

Studies on fecundity and some physiological features for the ovaries of Nile Tilapia, *Oreochromis niloticus* treated with different concentrations of Ethylenediaminetetraacetic Acid (EDTA).

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ABSTRACT

The present study aims to determine the effect of different concentrations of EDTA (0 g EDTA/L, 0.1 g EDTA/L and 0.3 g EDTA/L) on fecundity, survival rate and biochemical responses of the adult female of Nile tilapia, *Oreochromis niloticus* reared in glass tanks. Results showed that, the fecundity increases with the increasing in the concentrations of EDTA compared with control. Survival rate are affected by high concentration of EDTA (0.3 g EDTA/L) and reached to 33.3 % at the end of third week. However, an addition of EDTA to aquaria containing adult females increased the biochemical parameters at high concentration compared with control and low concentration samples except total proteins showed decreasing to lowest level. Statistical analysis mentioned a significant difference between the low concentration of EDTA and both of high concentration and control in all parameters. Otherwise, total carbohydrates exhibit non significant differences between all of them.

Keywords: EDTA; fecundity; physiological features; *Oreochromis niloticus*.

INTRODUCTION

EDTA has been used extensively in medicine as a chelating agent for removal of the toxic heavy metals. The disodium salt of EDTA is a common component in many eye drops and contact lens wetting and cleansing solutions. EDTA is also used in a number of personal care and hygiene products, such as shampoos, liquid soaps, creams, and lotions. Household disinfectants often contain EDTA, especially if fatty acid soaps are used in the disinfectant formulation. These soaps are sensitive to calcium and magnesium, and the chelating agent prevents the formation of hard-water soap curds (Hart, 1984). EDTA is a common sequestrate and antioxidant added to foods, body care, and household products. It binds trace minerals such as lead, copper, iron, cadmium and nickel that may be present in the product. When EDTA adds as an antioxidant, it prevents oxygen from causing color changes and rancidity (Ben-Best, 2009). EDTA has two advantages with respect to other compounds—its relative low biodegradability in groundwater systems (Nowack, 1996) and its strong complexing capacity with heavy metals (Kedziorek and Bourg, 2000).

Fish are often the top of aquatic food chain and is an important source of protein for human, which may absorb large amounts of some metals such as Cadmium, Copper, Iron, Nickel, Lead and Zinc through epithelial or mucosal surface of the skin, gills and gastrointestinal tract. One of the important functions of serum protein is the maintenance of osmotic balance between the circulating blood and the tissue fluids (Haper *et al.*, 1977). The influence of toxicants on the total protein concentration of fish has been also taken into consideration in evaluating the response to stressors. The total protein level is a frequently parameter of metal poisoning in fish.

Tilapia is an ideal candidate for warm water aquaculture. They spawn easily in captivity, use a wide variety of natural foods, as well as formulated feeds, tolerate

poor water quality and grow rapidly at warm temperatures. These attributes, along with relatively low input costs have made tilapia widely cultured freshwater fish in tropical and subtropical countries (Biswas *et al.*, 2005; Borgeson *et al.*, 2006; Tahoun, 2007; Khalaf-Allah *et al.*, 2013 and Ghanem *et al.*, 2014). In Egypt the Nile tilapia, *Oreochromis niloticus* aquaculture production about 870938 tons which form 63.48 % from our total production and put Egypt as a second biggest productive for Nile Tilapia after China. The total production of tilapia fry from hatcheries or fish farms in 2012 attained 242.558 million fry of *O. niloticus* and *O. aureus* dominate the fry production (GAFRD, 2012 and FAO, 2014).

The effect of chelating agent (EDTA) as benefit in reduce lead and cadmium toxicity with improve the physiological and biochemical profiles of the fish and its impact on fecundity were investigated by few authors (Shalaby, 2007; Shalaby *et al.*, 2011; Tonsy & Abdel-Rahman, 2012 and Ghanem *et al.*, 2014).

Physiological and biochemical parameters reflects physical and chemical changes occurring in an organisms, therefore detailed information can be obtained on general metabolism and physiological status of the fish (Kocabatmaz & Ekingen, 1978 and Tavares-Dias & Silva-Sandrim, 1998).

Therefore, the present study aimed to evaluate the effect of different concentrations of Ethylenediaminetetraacetic acid (EDTA) on fecundity, survival rate and biochemical status of Nile tilapia, *Oreochromis niloticus*. Such investigations may lead to a better understanding of some biological and physiological aspects.

MATERIALS AND METHODS

A total of 36 specimens of *O. niloticus* adult females, with a good condition were obtained from El-Hadad private farm at Kafr El-Sheikh governorate during June, 2014, after graduating from the ovulation cycle. It acclimatized at the laboratory for two weeks in well aerated large glass tank and fed daily on a commercial fish diet with optimum temperature for entered a new ovulation cycle.

The experimental work was conducted using 9 separate tanks each tank (100X50X50 cm) was filled with about 40 Cm in depth of the fresh dechlorinated water. In each tank, 3 fish were randomly taken from the stock tank. Also, three groups were chosen, each has 3 tanks. The first and second groups were treated with 0.1 g and 0.3 g EDTA/L respectively, while the control group has also 3 tanks without any treatment. The tanks were provided with aeration. The change of water occurred twice weekly and the aquaria were cleaned. Fish in each aquarium were fed twice daily seven days per week by commercial diet containing 30% protein. Fish were fed at a rate of 0.5 % of average body weight. The experiment was conducted for 3 weeks; length and weight for each treatment were recorded at the beginning and at the end of the experiment.

The environmental factors in all experiments including dissolved oxygen, hydrogen ion concentration (pH) and water temperature were recorded and adjusted as the following:

- (A) Dissolved oxygen was varied from 7.3 to 9 mg / L.
- (B) pH values fluctuated between 7 to 8.5.
- (C) Water temperature was $28 \pm 1^{\circ}\text{C}$.

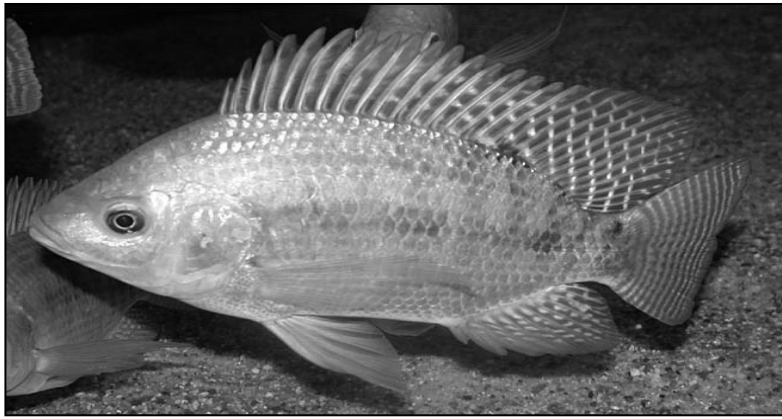


Fig. 1: Lateral view of the cichlid fish, *O. niloticus*.

A-Biological studies:

1-Total length:

Total length of each fish was measured to the nearest millimeter by steel ruler.

2-Total body weight:

The total body weight was taken using electronic balance to the nearest 0.1 gram and recorded.

3-Fecundity:

To study fecundity, females from control and both treatments were dissected to exit ovaries at the end of experiment. The ovaries were weighted to the nearest 0.1 gm and preserved immediately in saline solution. Samples from three different parts of each ovary were taken, weighed and placed in a Petri-dish. Ova were separated from the ovarian tissues with the aid of a dissecting needle and counted. Fecundity was calculated according to the following equations suggested by Nikolosky (1963).

$$\text{Absolute fecundity} = \frac{\text{Average number of ripe egg}}{\text{wt. of sample (gm)}} \times \text{wt. of ovary (gm)}$$

$$\text{Relative fecundity} = \frac{\text{no. of eggs}}{\text{body weight of fish (gm)}} \times 100$$

The gonadosomatic index (GSI) was calculated as the following:

$$\text{GSI} = \frac{\text{GW}}{\text{W}} \times 100$$

Where: GW: Weight of ovary (g).

W: Total body weight of fish (g).

4-Oocyte diameter:

To study oocytes diameter, so oocytes from each ovaries of control and both treatments were preserved immediately in saline solution, placed on a glass slide and measured with an ocular micrometer. The diameters of oocytes were determined by taking the mean of the maximum and minimum diameter.

B-Physiological studies:

After dissection of the fish, *O. niloticus*, a known weight from the ovaries of both treated with 0.1 and 0.3 of EDTA and untreated fish were kept under freezing condition until the biochemical determination.

▪ Tissue preparation:

For determination of the total proteins, total lipids, total carbohydrates and enzymes activities including aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT), a known weight of each ovary was homogenized in saline solution by using the electric homogenizer for 2 min. The homogenated specimens were centrifuged

4000 r.p.m. for 15 min. at 2 C⁰ in a refrigerator centrifuge. The supernatant solution was used directly or stored at 4 C⁰ until the latter examinations.

1- Determination of the total proteins:

Total protein content in the ovaries were determined according to the method of Doumas (1975) using a kit of Vitro Scient Company.

2- Determination of the total lipids:

Total lipids content in the ovaries were determined according to the method of Kaplan (1984), using a kit of Reactivos GPL Company.

3- Determination of the total carbohydrates:

Total carbohydrates content in wet ovaries were determined according to the method of Singh and Sinha (1977) as follows:

Reagents:

i) Anthrone reagent was prepared by addition of 72 ml sulfuric acid to 28 ml distilled water. While this mixture was still warm, 50 mg of anthrone and 1gm of thiourea were added with a vigorous shaking, then it was used directly after slowly cooling at the room temperature then it stored at 8 C⁰.

ii) Standard solution was prepared by addition of 50 mg glucose to 100 ml distilled water.

• Procedure:

▪ To determine the total carbohydrate content, 0.1 ml of the aqueous samples was added into a test tube and diluted with 1 ml distilled water then follow with 5 ml freshly prepared antherone reagent.

▪ The blank was prepared by adding 5 ml of antherone reagent to 1.1 ml of the distilled water.

▪ The standard solution was prepared by adding 0.1 ml of standard solution to 1 ml distilled water and then add 5 ml of the anthrone reagent.

All test tubes of blank, standard and samples were placed in a boiling water-bath for 10 min. Then, it was leaved to cool for 15 min. at the room temperature within a dark place. Reading of absorbance photometrically was recorded for the standard and the samples against blank at wavelength 620 nm.

Calculation: Total carbohydrates (mg/100ml) = $\frac{A_s}{A_{st}} \times n$

Where: A_s= Absorbance of the samples.

A_{st}= Absorbance of the standard solution.

n= Concentration of standard solution= 0.05 then, results were converted into mg/gm tissue.

4-Determination of ASAT and ALAT activities:

ASAT and ALAT of wet ovaries were determined according to the method of Reitman and Frankel (1957) by using a kit of Bioadwic Company.

Statistical analysis: Results were expressed in tables as mean ±S.D. Data were analyzed by using analysis of variance (ANOVA) according to Bailey (1981).

RESULTS

Fecundity:

Data showed that, the fecundity increases with the increasing in the concentrations of EDTA compared to control. The number of eggs /100 g body weight is 819 in control, 818 in the fish treated by 0.1 gm EDTA/L and 851 in the fish treated by 0.3 gm EDTA/L. The oocyte diameter ranged between 1.80– 2.80 mm in all treatments and averaged with mean 2.07±0.15 in control increased to 2.10±0.25

and 2.12 ± 0.19 in the fish treated by 0.1, 0.3 g EDTA/L, respectively. Survival rate are affected by high concentration of EDTA (0.3 g EDTA/L) and reached to 33.3 % at the end of third week (Table, 1 and Figures, 2 -5).

Table 1: Effect of different concentrations of EDTA on fecundity, Oocyte diameter and survival rate of *O. niloticus*.

| Items | Treatment | | |
|--|-----------------|-----------------|-----------------|
| | Control | 0.1 g EDTA/L | 0.3 g EDTA/L |
| Final average body weight (g) | 140 ± 10 | 141 ± 9 | 142 ± 10 |
| Total fish length (Cm) | 20.5 ± 1.5 | 20.7 ± 2.3 | 22.1 ± 1.1 |
| Ovary weight | 5.7 ± 0.2 | 5.8 ± 0.2 | 6.1 ± 0.2 |
| No. of egg /g of ovary | 201 ± 15 | 199 ± 13 | 198 ± 10 |
| Absolute fecundity | 1146 | 1154 | 1209 |
| Relative fecundity egg /100 g weight of fish | 819 | 818 | 851 |
| Gonadosomatic index | 4.1 | 4.1 | 4.3 |
| Oocyte diameter range | 1.8 – 2.5 | 1.8 – 2.8 | 1.8 – 2.8 |
| Average of oocyte diameter (mm) | 2.07 ± 0.15 | 2.10 ± 0.25 | 2.12 ± 0.19 |
| Survival rate % | 100 | 100 | 33.3 |

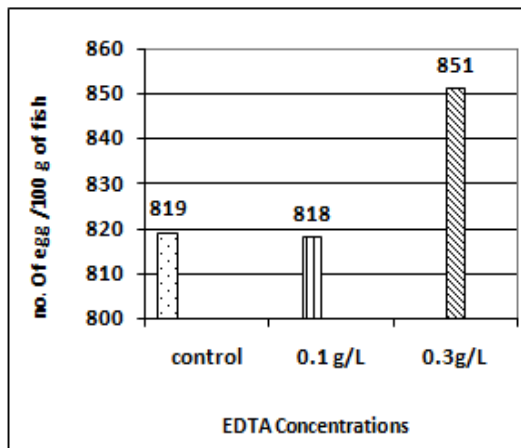


Fig. 2: Effect of different concentrations of EDTA on gonado-somatic index of *O. niloticus* females.

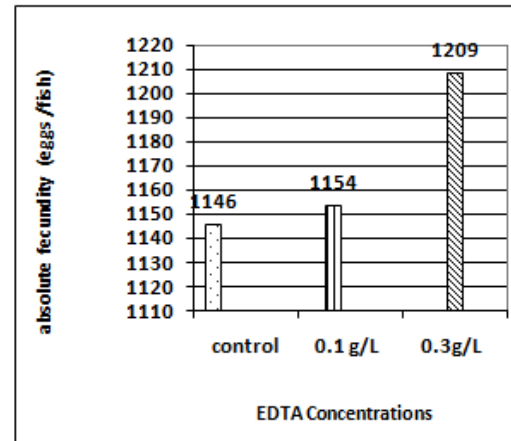


Fig. 3: Effect of different concentrations of EDTA on absolute fecundity of *O. niloticus* females.

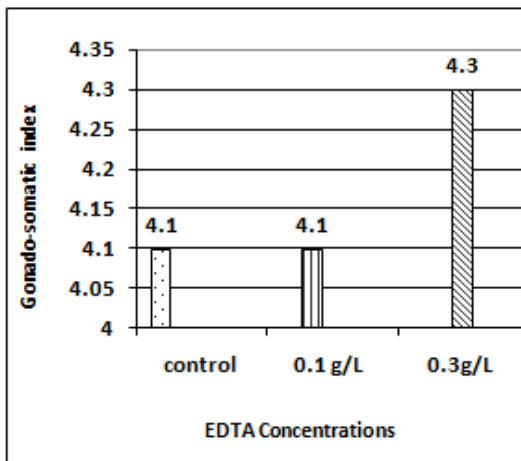


Fig. 4: Effect of different concentrations of EDTA on relative fecundity of *O. niloticus* females.

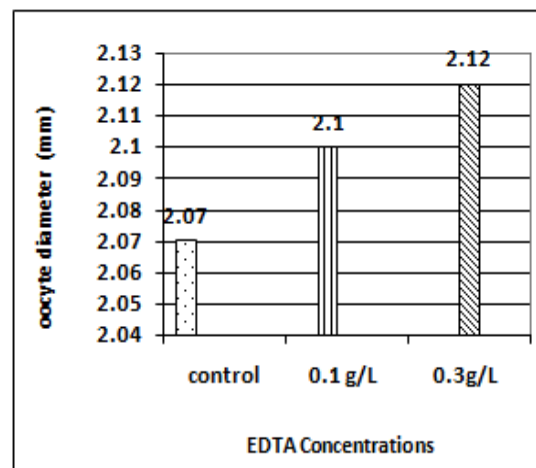


Fig. 5: Effect of different concentrations of EDTA on oocyte diameter of *O. niloticus* females.

Biochemical studies:

Biochemical analyses for adult female's ovaries of *O. niloticus*, reared in glass aquaria for 21 days are shown in Table (2) and are graphically represented in Figures (6-10).

Table 2: Biochemical analyses in the ovaries of adult females, *Oreochromis niloticus*, treated with different concentrations of EDTA.

| Concentrations (g/L EDTA) | Total proteins (mg/ g wet wt.) | Total lipids (mg/ g wet wt.) | Total carbohydrates (mg/ g wet wt.) | ASAT (U/g wet wt.) | ALAT (U/g wet wt.) |
|---------------------------|--------------------------------|------------------------------|-------------------------------------|--------------------------|--------------------------|
| Control | 267.67 ± 9.29 ^a | 63.50 ± 5.89 ^a | 4.73 ± 0.57 ^a | 0.63 ± 0.06 ^a | 0.13 ± 0.03 ^a |
| 0.1 g/L | 293.33 ± 5.28 ^a | 43.33 ± 10.32 ^b | 4.33 ± 0.65 ^a | 0.47 ± 0.06 ^b | 0.08 ± 0.02 ^b |
| 0.3 g/L | 241.67 ± 9.61 ^b | 64.83 ± 13.93 ^a | 5.23 ± 0.75 ^a | 0.73 ± 0.06 ^a | 0.16 ± 0.02 ^a |

Note: Mean values in the same row having the same letters are not significantly different ($P > 0.05$).

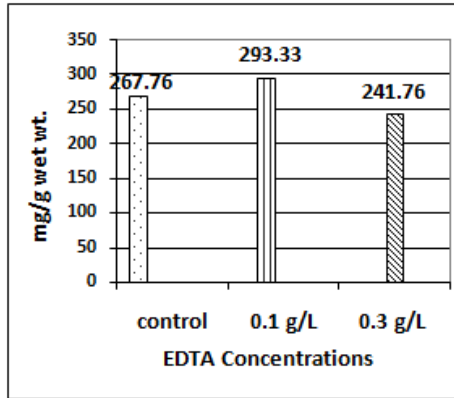


Fig. 6: Changes in total proteins in the ovaries of *Oreochromis niloticus*, treated with different concentrations of EDTA.

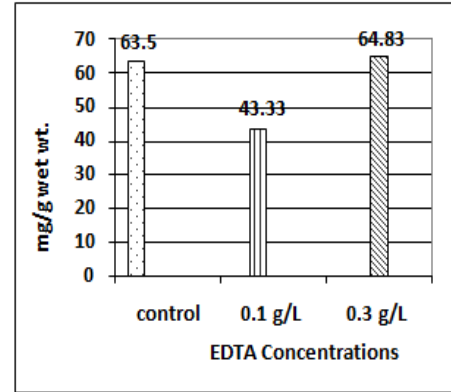


Fig. 7: Changes in total lipids in the ovaries of *Oreochromis niloticus*, treated with different concentrations of EDTA.

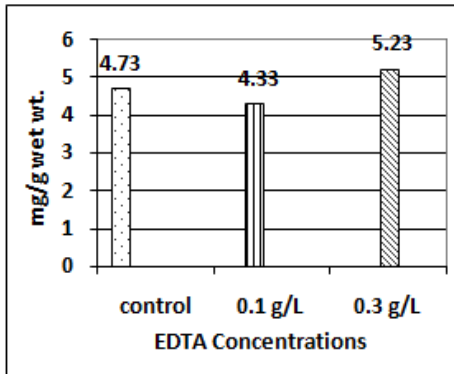


Fig. 8: Changes in total carbohydrates in the ovaries of *Oreochromis niloticus*, treated with different concentrations of EDTA.

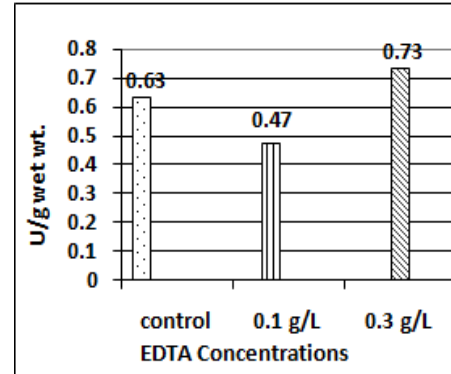


Fig. 9: Changes in ASAT activity in the ovaries of *Oreochromis niloticus*, treated with different concentrations of EDTA.

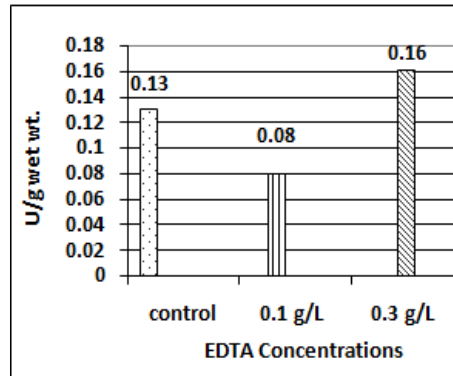


Fig. 10: Changes in ALAT activity in the ovaries of *Oreochromis niloticus*, treated with different concentrations of EDTA.

The present data showed that, an addition of EDTA to aquaria containing adult females increasing the biochemical parameters at high concentration compared with control and low concentration samples except total proteins showed an increasing at low concentration.

Table (2) and Figure (6) show a significant difference in total proteins in the ovaries of *O. niloticus*. Protein level increased gradually from 267.67 ± 9.29 in control group to reach its highest value (293.33 ± 15.28 mg/g wet wt.) in the samples treated with 0.1 g /L of EDTA, but decreased again to 241.67 ± 9.61 mg/g wet wt at the samples treated with 0.3 g /L. Statistically, a significant increase between the low concentration of EDTA and control were observed. On the other hand, total lipids concentration in the ovaries of *O. niloticus* exhibited acute declined (43.33 ± 10.32 mg/g wet wt.) at the low dose (0.1 g/L) of EDTA compared with control group and reached its maximum value at high dose (0.3 g/L) of EDTA (64.83 ± 13.93 mg/g wet wt). Statistical analyses showed a significant decrease between the low concentration of EDTA and both of control and high dose groups (Table, 2 and Figure, 7).

Concerning total carbohydrates, the present study show a slightly increasing of total carbohydrates in the ovaries of *O. niloticus* exposed to high concentration of EDTA (0.3 g/L) compared with control and exhibited a slightly decreasing at low dose (0.1 g/L); The carbohydrates values being 5.23 ± 0.75 , 4.73 ± 0.57 and 4.33 ± 0.65 mg/g wet wt. for high dose, control and low dose respectively. Analyses of variance exhibited a non significant difference between the low concentration of EDTA and other control and high dose groups (Table, 2 and Figure, 8).

Moreover, Table (2) and Figure (9) showed a slightly variations of aspartate aminotransferase (ASAT) activity in the ovaries of *O. niloticus* treated with EDTA from concentration to another one. The maximum value of ASAT activity in the ovaries of adult females (0.73 ± 0.06 U/g wet wt.) was recorded in the samples exposed to 0.3 g/L of EDTA compared with 0.63 ± 0.06 U/g wet wt. for the control samples and the minimum value (0.47 ± 0.06 U/g wet wt.) occurred at the samples treated with 0.1 g/L of EDTA. A significant decrease was recorded at the low concentration of EDTA compared with the other treatments.

The present study exhibited an increasing level of alanine aminotransferase (ALAT) activity in the ovaries of *O. niloticus* treated with high concentration of EDTA (0.16 ± 0.02 U/g wet wt.) compared with control samples (0.08 ± 0.02 U/g wet wt.) while the lowest value was detected at the low dose (0.13 ± 0.03 U/g wet wt.)). Statistical analyses showed significant decrease between low concentration of EDTA and other treatment (Table, 2 and Figure, 10).

DISCUSSION

An addition of EDTA to aquaria contains adult females stage of *O. niloticus* affects on fecundity, survival rate biochemical parameters due to enhancement in metabolic parameters and enzymatic activities at different concentrations compared with control samples.

Reproduction in fish is one of the basic biological features enabling the maximum survival and continuation of species. In the present study, Adult female obtained from high concentration of EDTA (0.3 g EDTA /L) showed slightly increase in ova diameter, absolute fecundity, relative fecundity and gonado-somatic index than those of low concentration of EDTA (0.1 g EDTA /L) and control group. The obtained data may be resulted from death of two-third of samples treated with high concentration of EDTA and remaining sample being more tolerate to the harmful

effect of EDTA. The same result of fecundity were obtained by previous studied which had been done on Nile tilapia, *O. niloticus* in Lake Edku and man-made Lake Abu-Zaabal and Damietta branch of the Nile River (Bakhoum, 2002; Shalloof & Salama, 2008 and El-Kasheif *et al.*, 2013). Also, the high mortality of *O. niloticus* adult females reared at high concentration of EDTA (0.3 g EDTA /L) may be due to the bad effect of EDTA on immune system during oogenesis in spite of the same concentration have a good physical and biochemical effect in rearing fry and fingerling stages (Hassan, 2015).

A significant difference of total proteins level is a frequently parameter of a healthy status in the studied fish. Total protein in *O. niloticus* ovaries showed gradually increasing to reach its highest value in the samples exposed to 0.1 g /L of EDTA, however, it decreased at the samples exposed to 0.3 g /L compared with the control. This may be attributed to the addition of EDTA by low concentration (0.1 g /L) to the media isolate the poisonous substances from the different tissues. These findings are agreed with results reported by Morel *et al.* (1987); Kargin (1996); Abdel-Rahman *et al.* (2009), Tonsy & Abdel-Rahman (2012) and Shalaby (2003).

However, total lipids indicated acute declined in the ovaries of adult, *O. niloticus* treated with 0.1 g/L of EDTA and appeared increasing at dose 0.3 g/L compared with control. This may be due to EDTA application and its effects were more pronounced at 0.1 g/ L EDTA, which are considered as the optimum dose that improve the health status and biochemical parameters of the fish in this experiment. Similar observations were mentioned that, all the tested biochemical parameters were improved due to EDTA application and their effects were more evident at 1.5% EDTA/ kg diet (James & Sampath, 1999 and Nicula *et al.*, 2011) and Shalaby (2007) on *O. niloticus* and Shalaby *et al.* (2011) on *Clarias gariepinus*, who reported that, the addition of EDTA lowered total lipids concentration in the fish, exposed to cadmium toxicity to be similar to that of the control fish.

In the present results, total carbohydrates in the ovaries of *O. niloticus* exhibited a slightly increasing in the samples exposed to high concentration of EDTA (0.3 g/L) was detected compared with control and a slightly decreasing at low dose (0.1 g/L) was recorded. The increase in carbohydrates level by treating with high concentration of EDTA (0.3 g/L) attributed to the increment in tissues glycogen resulted from unused energy caused by impairment and lethargy for the fish under stress conditions. Similar observations was obtained by Dheer *et al.* (1986); Al-Akel *et al.* (1988) and Ghanem (2006) who confirmed the fact that, all stress conditions invariably lead to retardation of growth and alter the physiological mechanism. If the stressed condition continuous, long enough mortality ensues.

Moreover, ASAT and ALAT activities in the ovaries of *O. niloticus* treated with EDTA were decreased with decreasing the dose of EDTA. The significant increase in activities of the different enzymes may be due to the tissues impairment caused by stress conditions. Similar observations were obtained by James *et al.* (1991), Svoboda (2001) and Shalaby *et al.* (2011), Yamawaki *et al.* (1986) and Shalaby (1997) who stated that, the increase of ASAT and ALAT may be attributed to the hepato-cellular damage or cellular degradation in liver, heart or muscle.

In conclusion, the biochemical results of Nile tilapia, *O. niloticus* are slightly good pattern in the fish treated with 0.1 g EDTA /L These observations suggest that, the exogenous treatment (0.1 g EDTA /L) may have practical utility in the fish culture of Nile tilapia, *O. niloticus*.

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ARABIC SUMMARY

دراسات على معدل الخصوبة وبعض الخصائص الفسيولوجية لمبايض إناث البلطي النيلى "أوريوكروموس نيلوتيكس" المعامل بتركيزات مختلفة من مادة الإيثيلين ثنائى الأمين رباعي حمض الخليك (إيدتا)

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تهدف هذه الدراسة إلى تحديد تأثير التركيزات المختلفة من مادة إيثيلين ثنائى الأمين رباعي حمض الخليك (إيدتا) ((٠ جم إيدتا / لتر) (٠,١ جم إيدتا / لتر) (٠,٣ جم إيدتا / لتر)) على معدلات الخصوبة ومعدل البقاء وكذلك تقدير التغير فى بعض الخصائص البيوكيميائية فى مبايض إناث أسماك البلطي النيلى والتي تم رعايتها فى أحواض زجاجية.

أوضحت النتائج وجود زيادة فى الخصوبة بزيادة التركيز من مادة الإيدتا مقارنة بالمجموعة الضابطة. كما بينت النتائج حدوث نقص فى معدل البقاء عند التركيز الأعلى (٠,٣ جم من الإيدتا / لتر) حيث وصل معدل البقاء الي ٣٣,٣ % فى نهاية الأسبوع الثالث من التجربة. كما أظهرت النتائج حدوث زيادة فى قيمة العوامل البيوكيميائية عند التركيز الأعلى مقارنة بالأقل تركيزا وكذلك المجموعة الضابطة فيما عدا نتائج البروتين الكلى الذي أظهر اعلي قيمه عند معاملة الأسماك بالتركيز الأقل من الإيدتا (٠,١ جم من الإيدتا / لتر). كما بينت التحاليل الإحصائية وجود فروق معنوية بين التركيز الأقل وكلا من الأعلى تركيزا و المجموعة الضابطة فى كل القياسات. إلا أن نتائج الكربوهيدرات الكلية أظهرت عدم وجود فروق معنوية بين كل تركيزات الدراسة.