



Effects of Ni and Cd contaminated fish meat on chromosomal aberrations and sperm morphology of Swiss albino mice

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Abstract: two heavy metals namely Ni and Cd were determined in a freshwater fish, *Barbusxanthopterus* using 700-atomic absorption: the highest values were found to be ($82.112 \mu\text{g g}^{-1}$), and ($4.702 \mu\text{g g}^{-1}$), respectively. Flesh of this fish was fed to mice in two groups for each two periods (1 and 2 months) to monitor chromosomal aberrations and sperm morphology. In Group (1) first period: the ring chromosome (3.2 ± 0.200) and centromeric gap (2.9 ± 0.314) have the highest value of aberration type, while the lowest value was chromatid break (0.0 ± 0.000), in second period: the centromeric gap (4 ± 0.333) and chromatid gap (3.4 ± 0.266) have the highest value of aberration type, while the lowest value was polyploidy (0.6 ± 0.266). In Group (2) first period: the highest value of abnormality type was sperm without tail (4.9 ± 0.276) swollen head sperm (4.1 ± 0.622) and sperm without hook (3.9 ± 0.433), while the lowest value was blunt hook sperm (0.0 ± 0.000). In second period: the highest value of abnormality type was defective head sperm (4.2 ± 0.416) and sperm without tail (3.7 ± 0.488), swollen head sperm (3.7 ± 0.667), and the lowest value was blunt hook sperm (1.0 ± 0.421).

Key words: fishes, Ni, Cd, mice

I. Introduction

Human activities, such as industrial and traffic emissions and various land-use practices may increase heavy metal loading into aquatic ecosystems [1]. Tulonen *et al.*, (2006) reported that in aquatic systems, the natural concentrations of metal ions are principally dependent on the ambient distribution, weathering and leaching of these elements from the soil in the catchments area. They also stated that heavy metals are carried to the lakes through atmospheric deposition and/or discharge. The characteristics of water, such as acidity or the amount of organic matter, are known to be important factors in determining the fate of heavy metals in lakes. It is well known that the heavy metals from man-made pollution sources are continually released into aquatic ecosystem. The contamination of heavy metals is a serious threat because of their toxicity, long persistence, bioaccumulation and biomagnifications in the food chain [2] (Authman, 2008). [2] Authman (2008) also believe that fishes can be considered as one of the most significant biomonitors in freshwater systems for the estimation of metal pollution concentration. In addition, fishes are located at the end of the aquatic food chain and may accumulate metals and pass them to human beings through food causing chronic or acute diseases (Al-Yousif *et al.*, 2000) [3]. The heavy metal concentration in fish tissues reflects past exposure via water and/or food and it can demonstrate the current situation of the animals [4] (Birungiet *et al.*, 2007).

It was revealed from the studies from both field and laboratory experiments that accumulation of heavy metals in a tissue is mainly dependent upon water concentrations of metals and exposure period; although some other environmental factors such as salinity, pH, hardness, and temperature play significant roles in metal accumulation. Ecological needs, sex, age, size, feeding habits as well as biological conditions of the fish affect the heavy metals accumulation in their tissues [5] (Canli and Atli 2003).

Due to the deleterious effects of metals on aquatic ecosystems, it is necessary to monitor their bioaccumulation, because this will give an indication of the temporal and spatial extent of the process, as well as an assessment of the potential impact on organism health [6] (Fernandes *et al.*, 2007) and in order to check for those hazardous to human health [2] (Authman, 2008). Recently, [7] Sofi (2013) found administration of cadmium chloride generally produced chromosomal aberrations as well as sperm abnormalities compared to the control.

The aim of this study was to determine the levels of toxic metals (Ni and Cd) in the water, and fishes and to determine their potential effects on chromosomal aberration, sperm abnormalities and on mice fed flesh of *Barbusxanthopterus* (Gatan) contaminated with (Ni and Cd).

II. Material and methods

Local Fish Samples were collected from some shops in Erbil and Sulaimani cities, and then analyzed for determination of Ni and Cd, by 700-atomic absorption spectrophotometer [8] (APHA, 2005).

Adult male laboratory mice *Mus musculus* strain BALB/c (8-10 weeks) in age, used in the present study. Diet containing prepared fish muscle instead of protein was produced, and then was given to two groups of mice for each two periods (1 and 2 months). The control groups (10 for each group) were taken the standard diet for the same periods. Chromosomal preparations from bone marrow cells were done by standard method of [9]

(Evans *et al.*, 1964). All slides were evaluated blindly at 1000X magnification for chromosomal aberrations. Hundred well spread complete metaphases were scored per each slide. Sperm was taken from epididymis using method of [10] (Karanowska, 1976) and [11] (Wyrubek and Bruce, 1978). The prepared slides underwent microscopic examination to determine number of normal sperms and abnormal sperm out of 100.

III. Statistical analysis

Statistical analysis was carried out using statistically available software (SPSS version 17). Comparisons between groups were made using one-way analysis of variance (ANOVA) in combination with Duncan t-test post hoc analysis. Duncan t-test treats one group as a control, and compares all other groups against it. P values <0.01 were considered significant.

IV. Results and discussion

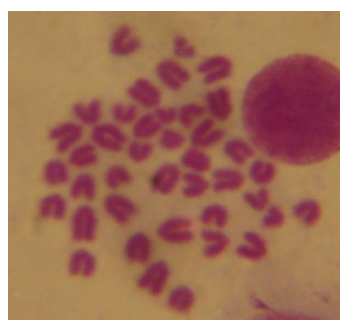
As indicated in the Table (1), the highest value of Ni of Gatan, was (82.112 $\mu\text{g g}^{-1}$), while the lowest value was (29.557 $\mu\text{g g}^{-1}$). The highest value of Cd of the same fish was (4.702 $\mu\text{g g}^{-1}$), but the lowest value was (1.187 $\mu\text{g g}^{-1}$). The heavy metals in this fish may depend on their food intake especially food chain of fish which is not known yet. Other possibility is their capability of ions absorption from surrounding water which again needs *in situ* investigation. The third possibility is the intrinsic physiology of fish in its pond, especially metabolic activity. Anyhow, pollution by heavy metals is reported from other regions of Iraq, as bioaccumulation of copper, cadmium, lead and zinc is reported in *Barbusdelayewi* and *Barbusgrypus* in Diyalariver at Rustemyia an area where municipal and industrial sewage is thrown into the river [12](Coad, 2010).[1]Tulonen *et al.*, (2006) found that metal concentration in perch (*Percafluviatilis*) were higher in a humic and acid lake than in a slightly humic lake partly be explained by varying dietary regime of perch. Anyhow, further investigation is required to explore the diet of the present fish, *B. xanthopterus* especially at the site of catchment.

Table (1): showing the amount of heavy metals (Ni and Cd) represented in μg present in the muscles of Gatan, *Barbusxanthopterus* examined.

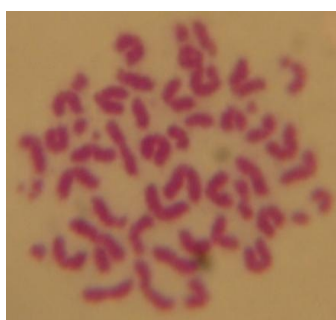
Heavy metal	Fish types	Minimum ($\mu\text{g gm}^{-1}$)	Maximum ($\mu\text{g gm}^{-1}$)
Ni	<i>Barbusxanthopterus</i> ,	29.557	82.112
Cd	<i>Barbusxanthopterus</i> ,	1.187	4.702

A. Effects of fish meat on chromosomal aberration in bone marrow cells of male albino mice

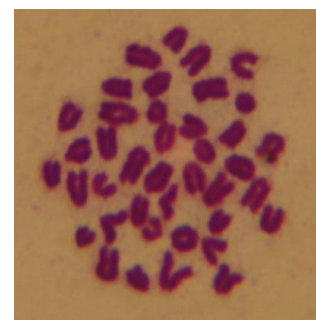
The results of chromosomal aberrations were summarized in Table (2) which indicate the significant differences ($P < 0.01$) between treated groups with fish meat and negative control (PBS) in total normal chromosome, total abnormal chromosome, centromeric gap, centromeric break, chromatid gap, chromatid break, ring chromosome, dicentric chromosome, pulverization and polyploidy (Figure 1), but there were no significant difference ($P < 0.01$) in chromatid break in (G1) (in first period) and pulverization in (G2) (in second period) when compared with the negative control. The ring chromosome (3.2 ± 0.200) and centromeric gap (2.9 ± 0.314) have the highest value of aberration type in (G1), while the lowest value was chromatid break (0.0 ± 0.000). While the centromere gap (4 ± 0.333) and chromatid gap (3.4 ± 0.266) has the highest value of aberration type in (G2), while the lowest value was polyploidy (0.6 ± 0.266).



Normal metaphase



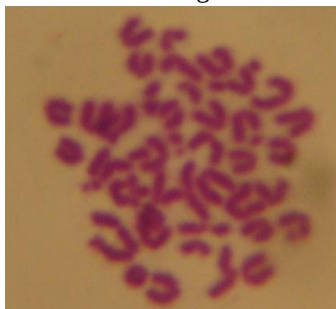
Acentric fragment



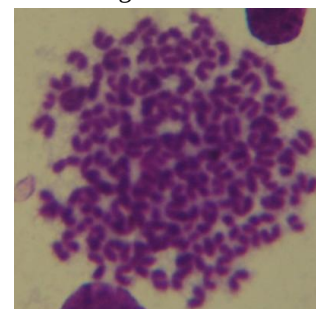
Ring chromosome



Pulverization



Chromatid gap & break



Polyploidy

Figure (1): Types of structural and numerical chromosomal aberrations (1000 X)

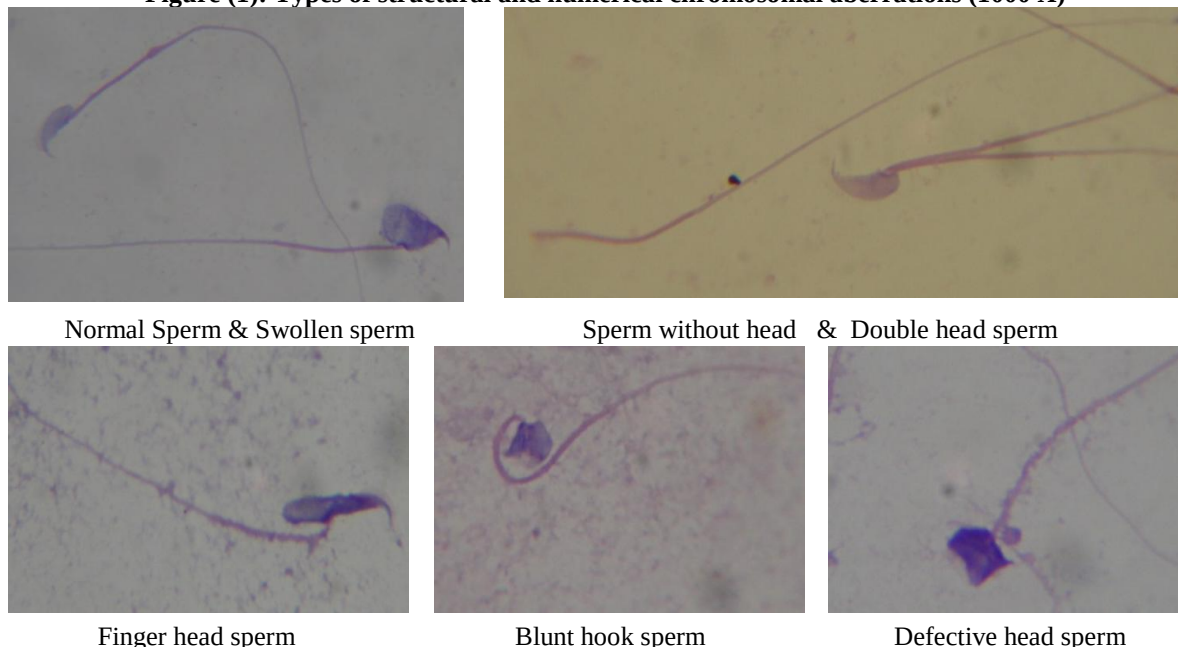


Figure (2): Types of sperm abnormalities (1000 X)

Table (2): Effects of fish meat on chromosomal aberrations in male albino mice (Mean $\pm$ SE) (P<0.01).

Treatments	Total normal Chromo.	Total abnormal Chromo.	Centromeric Gap	Centromeric Break	Chromatid gap	Chromatid Break	Ring Chromo.	Dicentric Chromo.	Pulverization	Acentric Fragment	Polyploidy
Control	94.666 $\pm$ 0.333 ^b	5.333 $\pm$ 0.333 ^a	1 $\pm$ 0.258 ^a	0.333 $\pm$ 0.210 ^a	1.333 $\pm$ 0.210 ^a	0.333 $\pm$ 0.210 ^a	0 $\pm$ 0.000 ^a	0 $\pm$ 0.000 ^a	0.833 $\pm$ 0.166 ^a	1.166 $\pm$ 0.166 ^a	0.166 $\pm$ 0.166 ^a
Group 2 Period I (1 Month)	82.2 $\pm$ 1.209 ^a	17.8 $\pm$ 1.209 ^b	2.9 $\pm$ 0.314 ^b	2.1 $\pm$ 0.233 ^b	2.4 $\pm$ 0.371 ^{ab}	0 $\pm$ 0.000 ^a	3.2 $\pm$ 0.200 ^b	1.4 $\pm$ 0.400 ^{ab}	2.5 $\pm$ 0.307 ^b	2.2 $\pm$ 0.290 ^a	1.1 $\pm$ 0.214 ^a
Group 2 Period II (2 Months)	78.9 $\pm$ 0.993 ^a	21.1 $\pm$ 0.993 ^b	4 $\pm$ 0.333 ^b	2.8 $\pm$ 0.200 ^b	3.4 $\pm$ 0.266 ^b	2.4 $\pm$ 0.163 ^b	2.9 $\pm$ 0.276 ^b	1.7 $\pm$ 0.366 ^b	1.3 $\pm$ 0.300 ^{ab}	2.1 $\pm$ 0.314 ^a	0.6 $\pm$ 0.266 ^a

Note: Similar letters in each column refer to non significant difference while different letters refer to significant difference between them.

Treatments	Total normal sperm	Total abnormal sperm	Sperm without head	Sperm without tail	Sperm without hook	Double head sperm	Double tail sperm	Swollen head sperm	Defective head sperm	Blunt hook sperm
Control	92 $\pm$ 0.447 ^b	8 $\pm$ 0.447 ^a	2.333 $\pm$ 0.333 ^a	2.5 $\pm$ 0.223 ^a	0.667 $\pm$ 0.210 ^a	0.166 $\pm$ 0.166 ^a	0.166 $\pm$ 0.166 ^a	0.833 $\pm$ 0.307 ^a	0.666 $\pm$ 0.333 ^a	0.333 $\pm$ 0.210 ^a
Group 2 Period I (1 Month)	78.9 $\pm$ 1.852 ^a	21.5 $\pm$ 1.641 ^b	3.1 $\pm$ 0.276 ^a	4.9 $\pm$ 0.276 ^b	3.9 $\pm$ 0.433 ^b	1.5 $\pm$ 0.521 ^{ab}	1.2 $\pm$ 0.326 ^a	4.1 $\pm$ 0.622 ^b	2.8 $\pm$ 0.249 ^b	0 $\pm$ 0.000 ^a
Group 2 Period II (2 Months)	77.5 $\pm$ 1.984 ^a	22.5 $\pm$ 1.984 ^b	3.2 $\pm$ 0.249 ^a	3.7 $\pm$ 0.448 ^{ab}	3 $\pm$ 0.149 ^b	2.1 $\pm$ 0.406 ^b	1.6 $\pm$ 0.498 ^a	3.7 $\pm$ 0.667 ^b	4.2 $\pm$ 0.416 ^b	1 $\pm$ 0.421 ^a

Table (3): Effects of fish meat on sperm abnormalities in male albino mice. (Mean $\pm$ SE) (P<0.01).

Note: Similar letters in each column refer to non significant difference while different letters refer to significant difference between them.

It was clear from the Table (2) that both treatment periods of fish meat were increased the quantity of chromosome aberration, but the most effective time was second period (after 2 months).

It was found from experiments on male Wistar rats received NiCl_2 in the dose of 50, 150, and 300 mg/kg that chromosome aberrations were increased significantly by NiCl_2 300 mg/kg as compared to the control group ($p < 0.001$) [13](Tarasub and tarasub,1992). Furthermore, Ni compounds have been shown to produce single-strand breaks in cellular DNA, as well as chromosomal aberrations and DNA-protein crosslinks ([14 & 15]Patierno and Costa, 1985;Misra et al., 1993). It has been suggested that Ni was not directly involved. Furthermore, in an interesting experiments of[16] Fahmy and Aly(2000) by using cadmium chloride they found a significant increase in the percentage of chromosomal aberrations in mouse bone marrow at the dose 5.7 and 9.5 m Kg-1.

B. Effects of fish meat on sperm morphology in male albino mice

The results of sperm morphology were presented in Table (3) which indicated significant difference ($P < 0.01$) between treated groups with fish meat and negative control in most parameters analyzed (total normal sperm, total abnormal sperm, sperm without tail, sperm without hook, double head sperm, double tail sperm, swollen head sperm and defective head sperm (Figure 2).

The highest value of abnormality type in G(1) was sperm without tail (4.9 ± 0.276) swollen head sperm (4.1 ± 0.622) and sperm without hook (3.9 ± 0.433), while the lowest value was blunt hook sperm (0.0 ± 0.000). The highest value of abnormality type in G(2) was defective head sperm (4.2 ± 0.416) and sperm without tail (3.7 ± 0.488) and the lowest value was blunt hook sperm (1.0 ± 0.421).

It was concluded from the experiments of [17]Wadi and Ahmad(1998) that lead targets testicular spermatogenesis and sperm within the epididymis to produce reproductive toxicity rather than acting at other sites within the hypothalamic-pituitary-testicular axis after administering the mice with two concentrations of lead (0.25% and 0.5%) via drinking water for 6 weeks.

On the other hand Chromium dichromate was significantly decreased the body weight gain, food and fluid intake, and epididymal sperm number, but had no effect on testis weight compared to control group after treated male mice (CD-1) with 150 ppm potassium dichromate for 12 weeks as indicated by the experiments of [18] Afonneet *al.*, (2002). In previous experiments, [17]Wadi and Ahmad(1999) found after administering two concentrations of lead(0.25% and 0.5%) via drinking water in mice, the low dose significantly reduced the number of sperms within epididymis while the high dose reduced the count percentage of motile sperms and increased the percentage of abnormal sperms within the epididymis. Furthermore, reduction of spermatocytes was observed after administration of cadmium chloride(0.9, 1.9, 5.7 mg Kg⁻¹(Fahmy and Aly, 2000)[16].

The possible mechanism in heavy metal toxicity includes the DNA-protein crosslinks by the formation of oxygen radicals (Kasprzak, 1991; Lin *et al.*, 1992; Tarasub and Tarasub,1992)[13,19, 20 & 21].

V. Conclusions and Recommendations:

1. There is a positive relation between contaminated fish meat and chromosomal aberrations as well as sperm abnormalities in albino mice compared to the control.
2. People must be aware for protecting environment in general and water resources in special.
3. Monitoring the water resources periodically so as to control any change that occur in its quality.
4. Treatment of waste water before its discharge to the lakes and streams.
5. Monitoring Fish quality and quantity.
6. Controlling all fish aquariums in the area.

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