Assessment of DNA damage in Fish collected from Nile River

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Abstract—DNA damage has been introduced as a useful tool for genotoxicity evaluation in polluted environment. In this study, the influence of environmental pollutants on the DNA damage was assessed in tilapia fish collected from three different sites including non polluted (served as control site), agricultural mixed point and industrial mixed point with Nile River Damage in DNA was assessed by calculating DNA fragmentation percentage. Results demonstrated that the collected samples from the mixed point of waste water represented the highest degree of DNA damage followed by the mixed point of agriculture drain when compared with samples collected from control site. The results suggested a genotoxicity of the aquatic environment of some regions at River Nile. Additionally, the DNA fragmentation in fish provided adequate sensitivity to be utilized as a tool in the monitoring of water pollution and environmental risk assessment.

Index Terms: Fish, River Nile, DNA fragmentation, Genotoxicity, DNA damage.

1 INTRODUCTION

The River Nile pollution depends on the quantity of wastes and on the nature of chemicals shed down [1]. The Nile had been threatened by agricultural and industrial effluents [2]. Due to increase in population density, industrial and agricultural activities which lead to high discharge rate in River Nile. Many trials have been done by many researchers since 1930 to find a relation between chemical pollution and acute toxicity in fish [3]. For investigation of the impact of toxic compounds on DNA function many biomarkers have been used as parameters forinvestigation of genotoxicity exposure. Such as DNA fragmentation, chromosomal aberrations and micronucleus test. Previous studies showed that fish can be used as a biomarker to investigate environmental changes in aquaculture [4]. Tilapia considered as a good example in ecotoxiclogical studies [5].

Apoptosis, programmed cell death, is identified by morphological and biochemical norms. Biochemically, the essential key of apoptosis is nucleosomal DNA fragmenta-tion corresponding to nucleosomal size [6]. Majority of the DNA breaks rather occur due to transformation in cell metabolic, recombination of DNA, repair or stress-relaxation during mitotic division. A stress stimulated by bacterial infection, changing in growth conditions, drugs, etc. leads DNA breaks at specific sites in different cells. Several studies have concentrated on fragmentation of DNA as a hallmark of apoptosis [7].

Bacterial pathogens induce apoptosis in immune cells of fish, humans and mammals. Some

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types of pathogenic bacteria such as Pseudomonas spp., Escherichia coli and Aeromonas hydrophila induce apoptosis using their exotoxins. A. hydrophila eliminate host immune cells (lymphocytes) through apoptosis [8]. In many bacteria, plasmids have an essential role in the stimulation of apoptosis in host cell such as Yersinia, Shigella and Salmonella which induce apoptosis in macrophage host cell according to proteins encoded by these bacterial virulence plasmids [9].

2 MATERIALS AND METHODS

2.1 Sampling Sites and Collection

Water samples were collected monthly for 1 year from three different sites in River Nile:

i- River Nile main stream from El Qanater, Qalubya Governorate.
ii- Agriculture drainage mixed with River Nile from El-Mansouria canal (Giza governorate)
iii- Wastewater drainage mixed with River Nile from El Mouheet drain (Giza governorate).

Nile Tilapia fish samples were collected and transferred alive to the laboratory. According to legal procedures fish were killed by decapitation. Fish samples were first decontaminated through washing several times with distilled H2O then with (70%) ethanol. Fish were aseptically dissected where liver, gills and muscles were isolated for DNA damage examination.

Fragmentation of DNA was conducted as described by Sellins, K.et al.1987 [10]. Briefly one ml of homogenization fish sample (liver, gills, muscle) was added to saline solution (0.9%Nacl), then it was centrifuged at 1500 rpm at 4°C for 10 min in labeled tube with I The supernatant was discarded, and then 1ml Triton X-100 Tris EDTA (TTE) solution was added to the pellet and vortex vigorously. This procedure permits the fragmentation of chromatin from nuclei, after cell disintegration and disruption of the nuclear structure.

Separation of DNA fragment from intact chromatin was accomplished by centrifuging at 14,000 rpm in 4°C for 10 min. Then supernatant was transferred to new eppendorf labeled tube with "F". To the pellet, 1ml TTE solution was added, then 1ml from (25%) Trichloroacetic-acid (TCA) was added to tubes "I" and "F" then tubes were vortexed vigorously.

Afterward, the tubes were incubated overnight at 4°C, then the DNA precipitation was recovered by centrifuging the tubes for 10 min in 4°C at 14.000 rpm and supernatant was discarded. DNA was hydrolysed by adding TCA 160 µl of 50% to each pellet and heated for 15 min at 90°C in heating block. A blank was prepared with TCA 160 µl of 5% alone. A freshly prepared diphenylamine (DPA) solution (320 µl) was added to each tube, then vortexed.

The color was allowed to be produced at 37°C for 4 hours approximately or at room temperature overnight. Two aliquots of colored solution (200 µl) were transferred from each tube to a well of a 96-well micro plate. The absorbance of the mixture at 600 nm was determined using ELISA micro plate reader.

2.2 Calculation:

Fragmented DNA percentage formula = F × 100 / (F+I).

2.3 Statistical Analysis:

All numerical data of DNA fragmentation were analyzed statistically using one-way analysis of variance (ANOVA) and by regression correlations using SPSS, 10. Data were expressed as Mean ± S. E for all experiments and the levels of significance were expressed in which $P < 0.05$.

3 RESULTS AND DISCUSSION

Environmental pollution was assessed by DNA damage [11]. Some of these pollutants may be carcinogenic or mutagenic that cause DNA damage. In this study, we demonstrated that fish in River Nile mixed with agricultural and wastewater exhibited to apoptosis as revealed by DNA fragmentation.

Results of DNA fragmentation (%) in Nile tilapia fish during a period of study February 2009 to

January 2010 were represented in Table (1). Table (1) represents the relationship between DNA damage and aquatic organisms in River Nile mixed with agricultural and wastewater. DNA damage showed significant difference in the muscles, gills, and liver which confirmed presence of pollutants in River Nile. This finding agrees with Mahmoud, A et al. 2010 [12], who reported that there was a significant difference between fish collected from polluted areas (Shanawan drainage canal) and the control area (Baher Shebeen canal). In the current study, both agricultural and wastewater caused an increase in DNA fragmentation percentage. Moreover DNA fragmentation percentage caused by wastewater was higher than that caused by agricultural water, which may be attributed to high heavy metals concentration. This observation agreed with the work of Birungi, Z et al. 2007[13], who reported that the relative accumulation of metals in tissue was of the order gills > liver > muscle. In other words, the highest percentage of DNA fragmentation in tilapia collected from wastewater sites was attributed to high level of pollution [14]. This finding was confirmed with a study which showed a significant percentage of DNA damage in the hepatic cells of Tilapia zillii inhabiting some polluted areas of River Nile in Egypt [15]. However, they found that the percentage of DNA damage resulted from pollution caused by industrial activity was higher than that pollution caused by agricultural activity [15].

Table (1): Mean values of DNA fragmentation (%) in fish organs of Tilapia collected from different polluted sites of Nile River for one year (February 2009 to January 2010)

Sample Sites	Fish Organs	DNA Fragmentation %	
		Mean	S.E.M
River Nile Main stream (before branches)	Muscle	23.76	1.03
	Liver	31.97*	1.31
	Gills	37.21*	0.72
Mixed point of agriculture drain	Muscle	34.94	0.48
	Liver	41.68*	1.27
	Gills	45.99*	1.12
Mixed point of wastewater	Muscle	39.08	0.28
	Liver	45.39*	0.95
	Gills	51.37*	1.21

Data are demonstrated as Mean ± Standard Error Mean for 12 reading of fish / group; * significant difference from the control group at $p<0.05$.

In this research, the percentage of DNA damage in the gills and liver of tilapia was higher than that in the muscles of tilapia collected from the same sites. This was confirmed by a study of Abdul Shakoor et al. 2010 [16], which revealed that intact DNA molecular weight in fish muscles was higher than DNA in fish gills at similar locations which show high pollution level in Indus River in the Mianwali District of Pakistan. From that study we can observed that DNA kept intact and not degradable due to lower metabolic activity of muscles. In addition Abdul Shakoor et al. 2010 [16] reported that metal concentration in the muscles was lower than that was found in the gills.

The present results are in agreement with Vittoria, S. et al. 2004 [17], who studied the DNA damage level and concluded that the DNA damage at the GÖteborg harbour area, Sweden was so elevated and attributed this to high chemical pollution level in the harbor. This was confirmed with another study at which tilapia fish, Oreochromis niloticus exposed to effluents showed consistently greater DNA damage [18].

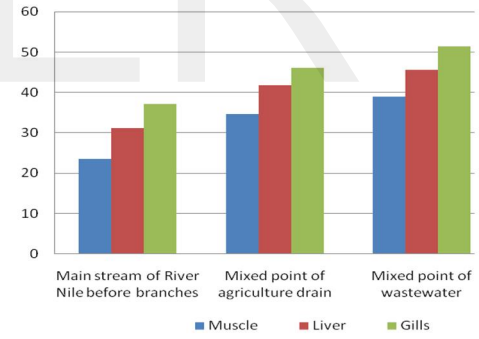

Figure (1): Mean values of DNA fragmentation (%) in fish organs of Tilapia collected from diffe polluted sites of Nile River for one year (February 2009 to January 2010)

From our results, DNA damage in the gills, liver and muscles of tilapia might be attributed to the contamination of River Nile with pathogenic bacteria. In agreement with our results, Tanmay, M.et al. 2009 [19]. Demonstrated that plasmid bearing Aeromonas hydrophila isolates induced the cellular alterations associated DNA fragmentation. Additionally, in several studies, it has been observed that some pathogenic bacteria, such as Staphylococcus aureus, Escherichia coli,

and A. hydrophila induced apoptosis and DNA fragmentation in freshwater fish species by their encoded toxins [20; 21 and 8].

Furthermore, in our results, the higher percentage of DNA damage in the fish gills which was more than in liver and in muscles of fish may be attributed to the higher bacterial count in the fish gills more than in the liver cells and muscles of tilapia. This was confirmed with Yagoub, S.et al. 2009 [22], who reported that the gills showed the highest number of bacteria; this was followed by muscles. In the present study, there was a significant percentage of DNA damage in the muscles, gills, and liver of tilapia surviving in River Nile which confirmed presence of pollutants in River Nile may be due to harsh condition facing fish population. We observed in our study DNA integrity was changed in fish organs (liver, gills and muscles) may be due to heavy metal concentration in fish. Many toxic materials and environmental conditions considered to be responsible for chromosomal DNA fragmentation as an integrative response. In our results, a high percentage of the DNA damage in the gills, liver and muscles of tilapia may be attributed to a high concentration of pollutants and long exposure duration. Therefore, this reaction depending on both toxic material concentration and duration of exposure. This was confirmed with a certain study which illustrate the long period of exposure to pollutant lead to DNA damage [23].

DNA changes may be permanent but possibility of DNA self-repairing might affect on the accurate interpretation of the related bioassays [24]. In our results, tilapia exposed to highly polluted sites might not induce DNA repair mechanisms because of higher percentage of DNA fragmentation in the gills, liver and the muscles of tilapia.

From the current study, it has been concluded that water contamination with either agricultural or industrial wastes may cause genetic disorders such as DNA damage and it is an indication of a polymorphism of fish liver expressed polypeptides. We can recommend that you should reduce the environmental risk, which is the source of the Nile River pollution and to preserve the life of aquatic organisms.

Further investigation should be done to know exactly types of pollution (chemical pollution or biological pollution) in River Nile mixed point with (agricultural drainage / wastewater).

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