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Evaluation of γ- radiation induced DNA damage in two species of bivalves and their relative sensitivity using comet assay

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ABSTRACT:

Ionizing radiation is known to induce genetic damage in diverse groups of organisms. Under accidental situations, large quantities of radioactive elements get released into the environment and radiation emitted from these radionuclides may adversely affect both the man and the non-human biota. Present study is aimed (a) to know the genotoxic effect of gamma radiation on aquatic fauna employing two species of selected bivalves (b) to evaluate the possible use of 'Comet assay' for detecting genetic damage in haemocytes of bivalves as a biomarker for environmental biomonitoring and also (c) to compare the relative sensitivity of two species of bivalves *viz. Paphia malabarica* and *Meretrix casta* to gamma radiation. The comet assays was optimized and validated using a different concentrations (18, 32 & 56 mg/l) of ethyl methanesulfonate (EMS), a direct-acting reference genotoxic agent, exposed to various time intervals (24, 48 and 72h). Bivalves were irradiated (single acute exposure) with 5 different doses (*viz.* 2, 4, 6, 8 and 10 Gy) of gamma radiation and their genotoxic effects on the haemocytes were studied using the comet assay. Haemolymph was collected from the adductor muscle at various time intervals of 24, 48 and 72h of both EMS exposed and irradiated bivalves and comet assay was carried out using standard protocol. A significant increase in DNA damage was observed as indicated by an increase in % tail DNA damage at different concentration of EMS and all the doses of gamma radiation as compared to controls in both bivalve species. This showed a dose-dependent increase of genetic damage induced in bivalves by EMS as well as gamma radiation. Further, the highest DNA damage was observed at 24 h which gradually

decreased with advancement of time i.e. at 48 and 72 h post irradiation of both species of bivalves. This may indicate repair of the damaged DNA and/or loss of heavily damaged cells as the post irradiation time advanced. The present study reveals that gamma radiation induces single strand breaks in DNA as measured by alkaline comet assay in bivalves and comet assay serves as a sensitive and rapid method to detect genotoxicity of gamma radiation. This study further indicates that both *Meretrix casta* and *Paphia malabarica* exhibit almost the same level of sensitivity to gamma radiation as measured by DNA damage.

Keywords: γ-radiation; genotoxicity; comet assay; *Paphia malabarica; Meretrix casta;* haemocyte.

Abbreviations: Gy- Gray, h- Hour, γ- Gamma, EMS- Ethyl methanesulfonate

1. Introduction:

Applications of nuclear technologies have made a very significant contribution to modern civilization. It is useful to humans in several ways, including as a source of power, as a medical diagnostics and also as an industrial tool. Exploding human population and his changing needs / lifestyle have resulted in a drastic increase in the production, consumption and disposal of chemical contaminants into our environment. The aquatic environment is often the ultimate recipient of a wide range of contaminants including chemical and radioactive wastes, a large proportion of which could be potentially genotoxic and carcinogenic ((Moore et al., 2004; Jha et al., 2000a). Radioactive wastes emit radiation in different forms e.g. α-, β-particles and gamma rays, which could pose a potential risk to human health and also to our environment (Dallas et al., 2012a). Exposure of a moderate-dose of 1-10 Gy of gamma radiation may occur in an environment / human exposure as a result of radiation accidents or nuclear/radiological terrorism

alone or in conjunction with bioterrorism (Coleman et al., 2003). During nuclear accidents, radionuclides are released into the environment, either in modest amounts or on a larger scale, such as that caused by the Chernobyl NPP (Ukraine, formerly USSR) in 1986 or the earthquake-tsunami at Japan's Fukushima Daiichi NPP in 2011. The risk to non-human biota due to ionizing radiation exposure is considerable current interest to both the International Commission on Radiological Protection (ICRP) and the International Atomic Energy Agency (IAEA) and they recommend the impact assessment of radiation on those organisms (IAEA, 1992; ICRP 1991, 2007). Very little information is available on the potential detrimental effects of ionizing radiation on aquatic invertebrates (Dallas et al., 2012a). They are very important human food source (Barnes and Rawlinson, 2009; Ren et al., 2010) and are also a source of food for various commercially important fish species (Pederson et al., 2008). Further, they are frequently used as model organisms for toxicological tests (Depledge, 1998).

Haemocytes, the cells of the open vascular system of mussels, have been extensively used for genotoxicological studies, including for monitoring cytogenetic damage (Mersch et al., 1996, Pavlica et al., 2001). The usage of haemocytes provides a relatively non-invasive source of material for bio-monitoring (Fossi et al., 1994; Mitchelmore and Chipman, 1998; Taddei et al., 2001). Further, these cell types are suitable for the comet assay and the MN assay because they can be rapidly and easily sampled without any need for cell dissociation (Belpaeme et al., 1998; Jha, 2008; Canty et al., 2009). These advantages have the benefit of shortening the time required for slide preparation and facilitating the sample process. The comet or single-cell gel electrophoresis assay is used as a very quick, sensitive method for measuring DNA damage in eukaryotic cells for the study of genetic damage associated with exposures to potentially genotoxic agents (Lovell and Omori, 2008). It is also used in regulatory and biomonitoring

studies in a range of mammalian and non mammalian both under in-vitro and in-vivo systems (Chaubey et al., 2001; Collins, 2004; Brendler et al., 2005; Malladi et al., 2007). It offers considerable advantages over several other cytogenetic methods used in DNA damage detection, such as sister chromatid exchange studies, micronucleus test and the chromosomal aberrations. Further, there is no need for cells to be in a dividing state. Other advantages include its rapidity i.e. results can be obtained in a single working day and wide applicability to virtually any nucleated cell type (Jha, 2008; Canty et al., 2009).

In addition to the detection and quantification of contaminants in the environment in order to reveal their environmental impact, it is very essential to identify their toxic effects on living systems, which are the ultimate recipients of toxicant-induced mutation (Claxton et al., 1998). Mussels are globally used as bioindicators for pollution of coastal and estuarine environments by metals and radionuclides (Lonsdale et al., 2009). Hagger et al. (2005) have reported the β- radiation induced genotoxic, cytotoxic, developmental and survival effects of tritiated water in the early life stages of the marine mollusc, *Mytilus edulis*. Further, the genotoxic effects of tritium (^{3}H) in the adult life stage of *Mytilus edulis* have been evaluated by employing micronucleus (MN) test and comet assay (indicating DNA single strand breaks/alkali labile sites) in the haemocytes of exposed individuals (Jha et al., 2005, 2006). Tritiated water (HTO) and tritiated glycine (T-Gly) induced a significant number of micronuclei in the haemocytes of *Mytilus edulis* (Jaeschke et al., 2010). External and internal dose rates of ionizing radiation altered the DNA strand breakage and *RAD51* mRNA expression in marine mussel *Mytilus edulis* which was observed using gene expression study and comet assay (Alamri et al., 2012). However, studies on the genotoxic potential of radiation in the estuarine bivalves are lacking. Hence, present study is aimed (a) to know of genotoxic effect of gamma radiation on

estuarine bivalves (b) to evaluate the use of 'Comet assay' for detecting genetic damage in haemocytes of bivalves as a biomarker for biomonitoring and also (c) to compare the relative sensitivity of two species of bivalves *Paphia malabarica* and *Meretrix casta* to gamma radiation.

2. **Materials and methods:**

2.1. Experimental bivalve specimens**:**

Two species of estuarine bivalves, *Paphia malabarica* and *Meretrix casta* which are abundant in Goa and used as a common sea food by locals were selected as the experimental animals. Healthy specimens were procured from an unpolluted site at Siolim, Goa and brought to the laboratory. Animals measuring an average length of 3.0 ± 0.4 cm were selected for the current study. They were maintained in aerated glass aquaria with sand, pebbles and estuarine water, procured from the same above cited unpolluted site. Water in the aquaria was replaced once in every day. These bivalves were acclimatized under the laboratory conditions for 2 weeks in semi static systems before exposure to reference agent as well as to different doses of gamma radiation.

2.2. Validation of Comet assay:

Prior to the evaluation of genotoxic effects of gamma radiation the comet assay was fully optimized and validated against a direct-acting reference genotoxin, ethyl methanesulfonate (EMS; Sigma, UK). The range of EMS concentrations used in the study was based on earlier studies (Jha et al., 2005). Both species of bivalves (10 animals per group) were exposed to different concentrations of EMS (18, 32 and 56 mg/l) dissolved in estuarine water.

2.2.1. Sample collection from bivalves:

Four hundred (400) µl of haemolymph was collected from the sinus region (located near the posterior adductor muscle) of each of the control and EMS exposed bivalves using a hypodermic syringe under dim light at various exposure time intervals of 24, 48 and 72h. Each sample was transferred to a micro centrifuge tube placed on ice in an ice box to prevent endogenous DNA damage occurring during sample preparation and also to inhibit DNA repair in the nfixed cells (Siu et al., 2004).

2.2.2. Cell viability Assay:

Prior to the Comet assay, the cell count and the cell viability were checked to ensure that there were an optimum number of living cells to perform the assay. The cell count and viability assessment were conducted with a haemocytometer and trypan blue dye exclusion test. Haemolymph samples showing more than 90% viability and a cell count of 10^6cells/ml were used for the comet assay.

2.2.3. Comet assay:

The technique of lysis, unwinding and electrophoresis were standardized for haemocytes by adopting the protocol of Singh et al. (1988) and (Tice, 1995) with slight modifications, prior performing comet assay. The comet assay was carried out in a dark room with dim red light. Haemolymph (15 µL) was mixed with 75 µL of 0.5% low melting point (LMP) agarose at 37°C and rapidly spread on a frosted microscope slide (Fisherfinest premium) pre-coated with 1% normal melting point (NMP) agarose. Cover slip was applied on the smear and the slide was allowed to solidify for 5 min in a freezer at 0°C. The cover slip was then gently removed and the slide was immersed in a fresh cold lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, pH 10, 10% DMSO 1% and Triton X-100) placed in a petriplate for 2 h at 4°C. Comet slides

were later immersed in electrophoresis buffer (300 mM NaOH, 1 mM EDTA, pH > 13) in a horizontal gel box for 30 min for the DNA to unwind. These slides were subjected to electrophoresis by applying 20 V and 275 mA for 20 min and later on the unwound DNA in the slides were neutralized in cold neutralization buffer (0.4 M Tris–HCl, pH 7.5) for 10 min in a petriplate, rinsed in distilled water and neutralized again. The DNA was stained with 15 µg/ml ethidium bromide and examined under a BX53 Olympus fluorescence microscope (Japan) at 200X magnification. Two slides per specimen and a total of 500 comets per group (25 cells per slide) were selected for analysis. The images of these comets were captured using ProgRes® Capture Pro 2.7. The captured images were analyzed using CASP (Konca et al., 2003) image analysis software and the percentage of tail DNA content (% tail DNA) was measured as an indicator of DNA damage (single strand breaks) (Kumaravel and Jha, 2006).

2.3. Selection of the radiation doses:

A dose range of 2-10 Gy of gamma radiation is selected for the present study as this quantum of radiation is suspected to be released into our environment during any nuclear accident.

2.4. Irradiation of bivalves:

Bivalves, *Paphia malabarica* and *Meretrix casta* (10 animals per group) were exposed to a whole body irradiation of a single dose of 2, 4, 6, 8 and 10 Gy allowing 0.5 cm deviations at the center of the dose rate from a gamma radiation source of a Cobalt Teletherapy Unit at Goa Medical College, Goa. Unirradiated bivalves were employed as control animals. After each time intervals (24, 48 and 72 h) of post irradiation of different doses of gamma radiation and the control group without irradiation, 400µl samples of haemolymph were taken from each bivalves,

the cell count and cell viability were checked as described above, and the rest of the sample was used for the Comet assays.

3. Statistical analysis:

Statistical analysis was performed by using PRISM, STATISTICA 6.0 packages. Data was analysed with student's t-test and ANOVA. Within the single experiment, the significance of the data for each dose against that of the respective control was evaluated by the Student's t-test. Dose respondents as well as time response of gamma irradiation on bivalves were determined by one-way ANOVA. The difference of DNA damage between the *Paphia malabarica* and *Meretrix casta* was analyzed by student's t-test. A level of probability of $P < 0.05$ was considered as statistically significant data.

4. Results:

4.1. Validation of Comet assay:

The DNA damage (% tail DNA) induced by different concentration of EMS (18, 32 and 56 mg/l) in the haemocytes of *Paphia malabarica* and *Meretrix casta* at various time intervals (24, 48 and 72h) are shown in fig. 1 (A&B). EMS induced a dose dependent increase of DNA damage in both the species of bivalves. The highest amount of DNA damage (% of tail DNA) was observed in both the species of bivalves exposed to the highest concentration of EMS (56 mg/l). Further, a time dependent increase of DNA damage was observed for all the concentration of EMS. All the doses of EMS induced a significant increase (t' test) of DNA damage in comparison with the controls in both bivalve species.

4.2. Irradiation of bivalves:

4.2.1. DNA damage in *Paphia malabarica*

The dose and the time dependent increase of DNA damage induced by gamma radiation and their statistical significance are represented in Fig. 2A and 3A respectively.

4.2.1.1. Dose-response assay:

Significant DNA damage ($P<0.001$) was seen in all individuals of *Paphia malabarica* irradiated by various doses of gamma radiation in comparison to their respective controls as per the t-test (Fig. 2A). Further, a dose dependent increase of the mean % tail DNA was observed, with a minimum (60.57±8.30) at the lowest dose (2 Gy) and the maximum (75.21±9.05) at the highest dose (10 Gy) at 24h of post irradiation. One way ANOVA of the data on the dose dependent DNA damage observed in the control and treated animals showed significance at $P<0.0001$ ($F=54.8$, 59.8 & 27.3) at all the time intervals studied (24, 48 &72 h respectively).

4.2.1. 2. Time-response assay:

Time response study indicated significant DNA damage at all the time intervals for all the doses of gamma radiation studied as per the t-test (Fig. 3A). The highest DNA damage (60.57±8.30) was observed at 24h post treatment, which decreased considerably in the later time intervals and reached the minimum (29.4±8.0) at 72 h (2 Gy). A similar trend was observed for the other doses too i.e (4, 6, 8 & 10). One way ANOVA of the DNA damage observed at different time intervals showed significant decrease in the DNA damage with the $P<0.0001$ ($F=34.10$, 21.20, 16.59, 16.08 and 9.96) for all the doses studied (2, 4, 6, 8 and 10 Gy respectively).

4.2.2. DNA damage in *Meretrix casta*

The dose response and the time dependent DNA damage induced by gamma radiation and their statistical significance are represented in Fig. 2B and 3B, respectively.

4.2.2.1. Dose-response assay:

A significant quantum of the DNA damage ($P<0.001$) was induced by all the doses of gamma radiation in the haemocytes of bivalves, in comparison to their respective controls as per the t-test at all the time intervals studied (Fig. 2B). Results also indicated a dose dependent increase of % tail DNA in these bivalves. The minimum % tail DNA (68.52±10.25) was observed at the lowest dose (2 Gy) and the maximum (82.43±9.11) was observed at the highest dose (10 Gy) at 24h of post irradiation. One way ANOVA of the DNA damage observed in the control and treated animals showed significance at $P<0.0001$ ($F=80.\ 0, 39.7$ & 26.5) at all the time intervals studied (24, 48 &72 h respectively).

4.2.2.2. Time-response assay:

The significant DNA damage was observed at all the time intervals for all the doses of gamma radiation studied as per the t-test (Fig. 3B). Maximum % tail DNA (68.52±10.25) was observed at 24 h which gradually declined (34.4±6.24) till 72 h (2 Gy) in a time-dependent manner. A similar trend was observed for the other remaining doses too. One way ANOVA of the DNA damage observed at different time intervals showed significance at $P<0.0001$ ($F=33.\ 6, 27.5, 33.3, 47.7$ and 37.5) for all the doses studied (2, 4, 6, 8 and 10 Gy respectively).

4.3. Comparison between *Paphia malabarica* and *Meretrix casta*:

4.3.1. Dose-response assay:

The DNA damage induced by various doses (*viz.* 2, 4, 6, 8 & 10 Gy) of gamma radiation in the haemocytes of *Paphia malabarica* and *Meretrix casta* at 24, 48 and 72h time intervals are compared each other and represented graphically in Fig. 4 (a, b and c).

4.3.1.1. 24 h post- treatment study:

Data on DNA damage at 24 h post treatment with gamma radiation is represented graphically in Fig. 4a. A linear increase in DNA damage in the form of % tail DNA was observed with the increasing doses of gamma radiation in both the species of bivalves. The *Meretrix casta* showed more DNA damage when compared with *Paphia malabarica* with all the doses of gamma radiation at 24 h post treatment, although the difference was not statistically significant.

4.3.1.2. 48 h post- treatment study:

The DNA damage at 48 h post treatment with gamma radiation is represented in Fig. 4b. Interestingly *Paphia malabarica* showed almost the same quantum of DNA damage as that of *Meretrix casta* for all the doses of the gamma radiation.

4.3.1.3. 72 h post- treatment study:

Gamma radiation induced DNA damage at 72 h post treatment is represented graphically in Fig. 4c. At 72h of post irradiation *Meretrix casta* showed a gradual, linear increase of DNA damage with increasing doses, whereas *Paphia malabarica* showed comparatively less DNA damage at lower doses (2 Gy to 6 Gy) but at higher doses (8 Gy to 10 Gy) it showed a drastic increase of DNA damage as compared to *Meretrix casta*. However, the differences observed were not statistically significant, neither at lower doses nor at higher doses.

4.3.2. Time-response assay:

Comparison between the DNA damage induced by various doses (*viz.* 2, 4, 6, 8 and 10 Gy) of gamma radiation at 24, 48 and 72h time intervals of gamma radiation in the haemocytes of *Paphia malabarica* and *Meretrix casta* are compared each other and represented graphically in Fig. 5 (a, b, c, d and e). Data on DNA damage at 24h post treatment with various doses of gamma radiations in *Meretrix casta* showed slightly higher quantum of genetic damage when compared with *Paphia malabarica*, although the difference is not statistically significant. The overall comparison between *Paphia malabarica* and *Meretrix casta* has shown that there is considerable difference in DNA damage induced by gamma radiation in these bivalves, although it is not statistically significant.

5. Discussion:

5.1. Validation studies of Comet assays:

The increased DNA damage observed in the present study in the haemocytes of EMS exposed bivalves (both species) indicates their sensitivity to genotoxic agents as well as their possible use for genotoxicity studies using the comet assay. Many of earlier studies have shown that the comet assay is a sensitive and reproducible method to detect genotoxic effects in the haemocytes of bivalves following exposure to a variety of direct and indirect-acting genotoxins (Dixon et al., 2002; Jha et al., 2005, 2006). We could find a similar observation in EMS exposed bivalves (both the species) in the present study. Further, similar to other reports, our study also suggests that haemocytes of bivalves are a sensitive and reliable cell type to evaluate the genotoxic effects of reference and environmental agents.

5.2. Gamma radiation induced DNA damage:

Significant increase of mean % tail DNA damage observed by comet assay in the present study at all the doses of gamma radiation when compared to controls in both bivalve species indicate the genotoxic potential of gamma radiation in these bivalves viz. *Paphia malabarica* and *Meretrix casta*. Bivalves being a member of phylum Mollusca which is the second largest group among the invertebrates, have largely been exploited worldwide for food, ornamentation, pearls, etc. and also used for biomonitoring of pollutants. Most of the earlier studies reported radiation induced developmental and survival toxicity in the early stages of life in aquatic invertebrates (Hingston et al., 2003; Dallas et al., 2012a). However, reports on the use of the comet assay in the cells of a bivalve to study the effect of gamma radiation are very scanty (Jha et al., 2005; Jaeschke et al., 2010; Alamri et al., 2012).

5.2.1. Dose-response assay:

The dose-dependent increase of DNA single-strand breaks, in the form of comet induction (% tail DNA) induced by irradiation in bivalves in the present study is on par with the observations of Jha et al. (2005, 2006) in which they exposed *Mytilus edulis* to a series of concentrations of tritium and consequently observed a dose dependent increase of MN and DNA damage in their haemocytes. Larger radioactive particle showed increased comet % of tail DNA and frequency of MN in the haemolymph of *Mytilus edulis* (Jaeschke et al., 2012). Radiation induced DNA damage has been reported recently in the haemocytes of *Mytilus edulis* using the comet assay and MN test by (Jaeschke et al., 2010; Alamri et al., 2012).

5.2.2. Time-response assay:

The significant genetic damage induced by gamma radiation at 24h time intervals which decreased by the 48h and further declined by 72h in both the species of bivalves in the present

time dependent study may suggest that the genotoxic effect of gamma radiation may not last for a long period. This is in agreement with the observation of Jaeschke et al. (2010) where in the frequency of MN in mussels exposed to HTO became insignificantly different from the control value, after 21 days of depuration. It is also par with similar reports by several scientists using chemical toxicants (Miyamae et al., 1997; Wong et al., 2001; Rank and Jensen, 2003; Sharma et al., 2007). Decrease in genetic damage at later time intervals may indicate either repair of damaged DNA or loss of heavily damaged cells or both (Banu et al., 2001; Preeti and Shyama, 2009).

5.3. Comparison between *Paphia malabarica* and *Meretrix casta*:

Almost similar quantum of DNA damage induced by various doses of gamma radiation in the haemocytes of *Paphia malabarica* and of *Meretrix cast* in the present study may indicate that both of these bivalves show the same level of sensitivity to gamma radiation induced DNA damage. Varying levels of relative sensitivity are reported in ecologically relevant invertebrates exposed to various contaminants under laboratory conditions (Jha et al., 2000b; Cheung et al., 2006; Canty et al. 2009) and in situ (Dallas et al., 2012b). In the present study, we observed an interesting dose response at 72h in *Paphia malabarica*. It showed comparatively less DNA damage at lower doses (2 Gy to 6 Gy) which increased drastically later on at higher doses (8 Gy to 10 Gy) as compared to *Meretrix casta*. *Meretrix casta* did not show a drastic increase of DNA damage with the increased doses of irradiation. However, this interesting trend doesn't show significant difference between the two species. Present observation on the relative sensitivity between two bivalves may suggest that the same doses of gamma radiation may produce similar amount of genotoxic response in different closely related species. This may account either due to

their close phylogenetic relationship or could be accounted for similar antioxidant defense mechanisms or both.

6. **Conclusion:**

The result of the comet assay-based study presented here has indicated the positive mutagenic effect of gamma radiation on bivalves. Further, a dose-related increase and a time-dependent decrease of genotoxicity of gamma radiation were also observed in the haemocytes of both bivalve species. These responses indicate that haemocytes can be used as sensitive indicators (biomarkers) of a genotoxicant within an environmentally realistic range. Overall, the assay provides a convenient, highly sensitive and non-invasive monitoring tool of environmental exposure to genotoxicants. Further, the alkaline comet assay appears to be a promising technique to assess the genotoxic potential of gamma radiation in estuarine bivalves. This study also showed that *Paphia malabarica* and *Meretrix Casta* has shown a similar level of sensitivity to gamma radiation.

Conflict of interest statement:

None

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Reference:

Alamri, O. D., Cundy, A. B., Di, Y., Jha, A.N., Rotchell, J.M., 2012. Ionizing radiation-induced DNA damage response identified in marine mussels, *Mytilus sp.* Environmental Pollution, Volume 168, Pages 107–112

Banu, S.B., Danadevi, K., Rahman, M.F., Ahuja, Y.R., Jamil, K., 2001. Genotoxic effect of monocrotophos to sentinel species using comet assay. Food and Chemical Toxicology 39, 361–366.

Barnes, D.K.A., Rawlinson, K.A., 2009. Traditional coastal invertebrate fisheries in south western Madagascar. Journal of the Marine Biological Association of the United Kingdom 89, 1589–1596.

Belpaeme, K., Cooreman, K., Kirsch-Volders, M., 1998. Development and validation of the *in vivo* alkaline comet assay for detecting genomic damage in marine flatfish. Mutation Research 415, 167–184.

Brendler-Schwaab, S., Hartmann, A., Pfuhler, S., Speit, G., 2005. The *in vivo* comet assay: use and status in genotoxicity testing. Mutagenesis 20, 245–254.

Canty, M. N., Hutchinson, T. H., Brown, R. J., Jones, M. B., Jha A. N., 2009. Linking genotoxic responses with cytotoxic and behavioural or physiological consequences: Differential sensitivity of echinoderms (*Asterias rubens*) and marine molluscs (*Mytilus edulis*). Aquatic Toxicology 94, 68–76.

Chaubey, R.C., Bhilwade, H.N., Rajagopalan, R., Sanjay, V.B., 2001. Gamma ray induced DNA damage in human and mouse leucocytes measured by SCGE-Pro: A software developed for automated image analysis and data processing for Comet assay, Mutation Research, 490 (2) 187-197.

Cheung, V.V., Depledge, M.H., Jha, A.N., 2006. An evaluation of the relative sensitivity of two marine bivalve mollusc species using the Comet assay. Marine Environmental Research 62, 301–305.

Claxton, L.D., Houk, V.S., Hughes, T.J., 1998. Genotoxicity of industrial wastes and effluents. Mutation Research 410, 237–243.

Collins, A.R., 2004. The comet assay for DNA damage and repair: principles, applications and limitations. Molecular Biotechnology 26, 249–261.

Dallas, L. J., Keith-Roach, M., Lyons, B.P., Jha, A.N., 2012. Assessing the impact of ionizing radiation on aquatic invertebrates: A critical review. Radiation Research 177, 693−716.

Dallas, L.J., Cheung, V. V., Fisher, A.S., Jha, A.N., 2012b. Relative sensitivity of two marine bivalves for detection of genotoxic and cytotoxic effects: a field assessment in the Tamar Estuary, South West England. Environmental Monitoring and Assessment 1-16

Depledge, M.H., 1998. The ecotoxicological significance of genotoxicity in marine invertebrates. Mutation Research 399, 109–122.

Dixon, D.R., Pruski, A.M., Dixon, L.R.J., Jha, A.N., 2002. Marine invertebrate eco-genotoxicology: a methodological overview. Mutagenesis 17, 495–507.

Fossi, M.C., Leonzio, C., Peakall, D.B., 1994. The use of non-destructive biomarkers in the hazard assessments of vertebrate populations. CRC Press, Boca Raton, 1–28.

Hagger, J.A., Atienzar. F.A., Jha, A.N., 2005. Genotoxic, cytotoxic, developmental and survival effects of tritiated water in the early life stages of the marine mollusc, *Mytilus edulis*. Aquatic Toxicology 74, 205–217.

Hingston, J.L., Copplestone, D., McDonald, P., Parker, T.G., Iaea., 2003. The use of biomarkers in the assessment of biological damage in the lugworm (*Arenicola marina*) and the lobster (*Homarus gammarus*) due to environmental contamination. In protection of the environment from ionising radiation – the development and application of a system of radiation protection for the environment. Vienna: International Atomic Energy Agency, 60–68.

IAEA, 1992. Effects of ionizing radiation on plants and animals at levels implied by current radiation protection standards, Technical Reports Series No. 332. Vienna: International Atomic Energy Agency.

ICRP, 1991. 1990 Recommendations of the International Commission on Radiological Protection. ICRP Publication 60. Annals ICRP 21, 1-3.

ICRP, 2007. The 2007 Recommendations of the International Commission on Radiological Protection. ICRP Publication 103. Annals of ICRP 37, 2-4.

Jaeschke, B.C., Millward, G.E., Moody, A.J., Jha, A.N., 2010. Tissue specific incorporation and genotoxicity of different forms of tritium in the marine mussel, *Mytilus edulis*. Environmental Pollution 159 (1), 274-280.

Jaeschke, B.C., 2012.Exploring phenomena that affect the fate and impact of radioactive materials in the blue mussel. Printed in Sweden by US-AB, Stockholm, 1-93.

Jha, A.N, Cheung V.V, Foulkes M.E, Hill S.J, Depledge M.H., 2000a. Detection of genotoxins in the marine environment: adoption and evaluation of an integrated approach using the embryo-larval stages of the marine mussel, *Mytilus edulis*. Mutation Research 464:213–28.

Jha, A.N., Hagger, J.A., Hill, S.J., Depledge, M.H., 2000b. Genotoxic, cytotoxic and developmental effects of tributyltin oxide (TBTO): an integrated approach to the evaluation of the relative sensitivities of two marine species. Marine Environmental Research 50, 565–573.

Jha, A.N., Dogra, Y., Turner, A., Millward, G.E., 2005. Impact of low doses of tritium on the marine mussel, Mytilus edulis: Genotoxic effects and tissue-specific bioconcentration. Mutation Research 586, 47–57

Jha, A.N., Dogra, Y., Turner, A., Millward, G.E., 2006. Are low doses of tritium genotoxic to *Mytilus edulis*?. Marine Environmental Research 62, 297–300.

Jha, A.N., 2008. Ecotoxicological applications and significance of the comet assay. Mutagenesis 23, 207–221.

Konca, K., Lankoff, A., Lisowska, H., Kuszewski, T., Gozdz, S., Koza, Z., Wojcik, A., 2003. Cross-platform public domain PC image-analysis program for the comet assay Mutation Research 534, 15–20.

Kumaravel, T.S., Jha, A.N., 2006. Reliable Comet assay measurements for detecting DNA damage induced by ionising radiation and chemicals. Mutation Research Genetic Toxicology Environment Mutagen 605, 7–16.

Lonsdale, D.J., Cerrato, R.M., Holland, R., Mass, A., Holt. L., Schaffner, R.A., Pan, J., Caron, D.A., 2009. Influence of suspension-feeding bivalves on the pelagic food webs of shallow, coastal embayments. Aquatic Biology 6, 263–279.

Lovell, D.P., Omori, T., 2008. Statistical issues in the use of the comet assay. Mutagenesis 23, 171–182.

Malladi S.M., Bhilwade, H.N., Khan, M.Z. and Chaubey, R.C. 2007. Gamma ray induced genetic changes in different organs of chick embryo using peripheral blood micronucleus test and comet assay, Mutation Research. 630, 20-27.

Mersch, J., Beauvais, M.N., Nagel, P., 1996. Induction of micronuclei in haemocytes and gill cells of zebra mussels, *Dreissena polymorpha*, exposed to clastogens. Genetic Toxicology 371, 47–55.

Miyamae, Y., Zaizen, K., Ohara, K., Mine, Y., Sasaki, Yu, F., 1997. Detection of DNA lesions induced by chemical mutagens by the single cell gel electrophoresis (comet) assay: 1. Relationship between the onset of DNA damage and the characteristics of mutagens. Mutation Research 393, 99–106.

Mitchelmore, C.L., Chipman, J.K., 1998. DNA strand breakage in aquatic organisms and the potential value of the comet assay in environmental monitoring. Mutation Research 399, 135–147.

Moore, M.N., Depledge, M.H., Readman, J.W., Paul Leonard, D.R., 2004. An integrated biomarker-based strategy for ecotoxicological evaluation of risk in environmental management. Mutation Research, Fundamental and Molecular Mechanisms of Mutagenesis, 552(1–2), 247–268.

Coleman, C.N., Blakely, W.F., Fike, J.R., MacVittie, T.J., Metting, N.F., Mitchell, J.B., Moulder, J.E., Preston, R.J., Seed, T.M., Stone, H.B., Tofilon, P.J., Wong, R.S., 2003. Molecular and Cellular Biology of Moderate-Dose (1 -10 Gy) Radiation and Potential Mechanisms of Radiation Protection Report of Workshop at Bethesda, Maryland December 17-18, 2001. Radiation research 159, 812-834.

O'Neill, P., Fielden, E.M., 1993. Primary free radical processes in DNA. Advance Radiation Biology 17, 53–120.

Pavlica, M., Klobucar, G.I.V., Mojas, N., Erben, R., Papes, D., 2001. Detection of DNA damage in haemocytes of zebra mussel using comet assay. Mutation Research 490, 209–214.

Pedersen, T., Nilsen, M., Nilssen, E.M., Berg, E., Reigstad, M., 2008. Trophic model of a lightly exploited cod-dominated ecosystem. Ecological Modelling 214, 95–111.

Preeti, R.R., Shyama. S.K., 2009. Genotoxic effects of monocrotophos, an organophosphorous pesticide, on an estuarine bivalve, *Meretrix ovum.* Food and chemical Toxicology 47, 1618-1623.

Ren, J.S., Ross, A.H., Hadfield, M.G., Hayden, B.J., 2010. An ecosystem model for estimating potential shellfish culture production in sheltered coastal waters. Ecological Modelling 221, 527–539.

Sharma, S., Nagpure, N.S., Kumar, R., Pandey, S., Srivastava, S.K., Singh, P.J., Mathur, P.K., 2007. Studies on the genotoxicity of endosulfan in different tissues of freshwater fish *Mystus*

vittatus using the comet assay. Archives of Environmental Contamination and Toxicology 53, 617–623.

Singh, N.P., McCoy, M.T., Tice, R.R., Schneider, E.L., 1988. A simple technique for quantitation of low-levels of DNA damage in individual cells. Experimental Cell Research 175, 184–191.

Siu, W.H.L.; Cao, J.; Jack, R.W.; Wu, R.S.S.; Richardson, B.J.; Xu, L.; Lam, P.K.S., 2004. Application of the comet and micronucleus assays to the detection of B[*a*]P genotoxicity in haemocytes of the green-lipped mussel (*Perna viridis*). Aquatic Toxicology 66, 381–392.

Taddei, F., Scarcelli, V., Frenzilli, G., Nigro, M., 2001. Genotoxic hazard of pollutants in cetaceans: DNA damage and repair evaluated in the bottlenose dolphin (*Tursiops truncatus*) by the comet assay. Marine Pollution Bulletin 42, 324–328.

Tice, R., 1995. The single cell gel/comet assay: a microgel electrophoretic technique for the detection of DNA damage and repair in individual cells. Environmental Mutagen 125, 315-339.

Wong, C.K.C., Yeung, H.Y., Woo, P.S., Wong, M.H., 2001. Specific expression of cytochrome P4501A1 gene in gill, intestine and liver of tilapia exposed to coastal sediments. Aquatic Toxicology 54, 69–80.

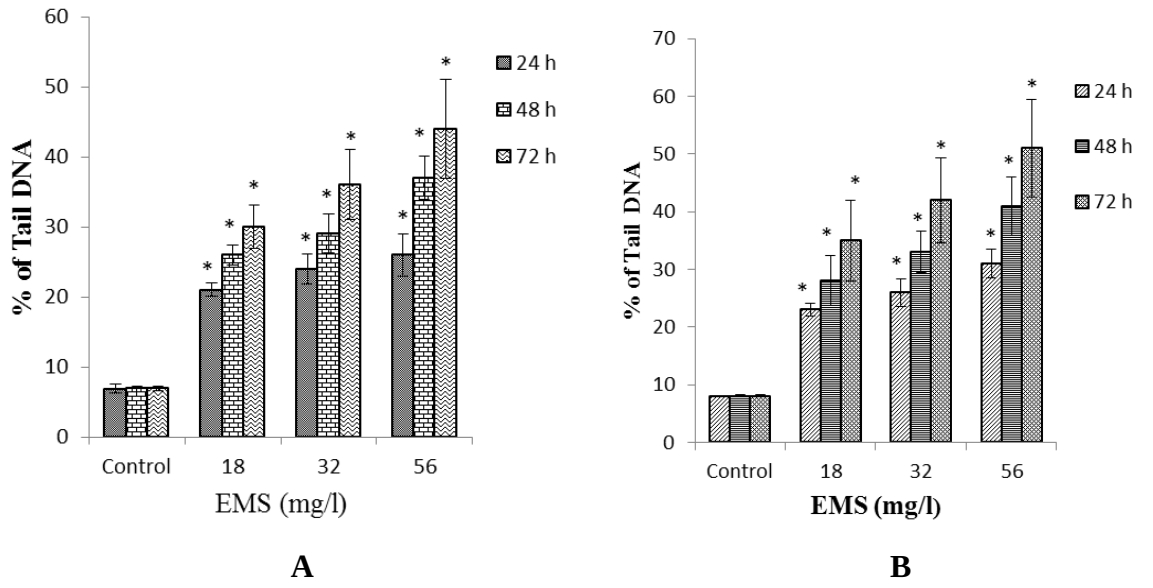


Fig. 1(A & B). Percentage of DNA damage in haemocytes of *Paphia malabarica* **(A)** and *Meretrix casta* **(B)** exposed to 18, 32 and 56 mg/l of EMS at various time intervals. Data are mean ± SD. (* P<0.001 denotes statistically significant difference from the control, Student's t-test significance

Dose-response :

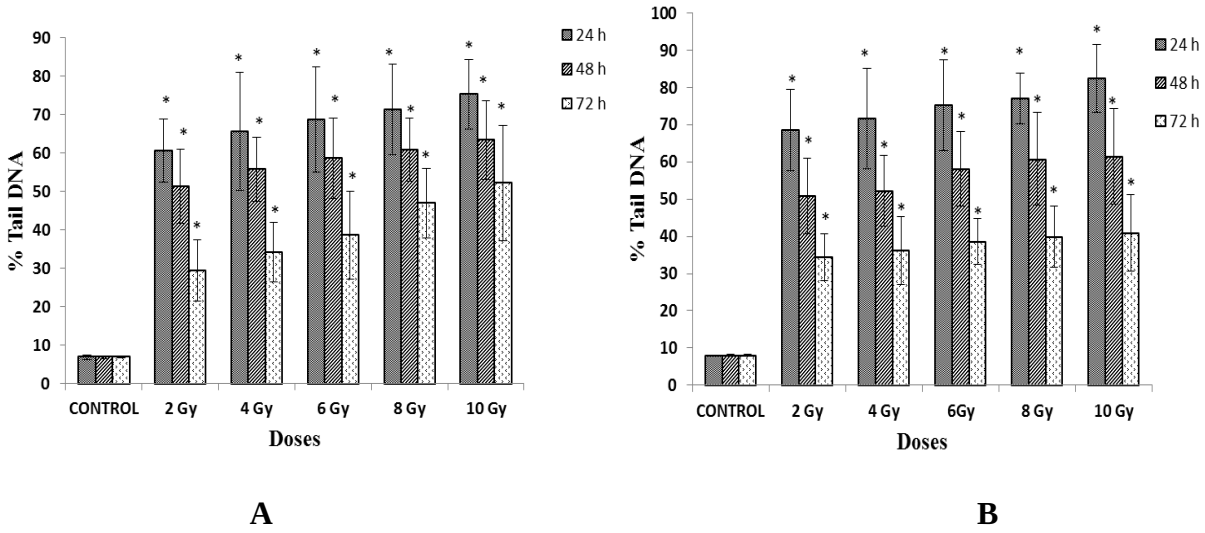
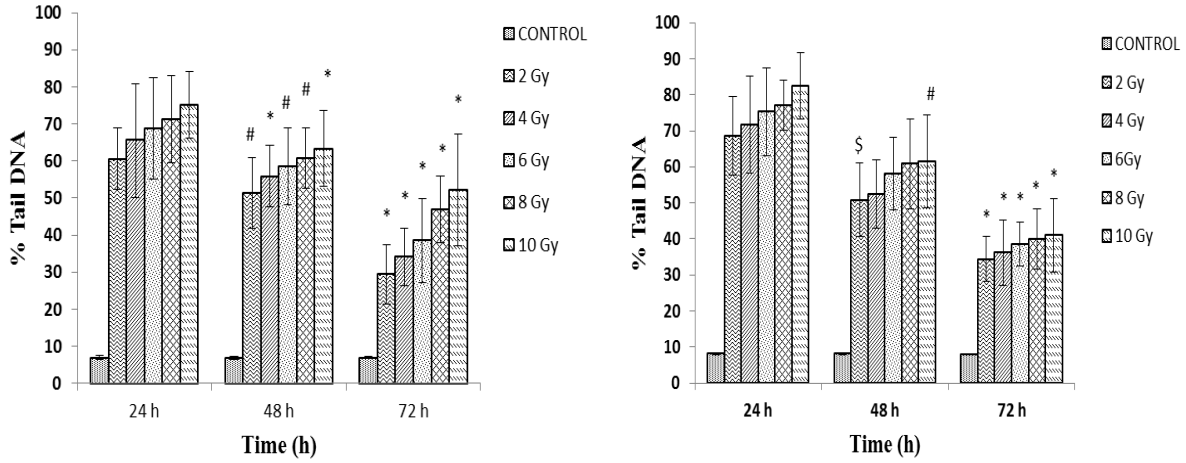


Fig. 2(A&B). Percentage of DNA damage in haemocytes of *Paphia malabarica* **(A)** and *Meretrix casta* **(B)** exposed to 2, 4, 6, 8 and 10 Gy of gamma radiation at various time intervals. Data are mean ± SD. (* P<0.001 denotes statistically significant difference from the control, Student's t-test significance).

Time-response :



A **B**

Fig. 3(A&B). Percentage of DNA damage in haemocytes of *Paphia malabarica* **(A)** and *Meretrix casta* **(B)** exposed exposed to various doses of gamma radiation at 24, 48 and 72h time intervals. Data are mean ± SD. ($ $P<0.05$ # $P<0.01$ * $P<0.001$ denotes statistically significant difference from the damage at 24h, Student's t-test significance).

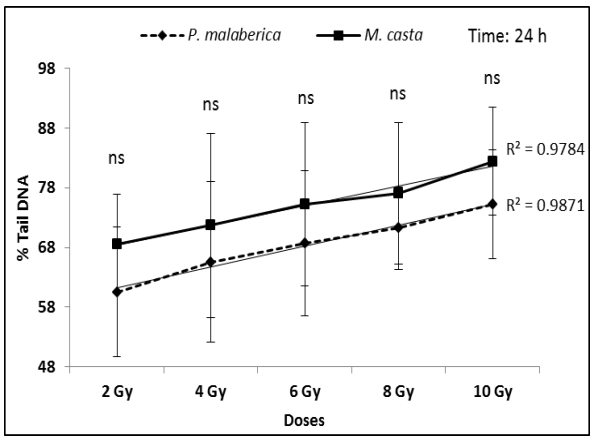


Fig. 4a

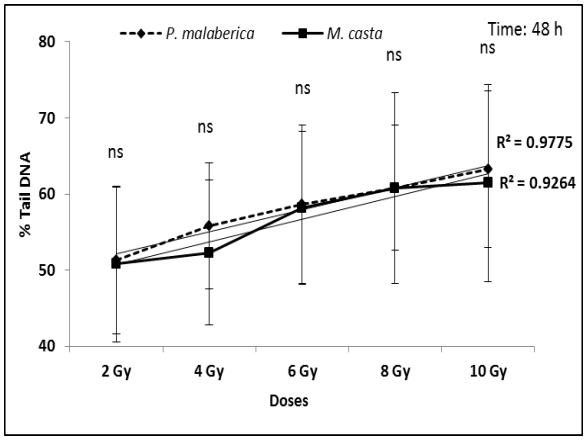


Fig. 4b

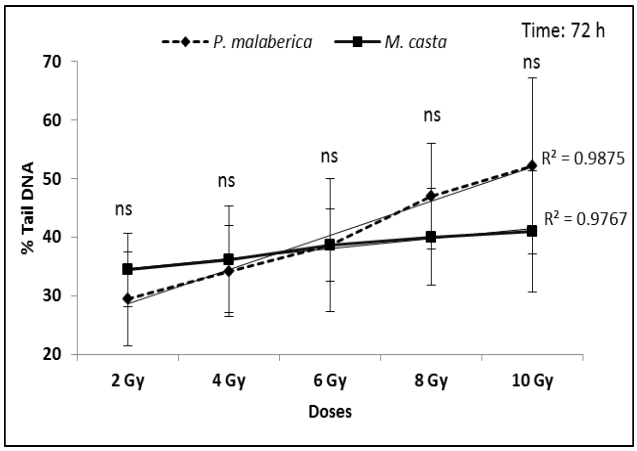


Fig. 4c

Fig. 4a, 4b & 4c. Comparison of the DNA damage induced by various doses (*viz.* 2, 4, 6, 8 & 10 Gy) of gamma radiation in the haemocytes of *Paphia malabarica* and *Meretrix casta* at 24h, 48h and 72h time intervals using t-tests. Each point indicates the mean ± SD of P*aphia malabarica* and *Meretrix casta*. Note: ns indicates a non-significant difference.

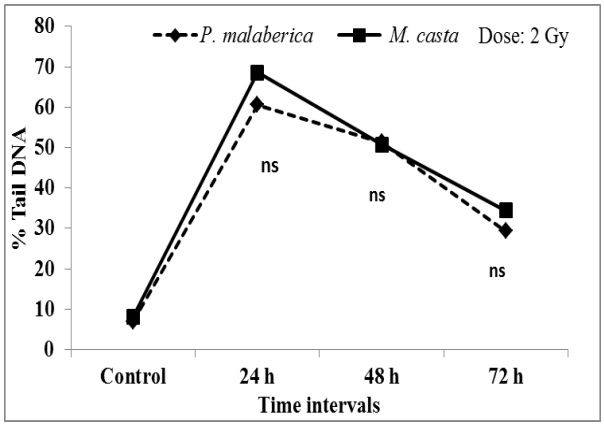


Fig. 5a

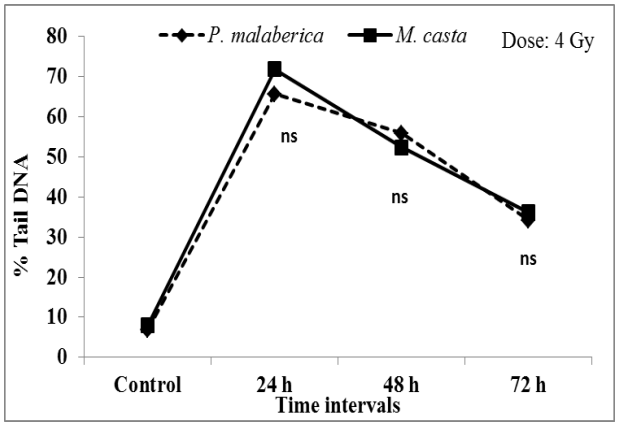


Fig. 5b

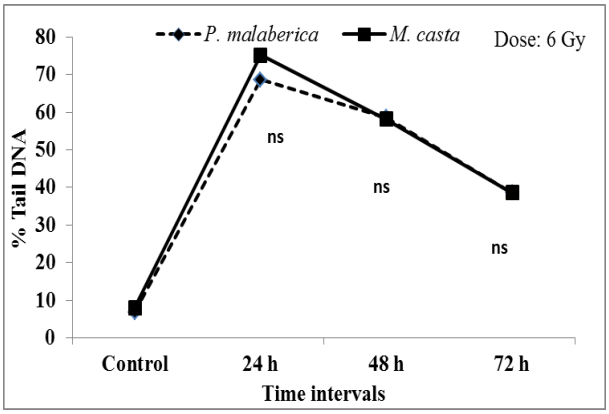


Fig. 5c

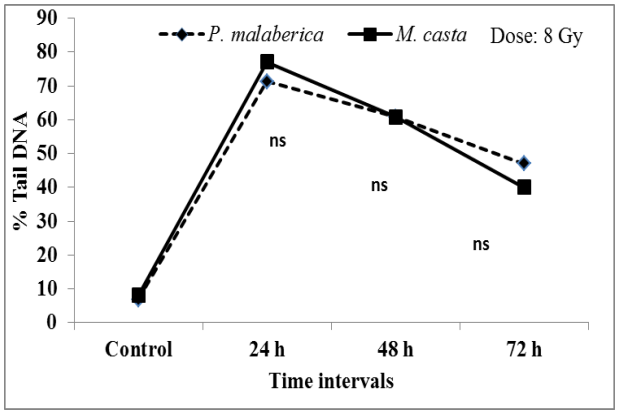


Fig. 5d

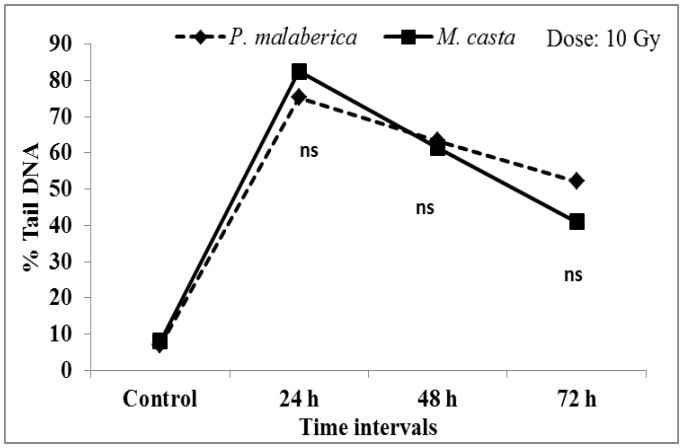


Fig. 5e

Fig. 5a, 5b, 5c, 5d & 5e. Comparison of the DNA damage induced at various time intervals of gamma radiation in the haemocytes of *Paphia malabarica* and *Meretrix casta* by various doses (*viz.* 2, 4, 6, 8 & 10 Gy). Each point indicates the mean ± SD of P*aphia malabarica* and *Meretrix casta*. Note: ns indicates a non-significant difference.