

DNA Damage in Common Carp Fish (*Cyprenus carpio* L.) Induced by Acute Exposure to Copper & Cadmium

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Abstract: This study was designed to detect the effect of heavy metals on DNA characteristics in common carp (*Cyprenus carpio* L.). Three concentrations (1ppm, 1.5ppm, 2ppm) have been selected for copper & cadmium respectively with acute exposure (96 hrs.). The results showed that high LT50 found in 1.5ppm while the lowest value of LT50 recorded in 2ppm. DNA damage fluctuated according to different exposure concentrations, therefore some lanes showed high fragmentation than another in both Fin and superficial tissues. Also, DNA extracted from fin is most fragmented than superficial tissues and Common carp DNA is more sensitive to low concentrations of heavy metals.

Key words: *Common carp* fish • DNA damage • Heavy metals • Toxicity • DNA fragmentation

INTRODUCTION

With industries development, pollution by heavy metals has considerable effect on aquatic organism. Metal ions with potential toxicity are known to cause huge defect on organ and blood levels in fish which they form metal complexes with structural protein, enzymes and nucleic acids and as consequence disturb their functions. Continuous accumulation of toxic heavy metals in common carp may effect hepatic function and cause cellular degeneration. [1]. Cadmium are widely distributed toxic environmental and industrial pollutants and may causes danger to growth and physiology of aquatic organism [2], it's already, copper toxicity to fish coincides with physicochemical parameters (pH, Temperature, E.C, Salinity, T.D.S and Dissolved oxygen) and this toxicity increase with water acidity.

[3] found through study the effect of zinc on lipids of erythrocytes from common carp with different temperature degrees, the heavy metals effect on aquatic organism by two means: a direct effect on the metabolic pathways cause changes in structure and physicochemical properties of fish membrane which leads to cell damage as well as long term functional and structural changes in the aquatic ecosystem. Evaluation of DNA damage in common carp has been done by [4]

for determination of possible pollution in Moagn (Ankara) by comet assay. The results showed that lake may be polluted by many genotoxic substances according to comet assay index. [5] Identified the expression of the genes of metal responsive Transcription factor -1 & GPX in common carp in addition to many studies interesting in common carp [6, 7].

Generally, common carp considered is one of major consumers as a food for Iraqi people and broadly used in estimation of genotoxicity studies. Although tolerant of most conditions, common carp prefer large bodies of slow or standing water and soft with preferring in groups of five or of pH water with brackish or slightly fresh climates in temperate more.

This research aimed to evaluate the DNA damage in common carp fish when exposed to acute different concentration of heavy metals and to determine the differences in DNA damage between Fin and superficial tissue.

MATERIALS AND METHODS

Exposure Protocol: *Cyprenus carpio* L. samples were collected from Al-Furat fish farm in Babylon province, once arrival to laboratory, they acclimated in aquarium full with dechlorinated tap water for 7 days and starved for

24 hrs. with photoperiod 12:12 light & dark cycle. The samples divided into different groups according to different concentration of copper & cadmium (1ppm, 1.5ppm, 2ppm) respectively and exposure extended to 96hrs, as acute exposure in aquarium under control conditions. Standard concentrations of copper & cadmium prepared from $\text{Cu}_2\text{SO}_4 \cdot 5\text{H}_2\text{O}$ & $\text{CdNO}_3 \cdot 4\text{H}_2\text{O}$ respectively.

LT50 (Median Lethal Time): Median lethal time was determined using a log probable paper according to [8].

DNA Damage Evaluation: Immediately soft tissue & fin specimens were collected from common carp from fin and superficial tissue and processed according the protocol clarified by Promega Corporation, Madison, WI, U.S.A. After extraction, DNA samples were tested by UV Trans illuminator type (CS-Cleaver scientific Ltd., U.K).

RESULTS AND DISCUSSION

The risk of heavy metals pollution returned to their persistency in aquatic ecosystem. The results showed that (3548.1) is a highest value of LT50 for 1.5ppm and (0.161) is a lowest value of LT50 for 2ppm and this return to variation in fish response to different concentration and in some time, some species don't appear any response due to their acclimation to stress factors such as heavy metals. Common carp may be able to adjust themselves and adapted to low oxygen levels and poor food environment and has high ability to store heavy metals

[9]. DNA fragmentation recorded mostly in fin than superficial tissue due to fin regards as important parts in fish in movement and swimming and highly exposed to pollutants such as heavy metals and fluctuation between different concentration has been detected (Fig. 1) & (Table 1). These fluctuations returned to some factors effect on DNA damage positively or negatively, such as organic substances in water influences the availability of metal to fish and reduce metal toxicity and it's naturally that antioxidants enzymes reduce the heavy metals inhibitory effect such as GSH playing fundamental role as a first line defense against heavy metals toxicity.

Due to Common carp have the high repair capacity to protect DNA integrity and according to previous reasons. The results showed less DNA fragmentation in high concentration. On the other hand, common carp has adaptive response in polluted aquatic environment and heavy metals cause a harmful effect by generating reactive oxygen species that leads to sever damage to cell by shifting the fluidity balance and make molecular complexes with cell protein and develop toxic effect on the cell towards dysfunction [10].

Heavy metals are vital inducers of oxidative stress in aquatic species which lead to formation of reactive oxygen species through two routes either by potential redox or without potential ability of redox [11] and as consequences, causes DNA damage. As we mentioned that High concentration of copper toxicity increase with low pH and going to be fatal to fish with increasing mobility and releasing into water column and the presence

Table 1: Quantitative variations of DNA damage of common carp fish for different concentration of copper & cadmium.

Lane number	Treatment	Lyses type	DNA lyses level
1	DNA Ladder	DNA MARKER	1250-100 bp
2	Fin 1ppm Cu	Smear	600
3	Tissue 1ppm Cu	No lyses	-
4	Fin 1.5 ppm Cu	3 bands Smear	200, 400, 500 700
5	Tissue 1.5ppm Cu	smear	500
6	Fin 2ppm Cu	3 bands Smear	200, 400, 500 800
7	Tissue 2ppm Cu	smear	500
8	Fin 1.5 ppm Cd	4 bands Smear	200, 400, 600, 800 800
9	Tissue 1.5 ppm Cd	3 bands Smear	200, 400, 600 600
10	Fin 1ppm Cd	Smear	500
11	Tissue 1ppm Cd	Smear	300
12	Fin 2ppm Cd	3bands Smear	200, 400, 500 500
13	Tissue 2ppm Cd	Smear	400

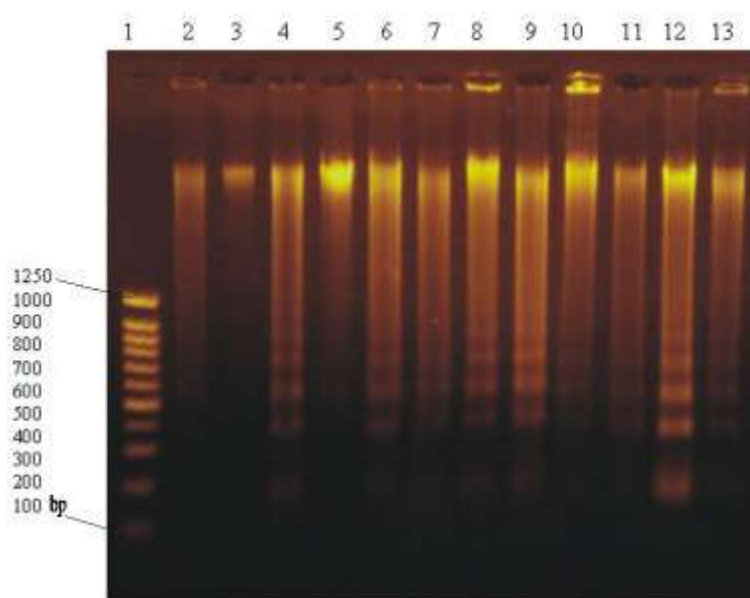


Fig. 1: DNA profile from fin & superficial tissue of common carp fish (*Cyprinus carpio L.*) which induced by acute exposure (96 hrs.) by different concentration of Cu & Cd.

Lane (1) DNA Marker, Lane (2) Fin 1ppm Cu, Lane (3) Tissue 1ppm Cu, Lane (4) Fin 1.5 ppm Cu, Lane (5) Tissue 1.5ppm Cu, Lane (6) Fin 2ppm Cu, Lane (7) Tissue 2ppm Cu, Lane (8) Fin 1.5 ppm Cd, Lane (9) Tissue 1.5 ppm Cd, (10) Fin 1ppm Cd, Lane (11) Tissue 1ppm Cd, Lane(12) Fin 2ppm Cd. Lane (13) Tissue 2ppm Cd.

of toxic metal has strong influence on the hematological parameters in common carp. Cd can accumulate primarily in major organ tissues of fish such as liver, stomach & gill with low concentration of copper in muscles compared to those found in gill [12, 13].

In spite of common carp ability to accumulate heavy metals but high a mount induce biochemical metabolism and lead to regeneration of reactive oxygen species and as consequences cause Sever DNA damage [14, 15].

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