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A Genotoxicological Study in Persian Gulf on Rock Oyster (Soccostrea cucullata) using Micronuclei and RAPID Assays

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ABSTRACT: Both micronuclei (MN) and binuclei (BN) as well as RAPD (Random Amplifying Polymorphism of DNA) assays are newly biomarkers which are well-introduced in toxic injury and related genotoxicity studies in bivalve, fishes and even humans. However, there is no record of such studies in Iranian Persian Gulf coast line so far. For this propose, we analyzed frequency of MN and RAPD patterns in gill cells of rock oyster (soccostrea cucullata) (n=30) collected from two area including Dayer (as reference area) and Mahshahr which was already shown oil contaminant are relatively high in this area owing to be oil vessel terminal. Our results showed micronuclei frequency is significantly higher in rock oysters of Mahshahr than Dayer area ($p<0.05$). Binuclei cells were also observed in some individuals of Mahshahr. Besides, RAPD analysis indicated less diversity in polluted area individuals. The results of this study suggest that MN and RAPD analyses can be easily considered as a useful tool for assessment of diverse pollution on aquatic organism in coastal area of Persian Gulf.

Key words: Miconulei, Binuclei, RAPD, Rock oyster, Pollution

INTRODUCTION

Complex mixtures of pollutants as a result of the discharge of industrial, agricultural and domestic wastes into surface waters have increased concern about their undesirable effects on aquatic ecosystems (Taseli, 2009, Nasrabadi *et al.*, 2010, Nabi bidhendi *et al.*, 2007, Mahmoudi *et al.*, 2010, Nakane and Haidary, 2010). These pollutions are mostly genotoxic and carcinogen (Jha *et al.*, 2004; Ohea *et al.*, 2004; Chen and White, 2004). Rock oyster (Saccostrea cuculata Born, 1778) is a filter feeder mollusk lives in rocky shore and sessile on mangrove forest as well as in piers in Persian Gulf coast (Hosseinzadeh, 1991). Due to sessile form of living in this species they can't escape from pollution (Salzar and Salzar, 2001), also this animal has a weak detoxification system thus they can show pollutant effect clearly. The use of genetic indicators in this organism is new approach in pollution monitoring (Monserrat *et al.*, 2006). From another point of view, rock oyster is suitable candidate for culture purpose in some countries such as Iran.

Micronuclei can originate both from acentric fragments resulting from chromosomal breaks which are not incorporated into the main nucleus and from whole chromosomes delayed during cellular division anaphase. These characteristics allow the detection of damage provoked both by clastogenic and aneugenic chemicals (Venier *et al.*, 1997; Dollecti and Venier, 2002; Barsiene *et al.*, 2006). This analysis was used in different environmental assessment studies to detect genotoxic effects of pure chemicals or mixtures of chemicals, both under field and laboratory condition. These damages are appropriate indices of pollutant exposure because it is a quick, sensitive and reliable analysis to determine damage to the DNA (Dixon *et al.*, 2002). Micronuclei assay as cytogenetic damage carried out in aquatic animal such as catfish (Ateeq *et al.*, 2002), cyprinid (Cavas *et al.*, 2005), echinoderms (Saotome *et al.*, 1999), snails (Barsiene, 2002), bivalve (Venier, 2002; barcine 2002; barcine, *et al.*, 2006). This assay have been carried out for genotoxicity of Benzo pyren (Venier *et al.*, 1997; Siu *et al.*, 2004), PAH (Soloco *et al.*, 2000; Leonard and Hellou, 2001; Francoini *et al.*, 2005), Heavy metals (A-Sabti, 1994; Bolognesi *et al.*, 1999) and oil pollution in field (Barsine, 2002; Barsiene *et al.*, 2006; Bolognesi and Digan, 2001, Bolognesi *et al.*, 2006) or laboratory (Reid and McFarlen, 2003) study.

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Studies showed on mussel (Mytilus edulis) gill cells by receding from sewage effluent MN and BN cells and significant difference between distance from effluent point and MN frequencies were observed (Izquierdo *et al.*, 2002). This study suggest that MN is enough sensitive for detect pollution effect on estuarine ecosystems. MN and BN cells also observed in mussel (Mytilus spp) gill cells collected from Baltic Sea (Barsiene *et al.*, 2006). Study on mussels from Oriestano Gulf of Italy showed that MN frequency correlated with heavy metal pollution (Magni *et al.*, 2006). In a 4 years period study in Guanabara Gulf investigation of PAHs effect reported MN formation (Francoini *et al.*, 2007). MN formation and other aniogenic effects were visible near oil terminal (Barsiene, 2002). In Oristano Gulf, Italy, MN damage known correlated with oil pollution (Magni *et al.*, 2006). The effect of pollutant on genome is directly related with to their effects on structure of DNA molecule (Atienzar *et al.*, 1999). The RAPD has been used in determining of these effects in various studies such as Daphnia cladoceran (Atienzar *et al.*, 1999; Atienzar *et al.*, 2001; Atienzar *et al.*, 1999), marine microalgae (Atienzar *et al.*, 2000), barnacle larvae (Atienzar *et al.*, 2002), mussel (Mytilus edulis) (Hagger *et al.*, 2005) and zebrafish (Zhiyi and Haowen, 2006). Direct and indirect effects of pollutant on aquatic organism may be cause to demographic effects (Atienzar and Jha, 2006). The population change due to pollution could be measured by RAPD technique (Atienzar and Jha, 2006). Toxicant induced population genetic effects may arise from the direct action of the toxicant at the DNA level. If these damage and mutation occurred in somatic cells are not pass to next generation but damage in germ cells can cause embryonic and other consequent population effects (Wolf *et al.*, 2004).

In this study MN and BN formation as cytogenetic damage and RAPD as molecular damage test on oyster from two area of Persian Gulf coast, Iran, have surveyed. The objective of this study is detection of cytogenetic and molecular damage of pollution especially oil pollution on rock oyster in Persian Gulf coast and identification of importance of genetic damage as well as introduction of genotoxical study in above area.

Vessel transportation and other activity in Mahshahr port (pier) caused to release of pollutant to coastal water, unless Dayer is far from pollutant source and is a fishing port and has lower pollutant.

MATERIALS & METHODS

This study was performed in two area of Persian Gulf coast on rock oyster. Briefly, a total of 30 Soccostrea cucullata with the same size were analyzed during 2007. All specimens were randomly collected from two Iranian piers in Persian Gulf, called Dayyer with no oil population and Imam Khomeini pier as a polluted area which is regularly exposes to vessel transport and oil pollution discharge. The samples were then transported alive in blue ice to biotechnology laboratory, University of Tehran until assays.

Barsiene method (2006) used for slide preparation. Oysters were dissected, biopsy carried out from individual gills and after washing by HANK's solution placed on microscopic slide in a drop of iced Carnoy and good quality cell suspension attained by gently squashing with tweezers in 3 min and fixed with iced methanol in 10 min. after that slides washed and stained 10 min with 5% Gimsa solution in phosphate buffer. 2 slides pre animals prepared. Slides analyzed by light microscope (Leica) at final magnification of 1000 for nuclear abnormalities (MN and BN cells) and 1000 about gill cells scored. Only intact cells scored. Blind scoring was performed to minimized technical variation. Genomic DNA was extracted from a piece of gill using conventional phenol-chloroform method (Atienzar *et al.*, 1999) and its concentration were measured using gel electrophoresis and comparison with known concentrations of Lambda phage DNA lambda phage .RAPD profile were generated with 5ng genomic DNA (Atienzar *et al.*, 2002). The 10-mer primers used were OPA9, OPB1, OPB5, OPB6, OPB7, OPB8, OPB10, OPB11, OPB12, and OPB14 (used by Atyienzar *et al.*, 2005). Thermal cycling parameters and analysis of PCR products by electrophoresis have been described in table in details. PCR products were electrophoreses on 1.5% agarose gel at 90 V h, stained with ethidium bromide and visualized under UV light. The GeneRulerTM 100 bp DNA ladder plus (Qiuagene) was used. The Thermal condition of PCR reaction consisted of 40 cycles: 45 second denaturation at 95 æ%C (except for the first cycle: 5 min), 45 second annealing at 48 æ%C, and min extension at 74 æ%C (except for the last cycle: 10 min).

Data gathered by Microsoft office, Excell ver2007 and analyzed by SPSS15. Mann- Whitney U-Test applied whereas significant differences between MN in gills cells of individuals in two sites. Data obtained from RAPD also analyzed by Unweighted pair-group using Arthimetic average (UPGMA) in NTsys pc ver 2.02 software.

RESULTS & DISCUSSION

The result of our MN (fig.1). analysis showed in fig. 2. The number of MN (mean: 6.87+1.97/ 100cells) in gill cells of rock oyster collected from Mahshahr pier was significantly lower than MN frequency in Dayyer coast (2.8+0.92) ($p<.05$) which is clear area. Our result

is quite in agreement similar study in oil spill effect using Mn in Bivalve (Bolognesi *et al.*, 2002) also in studies carried out by Barsiene (2002) and Barsiene et al. (2006) on blue mussel in Baltic Sea.

Analysis of binuclei cells showed that BN cells fig.3. frequency s generally less than Mn frequency. Result also showed that BN cells in gill cells of rock oyster in Mahshahr (1.27+0.72) were significantly ($p< 0.05$) lower than its value for rock oyster in Dayyer (mean: 0.27), fig. 4. Totally in each individuals MN was more than BN cells.

It seemed that apoptetic cells were formed in only some individual collected from Mahshahr pier. (fig. 3). Maximal frequency of apoptosis in individual was 13 in 1000 cells that have high frequency of MN. It is important to note that there was no certainty for apoptosis in this study. Apoptesis used as a sensitive biomarker in fish (Bush *et al.*, 2003) and bivalve (Barsiene *et al.*, 2006) MN frequencies in studied area demonstrate genotoxin existence in both areas. This study shows that because of vicinity of oysters collected from Mahshahr pier to pollution sources, have higher MN. In Dayer oyster lower frequency of MN (2.8±0.92 in 1000cells) may point to lower genotoxin in this area. Similar results obtained from study in Liguaria coast of Italy that the most frequency of MN (8 in1000cells) was for mussel collected from oil polluted area of Geneva (Bolognesi and Digan, 2001). In study on Baltic Sea (Buting oil terminal), similar result obtained (Barsiene, 2002).

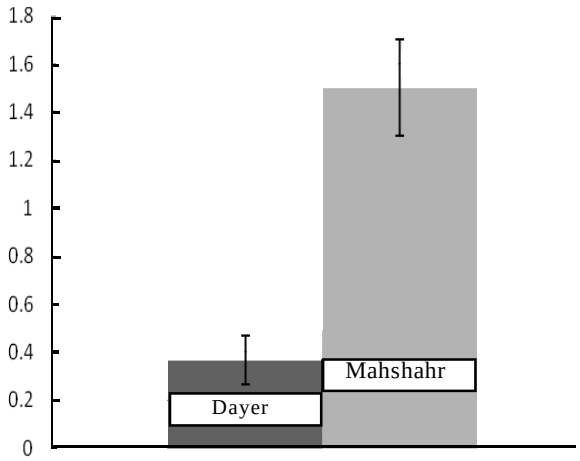

Fig. 1. Binuclei cell frequency in rock oysters gill cells of studied area (P<0.05)

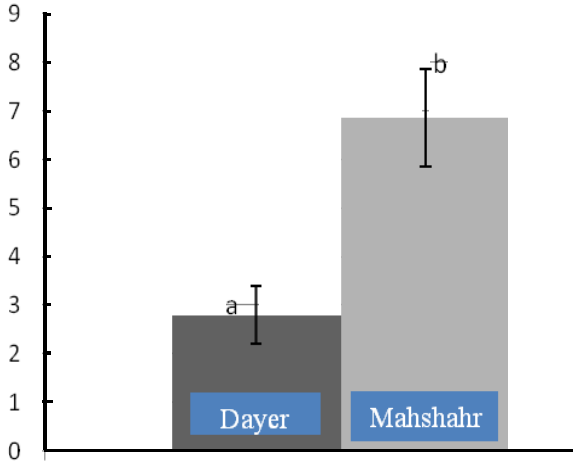

Fig. 2. Micronuclei frequency in rock oysters gill cells of studied area (P<0.05)

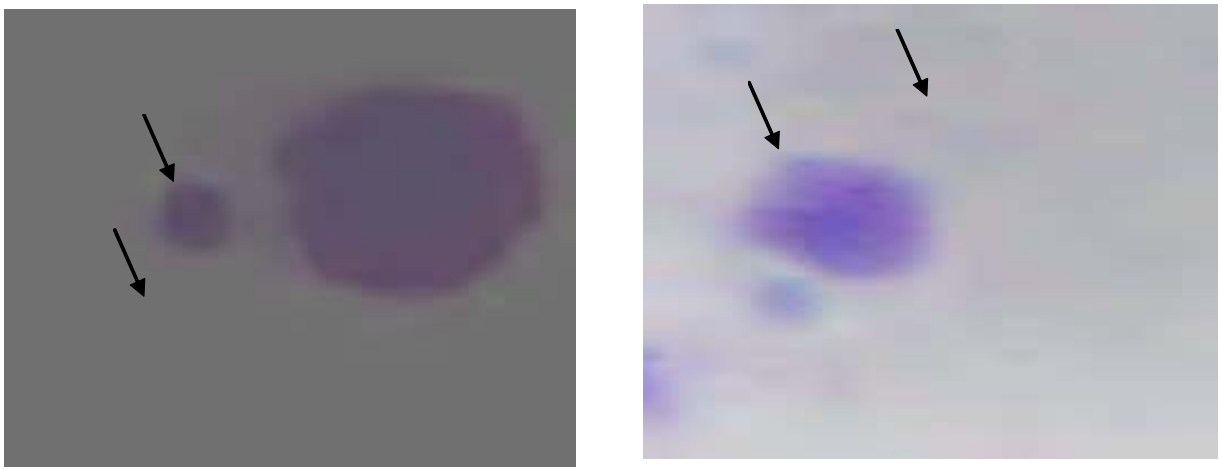

Fig. 3. MN in slides prepared from gill cells of rock oyster sampled from polluted area, MN has been showed by arrow beside main nucleus

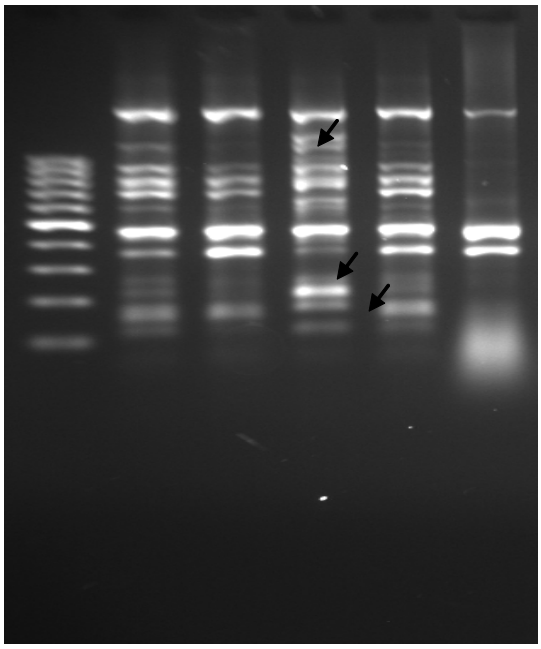

Fig 4. A RAPD Pattern in DNA of rock oyster in to studied area

RAPD analysis carried out based on band presence in individuals. In this study high annealing temperature proved reproducibility of RAPD bonds. The consistency showed by 2 time repetition. The results indicated that DNA extracted from oyster in Mahshahr produced RAPD profiles that differed from the control Dendrogram of similarity should that similarity of rock oyster Mahshahr is more than that in Dayyer (fig. 5). In both area differences in bond observed between animals. Between bands obtained from RAPD in animals of Dayer had higher order and higher difference. The band pattern doesn't show DNA damage obviously. But it seems that pollution had some demographic effect on polluted site. The high similarity between individuals of Mahshahr (sim coefficient= 0.8-0.99) than Dayer (sim coefficient= 0.7- 0.94) may because of decreased diversity and negative selection of some pollution. This approved by study of Nadig and Adams on sunfish population exposed to contaminants (Nadig and adams, 1998). Similar result also obtained from study in bay mussel (Mytilus galloprovincialis) and acorn barnacle (Balanus glandula) the authors of above study suggested that pollution can decrease genetic diversiry (Ma *et al.*, 2000). Our result is quite in agreement similar study in Daphnia exposed to Benzopyren (Atienzar *et al.*, 2004).

CONCLUSION

In conclusion this study showed that genotoxic damages occur in oysters collected from two studied area. This study was successfully indicated genomic damages. These damages include MN and BN cells that MN seems better indices to BN cells that this is because of more sensing of chromosomal damage. Because of high sensibility and relatively low cost, use of MN as pollution biomarker especially oil pollution in aquatic ecosystems can be useful. Studies needed to establish this biomarker in pollution effects on specific ecosystem. But for RAPD analysis it should use more individuals to determine molecular effect with certainty.

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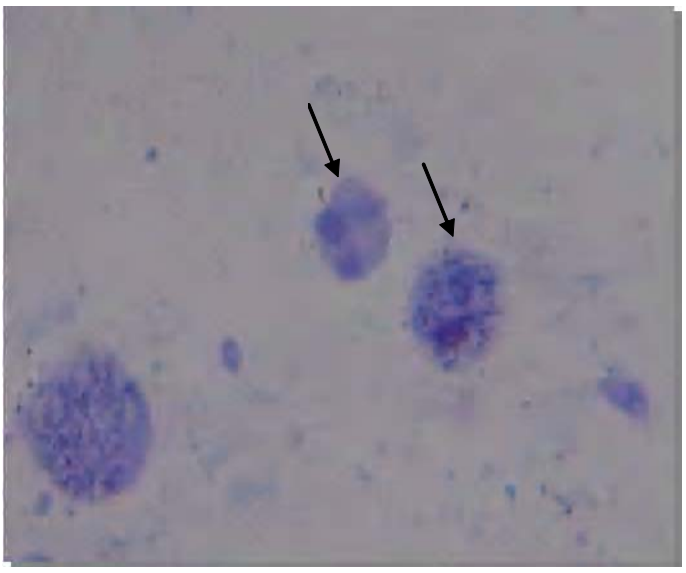

Fig. 5. BN cells and apoptotic cells showed by arrow in rock oyster gill cells of Mahshshr pier

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