

Thyroid hormones include triiodothyronine (T3) and thyroxin (T4) that regulate growth, development, differentiation, metabolism and maintenance of homeostasis in vertebrates as stated by Szischa *et al*. (2005). In all vertebrates, organogenesis and growth acutely depend on thyroid hormones (Power *et al*., 2001). In the present study, the first experiment was carried out to study the survival rate of fertilized eggs of *Oreochromis niloticus* at the different doses of total thyroxin (0.025-0.1 mg/L of water) for a period of 96 hrs of treatment.

The present findings showed that the best survival rate obtained after 96 hrs of eggs immersing in 0.025 mg/L, the survival rate reached 55%. However, the fertilized eggs exposed to high dose level of thyroxin (0.1 mg/L of water) at the same period, the survival rate decreased and reached 33%. In this respect, the thyroxin when treated at lower concentrations on four species of fishes, *labeo rohita*, *cyprinus carpio*, *catla catla*, *cirrhina mrigala*, the treatments were given in the dosages ranging from 0.025 to 0.2

mg/L showed that it has beneficial effect at 0.025 mg/L of water as described by Sawant and Belsare (1994). However, the treatment at higher dosages (0.15 to 0.20 mg/L) was found to reduce the survival and growth rates of chum salmon (*Oncorhynchus keta*) as reported by Dales and Hoar (1954) and Ali (1961).

In the present results, the concentrations of total thyroxin (T4) in the tissue of eggs were directly analyzed by ELISA before hatching reached 4.42±1.06. This value decreased after 10 hrs of post-hatching and the average was 1.98±0.32 ng/g wet wt. This value decreased gradually and reached to the minimum value in an average 0.30±0.04 ng/g wet wt. after 96 hrs of treatment ($p < 0.05$). It seems that, the changes in concentrations of the thyroid hormones in eggs, embryonic development and larvae of fresh water were differs according to type of fish species.

Several studies demonstrate that exogenous thyroid hormone treatment can stimulate yolk sac absorption, growth and survival rate as described in many of teleost species (Lam, 1994, Brown and Nunez, 1994). However, Tagawa and Hirano (1991) reported that, the reduction of thyroid hormones in eggs did not affect the embryogenesis and early development in medaka, *Oryzias latipes*. Nayak *et al*. (2001) studied thyroid hormones dynamics during the early development of *Heteropneustes fossils* and found that there was a steady decrease in both T3 and T4 levels during embryonic development. The level continued to decline after hatching until the yolk sac was completely absorbed. Khalil *et al*. (2011) study on Nile tilapia, *O. niloticus* larvae reported the effect of exogenous thyroxin, which might have been transferred from maternal circulation into oocytes and larvae. Similar results were obtained in our study in which the eggs were exposed to thyroxin (T4) at dose of 0.025 mg/L of water, however, Khalil *et al*. (2011) injected the female of broodstock with thyroxin hormone (1 to 10 µg T4 /g body wt) to transfer thyroxin from maternal circulation into oocytes and then to larvae. A role of thyroid hormones in embryogenesis and larvae development is further implied from studies in which thyroid hormones have been extracted and quantified from eggs and larvae. Moreover, T4 and T3 were detected in eggs of maternal origin as thyroid follicles were absent from embryo and early hatched larvae (Tagawa and Hirano, 1991).

In the present results, the immersion of fertilized eggs in hormone solution for 1.0 h found to be more effective for fish embryos and newly hatched larvae. Similar results were obtained by Nugegoda and Lam (1994) on fertilized egg in the tilapia, *Oreochromis mossambicus*. In this respect, Nayak *et al*. (2000) found that the treatment of fertilized eggs with thyroxin (T4) and cortisol (F) separately or together would produce an effect on embryogenesis and larval survival in fresh water in Indian major carp, *catla catla*.

In the present study, the concentration of total thyroxin (T4) in the tissues of larvae analyzed by ELISA showed that the larvae of total thyroxin (T4) at day zero of post hatching reached 4.03±1.25 ng/g wet wt.in average. This value decreased and reached to 3.53±90 ng/g wet wt.in an average after 3 days post hatching. The value of thyroxin gradually increased to 8.38±2.31 ng/g wet wt. after 17 days of post hatching. Then, these

values decreased gradually to the minimum value after 27 days post-hatching (1.97±0.79 ng/g wet wt.). The decrease of thyroxin after this period may be attributed to the larvae began to feed and need more quantity of thyroxin that is used for metabolism and growth. In Marble goby, *Oxyeleotris marmoratus*, larvae that studied by Abol-Munafi *et al*. (2005) and found that in the exogenous T4 treated larvae, the concentration of total T4 was 4-5fold higher than that of the control group. The authors added that the thyroid hormone levels decreased from 3-7 days of post-hatching 4.2 to 3.1 µg dL-1 whereas in control was from 1 to 0.7 µg dL-1

In the present study, the larval stage ended at 28 days of post hatching in which the larvae are able to swim in all directions inside the pond. The most organs became more developed and the gastric glands were completely formed. Similar results were observed in larvae of different fish species as stated by Balon (1975) and Abol-Munafi (2005). In the present study, the T4 increased and reached the peak value in 17 days of post hatching, after this period the level of T4 hormone declined but maintained constant level until 27 days post-hatching (1.79±0.97 ng/g wet wt.). This finding suggests that the thyroid hormones play a significant role during metamorphic phase and transformation of post larvae to juvenile.

In the present study, the effect of thyroid hormones on larval growth and survival rates showed that thyroxin treated larvae showed increased in the lengths and weights as in compared to the control group (p <0.05). The survival rate increased in treated larvae than those of control group and reached to 88.25% (p <0.05). The thyroid hormone treatment has been shown to promote growth, developmental and survival rates in several fish species (Lam, 1994; Brown and Nunez, 1994). Involvement of thyroid hormones in regulation of fish growth and development through the interaction with growth hormone and steroid hormones as was stated by Leatherland (1982). The decrease of survival rates occurs particularly in untreated larvae with thyroid hormone at the period of transition from endogenous nutrition to exogenous feeding. The main reason may be attributed to the lack of suitable food at the onset of first feeding (Watanabe and Kiron, 1994).

The thyroid gland in all vertebrates including fishes is composed of follicles, these organs appear as a ball like in structure which contain a single layer of cuboidal cells enclosing fluid filled lumens that is used as a source of thyroxin. Moreover, in teleosts, the absence of encapsulated glands is not restricted to the pharyngeal region and frequently migrated to unusual sites such as the kidneys, heart and sometimes the brain as stated by (Gorbman *et al*., 1983). The ontogeny of the thyroid gland during the teleosts development is poorly described (Leatherland, 1994). As a result of the small size of fish embryos and larvae, the disperse nature of the follicles within the developmental larval pharynx make identification is difficult (power, 2001).

In the present study, the functional unit of thyroid gland is spherical follicles cells that are lined with follicular epithelial cells surrounded the lumen containing colloid matrix. In small fry fish 3 DPH, the thyroid follicles were hardly detected. Few numbers

of small follicles cells appeared without any deposition of colloid material. Similar results were obtained by Power (2001) who stated that in small *S aurata*, no follicles were detected and instead isolated colloid containing epithelial cells were detected. In the present study, the thyroid follicles became more distinguished in the larvae after 7 DPH by examining sagittal section under the microscope. The follicle cells appeared round or tubular in shape, the lumens were small in size and surrounded by cubical epithelial cells. In this respect, the larvae of *sparus aurata* (7 DPH), despite the scarcity and the small size of the thyroid follicles, they were readily identified by using Cleveland – Wolf staining as with this method the colloid is rendered bright red (power, 2001).

The activity of the thyroid follicles may be influenced by numerous factors, but the principal factor controlling thyroid activity is thyroid stimulating hormone (TSH) which is produced by the pituitary gland (Barker, 1964; Leatherland and Barrett 1993). In the present study, after 10 DPH, the thyroid follicles increased in sizes and appeared embedded with connective tissue in the center of pharyngeal region. The epithelium surrounding the follicle cells appeared in cubical shaped. The appearance of colloid matrix within the lumen of the follicles may be used to give an indication of its activity. A densely staining uniformly eosinophilic colloid matrix is found next to squamous epithelium as described by (Gorbman *et al*., 1983). After 17 DPH as observed in the present study, the gel like viscous iodine began to appear in the center of the follicles. The colloid material was eosinophilic after staining with haematoxylin and eosin and appeared in red color. The layers of epithelial cells were basophilic and stained blue purple in color and appeared in squamous epithelium. Similar results were obtained by Power (2001) on the thyroid follicles of *sparus aurata* larvae. After 27 DPH, the follicles cells increased in size and have large lumen containing eosinophilic colloid materials. The epithelial cells appeared in columnar shaped and stained blue purple in color. Blood vessels scattered between the thyroid follicles and pigment cells distributed between the blood vessels. Similar results were obtained from thyroid gland development in *Sparus aurata* larvae after 36 days of post hatching (Power, 2001).

In the present study, the heights of thickness of the epithelial cells that surrounded the follicle cells were measured at different periods of treatment. In small fish larvae, the follicle cells appeared inactive stage and the layer of epithelial cells were increased and reached 17.5±0.025µm. The thickness of epithelial layer increased as the larvae increased in age and reached 27.2±0.08µm after 28 days of post hatching. Similar results were obtained from yellow fin sea bream, *Acanthopagus latus* as described by Salamat *et al*. (2012). It seems that the thyroid gland plays an important role in growth of the larvae and improves the survival rates of *O. niloticus* larvae.

It can be concluded that the present finding showed that the treatment group with thyroid hormone may play an important role in the fertilized eggs and newly hatched larvae. The eggs and small larvae exposed to small doses of thyroxin (0.025 mg/l of water) improve survival rate, growth and increase the development of thyroid follicles in

early life stage larvae in compared to the control larvae of *Oreochromis niloticus.* The present results also showed that the level value of thyroxin (T4) was higher in eggs and then gradually decreased and reached to 0.30±0.04 mg / g wet wt. at 96 hrs of treatment.

In larvae, the value was gradually increased after 17 days of post hatching and reached to 8.38±2.31 mg/ g wet wt. The value reached to the lowest value 1.97±0.79 mg/g wet wt. after 27 days of post hatching., The histological evidence of thyroxin in the thyroid gland, showed that the thyroid follicles in treated group have more development than that untreated group (control). These findings indicated that the thyroxin hormone may play an important role on fertilized eggs and newly hatched larvae and increased the survival rates.

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