FACULTY OF VETERINARY MEDICINE
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Molecular and Biochemical studies on 17α-Methyl Testosterone residues in Tilapia Nilotica

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By

Reham Masoud Kamel Mohamed Abo Hashem

(BVSc, Faculty of Veterinary Medicine, Alexandria University, 2011)

(MVSc, Faculty of Veterinary Medicine, Alexandria University, 2015)

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Molecular and Biochemical studies on 17α-Methyl Testosterone residues in Tilapia Nilotica

Tilapia Nilotica is considered one of the most important and cheapest sources of animal protein in Egypt, so Egyptian people are directed to depend on it due to low cost and high nutritive values.

In recent years, hormones and hormone like compounds have been frequently used in fish farms to enhance the growth in a shorter period of time for increasing the profits but the consumption of fish meat containing hormone residues especially synthetic hormones as 17 α- Methyltestosterone may be potentially hazardous to human consumers.

So the aim of this orkw was planned to detect the presence of MT residues in Tilapia Nilotica and to examine the effect of the exposure to this hormone on rat liver.

A pilot test was done for detection presence or absence of Methyl Testosterone residues in Tilapia Nilotica muscle. Fish samples were collected from Kafr El-Sheikh farms which producing Monosex Tilapia Nilotica.The samples were collected for detection of MT hormonal residues by using ELISA.

Forty male Wistar Albino rats weighing 180-200 g were kept in metal cages under environmental-controlled conditions according to the international ethical guidelines for the care and use of laboratory animals of Alexendria university. The rats were randomly assigned to four groups (10 rats each).

C group: control group (eat basal ration and water ad libitum).

F group: which take Tilapia Nilotica flesh that contains residues of Methyltestosterone previously estimated by ELISA about two kilograms fish mixed randomly with starter ration daily for 30 days,

A group: which take 20mg/kg body weight oral dose of Methyl testosterone was given by stomach tube daily for 30 days and

B group: which take 80 mg/kg body weight oral dose of Methyltestosterone was given by stomach tube daily for 30 days.

After the end of experiment, blood samples were collected for measuring biochemical parameters and liver exposed to a molecular, and a histopathological investigation.

All analyses were performed using the SPSS statistical software package (version 22.0 SPSS Inc., Chicago, USA). First, tests of normality were performed on raw data. For normally distributed variables, we used one-way analysis of variance (ANOVA) for the effect of group followed by Waller-Duncan's post-hoc. Non-normally distributed variables were compared with the non-parametric Kruskal-Wallis test with Mann–Whitney U-test for pairwise differences. All variables were presented as mean ± standard error of the mean. A probability value of 0.05 or less was considered statistically significant.

Results of the present study revealed the following:

- The mean value of MT residues in Tilapia Nilotica flesh was 2.55 ppb (µg/kg) and the standard deviation was 0.86.
- The hepatic CYT.P450E1 and TNF-α gene expression shows a dose dependant increase in fold change of expression of CYT. P450E1 and TNF-α genes. As the exposure of rats to MT leads to significant induction of gene expression of CYP P 450 E1 gene as the fold change on control group was one on both genes while in F group, A group, and B group (2.07 ± 0.13, 6.96 ± 0.19 and 13.2 ± 0.41) respectively and also TNF-α gene as F group, A group and B group was (2.85 ± 0.05, 8.84 ± 0.19, and 15.4 ± 0.32) respectively ($P \leq 0.05$).
- Liver function tests include ALT, AST, albumin, total protein, globulin, and A/G ratio in the rat exposed to different oral doses for 30 days, changes in liver function tests as shown in Table 3. ALT did not change in group F and A but increased signifcantly in group B when compared to control group 35.7±1.61 U/l, 37.8±2.79 U/l, 46.3±4.57 U/l and 37.0±1.81 U/l respectively ($P \leq 0.05$). AST also did not change in group F and A but increased signifcantly in group B when compared to control group 131±8.87 U/l, 158±11.7 U/l, 194±11.5 U/l and 143±8.21 U/l respectively ($P \leq 0.05$). Total protein did not change significanlty in F, Aand B groups compared to control group 6.13 ± 0.32, 5.92 ± 0.39 g/dl, 6.58 ± 0.22 g/dl, and 6.73 ± 0.10 g/dl respectively ($P \leq 0.05$). Albumin did not change significanlty in F, Aand B groups compared to control group 3.92±0.25 g/dl, 3.75±0.30 g/dl, 4.22±0.20 g/dl, and 4.20±0.04 g/dl respectively ($P \leq 0.05$). Globulin did not change significanlty in F, Aand B groups compared to control group 2.22±0.12 g/dl, 2.17 ±0.13 g/dl, 2.37±0.07 g/dl , and 2.53±0.07 g/dl respectively($P \leq 0.05$). Also A/G ratio did not change significanlty in F, Aand B groups

compared to control group 1.78 ± 0.11, 1.73 ± 0.11 , 1.79 ± 0.09, and 1.67 ± 0.04 respectively ($P \leq 0.05$).

- Effect of MT on total cholesterol, and high density lipoproteins; rats which exposed to high dose 80 mg/kg body weight for 30 days (group B) shows a lower cholesterol level but not in a healthy way as HDL-c was significantly decreased. Total Cholesterol decreased significantly in group B as in the control group was 70.5 ± 2.63 mg/dl and in group B 46.2 ± 2.32 mg/dl while F and A groups did not change significantly as their values were 70.8 ± 2.27 and 63.5 ± 4.81 respectively. Also, HLD-c was in the control group 51.5 ± 2.64 mg/dl, group F increased slightly becomes 57.7 ± 3.21 mg/dl and in group B decreased significantly become 40.0 ± 2.65 mg/dl while in group A decreased but not a significant decrease as it was 49.0 ± 5.06 mg/dl ($P \leq 0.05$).

- Histopathological examination of liver sections of control rats reveals normal hepatic architecture formed of lobules of hepatocytes separated by sinusoidal blood vessels and the portal tract contains inflammatory cells, while in F group reveals hepatocytes separated by sinusoidal blood vessels and the hepatocytes showing mild nuclear enlargement. In A group the hepatocytes showing mild to moderate nuclear enlargement, hyperchromatism, and coarse chromatin. Finally, in B group the hepatocytes showing hydropic changes also, it shows moderate nuclear enlargement and coarse chromatin.