An Evaluation of the Effect of 17α-Methyltestosterone Hormone on some Biochemical, Molecular and Histological Changes in the Liver of Nile Tilapia; *Oreochromis niloticus*

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Abstract: The present field investigation was designed to explain clearly why methyltestosterone is widely used by the producers of farmed tilapia. Also to demonstrate why there are no known risks to consumers, producers and on the environment from using this hormone provided the recommended best practices for methyltestosterone used in aquaculture of fish. In this study, all water quality parameters were within the acceptable range for fish growth. The present analyses showed no significant differences in plasma total protein, albumin, globulin, A/G ratio, AST, ALT, LDH, it showed highly significant differences in plasma CPK activities. Molecular biological analyses revealed that using of methyltestosterone was able to induce DNA fragmentation and molecular genetic variability (using RAPD-PCR fingerprinting pattern) in the liver tissues of the treated Nile tilapia; *Oreochromis niloticus*, which was higher in the first four studied months than the untreated control tilapia. Additionally, histopathological examination in liver sections of control fish showed normal structure followed by diffuse severe hepatocytic vacuolations, the treated fish showed diffuse vacuolar degeneration followed by mild and severe hepatocytic vacuolations .
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1. Introduction

Tilapias are among the most resistant fishes known against to diseases and relatively bad environmental conditions such as high stocking density of fish, lower water quality, organically pollutant water, and low dissolved oxygen level of the water (less than 0.5 mg/l). They have tolerance to salinity in wide range and are suitable for maintaining and feeding conditions in culture (Cruz and Ridha, 1994). Tilapia is a delicious, mild flavored fish that has become very popular because of its low price. This low price is achieved by converting the young females to males through the use of the hormone drug 17α-methyltestosterone.

Methyltestosterone treatment of tilapia fries is the most simple and reliable way to produce all male tilapia stocks, which consistently grow to a larger/more uniform size than mixed sex or all-female tilapias. It is highly effective on the Nile tilapia; *Oreochromic niloticus*, the main species farmed commercially worldwide, thus methyltestosterone treatment has become the standard technique to produce all-male tilapias.

Proteins act as transport substances for hormones, vitamins, minerals, lipids and other materials. Ahmad *et al*. (2002) found a significant reduction of plasma total protein was in fish fed 40 mg MT/kg, whereas it was insignificantly changed with other treatments. Albumin is synthesized by the liver using dietary protein. Its presence in the plasma creates an osmotic force that maintains fluid volume within the vascular space. Globulins are proteins that include gamma globulins (antibodies) and a variety of enzymes and carrier/transport proteins.

Most lipids are fatty acids or ester of fatty acid, act as energy storage, structure of cell membranes, thermal blanket and cushion, precursors of hormones (steroids and prostaglandins). Ahmad *et al*. (2002) reported that the *O. niloticus* showed the highest level in plasma total lipids at 40 mg MT/kg. Cholesterol is a chemical compound that is naturally produced by the body and is a combination of lipid (fat) and steroid. Cholesterol is a building block for cell membranes and for hormones like estrogen and testosterone. About 80% of the cholesterol is produced by the liver.

The enzyme alanine aminotransferase (ALT) is widely reported in a variety of tissue sources. The major source of ALT is of hepatic origin and has led to the application of ALT determinations in the study of hepatic diseases. Ahmad *et al*. (2002) found that, the activity of ALT was the highest with

control *O. niloticus* fish and that fed low doses of 0.5-2.5 mg MT/kg, while the less one was obtained with 40 mg/kg. Plasma AST is one of several enzymes that catalyze the exchange of amino and oxo groups between alpha-amino acids and alpha-oxo acids. Ahmad *et al*. (2002) reported that, AST activity was significantly increased with high methyltestosterone doses of 20 and 40 mg/kg, while there was no significant change among other treatments.

Lactate dehydrogenase is an enzyme that helps produce energy. It is present in almost all of the tissues and becomes elevated in response to cell damage. Determination of creatine phosphokinase and lactate dehydrogenase isoenzymes provides a definitive diagnosis of acute myocardial infarction.

Molecular Biological analyses:-

Genomic approaches have shown that different classes of toxicants operating through different mode of actions (MOAs) can induce unique and diagnostic patterns of gene expression in fish. The use of molecular markers has provided important advances in the characterization and genetic variation in many species, including yeast and mammals, as well as fish (Horng *et al*., 2004 and Assem and El-Zaeem, 2005).

The genotoxic effects were indicated by appearance of some changes in polymorphism band patterns including lost of stable bands or occurrence of new bands. There also exists a distinct distance between the band patterns of exposed fish and protected or control fish samples. (Mahrous *et al*., 2006).

The present study aims to assess the effect of this hormone and its environmental impacts on sex reversal of fish species. Also its impacts on some biochemical parameters as well as histological examination of vital organs of fish especially the liver. On the other hand, to assess the effect of this hormone on liver. Furthermore, the present study aims to assess the effect of this hormone on alternation of DNA structure that can lead to abnormal changes of DNA fingerprints (discrimination as well as estimation of genetic variation).

2. Materials and Methods

The present work was carried out on water and Nile tilapia fish; *Oreochromis niloticus*. Samples were collected directly from two sampling sites were chosen.

There are two groups:-

First group (Untreated Control): samples of tilapia fish (growing in natural condition away from the hormonal effect) were taken from World Fish Center Farm (WFC) in El-Abbassa, El-Sharkeya governorate. This group was with range length between (10.80±0.26 cm : 22.77±0.49 cm) and range weight between (19.2± 1.1 g : 256.7 ± 12.9 g).

Second group (Treated group): Samples of tilapia fish were taken from El-Nubaria farm belongs to National Research Center (NRC) which is previously used the oral administration of the synthetic androgen (17 α-Methyl testosterone) hormone to produce all male tilapia at 60 mg/kg feed to newly hatched tilapia fry (9-11mm total length) for a period of 28 days which results in populations comprising 97 to 100% phenotype males (Popma and Green, 1990). This group was with range length between (11.53± 0.23 cm – 25.93 ± 0.78 cm) and range weight between was (25.4 ± 1.9 g – 287.4 ± 10.5 g). Samples of fish were taken through different months (April till November 2009) and also water samples will be taken to analyze all possible parameters to know the water quality using in the two sources and its contents.

Water samples were collected from the different studied sites through different months during a period from April to November (2009) and placed in clean sampling glass bottles according to Boyd (1990), then taken to analyze all possible parameters to know the water quality of the sources and its contents. .

Fish samples were collected from the different studied sites through different months during a period from April to November (2009). Fish were dissected to get liver which were kept frozen (-20 ^{o}C) for determination of residual testosterone in muscle and DNA analyses of the Nile tilapia; *Oreochromis niloticus* collected from the different studied sites.

The blood samples were taken from caudal vein of an anaesthetized fish by sterile syringe using EDTA solution as anticoagulant (Ahmad *et al*., 2002). The blood samples were examined immediately for the following: plasma total protein, Albumin, Globulin, A/G ratio, total lipids, cholesterol, aspartate amino transferase (AST) , alanine amino transferase (ALT) activities, Lactate dehydrogenase, Creatine phosphokinase of the Nile tilapia; *Oreochromis niloticus* collected from the different studied sites.

The method described for the plasma total protein determination is based on the report of Weichselbaum (1946) and Gomal *et al.* (1949).The violet color developed is proportional to the number of peptide bonds in the protein and is nearly independent of the relative concentration of albumin and globulin (Cannon, 1974). It is measured photometrically at wavelength 550 nm.

Albumin, in the presence of bromocresol green at a slightly acid pH, produces a color change of the indicator from yellow-green to green-blue. The intense of the color formed is proportional to the albumin concentration in the sample (Young, 2001),

and is measured photometrically at wavelength 630 nm.

Plasma globulin is calculated by subtraction of the plasma albumin value from the plasma total protein value.

Plasma total lipids were determined according to Boutwell (1972). The quantitative determination of the total lipid index in plasma is applied using the sulfo-phospho-ovanillin colorimetric method. In this method, lipids react with sulfuric acid to form carbonium ions which subsequently react with the vanillin phosphate ester to yield a purple complex that is measured photometrically at wavelength (500-550 nm).

The enzymatic approach to cholesterol methodology was introduced by Flegg (1973) using cholesterol oxidase of bacterial origin following chemical saponification of the cholesterol esters. Roeschlau and Klin (1974) modified this technique and Allain (1974) published the first fully enzymatic assay, combining cholesterol oxidase and cholesterol esterase. The method presented is based on the Allain (1974) procedure and utilizes these enzymes in combination with the peroxidase/phenol-4-antipyrine reagent of Trinder (1969). The intensity of the final red color is proportional to total cholesterol concentration and is measured photometrically at wavelength (500 nm). Lipid Clearing Factor (LCF): a mixture of special additives developed by Stanbio is integrated into the cholesterol reagent to help minimize interference due to lipemia (Flegg, 1973 and Stein, 1986).

The enzyme reaction sequence employed in the Stanbio AST assay of aspartate aminotranseferase (AST) (Bergmeyer, 1978) is measured photometrically at wavelength 340 nm.

UV methods for ALT determination were first developed by Wroblewski and La Due in (1956). The method was based on the oxidation of NADH by lactate dehydrogenase (LDH). In 1980, the International Federation of Clinical Chemistry (IFCC) recommended a reference procedure for the measurements of ALT based on the Wroblewski and La Due (1956) procedures. The ALT reagent conforms to the formulation recommended by the International Federation of Clinical Chemistry (IFCC) (1980).

The procedure presented is essentially the Buhl and Jackson (1978) modification of Wacker (1956) which optimizes reaction conditions. LDH specially catalyzes the oxidation of lactate to pyruvate with the subsequent reduction of NAD to NADH. The rate at which NADH forms is proportional to LDH activity. The method described determined NADH absorbance increase per minute (Buhl and Jackson1978 and Wacker, 1956), and measuring was taken at wavelength 340 nm.

The kinetic procedure presented is a modification of Szasz (1975) of the Rosalki (1977) technique, which optimizes the reaction by reactivation of CPK activity with N-acetyl-Lcysteine (NAC). CPK specially catalyze the transphosphorylation of ADP to ATP, through a series of coupled enzymatic reactions, NADH is produced at a rate directly proportional to the CPK activity. The method determines NADH absorbance increase per minute at 340nm (Szasz, 1975 and Rosalki, 1977) and measuring was taken at wavelength 340 nm.

Molecular biological analyses:-

I- Quantitative analysis of DNA fragmentation

DNA (Deoxyribonucleic acid) fragmentation is an apoptosis marker. Hydrolysis of DNA leads to release of free deoxyribose that colorimetrically measured at (600 nm) after reaction with the diphenylamine reagent, as an indicator of cell death or apoptosis.

a- Diphenylamine reaction procedures:

The control and treated liver samples were collected immediately after sacrificing the Nile tilapia.

The proportion of fragmented DNA was measured by UV and calculated from absorbance reading at 600 nm using the formula:

$$\textbf{\%Fragmented DNA} = \frac{OD(S)}{OD(S) + OD(P)} \times \textbf{100}$$

OD(S) : Optical density of supernatants.

OD (P): Optical density of pellets.

b- DNA gel electrophoresis laddering assay :(Burton, 1956 and Lu et *al*., 2002)

Apoptotic DNA fragmentation was qualitatively analyzed by detecting the laddering pattern of nuclear DNA as described by Lu *et al*., (2002).

c- Molecular Analysis :(Mahrous *et al*., 2006 and Khalil *et al*., 2007)

The genomic DNA was isolated using phenol/chloroform extraction and ethanol precipitation method with minor modifications (Sambrook *et al.*, 1989).

II-Random Amplification of Polymorphic DNA (RAPD-PCR) analysis: DNA amplification reactions were performed under conditions reported by **Williams *et al*. (1990) and Plotsky *et al*. (1995).**

Histopathological Examination:

Liver of Nile tilapia; *Oreochromis niloticus* , collected from different studied sites were fixed in neutralized formalin, dehydrated, embedded in paraffin wax and sectioned at 5 µm then stained

with Haematoxylin and Eosin according to Carleton *et al*. (1967).

Statistical analyses:

The results were statistically analyzed using Duncan's multiple range tests to determine difference in means Statistical Analyses System (SAS, 2000) and Software Program of Statistical Analysis (SPSS, 2008). One way ANOVA test (Analysis of variance) comparing the treated and untreated control groups in all months. Differences in all the studied parameters were assessed by one way ANOVA.

3. Results and Discussion

The efficacy of an androgen is affected by the mode of administration and by its source, whether synthetic or naturally occurring (White *et al*., 1973). Synthetic androgens such as ethlytestosterone and methyltestosterone are more effective when administered orally than naturally occurring androgens like testosterone, androstenedione which are most potent when injected intraperitoneally. Androgen treatments at both levels (30, 60 mg/ kg feed of MT and ET) for 35 and 59 days produced 100% male populations, ET- 60 and MT-60 at 25 days also produced 100% male population but ET- 30 and MT-30 at 25 days also produced 98.4 % and 99.2% male, respectively (Tayamen and Shelton, 1978) .

We have been select tilapia fish for our study because tilapia in general reproduces with great rapidity (DaSilva *et al.,* 1973), which makes them suitable for commercial production. However, their prolific rate of reproduction leads to overcrowding and hence stunting when mixed sexes are cultured in ponds (Guerrero and Abella, I976).Various practical measures have been employed to control. The males of Tilapia grow faster than the females (Van Someren and Whitehead, 1960 and Holden and Reed, 1972). Traditionally, tilapias are cultured in fresh water or cages in inland waters (Ishak, 1979).

In Egypt, there is a considerable interest in extending the culture of the Nile tilapia; *Oreochromis niloticus*, which gives a good quality fish with a high marketability and excellent growth rates (Kheir *et al*., 1998).

The use of 17α-methyltestosterone (MT) to produce a monosex male population in tilapia has been extensively reviewed by Hunter and Donaldson, (1983). Many factors are known to effect the success of the androgen treatment in masculinizing tilapia females such as species, age at which hormone is administered, duration of treatment, and type and level of hormone used. Doses of 30 to 60 mg/kg given for 15- 60 days have reported in this connection (Hunter and Donaldson, 1983). Feeding swim –up fry to 10 to 60 mg methyl testosterone /kg feed for 21 to 28 days results in populations with 95 to 100% male (Clemens and Inslee, 1968; Hanson *et al*., 1983; Pompa and Green, 1990 and Muhaya, 1985).

The present field investigations include the study of water quality of water samples collected directly from the studied sites (Table 1), which include also fish samples taken from El-Nubaria farm belongs to National Research Center which is previously used 17α-methyltestosteronehormone to produce all male tilapia, but the another source was from World Fish Center Farm in El-Abbassa, El-Sharkeya governorate. It also concerned with the study of some physiological and biochemical parameters of fish reared in the different studied sites.

Analyses of plasma constituents have proved to be useful in the detection and diagnosis of metabolic disturbance and disease (Aldrin *et al*., 1982). Many factors affect the biochemical composition of fish such as fishing area, type of food, water quality and pollution (Wassef and Shehata, 1991; El-Ebiary *et al*., 1997; El-Ebiary and Mourad, 1998; El-Naggar *et al*., 1998 and Shakweer *et al*., 1998).

The metabolic pathways of fish could be distinguished throughout assessing some physiological parameters. The present study showed insignificant changes in plasma total protein, albumin, globulin, A/G ratio, total lipids, cholesterol, AST, ALT, LDH, CPK activities in the untreated control and treated fish. However, these results reflect the healthy status of the cultured fish at this treatment.

Protein plays an important role in the metabolism and regulation of water balance (Heath, 1995). It is the basic building nutrient of any growing animal and also used as an indicator of their state of health (Alexander and Ingram, 1980 and Lea-Master *et al*., 1990)

Regarding the plasma total protein of the Nile tilapia; *Oreochromis niloticus* collected from the different studied sites (Table 2), is clear that there is no significant difference in the plasma total protein in the untreated control and treated fish collected from the different studied sites with the highest value in fish collected in the last month of the study for untreated control fish and treated fish.

Chan and O'Malley (1976) and O'Malley and Tsai (1992) reported that, the plasma total protein was significantly decreased at high MT doses, and this result may be due to the fact that androgens regulate protein synthesis by binding to cytosolic or nuclear receptors for steroids that than modulates transcription. Ahmad *et al.* (2002) reported significant reduction of plasma total protein in fish fed 40 mgMT/kg feed, whereas it was insignificantly changed with other treatments.

Table (1): Water Quality Criteria for Water Samples collected from each fish farm for untreated control and treated sampling sites with 17 α methyl testosterone hormone of the Nile tilapia fish; *Oreochromis niloticus,* during April till November (2009).

Parameters	Water quality for untreated control Site (El-Abbassa)	Water quality for treated site (El-Nubaria)
Temperature (°C)	(19-24) 22.00 ± 0.68	(19-27) 23.3 ± 1.23
Dissolved Oxygen (mg/L)	(6.11-8.2) 6.86 ± 0.32	(5.8-7.3) 6.6 ± 0.24
pH	(6.82 – 7.8) 6.85 ± 0.26	(6.45-7.8) 7.3 ± 0.14
Ammonia (mg/L)	(1.1 – 1.4) 1.27 ± 0.05	(1.3- 2.4) 1.9 ± 0.14
Nitrate (mg/L)	(0.96 – 1.65) 1.26 ± 0.08	(1.43 – 1.94) 1.7 ± 0.08
Nitrite (mg/L)	(0.03 – 0.05) 0.04 ±0.002	(0.03 – 0.05) .045 ± 0.002
Total Hardness (mg/L as $CaCO_3$)	(111 – 132) 120.00 ± 3.24	(129 – 161) 147.2 ± 4.48
Total Alkalinity (mg/L as $CaCO_3$)	(186 -206) 189.33 ± 4.10	(196 – 228) 216.8 ± 4.53

- Data are represented as means of six samples ± SE.
- Student's t-Test between the two groups of the same parameter in the two studied sites for the whole studied period.

Table (2):Plasma total protein concentrations (g/dl) and Plasma albumin concentration (g/dl)for untreated control and treated Samples with 17 α methyl testosterone hormone of the Nile tilapia fish; *Oreochromis niloticus,* collected from El- Abbassa and El- Nubaria fish farms during April till November (2009).

Parameter / Months	Plasma total protein ntrol samples (El- Ab	Plasma total protein treated samples (El- Nuba		Plasma albumin in control samples (El- Abbassa)	Plasma albumin ated samples (El- Nu
April	5.81±0.13 [a]	5.97±0.14 [a]		1.50±0.03 [a]	1.45±0.05 [a]
May	5.65±0.18 [a]	6.09±0.11 [a]		1.53±0.04 [a]	1.58±0.03 [a]
June	5.52±0.18 [a]	6.07±0.12 [a]	*	1.59±0.04 [a]	1.57±0.06 [a]
July	5.61±0.23 [a]	6.43±0.29 [a]		1.51±0.03 [a]	1.59±0.07 [a]
August	5.51±0.25 [a]	6.20±0.33 [a]		1.51±0.09 [a]	1.58±0.04 [a]
September	5.33±0.18 [a]	6.26±0.15 [a]	**	1.54±0.07 [a]	1.54±0.05 [a]
October	5.58±0.23 [a]	6.25±0.21 [a]		1.55±0.09 [a]	1.61±0.05 [a]
November	5.62±0.23 [a]	6.43±0.20 [a]	*	1.53±0.08 [a]	1.60±0.04 [a]
F-Values	**0.412**	**0.593**		**0.157**	**0.937**

- Data are represented as means of six samples ± SE.
- Means with the same letter for each parameter in the same column between all months are non-significant different (P > 0.05); otherwise they do (SAS, 2000).
- Student's t-Test between the two groups in the same month for the whole studied period.
- One way ANOVA test (F-value) between all months in each group separately for the whole studied period.
- * Significant difference at P<0.05 ** Highly significant difference at P<0.01.

Regarding plasma albumin and globulin of the Nile tilapia; *Oreochromis niloticus* collected from the different studied sites (Table 2&3), there is no significance difference in the untreated control and treated fish, while there is a slight increase in the albumin concentrations of treated fish during the study, which indicates that there is no effect of the used hormone during the growth of fish.

Concerning A/G ratio of the Nile tilapia; *Oreochromis niloticus* collected from the different studied sites (Table 3), there is no significance difference in the A/G ratio of the untreated control and treated fish. Despite there is a relative stability in the A/G ratio in treated fish and a slight increase in the A/G ratio of untreated control fish during this study. This indicates that, there is no effect of the used hormone during the growth of fish.

Lipids, as an important source of energy, play an important role in toelest fish (Shatunovsky, 1971; Harris, 1992; Haggag *et al*., 1993 and El-Ebiary *et al*., 1997). In contrast to mammals fish prefer to utilize lipids rather than carbohydrates as a main source of energy (Black and Skinner, 1986). Lipids affected by spawning cycle, food viability, seasonal variations and biochemical activity of fish (Bayomy *et al*., 1993). Lipids are important metabolites for locomotory and reproductory activities of fish.

Table (3): Plasma globulin concentrations (g/dl) and A/G ratio for untreated control and treated samples with 17 α- methyl testosterone hormone of the Nile tilapia fish; *Oreochromis niloticus,* collected from El- Abbassa and El- Nubaria fish farms during April till November (2009).

Parameter / Months	Plasma globulin in control samples (El- Abbassa)	Plasma globulin in treated samples (El- Nubaria)		A/G ratio in control Samples (El- Abbassa)	A/G ratio in treated samples (El- Nubaria)
April	4.30 ±0.14 [a]	4.58 ±0.20 [a]		0.35±0.01 [a]	0.32±0.02 [a]
May	4.12 ±0.19 [a]	4.51 ±0.10 [a]		0.37±0.02 [a]	0.35±0.01 [a]
June	3.93 ±0.21 [a]	4.49 ±0.13 [a]		0.41±0.03 [a]	0.35±0.02 [a]
July	4.10 ±0.21 [a]	4.84 ±0.25 [a]		0.37±0.01 [a]	0.33±0.01 [a]
August	4.00 ±0.24 [a]	4.62 ±0.31 [a]		0.38±0.03 [a]	0.34±0.02 [a]
September	3.79 ±0.20 [a]	4.71 ±0.18 [a]	**	0.41±0.03 [a]	0.33±0.02 [a]
October	4.03 ±0.26 [a]	4.80 ±0.15 [a]	*	0.39±0.04 [a]	0.33±0.02 [a]
November	4.08 ±0.22 [a]	4.83 ±0.21 [a]	*	0.38±0.03 [a]	0.33±0.02 [a]
F-Values	**0.468**	**0.487**		**0.436**	**0.329**

Concerning the plasma total lipids and cholesterol of the Nile tilapia; *Oreochromis niloticus* collected from the different studied sites (Table 4), it is clear that, there was no significant changes in the plasma total lipids and cholesterol during the months of study in the untreated control fish samples and a gradual increase, relatively constant, in samples of treated fish. The present results of the plasma total lipids in the untreated control fish samples are similar to those reported by Ahmad *et al*. (2002) who reported no significant changes in plasma total lipids at moderate doses of MT (5, 10, 20 mg MT /kg feed) in Nile tilapia.

The decreasing in plasma total lipids reported by Dange and Masurekar (1984) in fish fed low levels of MT may be due to the increase of energy demand, which led to more consumption of protein and lipids. In contrast Ahmad *et al*. (2002) reported significant increase in plasma total lipids at 40 mg MT /kg feed in Nile tilapia.

Transamination represents one of the principal pathways for synthesis and deamination of amino acids, thereby allowing interplay between carbohydrates and protein metabolism during the fluctuating energy demands of the organism in various adaptive situations. They also are considered to be important in the assessment of the state of the liver as well as some of the organs (Verma *et al.*, 1981). Therefore, attention has been focused on the changes in AST and ALT activities, which promote gluconeogensis from amino acids, as well as the effects of changes in aminotransferase activities on the liver condition (Hilmy *et al*., 1981 and Rashatwar and Ilyas, 1983).

Determination of transaminases, (AST, ALT) has proven useful in the diagnosis of liver disease in fish (Maita *et al*., 1984 and Sandnes *et al*., 1988). Cell injury of certain organs leads to the release of tissue-specific enzymes into the blood stream (Heath 1995and Burtis and Ashwood 1996).The animo transferases (AST &ALT) are considered a good sensitive tools for detection of any variations in the physiological process of living organisms as reported by Nevo *et al*. (1978) and Tolba *et al*. (1997)

Comparing the present results of plasma AST activity of the Nile tilapia; *Oreochromis niloticus* collected from the different studied sites (Table 5) , it is clear that, there is no significant difference in the untreated control fish all over the studied period. But there is a significant increase in the activities of AST for the treated fish during the studied period, with special significance increase in September. This is in agreement with Ahmad *et al*. (2002) who reported that, AST activities was significantly increased with high MT doses (20 and 40 mg MT /kg feed), while there was no significant changes among other treatment. These results are confirmed by the present histopathological examination of the liver which showed some hepatocytic vacuolations in the liver tissues.

Comparing the results of plasma ALT of the Nile tilapia; *Oreochromis niloticus* collected from the different studied sites (Table 5), it is clear that, there is no significant differences between the ALT activities in untreated control and treated fish. In contrast Ahmad *et al*. (2002) reported that, the activities of ALT was highest in untreated control Nile tilapia fish and that fed low doses of MT (0.5-2.5 mg MT/kg feed), while the less activities was obtained with 40 mg MT /kg feed.

The present results are confirmed by Bhasin *et al.* (1998) who reported that high dosages of exogenous male hormones,

including methyltestosterone, are known to cause side effects, especially liver damage, but lower levels actually produce various health benefits. Our results are confirmed by the present histolpathological examination, which showed diffuse vacuolar degeneration in the liver tissues.

Table (4): Plasma total lipids concentrations (g/l) and Plasma cholesterol concentration (mg/dl) for untreated control and treated samples with 17 α- methyl testosterone hormone of the Nile tilapia fish; *Oreochromis niloticus,* collected from El- Abbassa and El- Nubaria fish farms during April till November (2009).

Parameter / Months	Plasma total lipids in control samples (El- Abbassa)	Plasma total lipids in treated samples (El- Nubaria)	Plasma cholesterol control samples (El- Abbassa)	Plasma cholesterol in treated samples (El- Nubaria)
April	23.13±0.20^{a}	22.03±0.59^{b}	156.97±3.24^{b}	154.60±6.21^{b}
May	22.71±0.49^{a}	24.11±0.56^{ab}	156.58±2.23^{b}	160.85±3.89^{b}
June	22.61±0.65^{a}	24.70±0.65^{ab} *	151.87±2.58^{b}	174.25±5.84^{ab} **
July	23.08±0.97^{a}	24.93±0.74^{ab}	150.60±3.57^{b}	182.00±5.62^{ab} **
August	23.53±0.53^{a}	25.31±1.16^{a}	146.67±5.69^{b}	189.17±4.60^{ab} **
September	23.31±0.85^{a}	25.71±1.18^{a}	151.27±5.66^{b}	183.42±6.36^{ab} **
October	22.38±1.18^{a}	26.11±0.95^{a} *	151.53±5.80^{b}	205.28±5.92^{a} **
November	23.98±1.24^{a}	25.91±0.1.35^{a}	150.02±5.81^{b}	215.47±7.04^{ab} **
F-Values	**0.388**	**1.945**	**0.553**	**1.226**

Table (5): Plasma AST activities (U/l) and Plasma ALT (U/l) activities ffor untreated control and treated samples with 17 α methyl testosterone hormone of the Nile tilapia fish; *Oreochromis niloticus,* collected from El- Abbassa and El- Nubaria fish farms during April till November (2009).

Parameter / Months	Plasma AST in control samples (El- Abbassa)	Plasma AST in treated samples (El- Nubaria)	ALT in Untreated control Samples (El- Abbassa)	ALT in Treated Samples (El- Nubaria)
April	73.30±1.29^{a}	76.70±2.42^{b}	31.95±1.21^{a}	32.43±2.13^{a}
May	74.16±2.11^{a}	83.23±3.09^{ab} *	31.06±1.11^{a}	33.88±2.58^{a}
June	70.91±1.79^{a}	85.43±2.58^{ab} **	31.78±1.68^{a}	34.13±2.93^{a}
July	75.66±2.71^{a}	85.06±4.39^{ab}	32.90±1.67^{a}	32.93±2.56^{a}
August	76.16±2.39^{a}	85.15±5.00^{ab}	32.43±1.53^{a}	36.41±4.14^{a}
September	75.08±3.50^{a}	94.51±5.45^{a} **	32.05±1.87^{a}	37.53±2.90^{a}
October	74.83±3.41^{a}	86.11±6.54^{ab}	32.30±2.42^{a}	38.60±2.70^{a}
November	77.26±3.26^{a}	87.96±4.96^{ab}	31.28±2.15^{a}	37.30±2.89^{a}
F-Values	**0.528**	**1.192**	**0.116**	**0.645**

- Data are represented as means of six samples ± SE.
- Means with the same letter for each parameter in the same column between all months are non- significant different (P > 0.05); otherwise they do (SAS, 2000).
- Student's t-Test between the two groups in the same month for the whole studied period.
- One way ANOVA test (F-value) between all months in each group separately for the whole studied period.
- * Significant difference at P<0.05 ** Highly significant difference at P<0.01.
-

Concerning plasma lactate dehydrogenase activities of the Nile tilapia; *Oreochromis niloticus* collected from the different studied sites (Table 6), it is clear that, there no significance difference in the (LDH) concentrations of the untreated control and treated fish.

Regarding plasma creatinine phosphokinase activities of the Nile tilapia; *Oreochromis niloticus* collected from the different studied sites (Table 6), there was no significant difference between the "CPK" activities in the untreated control fish, while there was a highly significant increase in the treated fish in October and November.

Molecular biological analyses:-

PCR-based techniques, such as RAPDs, have previously allowed the discrimination as well as estimation of genetic variation attributed to genotoxic elements. The exposure to genotoxic agents will give rise to alterations of DNA structure that can lead to abnormal changes of DNA fingerprints. Therefore, we have applied the random amplified polymorphism DNA (RAPD) method to evaluate the genotoxic effects.

The molecular biological results of the present study revealed that methyltestosterone was able to induce DNA fragmentation in liver (Table 7 and

Figs.1,2&3) of Nile tilapia; *Oreochromis niloticus*, in the first four studied months after MT treatment to induce sex reversal in farmed tilapias compared to the untreated control tilapia. In addition, the molecular genetic variability (using RAPD fingerprinting pattern) among the treated tilapia (in liver and testes tissues) was higher in the first four studied months after treatment than the untreated control tilapia.

Table (6): Lactate dehydrogenase activities (U/l) and Plasma creatinine phosphokinase activities (U/l) for untreated control and treated samples with 17 α- methyl testosterone hormone of the Nile tilapia fish; *Oreochromis niloticus,* collected from El- Abbassa and El- Nubaria fish farms during April till November (2009).

Parameter / Months	Lactate dehydrogenase in control samples (El-Abbassa)	Lactate dehydrogenase in treated samples (El-Nubaria)	Plasma creatinine phosphokinase in control samples (El-Abbassa)	Plasma creatinine phosphokinase in treated samples(El-Nubaria)
April	1445.63±21.11 [a]	1450.31± 6.79 [a]	10546.00 ±50.23 [a]	10587.50 ±209.53 [c]
May	1458.58±25.23 [a]	1457.63± 5.56 [a]	10546.83 ±50.72 [a]	10871.83 ±295.28 [bc]
June	1436.62±32.82 [a]	1465.66± 8.71 [a]	10538.50 ±88.08 [a]	10781.83 ±309.97 [c]
July	1456.55±39.32 [a]	1465.36±10.43 [a]	10526.50 ±57.68 [a]	10879.66 ±337.42 [bc]
August	1476.66±27.62 [a]	1467.73± 7.13 [a]	10552.16 ±56.27 [a]	10648.00 ±220.73 [c]
September	1474.60±49.24 [a]	1463.46± 11.60 [a]	10547.83 ±70.89 [a]	10879.00 ±370.69b [c]
October	1436.81±22.17 [a]	1476.40± 5.48 [a]	10574.33 ±56.01 [a]	11923.16 ±535.44 [ab] *
November	1457.11±41.10 [a]	1467.80± 7.85 [a]	10568.33 ±60.27 [a]	12360.33 ±435.98 [a] **
F-Values	**0.205**	**0.877**	**2.487***	**3.394****

Table (7): Effect of the 17 α methyl testosterone hormone on the DNA fragmentation ratio in liver tissues collected from Nile tilapia; *Oreochromis niloticus,* for several time intervals (April – November, 2009).

Parameter / Months	DNA fragmentation (%) liver tissues of untreated control sam (El- Abbassa)	DNA fragmentation (%) iver tissues of treated samples Nubaria)
April	10.66 ±0.33 [a]	13.33 ±0.33 [a] **
May	11.33 ±0.33 [a]	13.66 ±0.33 [a] **
June	10.66 ±0.33 [a]	13.66 ±0.33 [a] **
July	10.66 ±0.33 [a]	13.33 ±0.33 [a] **
August	11.33 ±0.33 [a]	12.33 ±0.33 [b]
September	11.66 ±0.33 [a]	12.66 ±0.33 [b]
October	11.66 ±0.33 [a]	11.66 ±0.33 [b]
November	10.33 ±0.33 [a]	11.66 ±0.33 [b] **
F-Values	**1.929**	**4.558 ** **

- Data are represented as means of six samples ± SE.
- Means with the same letter for each parameter in the same column between all months are non- significant different (P > 0.05); otherwise they do (SAS, 2000).
- Student's t-Test between the two groups in the same month for the whole studied period.
- One way ANOVA test (F-value) between all months in each group separately for the whole studied period.
- * Significant difference at P<0.05 ** Highly significant difference at P<0.01.

DNA gel electrophoresis laddering assay:-

Determination of the DNA fragmentation in liver tissues using DNA gel electrophoresis laddering assay in Nile tilapia; *Oreochromis niloticus* are summarized in figures (1 &2).

The results demonstrated that, the liver tissues collected from the Nile tilapia treated with testosterone showed DNA damage especially in the first four months after treatment (Fig. 1). In contrast, the liver tissues collected from untreated control Nile tilapia showed no changes in the genetic materials (Fig. 2). The DNA marker is in lane 1. Lane 2 to Lane 9 represent months of collection (April till November, respectively) of fish liver tissue samples treated with 17 α- methyltestosterone throughout the period of study.

For our knowledge, there are no data regarding the effect of methyltestosterone on the DNA damage in fish especially Nile tilapia. However, it could be postulated that the methyltestosterone residues were still existed in the fish tissues and/or in the fish

environment up to the first four months after treatment and then began to be disappeared, whereas the DNA fragmentation decreased after the first four studied months.

The action mechanism of testosterone treatment inducing genetic toxicity during the first months of age in tilapia (tilapia fry) is not investigated yet. In the present study, the negative effect of testosterone induced DNA damage may be attributed to the weakness in the immune system which may not be completed in growth yet. The main way in which steroid hormones interact with cells is by binding to proteins called steroid receptors. When steroids bind to these receptors, the proteins move into the cell nucleus and either alter the expression of genes (Lavery and McEwan, 2005) or activate processes that send signals to other parts of the cell (Cheskis, 2004) cause genetic toxicity. Beg *et al* (2008) reported that the possible genotoxicity of testosterone is depend on the metabolic activation. The first step of this mechanism may involve the aromatic hydroxylation catalyzed by cytochrome p450 as in the case of other steroids. Cytochrome p450 in liver fractions plays an important role in activating promutagens to proximate and/or ultimate mutagens.

The results of the present study revealed that the DNA damage attributed to methyltestosterone treatment was markedly disappeared after the first four studied months until it reached a relative stability rate similar to control untreated tilapia. It could be explained that the methyltestosterone residues in the fish tissues and/or in the fish environment were removed. Furthermore, disappearance of the DNA damage may be attributed to the increase of the immunity defense in the growing fish.

Traditionally, sex steroids are recognized as non-genotoxic carcinogens (Ho and Yu, 1993). To date, few studies have been reported on any aspect of DNA damage caused by testosterone treatment in organs of intact animals (Ho and Yu, 1993 and Ho and Roy, 1994). However, controversial results have been reported. Ho and Roy (1994) reported that testosterone combined with estrogen induced a dramatic increase in DNA strand breaks. In contrary, when female rats, converted to 'male type' by ovariectomy, treated with testosterone for one week, DNA damage caused by hepatocarcinogen (DL-ZAMI 1305) was completely abolished (Ragnotti *et al* ., 1987).

Marzin (1991) studied the mutagenicity of some synthetic androgen steroids, using number of genotoxicity tests *in vitro* and *in vivo* systems for gene mutations, chromosomal mutations and primary DNA damage demonstration. The results of this study showed no genotoxic activity attributed to these steroids. It is also found that 17α-alkylated steroids are directly toxic to hepatocytes, whereas the non-alkylated steroids show no effects at tested doses (Welder *et al*., 1995). Tsutsui *et al*. (1995) reported that testosterone did not induce gene mutations at the *hprt* or Na^+/K^+ ATPase locus. When testosterone was added at a final concentration of 2 mmol/l to DNA obtained from human surgical resections, rat liver, HepG2 cells, and calf thymus, did not form adducts with naked DNA. Furthermore, no adducts were observed in DNA isolated from HepG2 cells incubated with 10–100mol/l testosterone for 24 h (Seraj *et al*., 1996).

In agreement with our results, Hana *et al* (2008) reported that there were no significant differences in the frequency of total chromosomal aberrations between control and testosterone propionate-treated adult mice. In addition, they found that the molecular genetic variability using RAPD-PCR among the testosterone-treated adult mice was similar to control untreated mice. Whereas, all of the oligodecamers used revealed monomorphic bands in the control samples and those treated with testosterone propionate.

Additionally, Histopathological examination or biomarkers have been increasingly recognized as a valuable tool for field assessment of the impact of using 17 α-methyltestosterone hormone on fish organs (Heath, 1995; Schwaiger *et al*., 1996 and Teh *et al*., 1997). The investigated biochemical and physiological changes were confirmed by histopathological alterations of muscle, liver and testis of the Nile tilapia; *Oreochromis niloticus* collected from the two fish farms.

DNA gel electrophoresis laddering assay:-

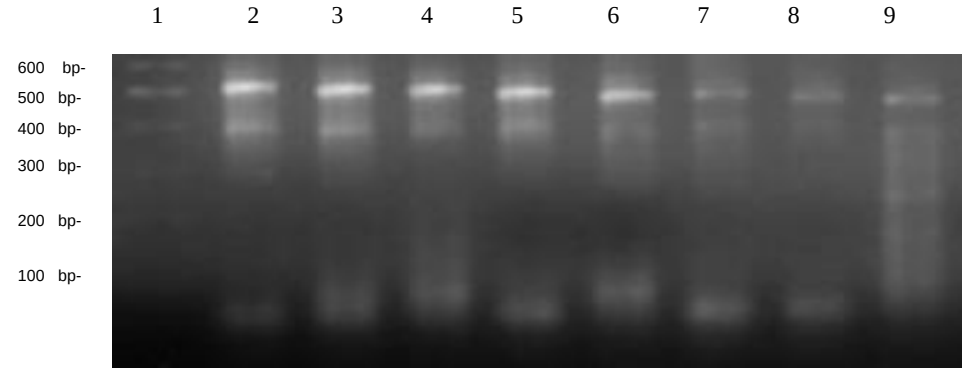


Figure (1): DNA fragmentation detected with agarose gel electrophoresis of tilapia DNA extracted from liver exposed to testosterone in different time intervals analyzed by DNA gel electrophoresis laddering assay. Lane 1 represents DNA ladder. Lanes 2 to 9 represent liver tissues collected from April to November (2009).

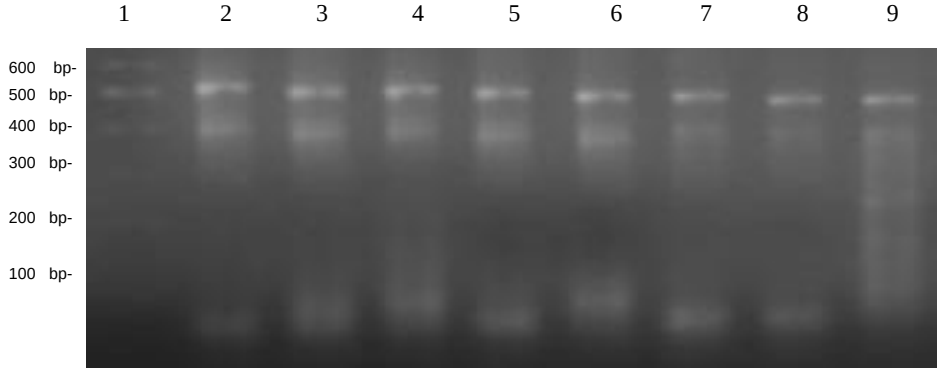
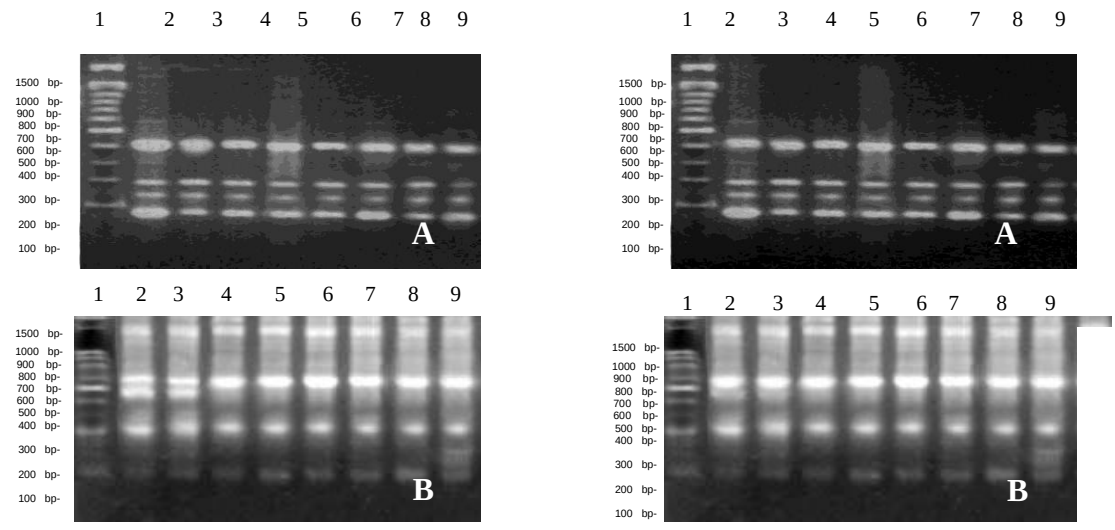


Figure (2): DNA fragmentation detected with agarose gel electrophoresis of tilapia DNA extracted from untreated liver in different time intervals analyzed by DNA gel electrophoresis laddering assay. Lane 1 represents DNA ladder. Lanes 2 to 9 represent liver tissues collected from April to November (2009).

Treated Liver **Untreated control Liver**



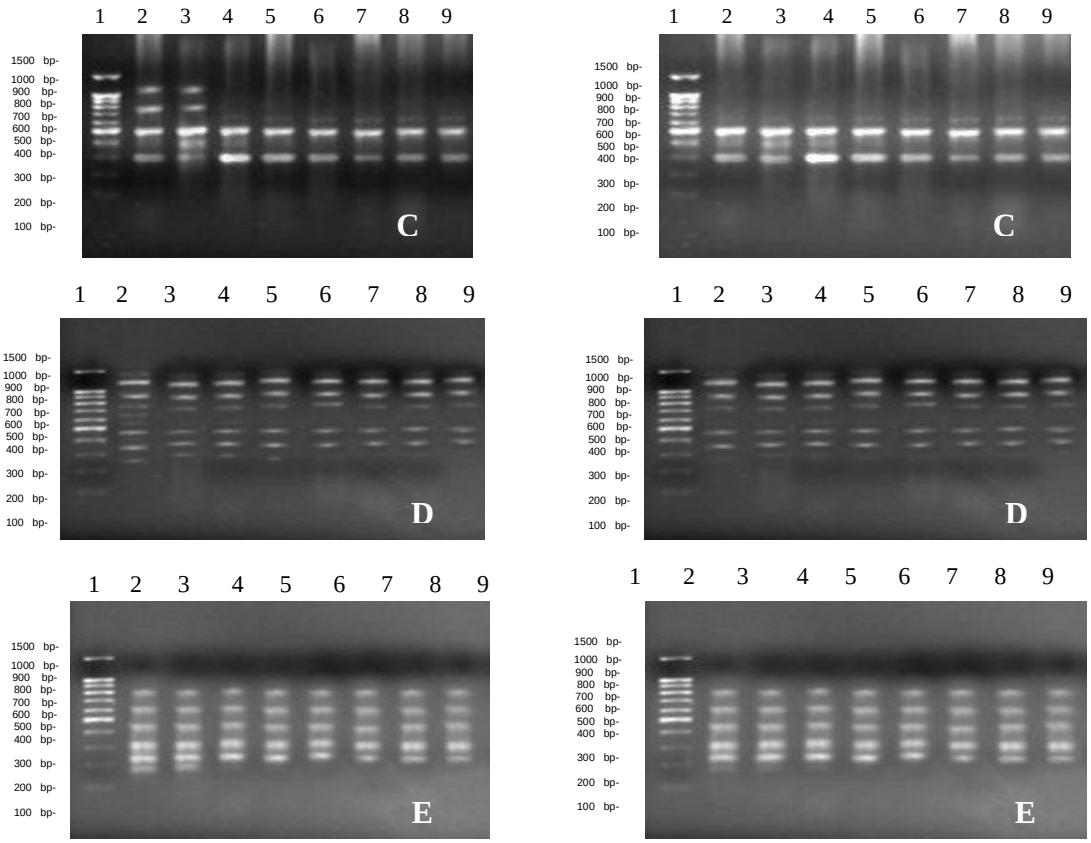


Figure (3)
Comparison of RAPD fingerprinting profiles of different tilapia genomic DNA: (A) Represents PCR products with primer A04, (B) Represents PCR products with primer A08, (C) Represents PCR products with primer A10,
(D) Represents PCR products with primer C09,(E) Represents PCR products with primer C12.

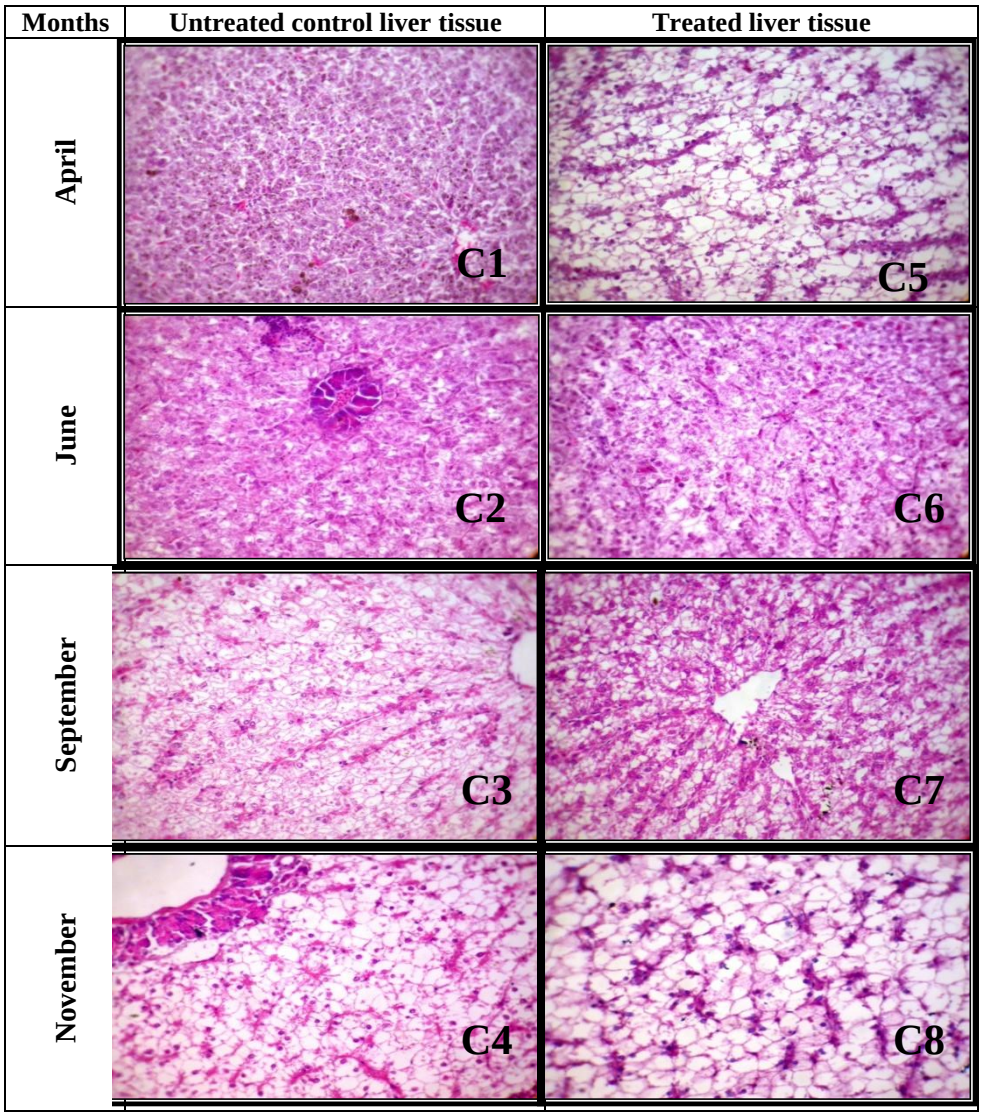
Liver:-

The liver section of the present studies (Photomicrograph 1) in the control group showed hepatic parenchymal arrangement consists of hepatocytes, which are rapidly arranged around central vein interconnecting laminae of two cells thickness, narrow straight sinusoid separating each lamina. This is in agreement with shown reported by Robert (2011).

Liver sections of untreated control fish (Sections, C1,C2,C3 &C4))showed the normal structure which followed by diffuse vacuolations at the end of the studied period, where diffuse severe hepatocytic vacuolations were appeared. On the other hand, liver sections of treated fish (Sections, C5,C6,C7 &C8)showed diffuse vacuolar degeneration followed by some hepatocytic vacuolations but at the end of the studied period severe hepatocytic vacuolations were appeared.

High dosages of exogenous male hormones, including methyltestosterone, are known to cause side effects, especially liver damage, but lower levels actually produce various health benefits, including reduced risks from cardio-vascular disease and cancer. Overall, it has been shown that the side effects of testosterone supplementation in humans are minimal when plasma testosterone levels are kept within the normal physiological range (Bhasin *et al.*, 1998).

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.



Photomicrograph (1):
Histological sections in Liver tissues of *Oreochromis niloticus* collected from the untreated control fish in El- Abbassa and treated fish in El-Nubaria fish farms from April till November (2009).

Untreated control
(C1) Liver showing normal structure (H&E 400X).
(C2) Liver showing normal structure (H&E 400X).
(C3) Liver showing diffuse severe hepatocytic vacuolations (H&E 400X).
(C4) Liver showing diffuse severe hepatocytic vacuolations (H&E 400X).

Treated
(C5) Liver showing diffuse vacuolar degeneration (H&E 400X).
(C6) Liver showing mild vacuolations (H&E 400X).
(C 7) Liver showing some hepatocytic vacuolations (H&E 400X).
(C8) Liver showing severe hepatocytic vacuolations (H&E 400X).

Also our results were in agreement with Khater (1998) who studied the effect of different doses (15, 30, 60, 90 mg) of 17α-methyltestosterone on the liver for 28 days and indicate that the hepatic parenchyma had diffused vacuolar degeneration. The central veins and hepatic sinusoids were congested.

Khater (1998) also reported that, liver tissue treated with 60 mg MT for 14 days, showed diffuse hydropic degeneration, the central vein was congested and hemorrhage was also seen in the hepatic parenchyma.

Finally, when 17 α-methyltestosterone is used for sex reversal treatment with dosage of 20 -40 mg/kg diet and the amount of 17 α-methyltestosterone ingested by tilapia is unlikely to exceed 10 µg/day. When tilapia are reared to a marketing weight of about 300 g, which under intensive culture conditions takes not less than 5 months (Melard and Philippart, 1981). Because the dose rates of 17 α-methyltestosterone used in human medicine ranged from 10 -50 mg daily (British Pharmacopoeia, 1980). Johanstone *et al*. (1983) concluded that, under normal circumstances, it would be unreasonable to suggest that hazardous levels of 17α-methyltestosterone might be ingested by consumption of adult fish treated as juveniles with this steroid.

Methyltestosterone treatment in tilapia farming is considered to be entirely safe provided the following recommended best practices are adopted by producers:

1. They restrict tilapia methyltestosterone treatment to the early fry stages, specifically to the first month from the time the fry are free-swimming/first-feeding.
2. They limit the dosage of methyltestosterone used to a maximum of 60 mg methyltestosterone /kg fry feed.
3. They rear methyltestosterone treated tilapia fry to adult size for at least five months after hormone treatment ends to ensure zero hormone residue remains in the fish.
4. As a precautionary measure, adopt safe handling protocols when preparing and administering methyltestosterone treated tilapia feed; use latex gloves and a protective face mask to avoid dermal contact or inhalation of methyltestosterone .
5. They keep a careful inventory of the amounts of methyltestosterone supplied to and used in each tilapia hatchery, and ensure that access of the hormone supply and record-keeping are controlled by the farm manager or hatchery supervisor.
6. They avoid direct release of hatchery water used for methyltestosterone treatment of tilapia fry into the environment. As a precautionary measure, tilapia hatcheries should utilize a gravel and sand filter, plus a shallow vegetated pond or an enclosed wetland, to receive and hold the hatchery wastewater for several days before discharge into the general environment.

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References

Ahmad, M.H.; Shalaby, A.M.E.; Khattab, Y.A.E. and Abdel-Tawwab, M. (2002): Effects of 17 α–methyltestosterone on growth performance and some physiological changes of Nile tilapia fingerlings (*Oreochromis niloticus* L.). Egypt. J. Aquat. Biol. Fish, 4(4): 295-311.

Aldrin, J.f.; Messager, G.L. and Baudin Laurencin, F. (1982): La biochemie clinique en aquaculture. Interest et prespectives. Cnexo. Actes. Colloq., 14: 291-326.

Alexander, J. B. and Ingram, G.A. (1980): A comparison of five of the methods commonly used to measure protein concentration in fish sera. J. Fish Biol., 16: 115-125.

Allain,C.C.(1974):Quantitative-enzymatic-colorimetric determination of cholesterol in [lasma .Clin. Chem., 20:470 473.

American Public Health Association (APHA), American Water Works Association and Water Pollution Control Federation (1995): Standard Methods for the Examination of Water and Wastewater. 19th edition, New York, N.Y.

Assem, S.S. and El-Zaeem, S.Y. (2005): Application of biotechnology in fish breeding. II: production of highly immune genetically modified redbelly tilapia; *Tilapia zillii*. Afr. J. Biotechnol., 4: 449–459.

Bayomy, M. F.; Khallaf, E.A. and Gaber, N.(1993): Studies on the fat contents and their relation to the production of *Clarias lazera* (CUV and VAL) in Bahr Shebeen Nile Canal. J. Egypt. Ger. Soc. Zool., 10 (B): 165-182.

Beg, T.; Siddique, Y. H.; Ara, G. ; Afzal, M. and Gupta, J. (2008): Antioxidant Effect of ECG on Testosterone Propionate Induced Chromosome Damage. Human Genetics and Toxicology Laboratory, Department of Zoology, Aligarh Muslim University, Aligarh 202002 (UP), India.Interna.J.Phamacol. 4 (4): 258-263.

Bergmeyer, H.U. (1978): Quantitative determination of Aspartate Aminotransferase (AST) in plasma. Principles of Enzymatic Analysis. Verlag Chemie.

Bhasin, S.; Bagatell, C.J.; Bremner, W. J.; Plymate, S.R.; Tenover, J.L.; Korenman, S.G. and Nielschlag, E. (1998): Issues in testosterone replacement in old men. J. Clin. Endocrinol. Metabol., 83: 3435-3448.

Black, D. and Skinner, E. R. (1986): Features of the lipid transport system of fish as demonstrated by studies on starvation in the rainbow trout. Prog. Fish-Cult., 156(B): 497-502.

Boutwell, J.H.M. (1972): Quantitative colorimetric determination of plasma total lipids. USD. H.E.W.

Boyd, C.E. (1990): Water Quality in Ponds for Aquaculture. Birmingham Publishing Co. Birmingham, Alabama.

British Pharmacopoeia (1980): Vol .1. Her Majesty's Stationary Office, London, pp 291- 292.

Buhl, S.N. and Jackson, K.Y. (1978): Quantitative determination of Lactate Dehydrogenase in plasma. Clin. chem., 24: 828-830.

Burtis, C.A. and Ashwood, E.R. (1996): Tietz fundamentals of clinical chemistry. Philadelphia, PA: Saunders.

Burton, K. (1956): The study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J., 62: 615–623.

Cannon, D.C. (1974): Quantitative colorimetric determination of plasma total protein. IN Clinical Chemistry- Principles and techniques, 2nd Ed. RJ Henry *et al*., Eds. Harper & Row, Hagerstown, MD, pp 411- 421.

Carleton, M.; Drary, Y.; Wallington, E.A. and Cammeron, H. (1967): Carleton's Histological Technique, 4 th ed., Oxford Univ. Press, New York, Toronto.

Chan, L. and O'Malley, B.W. (1976): Mechanism of action of the sex steroids. New Eng. J. Med. (June):1322-1328.

Chan,S.T.H. and Yeung, W.S.B. (1983): Sex control and sex reversal in fish under natural condition.In W.S. Hoar and D.J. Rondall and D.M. Donaldson (eds), Fish Physiology Vol.IX B, Academic Press, New York, pp. 171-213.

Cheskis, B. (2004): Regulation of cell signaling cascades by steroid hormones. J. Cellular Biochem., 93 (1): 20–7.

Clemens, H..P and Insleee, T. (1968): Production of unisexual broods by *Tilapia mossambica* sex-revered with methyl testosterone. Trans. Am. Fish. Soc., 97: 18-20.

Cruz, E. M. and Ridha, M. (1994): Overwintering tilapia; *Oreochromis spilurus* Gunther, fingerlings using warm underground sea water. Aquacul. Fish. Management, 25: 865- 871.

Dange, A.D. and Masurekar, V.B. (1984): Effect of naphthalene exposure on activity of some enzymes in Cichlid fish tilapia (*Sarotherodon mossambicus*) Peters. J. An. Morphol. Physiol., 31: 159-167.

Da Silva, A. B.; Sobrinho, A. C.; Fernandes, J. A. & Lovshin, L.L. (I973): Observation preliminaires sur l'obtention d'hydrides tous males des especes Tilapia hornorum et Tilapia nilotica.Notes et Documents sur la Peche et la Pisciculture n°7.

Deborah, A. S. (1990): The effects of the synthetic steroid 17-alpha-methyltestosterone on the growth and organ morphology of the channel catfish (*Ictalurus punctatus*), Aquacult., 84(1): 81-93.

EL-Ebiary, E.H. and Mourad, M.H. (1998): Response of florida red Tilapia (*Oreochromis urolepia hornorum* VS *Oreochromis hyprid*) fingerlings to different oil levels. Bull. Nat. Inst. of Oceanorg. And Fish. A.R.E., (24): 325-337.

El-Ebiary, E. H.; Zaki, M. A. and Mourad, M. H. (1997): Effect of salinity on growth, feed utilization and haematological parameters of Florida red Tilapia fingerlings. Bull. Nat. Inst. Oceanogr. Fish. A.R.E., (23): 203-216.

El-Naggar, G. O.; Zaghlol,K. H. ; Salah El-Deen, M.A. and Abo-Hegab, S.(1998): Studies on the effect of industrial water pollution along different sites of the River Nile on some physiological and biochemical parameters of the Nile tilapia, *Oreochromis niloticus*. 4th Vet. Med. Zag. Congress (26-28 August 1998 in Hurghada): 713-735.

Flegg, H.M. (1973): Quantitative-enzymatic-colorimetric determination of cholesterol in Plasma. Ana. Clin. Biochem., 10:79- 81.

Gomal, A.C.G.; Bradawil, C.J. and David, M.M., (1949): Quantitative colorimetric determination of plasma total protein. J.Biol.Chem., 177:751-753.

Guerrero, R.D. and Abella,T.A.(1976): Induced sex reversal of Tilapia nilotica using methyltestosterone. Fish.Res.J.Phillipp., (1): 46-49.

Haggag, A.M.; Marie, M.A.S.; Zaghloul, K.H. and Eissa, S.M.(1993): Treatment of underground water for fish culture in Abbassa farm, Sharkia. III Biochemical and histological studies. Bull. Fac. Sci. Cairo Univ., 61: 43-69.

Hana, Y.H.; Khalil, W. K.B.; Elmakawy, A. I. and Elmegeed, G. A. (2008): Androgenic profile and genotoxicity evaluation of testosterone propionate and novel synthesized heterocyclic steroids. J. Steroid Biochem. Mol. Biol., 110: 284–294.

Hanson, R.; Sinith Evman, R.O.; Shelton,W.H. and Dunham, R.A.(1983): Growth comparison of monosex tilapia produced by separation of sexes hybridization and sex reversal, p. 570. 579. In L. Fishelson and Z. Yaron (compsi). Proceedings of International symposium on Tilapia in Aquaculture. 8-13 May 1983. Nazareth, Isreal. Aviv University, Israel

Harris, C. L. (1992): Materials and mechanisms of cells. In: Concepts of Zoology. Harper Collins Publ. Inc., New York, 19-50.

Heath, A.G. (1995): Water Pollution and Fish Physiology. CRC. Press. Inc. Boca Raton, Florida. 359pp.

Heberer, T. (2002): Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. *Toxicol. Lett.*, 131: 5–17.

Hilmy, A.M.; Shabana, M.B. and Said, M.M. (1981): The role of serum transaminases (SGOT and SGPT) and alkaline phosphatases in relation to inorganic phosphorus with respect to mercury poisoning in Aphanius dispar Rupp (Teleos) of the red sea. Comp. Biochem. Physiol., 68C: 69-74.

Ho, S.M. and Roy, D. (1994): Sex hormone-induced nuclear DNA damage and lipid peroxidation in the

dorsolateral prostates of Noble rats. Cancer Lett., 84 :155–162.

Ho, S.M. and Yu, M. (1993): Selective increase in type II estrogen binding sites in the dysplastic dorsolateral prostates of Noble rats. Cancer Res., 53: 528–532.

Holden, M. and Reed, W. (1972): West African Fresh Water Fishes.Longman Group Ltd., London 5I p.

Horng, Y.M.; Chen, Y.T.; Wu, C.P.; Jea, Y.S. and Huang, M.C. (2004): Cloning of Taiwan water buffalo male-specific DNA sequence for sexing. Theriogenol., 62: 1536–1543.

International Federation of Clinical Chemistry (IFCC) (1980): Quantitative determination of alanine aminotransferase (ALT) in plasma. *J. Clin. Chem.Clin.Biol.*,18:5231-5233.

Ishak, M.M. (1979): Development and progress of aquaculture in Egypt with special reference to cage and open cultures. International Workshop on open and Cage Culture of Fish. Tigbauan, Iioilo, Philippines; IDRC and SEAFDEC, 11-22 Feb., pp.31- 32.

Johnson, B. (2007): 17-Alpha Methyltestosterone Clinical Field Trials - INAD 9332. Annual Summary Report on the Use of 17-Alpha Methyltestosterone in Field Efficacy Trials, U.S. Fish and Wildlife Service Bozeman National INAD Office Bozeman, Montana. 15p.

Johnstone, R.; Macintosh, D.J. and Wright, R.S., (1983): Elimination of orally administered 17α-Methyltestosterone by *Oreochromis mossambicus* (Tilapia) and *Salmo gairdneri* (rainbow trout) juveniles. Aquacul., 35: 249-257.

Khalil, W.K.B.; Mahmoud, M.A.; Zahran, M.M. and Mahrous,K.F. (2007): : A sub-acute study of metronidazole toxicity assessed in Egyptian Tilapia ; *Tilapia zillii. J. Appl. Toxicol.*, 27: 380–390.

Khater, A.M.M. (1998): Sex reversal in *Tilapia nilotica*. Ph.D. Thesis. Zagazig University, Agricultural Science (Poultry Production- Aquaculture- Fish hatching and sex reversal), pp. 144.

Kheir, M.T.; Mechail, M.M. and Abo-Hegab, S. (1998): Growth of adult and newly hatched fry of *Oreochromis niloticus* reared at different saline concentrations. Egypt. J. Zool., (30):107- 115.

Lavery, D.N. and McEwan, I.J. (2005): Structure and function of steroid receptor AF1 transactivation domains: induction of active conformation. Biochemical J., 391 (Pt 3): 449–64.

Lea- Master, B.R; Brock, J.A.; Fujioka, R.S. and Akamura, R.M. (1990): Haematological and blood chemistry values for *Sarotherodon melanotheron* and a red hybrid Tilapia in freshwater and sea water. Comp. Biochem. Physiol., 97(A): 525-529.

Luceri, C.; De Filippo C.; Caderni, G.; Gambacciani, L.; Salvadori, M.; Giannini, A. and Dolara, P. (2000): Detection of somatic DNA alterations in azoxymethane-induced F344 rat colon tumors by random amplified polymorphic DNA analysis. *Carcinogenesis*, 21: 1753– 1756.

Mahrous, K.F.; Khalil, W.K.B. and Mohamoud, M.A. (2006): Assessment of toxicity and clastogenity of sterigmatocystin in Egyptian Nile tilapia. Afr. J.Biotechnol., 5(12): 1180- 1189.

Maita, M.; Shiomitsu, K. and Ikeda, Y. (1984): Health assessment by the climogram of hemochemical constituents in cultured yellow tail. Bull. Jap. Soc. Scient., (51): 205-211.

Marzin, D. (1991): Trenbolone application of the Ames test. Recent data, Ann. Rech. Vet. 22 (3): 257–262.

Melard, C.H. and Philippart, J.C.(1981): Pisciculture intensive du tilapia Sarotherodon niloticus dan les effleuents thermiques d'une central nucleaire en Belgique. K. Tiews (Editor), " Aquaculture in heated effluents and recirculation system." Heenemann Verlagsgesellschaft, Berlin (EIFAC/ 80/Symp/ Doc.E/11).

Muhaya, B.B.M. (1985): Growth comparisons of tilapia nilotica males produced through oral administarion of methyltestoserone at varing levels and durations, Auburn University, Auburn, Alabama, USA.28 P.M.S. thesis.

Nagy, A.; Becsenyi, M. and Csanyi, V.(1981): Sex reversal in crap
(*Cyprinus carpio*) by oral administration of methyletestosterone. Can. J. Fish Aquat. Sci., 38:725-728.

Nevo, E.; Shimony, T. and Libin, M.(1978): Pollution selection of allozymes polymorphisms in branchtes. Experentia, 36: 1562- 1564.

Plotsky, Y.; Kaiser, M.G. and Lamont, S.J. (1995): Genetic characterization of highly inbred chicken lines by two DNA methods: DNA fingerprinting and polymerase chain reaction using arbitrary primers. Animal Genetics, 26: 163-170.

Popma, T.J. and Green, B.W. (1990):. Sex reversal of tilapia in earthen ponds. International Center for Aquaculture and Aquatic Environments Research and Development Series No. 35, Auburn University, Alabama, pp: 15.

Ragnotti, G.; Presta, M.; Maier, J.A.M.; Rusnati, M.; Mazzoleni, G.; Legati, F.; Chiesa, R.; Braga, M. and Calovini, D. (1987): Critical role of gonadal hormones on the genotoxic activity of the hepatocarcinogen DL-ZAMI 1305. Cancer Lett., 36:253–261.

Rashatwar, S.S. and Ilyas, R. (1983): Effects of chronic herbicide intoxication on the *in vivo* activities of certain enzymes in the liver of fresh water fish Nemacheilus denisonii (Day). Toxicol. Let., 16: 249-252.

Robert, B.M. (2011): Biology of Fish. California Animal Health and Food Safety Laboratory System University of California.

Roeschlau, P. and Klin, Z. (1974): Quantitative-enzymatic-colorimetric determination of cholesterol in plasma .Chem. Clin. Biochem., 12: 226-228.

Rosalki, S.B. (1977): Quantitative determination of creatine phosphokinase (CPK) in Plasma. J. Lab. Clin. Chem., 23: 646-650.

Sambrook, L.; Fritsch, E.F. and Manitatis, T. (1989): Molecular cloning: A laboratory manual. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.

Sandnes, K.; Lie, Q. and Waagbq, R. (1988): Normal ranges of some blood chemistry parameters in adult farmed Atlantic salmon; *Salmo salar*. J. Fish. Biol., (32): 129-136.

Schwaiger, J.; Fent, K.; Stecher, H.; Ferling, H. and Negele, R. D. (1996): Effect of sublethal concentrations of triphenyltriacetate on rainbow trout (*Oncorchynchus mykiss)*. Arch. Environ. Contam. Toxicol., (30): 327- 334.

Seraj, M.J.; Umemoto,A.; Tanaka,M.; Kajikawa, A.; Hamada K.and Monden,Y. (1996): DNA adduct formation by hormonal steroids *in vitro*. Mutat. Res., 370 (1): 49–59.

Shakweer, L.M; El-Ebiary, E.H. and Zaki, M.A. (1998): Comparative study on the major biochemical constituents in muscles of *Mugil cephalus* inhabiting the Mediterranean water, the north Delta lakes and fish farms of Egypt. Bull. Nat. Inst. Of Oceanongr. Fish. A.R.E., (24): 79-101.

Shatunovsky, M.I. (1971): Variations in different lipids and their relation to reproduction in the male fish *Clarias batrachus* (Linn). Indian J. Exp. Biol., (25): 639-640.

Software Program of Statistical Analysis, SPSS. (2008): Software Program of Statistical Analysis, Version 17.0 Edition for Windows. SPSS Inc., Chicago, IL, US.

Statistical Analyses System S.A.S. (2000): Statistical Analyses System, Version 6.2, SAS institution in corporation. Carry, NC27513 USA.

Stein, E.A. (1986): Quantitative-enzymatic-colorimetric determination of cholesterol in Plasma. in Textbook of Clinical Chemistry, NW Tiez, Ed. W.B. Saunders, Philadelphia, p.p. 879-886, 1818, 1829.

Szasz, G. (1975): Proceedings of the second international Symposium on clinical Enzymology, Chicago, October 1975.

Tayamen, M.M. and Shelton,W.L. (1978): Inducement of sex reversal in *Sarotherodon niloticus* (Linnaeus). Aquacul., 14:349-354.

Teh, S. J.; Adams, S. M. and Hinton, D. E. (1997): Histopathologic biomarker in feral freshwater fish populations exposed to different types of contaminant stress. Aquat. Toxicol., (37): 51- 70.

Tolba, M.R.; Mohammed, B. and Mohammed, A. (1997): Effect of some heavy metals on respiration, mean enzyme activity and total protein of the plumonate snails *Biomphalaria alexandria* and *Bulinus truncates*. J. Egypt.Ger.Soc.Zool., 24: 17-35.

Trinder, P. (1969): Quantitative-enzymatic-colorimetric determination of cholesterol in Plasma. Ann. Clin. Chem., 6: 24-26.

Tsutsui,T.; Konine,A.; Huff,J. and Barrett,J.C. (1995): Effects of testosterone, testosterone propionate, 17beta-trenbolone and progesterone on cell transformation and mutagenesis in Syrian hamster embryo cells, Carcinogenesis 16 :1329–1333.

Van Someren, V. D. and Whitehead, P. J. (1960): The culture of Tilapia nigra (Gunther) in ponds. The early growth of males and females at comparable stocking rates, and the length/weight relationship. East Africa. Argric. J., 25 (3): 69-I73.

Verma, S.R.; Rani, S. and Dalela, R.C. (1981): Isolated and combined effect of pesticides on serum transaminases in *Mystus vittatus*. Toxicol. Let., 9: 67-71.

Wacker,W.E.C.(1956): Quantitative determination of lactate dehydrogenase in plasma. New Engl. J. Med., 255:449-451.

Wassef, E. and Shehata, A.B. (1991): Biochemical composition of gilthead sea bream; *Sparus aurata* from Lake Bardawil. K.A.U. Mar. Sci., 2: 111-122.

Weichselbaum, T.E. (1946): Quantitative colorimetric determination of plasma total protein. Am. J. Clin. Patho., 7:40-42.

Welder, A.A.; Robertson, J.W. R. and Melchert, B. (1995): Toxic effects of anabolic–androgenic steroids in primary rat hepatic cell cultures, J. Pharmacol. Toxicol. Methods 33(4):187–195.

White, A.; Handler, P. and Smith, E.L. (1973): Principles of biochemistry, 5 edn. McGraw. Hill, New York, 1295pp.

Williams, J.K.G.; Kubelik, A.R.; Livak, K.J.; Rafalsky, J.A. and Tyngey, S.V. (1990): Molecular changes during gonad masculinization. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531-6535.

Wrobewski,F. and La Due, J.S.(1956): Quantitative determination of alanine aminotransferase (ALT) in plasma. Proc.Soc. Exper. Biol. Med., 91:569-572.

Young, D.S. (2001): Colorimetric Quantitative determination of albumin in plasma. Effect of disease on clinical lab. Tests, 4th Ed AACC.