

## GENOTOXIC AND BIOMARKER RESPONSE OF MUSSEL GILL CELLS, HAEMOCYTE AND A549 HUMAN LUNG CELLS TO RADIATION FROM X-RAYS

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### ABSTRACT

*A comparative study on the genotoxic effect of radiation from X-rays of different levels on Mussel gill cells, haemocyte and human lung cell A549 using the comet assay was carried out. The results revealed an increasing DNA damage with increasing doses of X-ray, single strand breaks and alkali labile sites in the haemocyte of the control had a mean value of  $5.92 \pm 1.27$  while the highest treatment group 15Gray (Gy) had a mean value of  $78.65 \pm 4.36$ , with significant differences ( $P < 0.05$ ). A mean value of  $21.10 \pm 13.10$  was recorded for Mussel gill cells in the control group while the highest treatment group 15Gray (Gy) had  $85.55 \pm 1.43$ . Statistical analysis revealed significant differences ( $P < 0.05$ ) for univariate tests of the control group and the treatment groups. The A549 human lung cells had a minimal damage in the control group  $2.30 \pm 0.30$  while the highest exposed group had a mean value from the median % tail intensity of  $43.10 \pm 2.81$ . Pair wise comparison of gills cells, haemocytes from mussels and A549 human lung cells exposed to 15 Gy of X-rays shows that the highest damaging effect was observed on the gill cells followed by the haemocytes with the least damaging effect on the A549 human lung cells exposed to the same dose rate of X-rays with significant differences ( $P < 0.05$ ) between them. This study revealed that A549 human lung cells were more resistant to DNA damage induced by the X-rays when compared to mussel gill and haemocyte cells and also indicates that mussel gill cells and haemocytes can serve as biomarkers of genotoxicity of radiation exposure using the comet assay.*

### INTRODUCTION

Radiations have been known to cause varying DNA damages depending on the dose rate to which a cell is exposed. These damages vary from rupture of DNA strands, alteration to bases, destruction of sugars, crosslinks and formation of DNA base dimers. X-ray dose of 1Gy causes about 1000 single strand breaks and about 50-100 double strand breaks in a

typical mammalian cell, while an exposure to 5Gy can cause about 50 % cell death (Nikjoo *et al.*, 2001; Watanabe *et al.*, 2015). Radiations have become a frequent occurrence in nature, as seen in the aquatic environment. Radionuclides and radioisotopes have been disposed into these environments via industrial activities and they are constantly disintegrating and giving off radiations that may have detrimental effects on the natural

biota of these ecosystems. Ultraviolet rays from the sun have been shown to be potent DNA damaging agents to exposed organisms (Lesser and Farrell, 2004), hence the need to constantly avoid radiations. UV is also absorbed in water, and the absorbance is increased if the water is contaminated. Hence, changing water quality as well as the depth of water over a marine organism may impact the UV-induced damage. Chronic occupational exposure to low-dose ionizing radiation has been investigated among hospital technicians in Turkey (Ünderger *et al.*, 1999a) and among industry workers in Poland (Wojewódzka *et al.*, 1998; Kruszewski *et al.*, 1998). The exposed hospital technicians had a trend toward a higher level of DNA damage based on the whole population of scored cells; (Ünderger *et al.*, 1999a). There was a 2-fold higher level of DNA damage among the Polish industry workers, but no difference in endonuclease III- or FPG-sensitive sites was seen between exposed and unexposed workers (Kruszewski *et al.*, 1998). This was unexpected because both endonuclease III and the FPG protein detect oxidative DNA damage although of different kind. However, the effect may be due to an adaptive response toward oxidative stress conferred by repeated exposures to ionizing radiation. For instance, it has been reported that leukocytes obtained from children living in the Chernobyl region are protected from (or have a lower responsiveness to) *ex vivo* damage of bleomycin (Frenzilli *et al.*, 1998). This study was therefore aimed at comparing the genotoxic effect of radiation from X-rays of different levels on mussel gill cells, haemocyte and human lung cell A549.

## MATERIALS AND METHODS

The A549 cells were seeded into 12 well plate culture dishes, with each well having a diameter of 22.6 mm and ( $17 \times 10^4$  cells/dish) after culture for 24h before the exposure. Semiconfluent cell cultures were exposed for 30 min, 24h and 48h to water soluble fractions of the three crude oils at two concentrations of full strength (FS) and 50% dilution.

Unexposed cells were used as negative control while sea water used in the dilution of the cells served as positive control. The concentration of Cells was determined with a haemocytometer and counting of cells was carried out visually with the aid of a microscope (Olympus Bx51 Japan) and a manual counter. Agarose was prepared according to the methods of Sambrook *et al.*, (1989). Mussel gill cells, haemocytes and A549 human lung cells were exposed to 225KeV X-ray (X-RAD 225 Pxi precision X-ray equipment) at doses ranging from 0, 1, 3, 5, 8, 10, 12 and 15 Gray (Gy) filtered through 0.5 mm copper and at a distance of 30cm. The trypan blue exclusion test was used to assess cells for cytotoxic damage after exposure to toxicants. A semi-automated scoring system was used for this experiment. The Comet assay IV software produced by Perceptive Instruments Ltd. UK, was used with Leica DLMB (light source Osram Mercury Short ARC HBO 50W/2) epi-fluorescence microscopes fitted with a 20x lens and a fabricated microscope stage having space to take one complete film with a large cover slip covering the entire surface of the film. The fluorescent SYBR Gold stain bound to the comet DNA emits visible light when illuminated with excitation light thus producing a light signal which is detected by the image analysis software to measure the integrated light intensity of the comet head and tail. The percent tail intensity relative to the head DNA intensity is used to evaluate the measure of damage, as increases are found to be linear with break frequency (Lovell and Omori, 2008). Statistical analysis of the data was done using the pair wise Duncan test for mean differences.

## RESULTS

### X-ray dose response of mussel gill cells

Gill cells of mussels exposed to X-rays showed a linear dose response with increasing damage observed at increasing doses of X-ray exposure (Figure 1). A mean value of  $21.10 \pm 13.10$  was recorded for single strand breaks and alkali labile sites in the control group.

Single strand breaks and alkali labile sites in the highest treatment group 15Gray (Gy) recorded a mean value of  $85.55 \pm 1.43$ . Statistical analysis revealed significant differences ( $P < 0.05$ ) for univariate tests of the control group and the treatment groups of gill cells exposed to X-rays.

### **Radiation Dose Response of Mussel Haemocytes**

A similar linear dose response was observed in mussel haemocytes with increasing damages recorded at increasing doses of X-ray exposure (Figure 2). Single strand breaks and alkali labile sites in the haemocytes of the control group had a mean value of  $5.92 \pm 1.27$  while the highest treatment group 15 Gray (Gy) had a mean value of  $78.65 \pm 4.36$ . Statistical analysis of univariate exposed samples revealed significant differences ( $P < 0.05$ ), the pair wise analysis also revealed significant differences ( $P < 0.05$ ) in a linear order with increasing exposure dose within the treatment groups and the control. Also, comparing data for bivariate controls (spun and un-spun) haemocytes, there were significant differences ( $P < 0.05$ ) between the two groups.

### **Effect of Centrifugation on Mussel Gills and Haemocytes**

Centrifugation at  $4^{\circ}\text{C}$ , 300xg for 5 minutes was observed to elicit some level of

DNA damage in the control group of centrifuged gill cells (Figure 1) and Haemocytes (Figure 2). A linear dose response was observed in un-centrifuged haemocytes with increasing damages observed at increasing X-ray doses (Figure 3). Un-centrifuged haemocytes from mussels revealed that there were lower damages in the control as compared to the control of the

centrifuged haemocytes. The control had a minimal damage of  $3.69 \pm 2.27$  while the highest treatment exposed to 15Gy X-rays had a mean value from the median % tail intensity of  $71.06 \pm 5.28$ . Results from univariate samples analyzed statistically revealed significant differences ( $P < 0.05$ ), while the pair wise analysis resulted in significant differences ( $P < 0.05$ ) in a linear order following the pattern of an increasing exposure doses within the control and the treatment groups.

### **X-ray Dose Response of A549 Human Lung Cells**

A549 human lung cells exposed to X-rays showed a linear dose response with increasing damage observed at increasing doses of X-ray exposure. The A549 human lung cells are more rigid and could withstand the effect of centrifugation with minimal damage in the control group  $2.30 \pm 0.30$  while the highest exposed group had a mean value from the median % tail intensity of  $43.10 \pm 2.81$  (figure 4). Pair wise comparison of gills cells, haemocytes from mussels and A549 human lung cells exposed to 15 Gy of X-rays shows that the highest damaging effect was observed on the gill cells followed by the haemocytes with the least damaging effect on the A549 human lung cells exposed to the same dose rate of X-rays. There were statistically significant differences ( $P < 0.05$ ) between them. Figure 5 Univariate samples statistical analysis revealed significant differences  $P < 0.05$ , while the pair wise analysis resulted in significant differences  $P < 0.05$  in a linear order following the pattern of an increasing exposure rate within the control and the treatment groups.

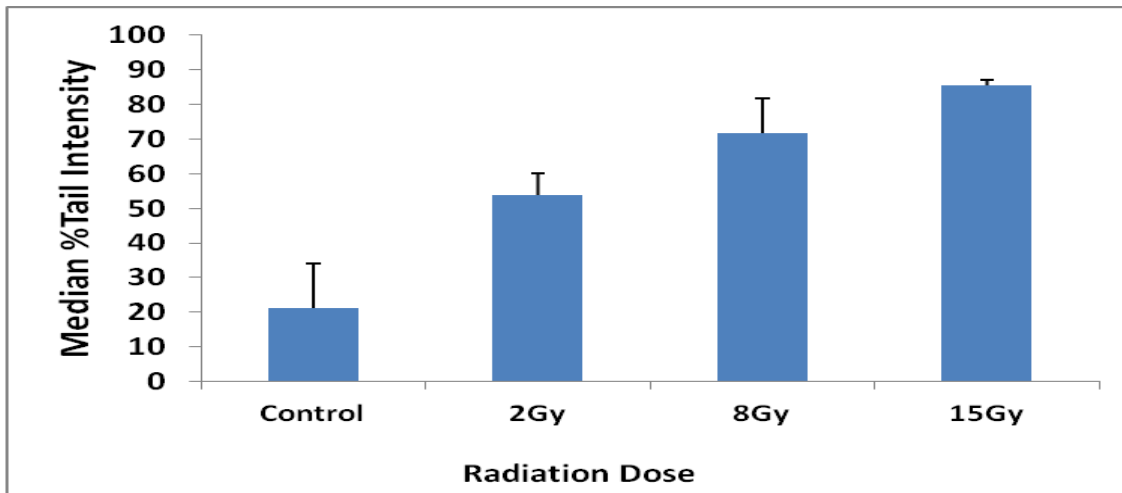


Figure 1: X-rays dose response of mussels gill cells

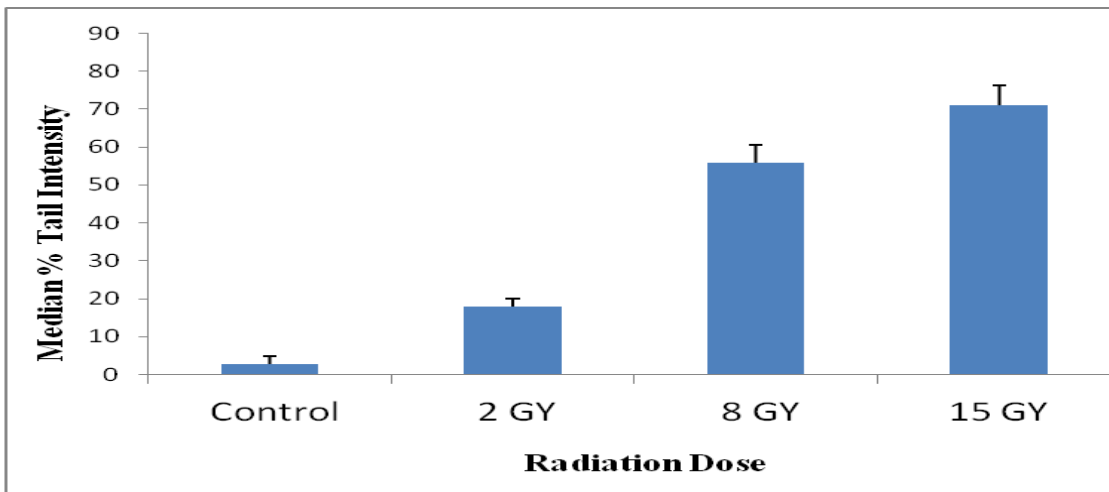


Figure 2: X-rays dose response of mussel haemocytes

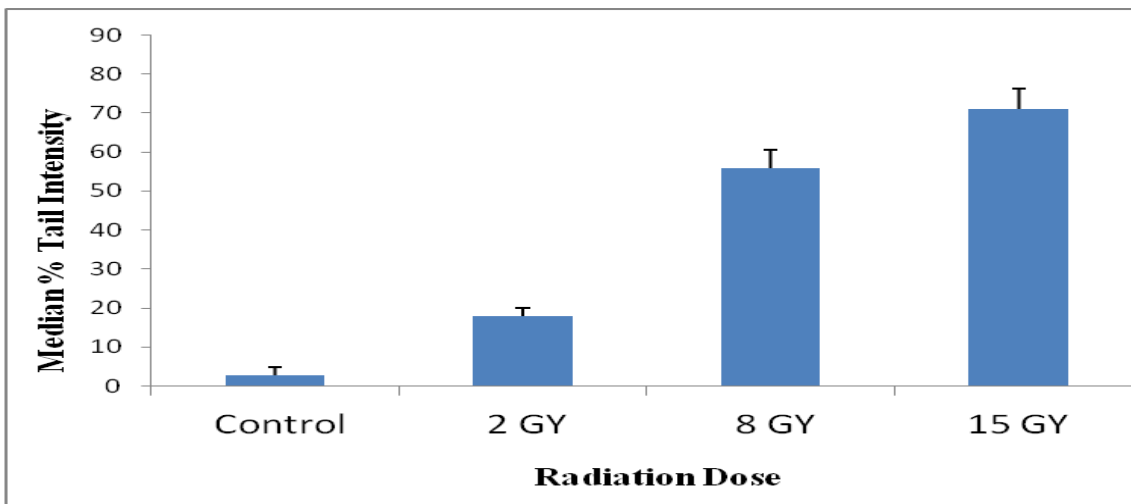


Figure 3: X-rays dose response of un-centrifuged mussel haemocytes

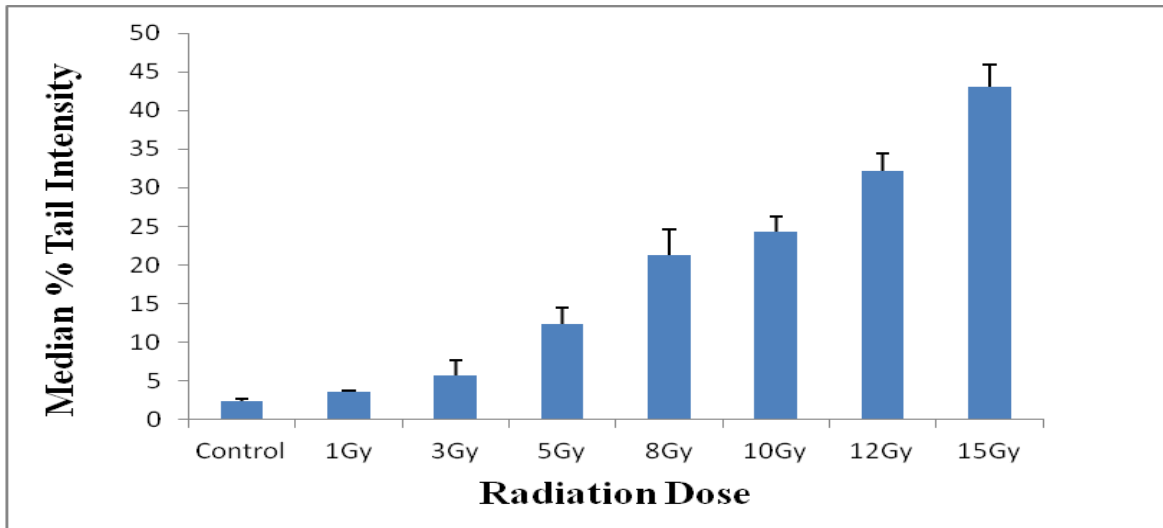


Figure 4: X-ray dose response of A549 human lung cells

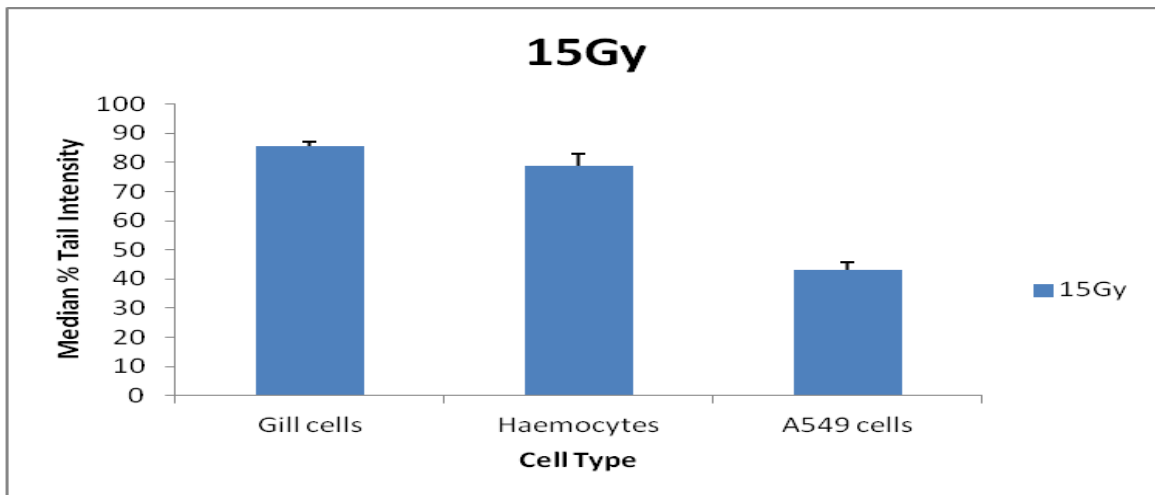


Figure5: Comparison of the effect of 15 Gy on mussel gill and haemocyte cells, and A549 human lung cells.

## DISCUSSION

X-rays discovered by Roentgen in 1895 are highly penetrating ionizing radiations emitted with various energies & wavelengths (Bowers, 1970). Ro 129786 also known as ethyl 7-oxo-7h-thieno [2,3-A]-quinolizine-8-carboxylate is an antipsychotic drug with photomutagenic effects, while formamidopyrimidine DNA glycosylase (FPG) is an enzyme used to evaluate oxidative DNA lesions in cells due to its ability to make breaks in the DNA at sites of the oxidised base (Collins *et al.*, 2004). In this study, varying concentrations of the FPG the titration step was shown not to have a significant influence in its ability to elicit similar oxidative DNA lesions under standard

duration of enzyme exposure, thus lower concentrations of the enzyme can be used to reduce cost. This study revealed that A549 human lung cells were more resistant to DNA damage induced by the X-rays when compared to mussel gill and haemocyte cells. Centrifugation step applied in this study had a significant DNA damaging effect on the control group of mussel's gill cells and haemocytes. The use of chemical and mechanical procedures to obtain cell suspension may also lead to DNA damage (Kosmehl *et al.*, 2006). The X-ray dose response experiment was done to demonstrate a linear induction curve using a controlled positive control, and to be able to calculate approximate numbers of lesions per cell since

this number is known for x-rays. A close to linear dose response curve with increasing dose rates of X-ray exposure was plotted from the data of single strand breaks and alkali labile sites in exposed mussel gills and haemocytes. This indicates that mussel gill cells and haemocytes can serve as biomarkers of genotoxicity of radiation exposure using the comet assay. Mussel gill cells and haemocytes exposure to X-rays indicate similar sensitivity though the choice of which to use should be based on the evaluation required (Rank and Klara, 2003). Mussel gill cells and haemocytes are commonly used in the comet assay, though haemocytes are easily isolated when compared to the procedure for isolating mussel gill cells (Nacciet *al.*, 1996; Steinert *et al.*, 1998b) anatomical and physiological functions further suggest gill cells are the most appropriate target cells because during the filter feeding process, the gill cells are directly exposed to the DNA damaging chemicals that are dissolved or bound to particles in water. A biomarker of radiation exposure in organisms at the molecular level must be able to measure response in the individual cell (Anderson *et al.*, 1994). The comet assay is able to evaluate such biomarkers. Similar evaluations of biomarker response to radionuclides in the environment have been conducted using the comet assay (Christopher *et al.*, 1997). The advantages of molecular or cellular biomarkers are that they respond to stress predictably and more rapidly, and are indicative of the mechanisms of toxicity thereby yielding a rudimentary characterization of the contaminants influencing them.

## CONCLUSION

Genotoxic compound and radiations pose a huge health risk to man his animals and the environment, detection employing the Comet assay through biomarkers in Mussel gill cells and haemocytes can enhance early detection thus preventing significant exposure and damage.

## REFERENCES

- Anderson, S., Sadinski, W. and Shugart, L. (1994). Genetic and molecular toxicology: a research framework. *Environ. Health Perspect.*, 102(12): 3–8.
- Christopher, M., Hardy, M. A., Dean, R. J., Leon, N. C., Matthew, J. M. and Diana, M. H. (2011). DNA barcoding to support conservation: species identification, genetic structure and biogeography of fishes in the Murray-Darling River Basin, Australia. *Marine and Freshwater Research*, 62(8): 887-901.
- Frenzilli, G., Lori, A., Panasiuk, G., Ferdeghini, M., and Barale, R. (1998). Comet assay on children's leukocytes 8 years after the Chernobyl disaster. *Mutat. Res.*, 415: 151-158.
- Kosmehl, T., Hallare, A. V., Reifferscheid, G., Manz, W., Braunbeck, T. and Hollert, H. (2006), A novel contact assay for testing genotoxicity of chemicals and wholesediments in zebrafish embryos. *Environmental Toxicology and Chemistry*, 25: 2097-2106.
- Kruszewski, M., Wojewódzka, M., Iwanenko, T., Collins, A. R., and Szumiel, I. (1998). Application of the comet assay for monitoring DNA damage in workers exposed to chronic low-dose irradiation II. Base damage. *Mutat. Res.*, 416: 37-57.
- Lovell, D. and Omori, T. (2008). Statistical issues in the use of the comet assay. *Mutagenesis*, 23(3): 171-182.
- Nacci, D. E., Cayula, S. and Jackim, E. (1996). Detection of DNA damage in individual cells from marine organisms using the single cells gel assay. *Aquat. Toxicol.*, 35: 197-210.
- Nikjoo, H., O'Neill, P., Wilson, W. E. and Goodhead, D. T. (2001). Computational approach for determining the spectrum of DNA damage induced by ionizing radiation. *Radiat. Res.*, 156: 577-583.
- Rank, J. and Klara, J. (2003). Comet assay on gill cells and haemocytes from the blue mussel *Mytilus edulis*. *Ecotoxicology and Environmental Safety*, 54: 323-329.

- Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). Gel electrophoresis of DNA. In: Sambrook, J., Fritsch, E. F. and Maniatis, T. (Eds.) *Molecular Cloning: A Laboratory Manual*. New York: Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA.
- Steinert, S. A., Streib-Montee, R. and Sastre, M. P. (1998b). Influence of sunlight on DNA damage in mussels exposed to polycyclic aromatic hydrocarbons. *Mar. Environ. Res.*, 46: 355-358.
- Ündeger, Ü., Basaran, N., Kars, A. and Güc, D. (1999a). Assessment of DNA damage in nurses handling antineoplastic drugs by the alkaline COMET assay. *Mutat. Res.*, 439: 277-285.
- Watanabe, R., Rahmanian, S. and Nikjoo, H. (2015). Spectrum of radiation-induced clustered non-DSB damage - A Monte Carlo track structure modeling and calculations. *Radiat. Res.*, 183: 525-540.
- Wojewódzka, M., Kruszewski, M., Iwanenko, T., Collins, A. R., and Szumiel, I. (1998). Application of the comet assay for monitoring DNA damage in workers exposed to chronic low-dose irradiation. I. Strand breakage. *Mutat. Res.*, 416: 21-35.