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Effect of Oxytetracycline and Florfenicol on the Cytogenetic Picture of Nile Tilapia (Oreochromis niloticus) Fish

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Abstract

Oxytetracycline (OTC) and florfenicol (FF) are antibiotics with high potency against the bacterial diseases. Therefore, they are commonly used against bacterial infection in fish farming. The present work aimed to study the effect of different doses of oxytetracycline (0, 40, 80, 120mg/kg basal diet) and florfenicol (0, 7.5 , 15 , 22.5 mg/kg basal diet) on chromosomal structure and micronuclei induction in RBCs of Nile tilapia fish. The results revealed that the dose of 40 mg OTC/kg basal diet and the dose of 7.5 mg FF/kg basal diet showed non-significant increases in chromosomal aberrations compared with control. The same dose of florfenicol caused significance difference in the frequency of micronuclei compared with control group. The other doses of OTC and FF were significant increases ($p<0.05$) in total chromosomal aberrations (11.40 & 15.60 and 16.80 & 19, respectively) and the frequency of micronuclei (5.4 & 6.8 and 6.4 & 8.5, respectively) compared with control group (8 and 2.4, respectively). The mutagenic influence of therapeutic and super- therapeutic doses of FF treatment was more obvious on chromosomal structure and micronuclei frequency compared to therapeutic and super-therapeutic doses of OTC treatment. These findings, in general, add to the fact that fish such as tilapia (Oreochromis niloticus) is a promising models in vivo for monitoring the mutagenic effect of substances on aquatic ecosystems.

Keywords: oxytetracycline - florfenicol – chromosomal aberrations – micronuclei- tilapia fish.

INTRODUCTION

Tilapia has become one of the most important fish species for fresh water culture [1]. The antibiotics oxytetracycline and florfenicol have been used in fish culture to control or treat bacterial infection in fish [2]. Florfenicol is a broad-spectrum, primarily bacteriostatic, antibiotic with a range of activity similar to that of chloramphenicol, including many gram-negative and gram-positive organisms; however, florfenicol does not carry the risk of inducing a plastic anemia that is associated with chloramphenicol [3].

Florfenicol is a fluorinated derivative of thiamphenicol that blocks the peptidyltransferase at the 50S ribosome subunit and acts against a wide variety of both Gram-positive and Gram-negative bacteria [4]**.** As a medicated feed, florfenicol has been used to treat a wide variety of fish diseases in various warm- and cold-water cultured fish species [5], [6].

Oxytetracycline (OTC) is a bacteriostatic compound with a broad antibacterial activity against both Gram-negative and Gram-positive microorganisms, and both aerobic and anaerobic species [7]. OTC has been approved for use in aquaculture by the US Federal Drug Administration [8]. Recent study by Zounkova et al., (2011) [9] reported that Oxytetracycline may have genotoxic and ecotoxic effects in aquatic ecosystems.

The Nile tilapia, Oreochromis niloticus, is a large genus in the cichlid family (cichlidae). Tilapia is native to Africa from Egypt south to East and Central Africa, and as far west as Gambia. It is also commercially known as mango fish, Nilotica or boulti. One of tilapia's greatest assets is the fact that it is so good at converting plant matter into high quality protein. Its herbivorous feeding habits help to alleviate the fishing pressure on wild fish stocks. Farming herbivorous fish, such as tilapia, increases the world protein supply. The normal chromosome number of tilapia fish is 44 chromosomes (22 chromosome pairs). The first and second pairs are of conspicuous size, especially the first on which is larger pairs (through to be the marker chromosome), the other twenty pairs are small with short arms and gradually decrease in size [10], [11], [12], [13].

Chromosomal analysis have been used as a tool for monitoring the mutagenic effect of substance in water [14]. Micronucleus test is a wider application using as biological marker for detection of the clastogenic pollution in the water environment [15], [16].

The aim of the present study was detection of the effect of different dose of oxytetracycline and florfenicol on the chromosomal structure and induction of micronuclei in Nile tilapia.

MATERIALS AND METHODS

Location

The experimental work of the present study was carried out at the Laboratory of Fish nutrition, Faculty of Agriculture, Moshtohor, Benha University.

Fish source

The experimental fish (Nile tilapia) were obtained from El-Manzala hatchery, Al-Dakahlya Governorate. The experimental fish were transported in 50-liter plastic bags filled with water and oxygen to the laboratory. After arrival to the laboratory, fish were adapted and distributed randomly into 7 groups and each group was replicate in three aquarium and each aquaria was stoked with 30 fish with initial weight of 7± 0.66g.

Experimental design

The experiment started on May 2011 and lasted in August of the same year (90 days). The oxytetracycline and florfenicol were added to the basal diet (as medicated feed during the whole experimental period) as follow:

Group1: control group (kept without treatment)

Group2: administered sub-therapeutic dose 40 mg oxytetracycline/kg basal diet

Group3: administered therapeutic 80 mg oxytetracycline/kg basal diet.

Group4: administered super-therapeutic 120 mg oxytetracycline/kg basal diet.

Group5: administered sub-therapeutic dose 7.5 mg florfenicol/kg basal diet

Group6: administered therapeutic dose 15 mg florfenicol/kg basal diet

Group7: administered super-therapeutic dose 22.5 mg florfincol/kg basal diet.

Composition and chemical analysis of the basal diet used in the experiment are presented in Table (1).

Chromosomes preparation

Samples for studying the chromosomal aberrations were taken from the anterior kidney of fishes after the exposure period. The fish were injected with yeast suspension at dose 1 ml /100 gm bw [18]. Twenty four hours after the first injection, specimens were injected intra muscular with 0.01 ml of 0.03 mg/g b.w freshly prepared colchicine and squash technique for kidney tissue was used for the preparation of chromosomes [19]. For every fish, at least 50 metaphase spreads were examined and the chromosomal aberrations were detected.

Micronucleus preparation

A drop of blood from the caudal vein was mixed with a drop of fetal calf serum, then directly smeared on slide and air dried. After fixation in absolute methanol for 5 min., they were stained with 5% Giemsa for 7 min. Two thousand erythrocyte per fish were analyzed to determine the frequency of micronucleated cells [16].

Statistical analysis

Statistical analysis of the obtained data was analyzed according to SAS (1996) [20]. Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's post hoc test for comparison between different treatments [21]. Results were reported as mean ± S.E. and differences were considered as significant when $P<0.05$.

Table 1. Composition and chemical analysis of basal diet.

Ingredient	**%**
Fish meal	20
Sobean meal	31
Yellow corn	32
Wheat bran	10.5
Corn oil	3
Vit & Min. Mix(1)	3.5
Sum	100
Proximate analysis	
Dry matter	95.23
Protein	30.12
Lipid	5.32
Ash	8.45
ME(Kcal/kg diet)[2]	3019
P/E ratio	99.78

1. Vitamin & mineral mixture/kg premix: Vitamin D_3, 0.8 million IU; A, 4.8 million IU; E, 4g; K, 0.8g, BI. 0.4g; Riboflavin, 1.6g: B6, 0.6g, B_{12}, 4mg; Pantothenic acid, 4g; Nicotinic acid, 8g; Folic acid, 0.4g; Biotin, 20mg, Mn, 22g; Zn, 22g; Fc, 12g; Cu, 4g; I, 0.4g. Selenium, 0.4g and Co, 4.8 mg.

2. Based on Kilocaloric values of $4.50g^{-1}$ protein, $8.51g^{-1}$ lipid and $3.49g^{-1}$ NFE [17].

RESULTS AND DISCUSSION

Florfenicol is an antimicrobial agent acting by inhibiting the peptidyl transferase reaction at the 50 S subunit of the ribosome, while oxytetracycline make protein synthesis inhibition at the ribosomal level (interfere with peptide elongation) as reported by [22], [23]. These actions lead to misinformation of DNA molecules [24] or inhibition of replication due to disturbed polymerase activity.

The mitotic chromosomes from the head kidney of the fish Tilapia niloticus were studied with an initiative to gain information about the nature and extent of the damage that may be produced by in vivo treatments of oxyteracycline and florfenicol.

The fish treated with different doses of oxytetracycline (0, 40, 80, 120mg/kg basal diet) and florfenicol (0, 7.5 , 15 , 22.5 mg/kg basal diet). The results of the Table(2) illustrated that the chromosomal aberration detected were gap, break, deletion, fragment and centromeric attenuation (figs 1-5). The

mean of chromosomal abnormalities observed in kidney cells of fish treated at dose of 40 mg OTC/kg basal diet and the dose of 7.5 mg FF/kg basal diet was non-significant compared with control.

The other doses (therapeutic and super-therapeutic) of OTC and FF caused significant increases ($p<0.05$) in total chromosomal aberrations (11.40 & 15.60 and 16.80 & 19, respectively) and the frequency of micronuclei (5.4 & 6.8 and 6.4 & 8.5, respectively) compared with control group (8 and 2.4, respectively). The mutagenic effect of therapeutic and super-therapeutic doses of FF treatment was obvious on chromosomal structure and micronuclei frequency compared to therapeutic and super-therapeutic doses of OTC treatment. Regarding to the types of chromosomal aberration recorded, centromeric attenuation (C.A) and deletion were significant increase compared with other aberrations induced by OTC and FF.

The mean value of micronuclei erythrocytes of tilapia fish had significant increase in different doses of OTC and FF compared to control. The mean of micronuclei erythrocytes of tilapia fish was ranged from 2.4 to 8.5 with significant differences between groups (Table 3, fig., 6). The higher micronuclei mean values 6.8 and 8.5 were obtained with fish fed on diet contained 120 mg OTC/kg basal diet (T_4) and 22.5 mg FF/kg basal diet (T_7).

As far as we are aware, no attempts have been done to study the clastogenic effects of oxytetracycline and florfenicol on the chromosomes of fish, despite of the various reports on cytogenetic of other antibiotics. Our results are in agreement with the studies had been done by [25], [26]. They found that flumequine at dose of 10 mg/kg body weight / day caused significant increases in total chromosomal aberration in Nile tilapia fish. Heijden et al., (1995) [27] reported that flumequine posses mitogenic properties in Europeans eels as well as Ueda et al., (1992) [15] who found increases in the frequency of chromosomal aberrations and chromosome gaps, break and exchange in adult bitter lings treated with mitomycin. The types of recorded aberrations in the present study were similar to types of aberrations induced by mitomycin which study by [28].

The results obtained are concurrent with **[29]** study**.** They found that the dose and time dependant increased the chromosomal aberrations after treatment with bleomycin, mitomycin and doxorubicin antibiotics in the gills of fish.

It has been pointed that drugs may present a potential hazard to mankind by causing gene mutation or chromosome aberrations. Genetic alterations in somatic cells can including cell death or transform into malignancy [30], [31]. The overall results of the present study revealed that these antibiotics (OTC and FF) induced clastogenic effects in the cells of Nile tilapia fish and the FF has mutagenic effect more than OTC. As so, the findings of this study, in general, add to the fact that fish such as tilapia niloticus is a promising models in vivo for monitoring the mutagenic effect of substances on aquatic ecosystems.

Table 2. Effect of different doses of oxytetracycline (OTC) and florfenicol (FF) on the structure chromosomes of Nile Tilapia.

Treatments	No.	Gap	Break	Deletion	Fragment	Centromeric attenuation	Aneuploidy	Total
T1 (Control)	5	0.80 ±.37 d	1.20 ±.58 c	1.20± .37 b	1.00 ±0..40	1.8 ±.24 c	2.00 ±.40	8.00 ±.60 d
T2 (40 mg OTC/kg basal diet)	5	0.80 ±.37d	1.0 ±.37c	1.60 ±.50 b	1.00 ±.30	2.20 ±.37 bc	2.20 ±.37	8.80 ±.58 d
T3 (80 mg OTC/kg basal diet)	5	1.20 ±.37bcd	0.80 ±.37 c	2.60 ±.24 ab	0.80 ±.37	3.40 ±.24 ab	2.60 ±.40	11.40 ±1.02 c
T4 (120 mg OTC/kg basal diet)	5	3.00 ±.44a	1.60 ±.40 bc	2.00 ±.37b	2.00 ±.73	3.40 ±.59 ab	3.60 ±.37	15.60 ±1.10 b
T5 (7.5 mg FF/kg basal diet)	5	1.00 ±.40cd	1.20 ±.49 c	2.00 ±.45 b	1.00 ±.32	3.00 ±.45 abc	2.20 ±.37	10.40 ±.68cd
T6 (15 mg FF/kg basal diet)	5	2.20 ±.49abc	2.60 ±.24ab	4.00 ±.84 a	1.20 ±.37	3.80 .49 a	3.00 ±..32	16.80 .66 ab
T7 (22.5 mg FF/kg basal diet)	5	2.40 ±.24ab	3.40 ±.51 a	4.00 ±.45 a	1.80 ±.37	4.00 .55a	3.40 ±.75	19.00 .71a

Data presented as means ± standard error (SE).
Means followed by different letters in each column are significantly ($P< 0.05$) different.

Table 3. Effect of different doses of oxytetracycline (OTC) and florfenicol (FF) on micronuclei erythrocytes.

Treatments	NO	Erythrocytes	Micronuclei erythrocytes
T1 Control	5	1000	2.60 ± .24e
T2 (40 mg OTC/kg basal diet)	5	1000	3.60 ± .50 ed
T3 (80 mg OTC/kg basal diet)	5	1000	5.40 ± .60 bc
T4 (120 mg OTC/kg basal diet)	5	1000	6.80 ± .66 ab
T5 (7.5 mg FF/kg basal diet)	5	1000	4.60 ± .60 cd
T6 (15 mg FF/kg basal diet)	5	1000	6.40 ± .60 b
T7 (22.5 mg FF/kg basal diet)	5	1000	8.50 ± .50a

Data presented as means ± standard error (SE).
Means followed by different letters in each column are significantly (P< 0.05)

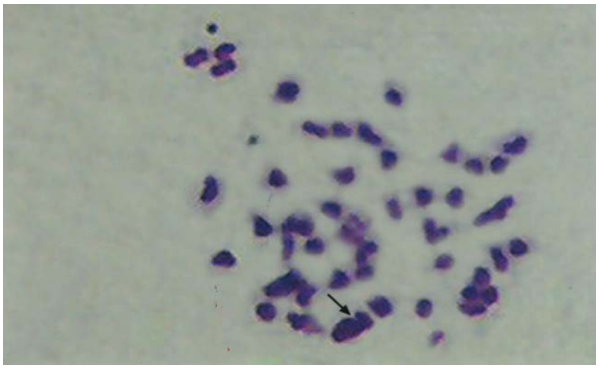

Figure 1. Metaphase spread showing gap arrow obtained from oxytetracycline 80mg/kg basal diet exposed group (1250x).

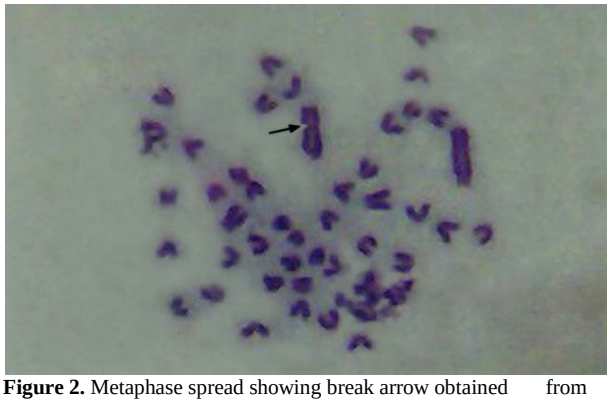

Figure 2. Metaphase spread showing break arrow obtained from florfenicol 22.5mg/kg basal diet exposed group (1250x).

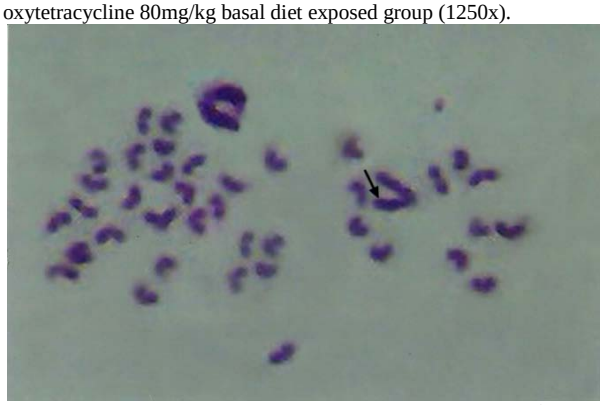

Figure 3. Metaphase spread showing deletion arrow obtained from florfenicol 22.5mg/kg basal diet exposed group (1250x).

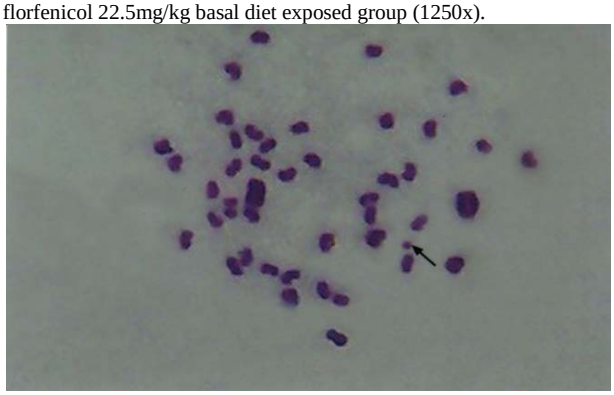

Figure 4. Metaphase spread showing fragment arrow obtained from oxytetracycline 120mg/kg basal diet exposed group (1250x).

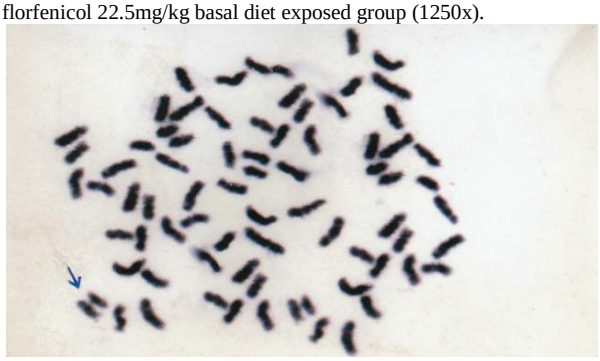

Figure 5. Metaphase spread showing centromeric attenuation arrow obtained from oxytetracycline 120mg/kg basal diet exposed group (1250x).

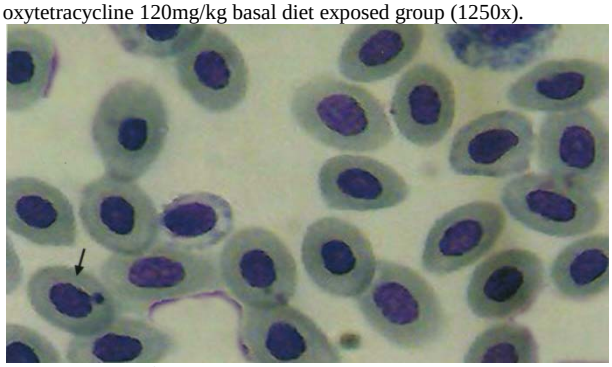

Figure 6. Blood film showing micronucleated erythrocyte

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