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High dietborne Cu and Cd induced genotoxicity of Nile tilapia (*Oreochromis niloticus*)

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ARTICLE INFO

Article history:
Received 24 January 2015
Received in revised form
29 March 2015
Accepted 4 April 2015
Available online 13 April 2015

Keywords:
Oreochromis niloticus
Dietborne metals
Bioaccumulation
Chromosomes

ABSTRACT

In this study, the effects of fish diet contaminated with Cu, Cd and Cu + Cd on Nile tilapia, was demonstrated by evaluating its bioaccumulation in the muscle and by testing the cytogenetic profile. Fish exposed to diet contaminated with Cu, Cd or their mixture had a significant increase in the number of chromosomal abnormalities and an inhibition of the mitotic index. Our study revealed high muscle Cu or Cd content in fish fed with diet contaminated with high dietborne Cu, Cd, Cu and Cd. It also revealed that the chromosomal abnormalities were higher for fish fed diet Cd contaminated and Cu + Cd contaminated diets than those fed diet Cu contaminated diet. Thus, maybe fish diets contaminated with Cu, Cd, Cu + Cd induced genotoxicity and mutation. Also, maybe high concentrations of Cu and Cd in fish tissue resulted from feeding on Cu and Cd contaminated diets, are dangerous for human consumption.

1. Introduction

Karyological studies of teleost fishes can contribute significantly to demonstrate the potential impact of metals on genotoxicity and cytotoxicity (Harabawy et al., 2014). Many waterborne pollutants have been known to cause chromosomal aberrations to fish (Pak et al., 2012). Several studies found an increase in cytogenetic abnormalities in fish and shellfish exposed to polluted environments (Mahmoud, 2006; Andriyasheva, 2011; Harabawy et al., 2014). Manna and Mukherjee (1986, 1989) found a higher frequency of chromosomal abnormalities in *Oreochromis mossambicus* treated with malathion, mercuric chloride, inorganic weedicide, and sodium arsenate. Diet contaminated with metals as Cu, Zn, Cd induce deleterious health effects on fish (Szczerbik et al., 2006; Kamunde and MacPhail, 2011; Mustafa et al., 2012). Mustafa et al. (2012) found that level of oxidative DNA damage (as determined by modified Comet assay) showed significant increase following exposure of carp (*Cyprinus carpio*) to dietary Cu level. For Nile tilapia, Shaw and Handy (2006) found that high dietary copper induces harmful physiological and histological alterations. Also, the dietary cadmium has been found to induce significant alterations of intestine and kidney ATPase activities of the discus fish (*Symphysodon* sp.) as a cichlid fish (Maunder et al., 2011).

Fish requires copper as an essential element (Watanabe et al., 1997) and can obtain it from either water or diet (Handy, 1996; Wood, 2001). Meanwhile, excess dietary Cu (16–730 mg Cu/kg dw feed) induces toxicity to freshwater fish (Clearwater et al., 2002). The normal values of Cu and Cd in freshwater are 1000 and 8.7 μg/l, respectively (Wahaab and Badawy, 2004; Toufeek, 2011). Cadmium (Cd) is a non-essential element that induces harmful bio-physiological changes in addition to cell

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http://dx.doi.org/10.1016/j.etap.2015.04.001
1382-6689/

injuries (Roméo et al., 2000; Kalman et al., 2010; Giusto et al., 2012; Liu et al., 2014).

Tilapias are commonly cultured fish in Egypt, as they primarily exhibit excellent growth rates even on low protein diets (El-Sayed, 2006). Extensive research efforts have been given to study their biological and environmental conditions related to improve their production (Khallaf et al., 2003; Fridman et al., 2012). Tilapia nutrition has been given special attention in order to maximize their growth and feed utilization (Abdel-Tawwab et al., 2010; Fortes-Silva and Sánchez-Vázquez, 2012).

Nile tilapia, *Oreochromis niloticus*, is one of the aquatic organisms affected by heavy metals, so in many cases, it is used as biomarker in toxicological studies (Handy, 2003; Grosell et al., 2007; Mustafa et al., 2012; Al-Bairuty et al., 2013). In wild habitat or in aquaculture, they subjected to ingest contaminated food which causes subsequent deleterious effects on their health (Friberg et al., 1986; Clearwater et al., 2002; Giusto et al., 2012). Among the most serious pollutants are Cu and Cd. Either in diet or in water, they are extremely toxic to fish (Roméo et al., 2000; Kalman et al., 2010). Worthy to mention that, knowledge of dietary Cu and Cd interaction still remains very scanty (Kamunde and MacPhail, 2011).

There is insufficient data concerning the toxic effects of dietary Cu, Cd and their mixture on tilapia, as one of the most important fish species all over the world. Therefore, the present work was intended to investigate genotoxicity of dietary Cu or Cd or their combination using chromosomal abnormalities of *O. niloticus*.

2. Materials and methods

2.1. Fish

Adult Nile tilapia *O. niloticus*, were obtained from, private fish farm close to El-Kanater El-Khairia, Qalubia, Egypt, and transported to faculty of science, Benha University. Their total length and total body weight ranged from 19.66 to 22.0 cm and 158.5 to 138.96 g, respectively. They were placed in well aerated four aquaria (58 cm length, 97 cm width, 85 cm height) the water temperature was kept at 22 ± 1 °C by using thermostat. Fish were acclimatized at laboratory for 10 days and fed on a standard fish diet, their ingredients is indicated in Table 1.

One hundred and eighty fish were used in the present study. They were divided into four groups; each one contained 45 fish as follows: the first Group (control), fish fed on standard diet, the second group (Cu group), fish fed on Cu Contaminated diet (2 g/kg dw diet), the third group (Cd Group), fish fed on Cd contaminated diet (10 g/kg dw diet) and the fourth group (Cu + Cd Group) Fish fed on Cu + Cd contaminated diet (2 g Cu + 10 g Cd/kg dw diet). The fish were fed to satiation two times every day for 30 days, the uneaten food was removed approximately after 4 h to avoid any possible food contamination. Half of each tank water was changed with dechlorinated tape water three times a week. The Cu and Cd in the water used in the experiment follow those of drinking water standard, not exceeding 1.3 and 0.005 mg/l, respectively (WHO, 2006). Every ten days 8 fish from each group were sacrificed for analysis.

Table 1 – Ingredients of fish feed.

Ingredients	%
Fish meal	9.1
Soybean flour	52.57
Corn flour	19.25
Starch	7.0
Corn oil	1.80
Cod liver oil	1.98
Vitamin premix[a]	2.00
Mineral premix[b]	2.00
Cellulose-α	3.30
Carboxy-methyl-cellulose	1.00

[a] Viatmin Premix (per kg of Premix), Thiamine, 2.5 g; riboflavin, 2.5 g; Pyridoxine, 2.0 g; inositol, 100 g; biotin, 0.3 g; pantothenic acid, 100.0 g; folic acid, 0.75 g; para-aminobenzoic acid, 2.5 g; choline, 200.00 g; nicotinic acid, 10.0 g; cyanocobalamine, 0.005 g; α-tocopheral acetate, 20.1 g; menadione, 2.0 g; retinol palmitate, 100,000 IU; Choleclaciferal, 500,000 IU.

[b] Mineral premix (g per kg of premix: $CaHPO_4 \cdot 2\ H_2O$, 727.2; $MgCO_3 \cdot 7H_2O$, 127.5 KCl, 50.0, NaCl, 60.0, $Fe\ C_6H_5O_7 \cdot 3H_2O$, 25.0; $ZnCO_3$, 5.5; $MnCl_2 \cdot 4H_2O$, 2.5 $CuCl_2$, 0.785).

2.2. Diet formulation

The normal diet ingredients were purchased from commercial suppliers at Benha city (about 40 km North Cairo) (see Table 1). Elevated level of Cu (2 g/kg dw diet) and Cd (10 g/kg dw diet) in diet were formulated using copper sulphate and cadmium chloride, respectively, as described by Szczerbik et al. (2006) and Shaw and Handy (2006).

2.3. Determination of Cu and Cd in fish muscle

Half gram of the dorsal epiaxial muscle of each of eight *O. niloticus* was digested in 3 ml of concentrated sulfuric acid and 3 ml distilled water using boiling water bath for 1 h. Then 4 ml of perchloric acid was added to complete the digestion process. The solution was then diluted to 25 ml with distilled water. The Cu and Cd contents were measured using PerkinElmer Atomic absorption spectrophotometer (model 2380 USA). It was standardized using 1000 ppm (μg/ml) of inorganic copper and cadmium in a nitric acid matrix.

2.4. Chromosomal abnormalities

Each of eight fish from each group was injected intramuscularly (IM) with 0.01 ml/g body weight of 0.03% of freshly prepared colchicine. The fish was then placed in a well-aerated glass aquarium for 4–6 h, and then was decapitated.

At the end of the experiment, the head kidney was carefully separated from the fish. It was used for chromosomal preparation using Al-Sabti et al. (1983) squash technique with some modifications. The isolated head kidney was washed with saline, cut into small pieces and well mixed in isotonic saline solution using Pasteur pipette. Then, it was centrifuged at 1000 rpm for 10 min. The supernatant was removed and the hypotonic solution (0.56% KCl) was added to the pellet of tissues and mixed thoroughly. The tissues were left in hypotonic solution for 40–50 min. In this step the cells were swollen and the chromosomes were separated. Latter, centrifugation was

Table 2 – Effect of diet contaminated with Cu, Cd and Cu + Cd on Cu and Cd contents (ppm) in the muscle of Nile tilapia (*Oreochromis niloticus*).

Groups	No	Cu		Cd	
	8	Mean ± SD	% Differences	Mean ± SD	% Differences
Control	8	0.542 ± 0.128	–	0.896 ± 0.156	–
Cu for 10 days	8	1.0 ± 0.153	84.502***	2.063 ± 0.177	130.245***
Cu for 20 days	8	0.575 ± 0.067	6.089	2.104 ± 0.212	134.821***
Cu for 30 days	8	0.416 ± 0.059	−23.247*	2.979 ± 0.156	232.477***
Cd for 10 days	8	0.958 ± 0.118	78.752*	1.646 ± 0.159	83.687***
Cd for 20 days	8	0.521 ± 0.059	−3.874	2.688 ± 0.306	199.944***
Cd for 30 days	8	1.208 ± 0.516	122.878**	3.021 ± 0.412	237.147***
Cu + Cd for 10 days	8	1.313 ± 0.540	142.250**	1.688 ± 0.270	88.337***
Cu + Cd for 20 days	8	0.448 ± 0.015	−17.343	3.021 ± 0.156	237.147***
Cu + Cd for 30 days	8	0.625 ± 0.285	15.314*	2.813 ± 0.335	213.950**

Data are mean ± SD. NO is the number of fish. *Significant difference from the control ($P < 0.05$), **Highly difference from the control ($P < 0.05$), ***Very highly difference from the control ($P < 0.05$), % Difference is the % difference from the control value.

carried out at 7000 rpm for 10 min and the supernatant was discarded.

Fixation was carried out in 8 ml of cold mixture of 1:3 (v:v) of glacial acetic acid, and methyl alcohol. The mixture was added drop-wise on the wall of the centrifuge tubes. The tissues were left in the fixative at 4 °C for about 30 min, then the tubes were centrifuged at 1000 rpm for 10 min, and the supernatant was discarded. This step was repeated for one or two times. At the last step, the supernatant was discarded and small amount of the fixative was added.

For every fish sample, cells suspension was concentrated and then spread by Pasteur pipette on clean-iced slides that were flamed on Bunsen burner. The chromosomal preparations were stained with 10% Giemsa for 45 min and then rinsed in distilled water and allowed to dry at room temperature.

For every fish at least 50 well metaphase spreads were examined for the chromosomal aberrations (structural and numerical) by research light microscope using oil immersion lens (Leitz, DiAlux 22 EB, Germany). Structural chromosomal aberrations were identified as the following: chromosome deletion as acentric fragment lost (Avers, 1980), chromosomal gap and breaks in which two chromaid appear are not of same length (Pai, 1985), chromosome fragmentation in which centromere is missed, end to end association in which two chromosomes are attached from one chromatid end (Hondt et al., 1981), centromeric attenuation that appear as separated chromatids (Darrance et al., 1975) and ring chromosome that appear in ring form (Avers, 1980). On the other hand, numerical chromosomal aberrations were assessed as polyploidy as the appearance of more than two sets of chromosomes (Avers, 1980) and chromosomes sickness that is related to improper folding of chromosme fiber into single chromatids and chromosome fibers are intermingled (Brogger, 1974). The mitotic activity was expressed as the mitotic index (Number of dividing cells/1000 cells).

2.5. *Statistical analysis*

The overall data are presented as mean ± standard deviation. Statistical analyses of the data were computed by SPSS (Version 10). Statistical analysis of the obtained data was computed to find the differences of fish exposed to each of Cu, or Cd or Cu + Cd contaminated diets for 10, 20 and 30 days from those of the control. Significant differences between every pairwise experimental groups were done using two paired Student's *t* test (Pipkin, 1984). It was presented at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ as a significant, high significant and very high significant differences, respectively.

3. Results

3.1. *Metals bioaccumulation*

The Cu level in flesh of *O. niloticus* was increased after 10 and 30 days of feeding on diet contaminated with Cu. Worthy to mention that, both Cu and Cd levels in fish flesh increased after 10 and 30 days due to feeding on diet contaminated with elevated levels of mixture of Cu and Cd (Table 2).

3.2. *Cytogenetic parameters*

The cytogenetic profile of the examined fish was tested by assessing the number of chromosomes, chromosomal abnormalities and a mitotic index for the head kidney cells of Nile tilapia (*O. niloticus*). The 22 chromosomes pairs displayed a normal pattern in fish fed on control diet (Fig. 1a).

The number of chromatid deletion in the head of kidney cells increased as a result of the diet contaminated with Cu, Cd and Cu + Cd (Table 3a, Fig. 1b). After 10 days, the Cd contaminated diet gave more drastic response followed by Cu + Cd and Cu contaminated diets and the incidence of chromatid deletion induced by metal contaminated diets was time dependent for Cu and Cu + Cd contaminated diets.

Diet contaminated with Cu induced significant increase of chromatid gap after 20 and 30 days (Table 3a, Fig. 1c). Chromatid gap revealed highly significant increases in their the mean values of fish fed on diet contaminated with Cd (after 30 d) and also fish fed on diet contaminated with Cu and Cd (after 20, 30 days).

Break of chromosome occurred when it has unstained area shorter than its diameter or equal to it (Fig. 1d). There were a significant ($P \leq 0.001$) increase of such chromosomes in the fish which were fed on diet contaminated with Cd and also fish

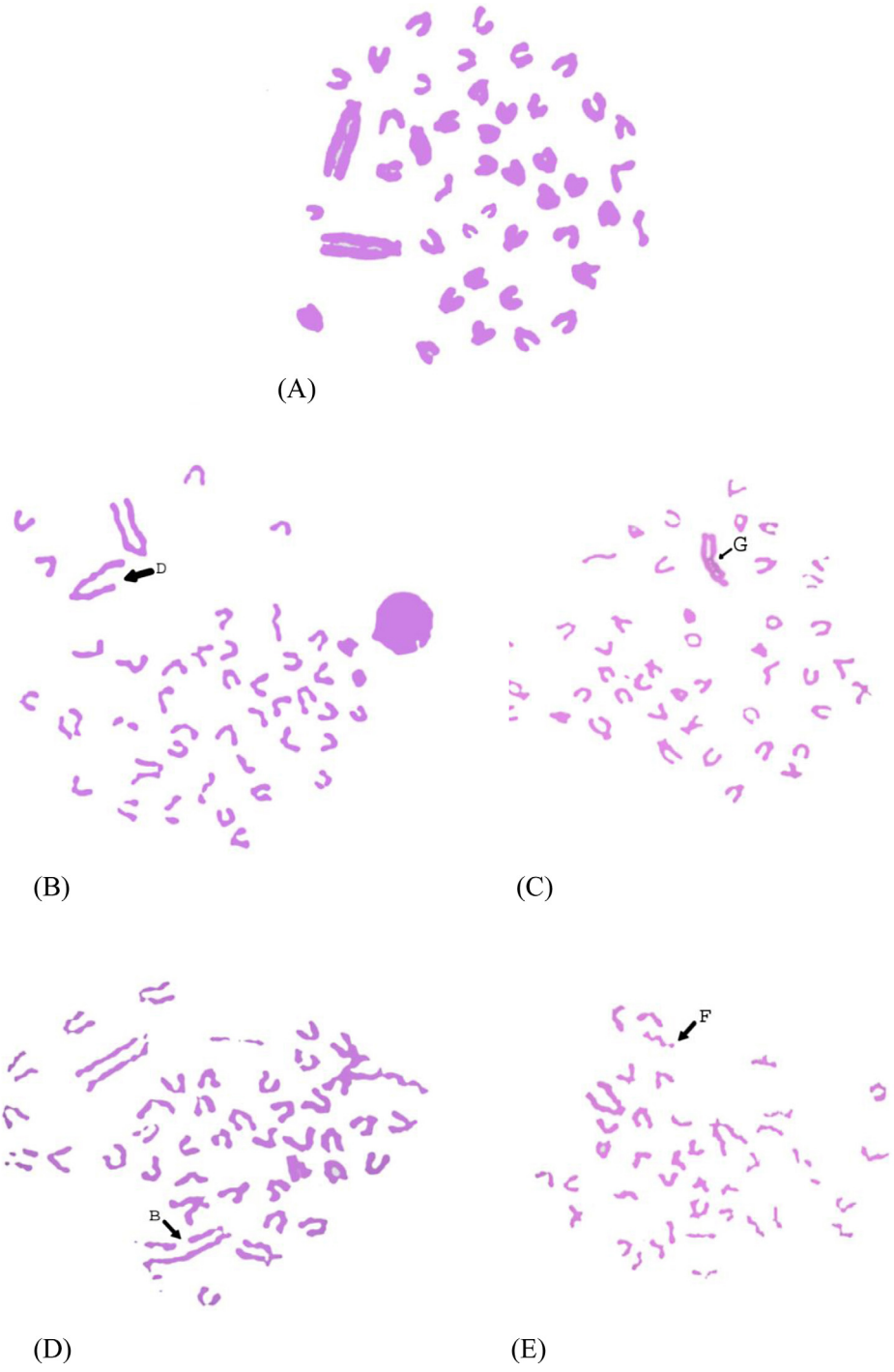

Fig. 1 – Photomicrograph of metaphase spread chromosomes (2000×) of head kidney cells of Nile tilapia (*Oreochromis niloticus*): (A) normal chromosomes from control fish, (B) abnormal chromosomes with chromatid deletion (D) from fish fed metal contaminated. (C) Abnormal chromosomes with chromatid gap (G) from fish fed metal contaminated. (D) Abnormal chromosomes with chromatid break (B) from fish fed metal contaminated. (E) Abnormal chromosomes with chromatid fragmentation (F) from fish fed metal contaminated.

which were fed on diet contaminated with mix group (Cu + Cd) (Table 3a).

Fragmentation of the chromosomes was observed when the chromatids lose its centromere (Fig. 1e). It is apparent that there were marked increases in their mean values at all experimental groups compared with control group (Table 3a). The mixture of Cu and Cd appear to induce additive effect in the chromatid fragmentation over those fed lonely on high dietborne Cu or Cd (Table 3a).

For Cu contaminated diet, there were significant increase in the mean incidence values of chromatid end to end association after 20 and 30 days over its normal range (Fig. 2a). Its

Table 3a – Effect of diet contaminated with Cu, Cd and Cu + Cd on chromosomal aberrations in the head kidney cells of Nile tilapia (*Oreochromis niloticus*).

Groups		Chromatid deletions	Chromatid gap	Chromatid break	Chromatid fragmentation	Chromatid end to end association
Control		2.4 ± 1.020	1.2 ± 0.748	1.2 ± 0.748	0.8 ± 0.748	0.4 ± 0.49
Cu contaminated diet	10 days	8.2 ± 2.227*	1.6 ± 1.020	2.6 ± 1.020	5.2 ± 1.166**	1.8 ± 1.166
	20 days	11.6 ± 3.262**	3.4 ± 1.020*a	6.4 ± 2.059**a	7.0 ± 1.414**	5.8 ± 3.311*
	30 days	14.6 ± 1.020***a	4.6 ± 1.625*a	6.8 ± 1.470**a	7.8 ± 1.720**	6.2 ± 2.561*a
Cd contaminated diet	10 days	10.4 ± 1.200***	2.2 ± 0.748	4.8 ± 1.720*	6.0 ± 1.414**	2.4 ± 1.020*
	20 days	13.6 ± 2.577**	7.2 ± 2.926*a	9.2 ± 2.482**a	7.8 ± 1.720**	6.8 ± 1.720**a
	30 days	15.4 ± 3.611**	7.8 ± 2.135**a	10.4 ± 2.577**a	16.0 ± 2.280***ab	10.0 ± 2.966**a
Cu + Cd contaminated diet	10 days	11.2.445**	3.4 ± 2.059	7.0 ± 1.414**	8.0 ± 2.449**	4.2 ± 1.166**
	20 days	14.6 ± 2.88**	9.8 ± 3.059**a	9.4 ± 2.245**	10.0 ± 2.0***	10.2 ± 2.040***a
	30 days	17.4 ± 2.728***a	10.6 ± 3.072**a	12.0 ± 2.191***a	17.0 ± 2.786***b	13.2 ± 2.040***a

Data are mean ± SD of the number of cells with chromosomal aberrations/50 metaphase cells for 5 fishes. *Significant difference from the control ($P < 0.05$), **Highly difference from the control ($P < 0.05$), ***Very highly difference from the control ($P < 0.05$), a significant difference from 10 days group ($P < 0.05$), b significant difference from 20 days group ($P < 0.05$).

frequency in head kidney cells of *O. niloticus* were elucidated a significant increase ($P \leq 0.001$) in fish raised on diet contaminated with Cu and Cd (Mix group) and also in fish fed on diet contaminated with Cd, over those of the control. At the same time, in this type of aberration the higher values of abnormalities appear for fish fed on diet contaminated with Cu than control (Table 3a). Thus, end to end chromosomal association was more drastic for fish fed diet contaminated with high Cd and Cu + Cd than those fed diet contaminated with high Cu.

There was a chromosomal break in the centromeric region, led to separation of the two chromatids (Fig. 2b). The numbers of chromosomes having centromeric attenuation in head kidney cells of *O. niloticus* were markedly increased due to feeding on different metal contaminated diets (Table 3b). In relation to the control group, there were very highly significant increases ($P \leq 0.001$) in the mean values of centromeric attenuation of fish fed on diet contaminated with Cd and on mixture of Cd and Cu (after 20 and 30 days). On the other hand, the difference between the average values of this chromosomal abnormality of control and fish fed on Cu contaminated diet was significant ($P \leq 0.01$) at most of the tested durations (Table 3b).

Ring chromosomes occurred when chromosomes lack free ends but form a continuous ring (Fig. 2c). Head kidney cells recorded number of ring chromosomes, which showed a significant ($P \leq 0.01$) increase in fish fed on diet contaminated with Cu (Table 3b). Similar results were observed for Cd contaminated group. Mostly, the recorded mean values of ring chromosomes for fish fed on Cu + Cd was very highly significantly increased, over those of the control fish. This revealed additive effect of mixture of Cu and Cd on induction of ring chromosomes. Further, the ring chromosomal abnormality may be sensitive for the additive effect of Cu and Cd.

Stickiness is due to improper folding of chromosome fiber into single chromatids and chromosome fibers are intermingled. As a result, the chromosomes become attached to each other (Fig. 2d). The incidence of chromosomes stickiness has increased for fish fed on all tested contaminated diets (Table 3b). For Cu contaminated diet, after 10 and 20 days, there were a significant increase in stickiness chromosomes compared with the control value. The recorded mean values of stickiness chromosomes of fish fed on contaminated diet showed a very highly significant increase at both groups of Cd and Cu + Cd over the control values.

There were highly significant increases in the incidence rate of polyploidy in Cu + Cd group and Cd group over those of the control. Only after 30 days, there was significant difference between control fish and fish fed on diet contaminated with Cu (Table 3b, Fig. 2e). Thus, the data of the assessment of different chromosomal aberrations evoked potentiality of dietborne Cd toxicity than dietborne Cu and the additive effect of their mixture. Also, the data declared the following toxicity order, Cd > Cu + Cd > Cu.

3.3. *Mitotic index*

It was evident that the mean values of the mitotic index of the treated fish (Cu, Cd and Mix groups) exhibited a marked decrease, than that of the control (Table 3b). For fish fed Cu contaminated diet, the reduction in the values of mitotic index from those of the control was significant, highly significant and very highly significant after 10, 20 and 30 days, respectively, i.e., time dependent effect. For Cd contaminated diet, its reduction was more severe. Its values were reduced with highly significant difference (10 days) and very highly significant difference (After 20, 30 days) from the control value.

4. Discussion

Metals accumulation in different fish tissues can serve as a bioindicator for aquatic pollution (Squadrone et al., 2015). Metals accumulation in vital organs affects fish health and distribute differently in their tissues (Squadrone et al., 2013). Generally, metals accumulate in higher extent in fish liver followed by gills and kidney (Ben Salem et al., 2014). The fish have a higher position in food chain that terminate by the human being (Fang et al., 2009. Therefore, metals accumulation in fish flesh provides useful data for human consumption (Squadrone et al., 2013). The present study reported higher accumulation of Cu and Cd in *O. niloticus* flesh when they feed

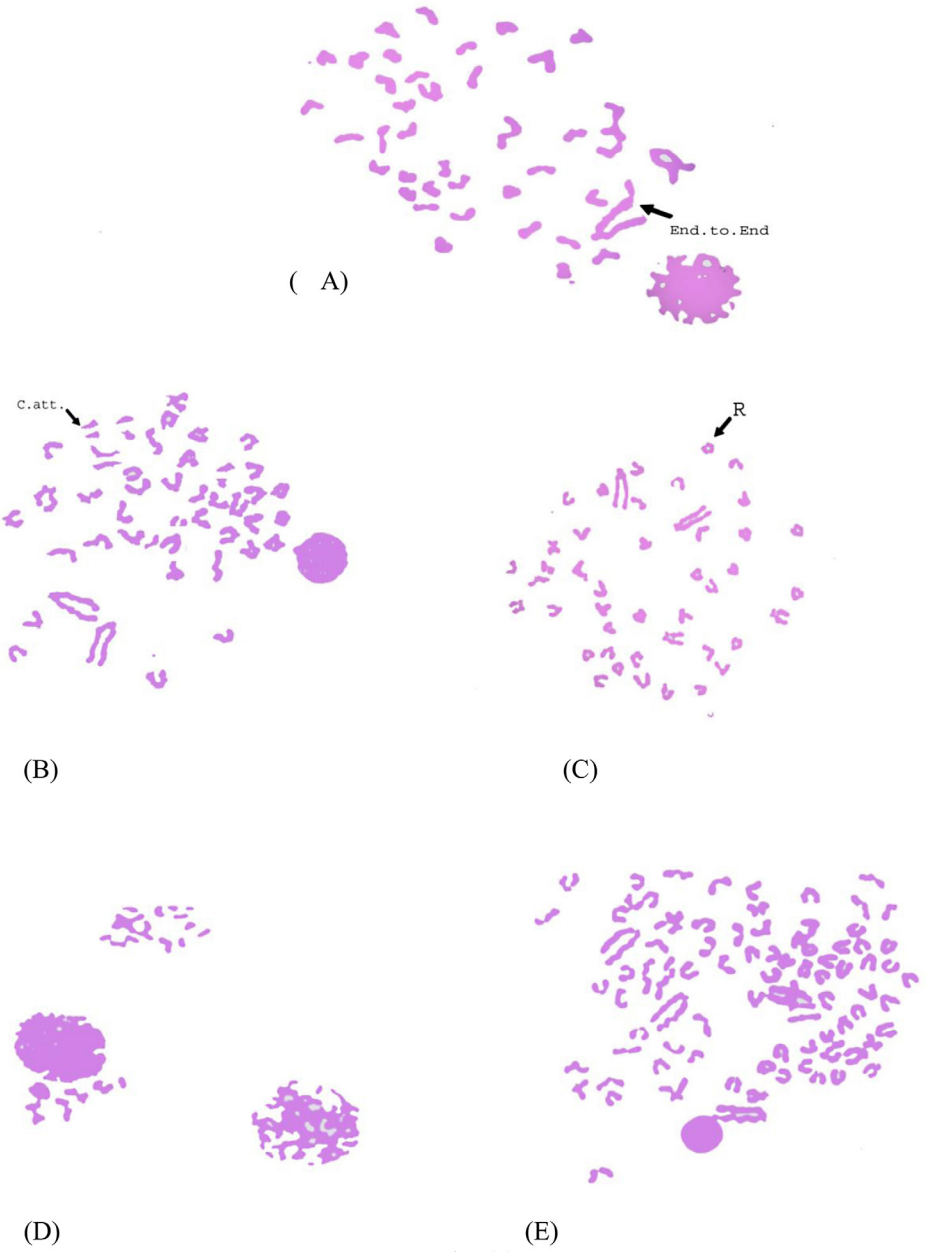

Fig. 2 – Photomicrograph of metaphase spread chromosomes (2000×) of head kidney cells of Nile tilapia (*Oreochromis niloticus*): (A) abnormal chromosomes with chromatid end to end association (end to end) from fish fed metal contaminated. (B) Abnormal chromosomes with chromatid centromeric attenuation (C. att.) from fish fed metal contaminated. (C) Abnormal chromosomes with chromatid ring (R) from fish fed metal contaminated. (D) Abnormal chromosomes with chromatid stickiness from fish fed metal contaminated. (E) Abnormal chromosomes with chromosomal polyploidy from fish fed metal contaminated.

on diet containing high Cu, Cd or their mixture. This can pose serious risk for human health through consumption of these fishes.

In the present study, various chromosomal aberrations were observed in the spreads of kidney cells of Nile tilapia (*O. niloticus*) at the different groups. These aberrations include centromeric attenuation, break, deletion, fragmentation, gap, end to end association, ring, polyploidy, and stickiness. It has been observed that the frequency of deletion in the chromosomes was very highly significantly increased at all groups, compared to that of the control group. Earlier, chromosomal abnormalities were reported for grass carp (*Ctenopharyngodon idella*) exposed to sevin insecticide (Mattar et al., 1992 Also, the heavy metals is known to induce high frequency of chromosomal aberrations (chromatid deletion, fragments, ring chromosome, stickiness, chromosome gap, chromatid gap, haploidy and polyploidy) on the catfish, *Channa punctatus* (Yadav and Trivedi, 2009).

The number of gap and polyploidy were highly significantly increased in fishes fed on contaminated diet with Cd and

Table 3b – Effect of diet contaminated with Cu, Cd and Cu + Cd on chromosomal aberrations in the head kidney cells of Nile tilapia (*Oreochromis niloticus*).

Groups		Chromatid centromeric attenuation	Chromatid ring	Chromatid stickiness	Chromosomal polyploidy	Mitotic index
Control		11.6 ± 3.007	0.8 ± 0.748	0.8 ± 0.748	1.0 ± 1.095	122.2 ± 3.429
Cu contaminated diet	10 days	29.4 ± 4.673**	7.2 ± 1.60**	1.6 ± 1.020	1.2 ± 1.166	114.6 ± 3.720*
	20 days	37.6 ± 2.059***	7.6 ± 1.497**	3.0 ± 1.095*	2.4 ± 1.020	102.4 ± 4.758**a
	30 days	43.0 ± 10.900**	10.8 ± 3.763**	3.6 ± 1.020*	4.0 ± 1.873*	95.8 ± 3.970***a
Cd contaminated diet	10 days	32.0 ± 3.406*	7.4 ± 1.020**	2.0 ± 1.095	1.8 ± 1.327	104.6 ± 4.030**
	20 days	41.2 ± 3.059***a	9.8 ± 3.970*	5.0 ± 1.095**a	5.6 ± 2.059*a	98.2 ± 4.118***
	30 days	49.8 ± 1.327***ab	14.4 ± 3.61**a	7.8 ± 2.713**a	6.0 ± 1.789**a	85.8 ± 3.868***ab
Cu + Cd contaminated diet	10 days	32.4 ± 4.079*	8.0 ± 2.098**	2.8 ± 1.166*	3.8 ± 1.166*	103.8 ± 5.946**
	20 days	41.8 ± 3.311***a	10.2 ± 1.60***	6.4 ± 2.154**a	7.6 ± 2.059**a	95.6 ± 5.851**
	30 days	50.2 ± 5.192***ab	14.6 ± 2.059***ab	10.0 ± 2.0***a	8.6 ± 1.855**a	84.6 ± 2.577***ab

Data are mean ± SD of the number of cells with chromosomal aberrations/50 metaphase cells for 5 fishes. *Significant difference from the control ($P < 0.05$), **Highly difference from the control ($P < 0.05$), ***Very highly difference from the control ($P < 0.05$), a significant difference from 10 days group ($P < 0.05$), b significant difference from 20 days group ($P < 0.05$).

mixture of Cd and Cu at all tested durations compared to that of the control group. These results are differed to those recorded by Al-Sabti (1985), Svetlana et al. (1994) and Pak et al. (2012) for different fish species exposed to environmental pollution. The frequencies of chromosomal aberrations (chromatid gaps and breaks) are the most dominant aberration in *O. niloticus* exposed to aquatic weedicide (Hamdoon and Seddky, 1995).

Although most toxicity data were expressed as dietborne Cu concentration but, characterizing dietborne Cu toxicity by daily doses (using daily rations) provides a clear data about its toxicity in relation to the duration of exposure (Clearwater et al., 2002). Dietary Cu at 34 and 691 mg Cu kg day^{-1} dry diet significantly increased intestinal cell proliferation, intestinal apoptosis and intestinal metalothionein levels and decreased growth rate of Atlantic salmon parr (*Salmo salar*) but do not significantly affect survival rate (Clearwater et al., 2002). They have been found that the dietborne Cu ranging 35–45 mg kg^{-1} body weight day^{-1} is at the threshold of chronic toxicity to rainbow trout (Clearwater et al., 2002). In contrast, another study on juvenile rainbow trout showed minimal negative impacts of 10,000 mg Cu kg^{-1} dry diet (70 mg Cu kg^{-1} day^{-1}) on growth and survival after 4 weeks exposure (Handy, 1993). On the other side, sub-lethal dietary Cd has been found to affect ionoregulation and cause physiological malfunctioning in fish (Baldisserotto et al., 2005; Ng et al., 2009). Both, dietary Cu and Cd have been found to induce oxidative stress on fish (Clearwater et al., 2002; Jiang et al., 2014).

It was apparent from the present study that the chromosomal break, end to end association, ring and centromeric attenuation of *O. niloticus* fed on diet contaminated with Cd and mixture of Cd and Cu were very highly significantly increased from the values of the control. Genotoxicity of heavy metals reported herein was recorded by Chandra and Khuda-Bukhsh (2004) for *O. mossambicus* exposed to $CdCl_2$. The results obtained herein were concurrent with the studies of Krishna and Gupta (2002) who found that the effects of sub-lethal levels of copper, cadmium, zinc and Aflatoxin on fingerlings of *Labeo rohita* at different durations increase the frequencies of chromosomal aberrations included break, acentric chromosomes, ring chromosomes and abnormally small and thick chromosomes. Mathew and Jahageerdar (2003) observed that increased percentage of chromosomal aberrations of *C. punctatus* with the increasing levels of mercuric nitrate in water. Mahmoud (2006) indicated that exceeding permissible levels of some heavy metals in River Nile at Qalubia Province (Egypt) induce chromosomal changes in Nile tilapia (*O. niloticus*). The present study confirms the previously reported results for using fish as a model in carrying out genotoxic investigations relating to contaminated food. Such phenomenon is also confirmed by Pak et al. (2012).

In conclusion, the obtained results provoked a genotoxicity of dietborne metals to *O. niloticus*. Therefore, the present study, advice to pay more attention of fish feed from the possible contamination with heavy metals.

Conflict of interest

The authors declare that there are no conflicts of interest in this article.

Transparency document

The Transparency document associated with this article can be found in the online version.

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