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Effects of gamma radiation on the early developmental stages of Zebrafish (*Danio rerio*)

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ABSTRACT

The zebrafish is gaining importance as a popular vertebrate model organism and is widely employed in ecotoxicological studies, especially for the biomonitoring of pollution in water bodies. There is limited data on the genetic mechanisms governing the adverse health effects in regards to an early developmental exposure to gamma radiation. In the present study zebrafish (*Danio rerio*) embryos were exposed to 1, 2.5, 5, 7.5 and 10 Gy of gamma radiation at 3 h post fertilization (hpf). Different developmental toxicity endpoints were investigated. Further, expression of genes associated with the development and DNA damage i.e. (sox2 *sox19a* and *p53)* were evaluated using Quantitative PCR (qPCR). The significant changes in the expression of sox2 sox19a and p53 genes were observed. This data was supported the developmental defects observed in the zebrafish embryo exposed to gamma radiation such as i.e. increased DNA damage, decreased hatching rate, increase in median hatching time, decreased body length, increased mortality rate, increased morphological deformities. Further, study shows that the potential ecotoxicological threat of gamma radiation on the early developmental stages of zebrafish. Further, it revealed that the above parameters can be used as predictive biomarkers of gamma radiation exposure.

1. Introduction

High profile disastrous incidents in nuclear facilities such as the nuclear reactor explosion in Chernobyl and the break-down of the cooling system in Fukushima have demonstrated that released radio-nuclides can be transported across the globe through the aquatic ecosystem (Han et al., 2014; Won and Lee, 2014). The impact of ionizing radiation on non-human biota including plants and animals are yet to be understood (Singhal et al., 2009). Early effects will be found at the molecular level, as this is one of the first targets in the cell (Moore, 2002). Thus, one approach to detect such radiation effects at an early stage is to study gene expression level (Weber et al., 2013; Wirbisky et al., 2014). The measurement of gene expression levels before and after exposure to a mutagen/chemical/physical agent can both provide information about the mechanism of action of toxicants as well as form a "genetic signature" from the pattern of gene expression (Freeman et al., 2014; Jaafar et al., 2013). Zebrafish embryos are ideal for evaluating radiation induced genotoxic stress (Choi and Yu, 2015; Freeman et al., 2014). FASSET has strongly recommended the need to undertake more systematic studies on the effect of radiation on fish eggs as a separate reference organism (FASSET Project, 2001).

Different development defects were observed in fish embryos exposed to X-ray (Ishikawa and Hyodo-Taguchi, 1997; Kuhne et al., 2009; Yasuda et al., 2006; Miyachi et al., 2003; Zhou et al., 2014). Bystander effects of ionizing radiation induction of DNA damage have been reported in zebrafish embryos (Pereira et al., 2014; Sophia et al., 2015). The majority of the available reports on the effect of gamma radiation on zebrafish embryos and zebrafish cell lines are mainly based on chronic exposures (Pereira et al., 2011; Ryan et al., 2008; Simon et al., 2011).

Sox genes play an important role in multitude of developmental and physiological processes, in vivo. The major functions of these genes include skeletogenesis (Smits et al., 2001), stem cell development in the embryo (Avilion et al., 2003), cardiogenesis (Akiyama et al., 2004), neurogenesis (Pevny and Placzek, 2005), sex determination (Polanco and Koopman, 2007) and hematopoiesis (Schilham et al., 1997) in

Abbreviations: Gy- Gray, h- Hour; hpf- hours post fertilization, γ- Gamma

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zebrafish. *Sox*2 gene has diverse obligatory roles to play in zebrafish embryonic development (Okuda et al., 2010). *Sox*2 gene encodes a transcriptional factor and is well known for its role in maintaining pluripotent stem cell population and differentiation during early development. The *sox19* gene play very important role in during embryogenesis. Abnormality in the tail region and central nervous system observed in zebrafish embryo when *sox2*, *sox3* and *sox19a* were knocked down (Okuda et al., 2010).

The p53 is a major transcription factor notably modulated by double stranded breaks (DBS). It binds directly to the regulatory sequences of its target genes which are involved in a variety of pathways, including DNA repair, cell cycle progression, cell death, transcription regulation, and other signaling pathways (Helton and Chen, 2007; Rashi-Elkeles et al., 2011).

The aim of this study is to investigate expression of *sox*2 *sox19a and p53* genes and effect on developmental toxicity endpoints after gamma radiation in relation to the genotoxic effect of gamma rays in zebrafish embryo. This study provides a better understanding on molecular mechanisms underlying the genotoxic effect of gamma rays to fish embryo.

2. Materials and methods

2.1. Fish maintenance and egg production

Adult zebrafish *(Danio rerio)* were procured from the Aquaculture farm (Margao, Goa), sexed and maintained separately as stock in aquaria (fitted with aerators and heaters) at 28 ± 1 °C with 14:10 h (light: dark) photoperiods and acclimatized for a week. Water was manually renewed by replacing 50% of the total volume once in every week with fresh water and also by refilling the evaporated water every day. Fishes were fed twice daily with live brine shrimps (*Artemia salina*) and commercial fish feed (Brand et al., 2002). As and when the eggs were needed for studies, 1 male and 2 females were placed in a hatching box in the aquaria in the late evening and allowed to breed for overnight. Spawning process was triggered in the early morning hrs of the day by switching on the lights which lasted around one h. Viable eggs were collected and rinsed thrice with E3 medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$ and 0.33 mM $MgCl_2$) prepared as per Brand et al. (2002) with pH 7.2–7.3, dissolved oxygen 6.3 mg/L, total hardness 65 mg/L (as $CaCO_3$) and temperature 28 ± 1 °C. All the chemicals used were of analytical grade (Sigma-aldrich, USA). In order to ensure developmental synchronization at the beginning of exposure, the embryos of 3 h post fertilization (hpf) (blastula stage) were employed for irradiation.

2.2. Irradiation

The assay was mainly based on the embryo test procedure developed by OECD guideline. In brief, eggs were transferred into a 96-well multiplate, one embryo per well filled with E3 medium. Embryos (3hpf) were irradiated at a dose rate 1.1633 Gy/min amounting to a total dose of 1, 2.5, 5, 7.5 and 10 Gy of gamma rays (single exposure) from a Co_{60} teletherapy unit at Goa Medical College, Goa. According to Jae et al. (2012) more than 1 Gy of radiation is suspected to prevail in the environment during any nuclear accident. However to ensure the safety at high doses which may occur at the time of accidental exposure, doses of upto 10 Gy were used. Parallel controls were mock irradiated by placing the samples in the irradiator. Both the control and irradiated embryos were maintained at 28 ± 1 °C with a 14:10 h (light: dark) photoperiod. E3 media were renewed regularly at 24 h intervals of time. The experiments were carried out in triplicates.

2.3. Endpoints

2.3.1. Developmental toxicity endpoints

Mortality, hatching rate, malformations and body length of the larvae were employed as the toxicological endpoints at the individual level for the present study. The embryos in the well were directly observed under a stereo microscope connected to a camera and the above endpoints were scored at 24, 48, 72, 96 and 120 hpf.

The mortality rate was calculated as the number of dead embryos at 120 hpf divided by the number of embryos used for the experiment at its beginning. HT_{50} was calculated as the (time necessary for 50% of the eggs to hatch in each experimental condition). The hatching rate is calculated as the total number of embryos hatched at 120 hpf divided by the number of embryos taken for the experiment. Larvae of 120 hpf were positioned on the lateral side, photographed and their body length was measured. The frequency of morphological deformities in embryos was calculated as the total number of larvae with morphological deformities at 120 hpf divided by the number of alive zebrafish.

2.3.2. Comet assay

The genotoxic effect induced by gamma irradiation in the early developmental stages of zebrafish at the DNA level was evaluated employing the alkaline comet assay as per Singh et al. (1988) with slight modification. Mechanical cell isolation, the first step of the comet assay, was carried-out as described by Kosmehl et al. (2006). Embryos which had a minimum of 90% cell viability were selected for the comet assay. Two hundred cells were scored from each of the five slides per group. Percentage tail DNA, which is considered as the most reliable parameter (Praveen Kumar et al., 2014) was recorded. All the experimental and control groups were represented in three replicates.

2.3.3. Quantitative PCR (q-PCR)

Total RNA was extracted from both the control (non-irradiated embryos) and 5 Gy of gamma radiated embryos (20 each) in triplicate at 24, 72 and 120 hpf using TRizol reagent (Invitrogen, USA) according to the manufacturer's instructions. The quality and quantity of these RNA were evaluated by NanoDrop 2000 spectrophotometer (Thermo Scientific, USA).

First-strand cDNA molecules were synthesized from 1 µg of total RNA using the SuperScript III Reverse Transcriptase (Invitrogen, USA) according to the manufacturer's instructions. The cDNA mixture was used as a template for real-time PCR. Genes that were studied in the present study have selected from Desai et al. (2011) and shown in Table 1. The *β actin* gene has been selected as housekeeping gene, due to its high stability (McCurley and Callard, 2008).

Real-time PCR reactions were carried out with one cycle at 95 °C for 10 min and 40 amplification cycles at 95 °C for 10 s, 58 °C for 15 s and 72 °C for 15 s. Reaction mixture (20 µL) contained 4 µL of reverse transcribed product (cDNA) as template, 10 µL of SsoFast EvaGreen supermix (Bio-Rad, USA), 1 µL each gene specific primer at a final concentration of 300 nM and 4 µL RNase free water. The amplification reaction was carried out using CFX connect Real-Time PCR Detection

Table 1
Target genes for q-PCR along with primers.

Gene name	Accession ID	Primer sequence	Reference
*sox*2	NM_213118.1	F: CTCGGGAAACAACCAGAAAAR: TCGCTCTCGGACAGAAGTTT	Desai et al. (2011)
p53	AF365873	F: GGGCAATCAGCGAGCAAA R: ACTGACCTTCCTGAGTCTCCA	
sox19a	NM_130908.1	F: TGTCAACAGCAACAACAGCAR: GTTGTGCATTTTGGGGTTCT	
β actin	NM_181601.3	F: CGAGCTGTCTTCCCATCCAR: TCACCAACGTAGCTGTCTTTCTG	

System (Bio-Rad, USA). The *β actin* gene was selected as reference gene for the present study, due to its high stability (McCurley and Callard, 2008). Further, the stability of the *β actin* towards gamma radiation was confirmed by experimentation. There was no statistically significant difference for expression of *β-actin* between control and exposed groups at each time interval; therefore, *β actin* was used as reference gene for normalization. Relative gene expression of *sox2 sox19a and p53* as compared to the reference gene, *β actin* was determined using CFX Manager™ software (Deepa et al., 2013). A standard curve of cDNA template was run on each plate to allow for within experiment plate normalization.

2.4. Statistical analysis

Analysis of the data obtained was carried out using the statistical package GraphPad Prism 5.0 (GraphPad Software, Inc. CA 92037, USA). The control and experimental values obtained at various time intervals (24, 48, 72, 96 and 120 hpf) for hatching rates and mortality were pairwise compared using pairwise chi square tests. In order to keep a global alpha risk at 5% level for each observation time, *p*-values of the pairwise Chi-square tests were adjusted according to the Holm-method (Holm, 1979). Comet assay data (% tail DNA) were arcsine transformed and tested for normality and homogeneity using the Shapiro-Wilk test and Levene's test respectively. Data of genotoxicity endpoints (comet assay), morphological deformities, body length, hatching rate and median hatching rate were analyzed by a one-way ANOVA, with a post hoc pairwise Tukey test to identify differences between specific treatment groups. Data of relative expression were analyzed using student's *t*-test. For gene expression analysis, the efficiency of qPCR was calculated from the standard curve for each plate. Samples from the same experiment run over multiple plates were adjusted to the plate with the efficiency closest to 1 by resolving for slope and intercept of the standard curve. The correlation between DNA damage and abnormalities in embryo development was analyzed by the Pearson coefficient test. A level of probability of $p < 0.05$ was considered as a statistically significant data.

3. Results

3.1. Mortality rate

The mortality induced by various doses of gamma radiation (1–10 Gy) in zebrafish embryos are shown in Fig. 1. The control group of embryos (0 Gy) exhibited normal survival status and showed an overall mortality of less than 2%. Significant mortality was seen in irradiated zebrafish embryos irradiated with various doses of gamma radiation compared to their respective controls. Further, a significant dose-dependent increase ($F = 900$, $p < 0.001$) of the mortality rate was observed, with a minimum (18.06 ± 1.04%) at the lowest dose (1 Gy)

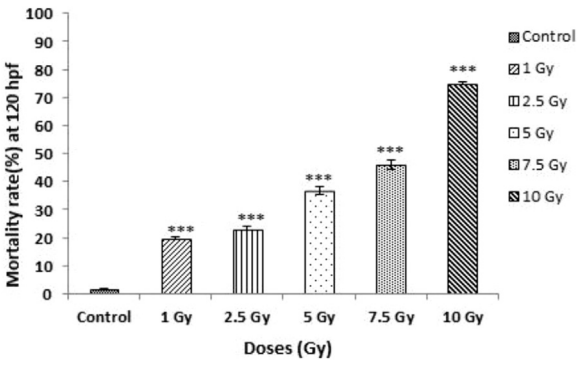

Fig. 1. Mortality rate of embryos irradiated with various doses of gamma radiation at different time intervals (Mean ± SD). (*** $P < 0.001$ denotes statistically significant difference from the control, Tukey's test of significance).

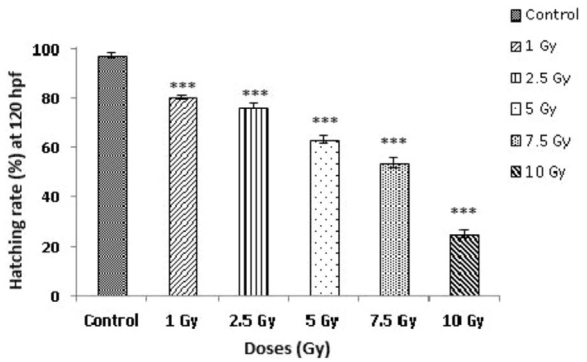

Fig. 2. Hatching rate of embryos irradiated with various doses of gamma radiation at 120hpf (Mean ± SD). (*** $P < 0.001$ denotes statistically significant difference from the control, Tukey's test of significance).

and maximum (73.27 ± 1.80%) at the highest dose (10 Gy) at 120hpf. Further, embryos irradiated with 1–10 Gy of gamma radiation exhibited significantly increased mortality at all the time intervals of study 24–120 hpf; Supplementary data: S1).

3.2. Hatching time and rate

The hatching process was not synchronous; the median hatching time [HT_{50} (95% CI)] for $= 167.5$, $p < 0.001$) with increasing doses of gamma radiation (Supplementary data: S2). The lowest median hatching time HT_{50} (67.43 ± 0.8%) hpf was observed in embryos irradiated with the lowest dose (1 Gy) and the highest (82 ± 2.72%) hpf in embryos irradiated with the highest dose (10 Gy). The hatching rates of zebrafish embryos exposed to various doses of gamma radiation at 120 hpf is shown in Fig. 2. A dose dependent decrease ($F = 702.8$, $p < 0.001$) of the hatching rates was observed, i.e. the hatching rate decreased with increasing doses of gamma radiation from 1 to 10 Gy. Further, the maximum hatching rate (80.21 ± 1.04%) was observed in embryos irradiated with the lowest dose (1 Gy) and the minimum (25 ± 9.05%) in embryos irradiated with the highest dose (10 Gy). Significant decrease in hatching rate of exposed eggs was noted at various time intervals and the surviving embryos hatched by 120hpf (Supplementary data: S3). This indicates the delay in the hatching process as a result of irradiation.

3.3. Total Body length

The size of the irradiated (1–10 Gy) and unirradiated (control) larvae attained by 120hpf (as measured by their total body length) are depicted in Supplementary data: S4. Irradiated larvae exhibited a dose dependent decrease ($F = 5.08$, $p < 0.01$) of total body length. Control larvae measured 4.02 ± 0.20 mm, whereas, the irradiated ones ranged from 3.23 ± 0.25 mm (1 Gy) to 3.03 ± 0.50 mm (10 Gy).

3.4. Morphological deformities

Frequency of morphological deformities induced by various doses of gamma radiation in zebrafish embryos at 120 hpf is represented in Fig. 3. Statistically significant increase of morphological deformities were seen in larvae irradiated with 1–10 Gy of gamma radiation as compared to controls ($F = 92.62$, $p < 0.001$). Malformations such as pericardial edema, Yolk sac edema, curved notochord and thin caudal fin were observed. The frequency of malformed larvae ranged from 6.48% to 58.73% in irradiated larvae as compared to the 0.71% in controls. Higher doses of gamma radiation induced severe malformations.

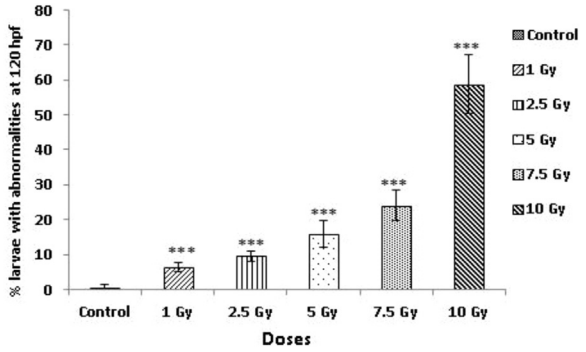

Fig. 3. Frequency of morphological deformities (Mean ± SD) induced by various doses of gamma radiation in zebrafish embryos at 120 hpf. (*** $P < 0.001$ denotes statistically significant difference from the control, Tukey's test of significance).

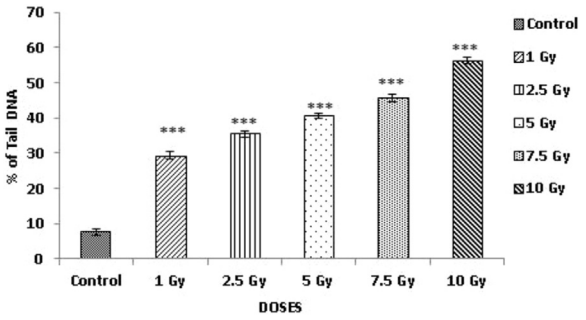

Fig. 4. DNA Damage (% of tail DNA) in embryos irradiated with various doses at 24 h after exposure. Data are (Mean ± SD). (*** $P < 0.001$ denotes statistically significant difference from the control, Tukey's test of significance).

3.5. Comet assay

The DNA single strand breaks expressed as the mean tail DNA content (% tail DNA) induced by different doses of gamma radiation (1–10 Gy) in zebrafish embryo at 24 hpf are given in Fig. 4. Significant DNA damage was noticed in all irradiated zebrafish embryos as compared to their controls ($F = 434.9$, $p < 0.001$). Interestingly, a dose dependent increase in the mean % tail DNA was observed, with a minimum (29.18 ± 1.35) at the lowest dose (1 Gy) and the maximum (56.52 ± 0.91) at the highest dose (10 Gy).

3.6. Gene expression

A significant difference in gene expression was observed for *sox*2 *sox19a and p53* gene in control (un-irradiated embryos) and irradiated embryos and is represented in Fig. 5 (A, B and C). Statistically significant decrease in the expression of *sox*2 gene was observed in the gamma irradiated embryos of zebrafish as compared to the unirradiated control embryos at all the time intervals. Further, statistically significant increase in the expression of *p53* and *sox19a* gene expression were also observed in the gamma irradiated embryos of zebrafish as compared to the unirradiated control embryos.

3.7. Correlation between comet assay and morphological deformities

The results of the correlation between comet assay and the morphological deformities induced by irradiation in zebrafish embryos are represented in Fig. 6. A significant positive relationship ($r = 0.8634$, $P = 0.0027$) was noted between the two parameters.

4. Discussion

Toxic effects of gamma radiation on aquatic biota and ecosystems are becoming the emerging concern of recent years. The present study revealed the adverse effects of the acute exposure of gamma radiation in zebrafish embryo as indicated by their hatching rate, mortality rate, DNA single strand breaks, body length and morphological deformities and up and down regulation in gene expression.

4.1. Mortality rate

Increased mortality of irradiated embryos observed in the present study suggests the toxicity of gamma radiation on zebrafish embryos. Similar increased mortality rate was reported by Bourrachot et al. (2008) in zebrafish embryos exposed to uranium radioactivity. Mortalities were also noted in zebrafish embryos, which were exposed to various chemicals (Fraysse et al., 2006) or metals like copper (Johnson et al., 2007). In the present study, mortality started to occur at significant level by 24 hpf or day 1 itself at all the doses studied. This is on par with the observations of McAleer et al. (2005) in zebrafish embryos at 24 hpf, which were exposed to various doses of X-rays. However, Freeman et al. (2014) failed to observe significant increase of mortality and Simon et al. (2011) observed a delay in the mortality of gamma irradiated zebrafish embryos. These may be due to the low doses and chronic exposure to gamma radiation which induces a metabolic shift from oxidative phosphorylation to aerobic glycolysis resulting in increased radiation resistance as observed in human cells by Lall et al. (2014). Present observation of increased mortality in irradiated embryos is supported and supported by additional observations of the present study such as increased DNA damage and change in the expression of developmental genes.

4.2. Hatching rate

Gamma radiation induced a significant increase of the median hatching time of the zebrafish embryos compared to the controls in the present study. Further, a significant decrease of the hatching rate was also noted in the gamma irradiated embryos. The decreased hatching rate observed in the present study are in agreement with the findings of Pereira et al. (2011) where they observed an impairment of hatching success in zebrafish embryos exposed to gamma radiation. Further, Rhee et al. (2012) also observed decreased hatching rate in the embryos of the fish *Kryptolebias marmoratus* exposed to gamma radiation. This indicates the similar developments toxicity observed in other fishes it may be because of similar manner of hatching process. Further, a linear dose effect of hatchability was observed by Egami et al. (1983) in embryos resulted from the mating between a normal female and an abnormal male Medaka with mutation induced by gamma rays. Hatching delay was also observed in a crustacean *Daphnia magna* by Gilbin et al. (2008), in which broods were chronically irradiated with gamma radiation. Similar findings were also reported in zebrafish embryos exposed to chemical or metal toxicants (Bourrachot et al., 2008; Fraysse et al., 2006; Johnson et al., 2007). However, Freeman et al. (2014) failed to observe significant alternation in hatching rate and Simon et al. (2011) observed accelerated hatching time, which may be because of the chronic exposure of 3–24 hpf zebrafish embryos to gamma radiation over a 20-day period in contrast to the acute exposure in our present study. A complex combination of biochemical and physical mechanisms are reported to be involved in the process of hatching of the zebrafish embryos (Inohaya et al., 1997). Chorion is digested by the hatching enzyme (proteolytic enzyme) which is secreted by hatching gland cells of the embryo. This hatching enzyme contains two constituent proteases: choriolysin H (HCE) and choriolysin L (LCE), which belong to the astacin protease family, a subfamily of zinc-proteases. Hatching delay and increase of median hatching time observed in the gamma irradiated zebrafish embryos in our study may be due to the delay/anomaly of the hatching enzyme and/or due to the hypoxia induced by radiological stress. This observation is complemented by the DNA damage and developmental gene observed in this study.

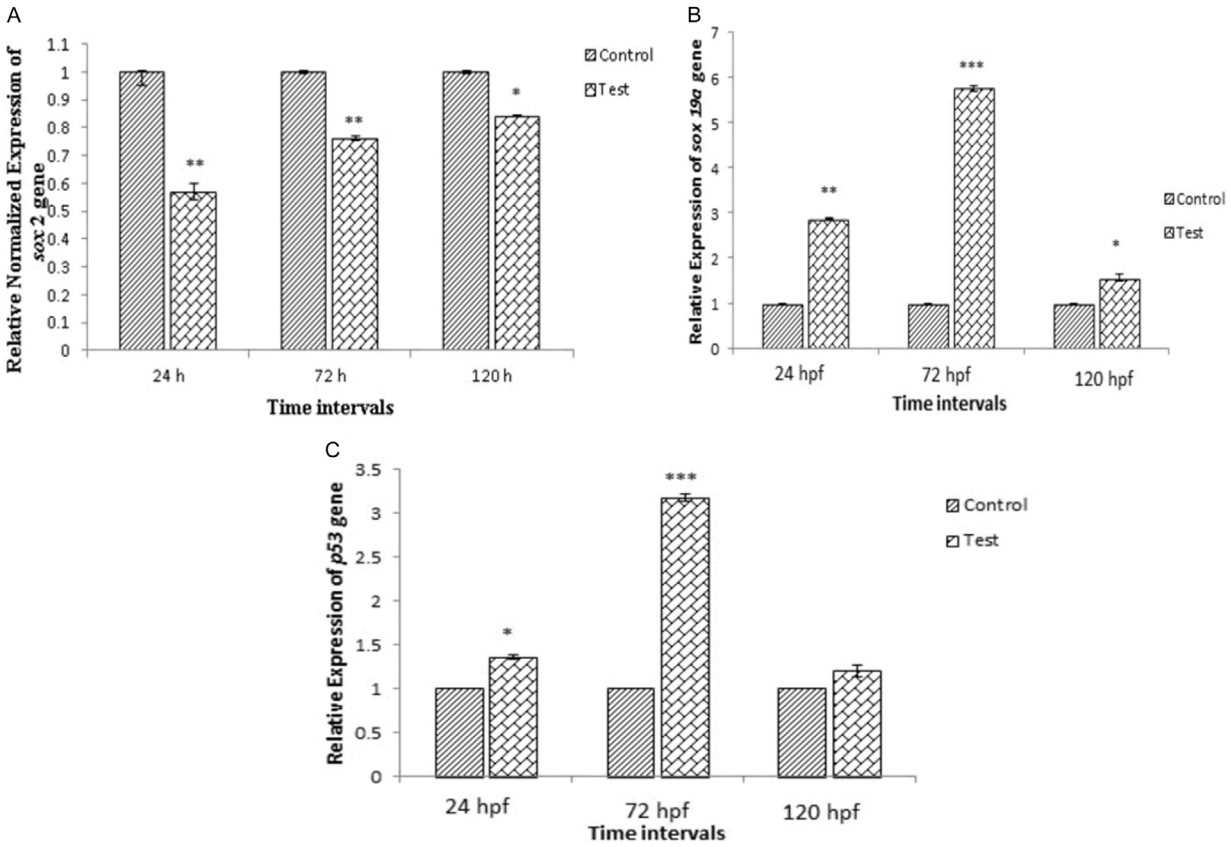

Fig. 5. (A, B and C). Effect of gamma radiation on *sox2*, *sox19a* and *p53* gene expression in zebrafish embryos. Data are Mean ± S.D). ***t*-test**: Control Vs Treated. Note: *** = P < 0.001, ** = P < 0.01, * = P < 0.05.

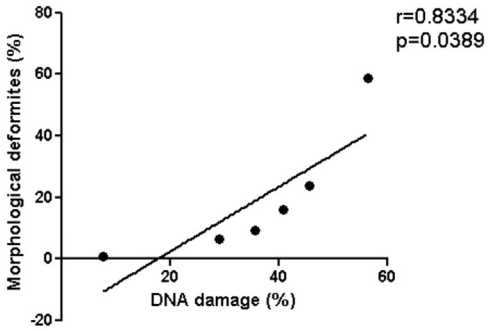

Fig. 6. Correlation between DNA damage and morphological deformities in zebrafish [Pearson correlation was used and the level of significance was set at 95%.

4.3. Body length

The significant reduction of the body length observed in the irradiated zebrafish in the present study is in line with the findings of Freeman et al. (2014) in which zebrafish embryos were exposed to gamma radiation doses (1, 2, 5, 10 Gy) at 26 h post fertilization (hpf). Further, Bourrachot et al. (2008) also observed in uranium exposed zebrafishes. The reduced body length observed by us may be because of the radiation stress induced by gamma irradiation. This is supported by our expression study of the developmental gene.

4.4. Morphological deformities

High frequency of morphological deformities was induced by various doses of gamma radiation ranging from 1 to 10 Gy in the larvae at 120 hpf of zebrafish. Increased morphological deformities observed in this study is on par with the findings of Torres et al. (2012) who observed several developmental abnormalities, mainly posterior/caudal notochord bending/torsion in zebrafish exposed to UV radiation. Bending of the caudal region was the major kind of morphological deformity note in the present study. Similar deformities were reported by Ishikawa and Hyodo-Taguchi (1997) in medaka (*Oryzias latipes*) exposed to X-rays. A similar observation was reported by Pereira et al. (2011) and Freeman et al. (2014). Further, Okuda et al. (2010) observed very severe developmental abnormalities knockdown of the four B1 sox genes sox2/3/19a/19b. Increased morphological deformities in zebrafish larvae may be due to radiation induced DNA damage (single/double strand breaks). Change in the expression of *sox2* and *sox19a* gene may also contribute to the induction of morphological deformities.

4.5. Genotoxic effects of gamma radiation (comet assay)

Significant increase of the radiation induced DNA single-strand breaks (% tail DNA) observed in zebrafish embryos in the present study at all the doses studied indicate the genotoxic potential of gamma radiation. Increased DNA damage was also reported by Pereira et al. (2011) where they observed significant DNA damage in gamma irradiated zebrafish embryonic cells (ZF4). The dose-dependent increase of DNA damage induced by irradiation in the zebrafish embryo in the present study is on par with the similar observations of Simon et al. (2011) in which they exposed 6 hpf zebrafish embryos to various doses of gamma radiation (from 1 to 1000 mGy/d). Jarvis and Knowles (2003) also reported that the zebrafish larvae (5–6 days post laying) exposed to 0.4, 1.2 or 7.2 mGy/h for 1 and 24 h showed a dose dependent increase of DNA damage.

Radiation may act either directly on the DNA molecules and induce mutations or indirectly on water molecules to induce water-derived free radicals. These free radicals in turn will react with the nearby molecules

in a very short time, resulting in breakage of chemical bonds or oxidation of the affected molecules. The major effect of radiation in cells is DNA breaks (Alizadeh et al., 2013).

4.6. Gene expression

Significant change in the expression level of *sox*2 and *sox*19a gene has been clearly revealed in gamma irradiated zebrafish embryos. Similar results were observed by Desai et al. (2011) where they studied the effect of chilling and subsequent warming on the expression of developmental genes *sox*2, *sox*3 and *sox*19a in zebrafish. Okuda et al. (2010) observed the sox gene proteins control a wide range of developmental regulators in the early embryo and suggest that the sox gene functions are central to coordinating cell fate specification with patterning and morphogenetic processes occurring in the early embryo. Freeman et al. (2014) have also observed a significant decrease in expression of *LIN7B* genes in zebrafish embryos exposed to gamma radiation. Expression level of DNA repair genes could also decide the extent of stability of zebrafish genome in a specific genotoxic stress condition. Damages which could not be repaired by their DNA repair system finally might have led to the morphological deformities in the zebrafish larvae (Pereira et al., 2011). Sandrini et al. (2009a) have observed activation of the DNA repair systems in the hepatocytes of zebrafish which were exposed to ionizing radiations. Several studies in fish have clearly revealed that gene expression can be modulated by exposure to chemical genotoxicants (Sandrini et al., 2009b; Geffroy et al., 2012). Significant down regulation of *sox*2 gene expression in the present study may be due to the sensitivity of the *sox* genes to gamma radiation. This result is supported by the increased DNA damage and morphological deformities in the present study. In this study, p53 was up-regulated at transcriptional level by irradiation at, 24 and 72hpf, but no change was found at other time points. The role that p53 played in radiation-induced apoptosis during zebrafish development remains unclear. Since 48–72 hpf is a critical period during the early embryonic development of zebrafish, the up-regulation of p53 at 72 hpf may be crucial in enabling the zebrafish to cope with stress and ensure normal development by inhibiting the induction of “abnormal” apoptosis, although the precise role of p53 is not known.

4.7. Correlation between DNA damage and Morphological deformities

Positive correlation between DNA damage (as observed by comet assay) and morphological deformities in zebrafish larvae observed in the present study may suggest the role of DNA single strand breaks in the induction of physical deformities in zebrafish embryo. DNA damage may also result in the down regulation of the developmental gene *sox*2, which together as may contribute all the developmental defects observed in the study. Similar gamma radiation induced DNA damage and impaired growth were observed in invertebrate model, in copepod (Han et al., 2014; Won and Lee, 2014).

5. Conclusion

The major effects of gamma radiation observed in the embryos of zebrafish include decreased hatching rate, increased median hatching time, decreased body length, increased mortality rate, increased morphological deformities, increased DNA damage and change in expression of development and DNA damage genes. This clearly demonstrated the positive mutagenic effect of gamma radiation on zebrafish embryos. These responses indicate that zebrafish embryo can be used as a sensitive bio-indicator of a genotoxicant within an environmentally realistic range. The alkaline comet assay appears to be a promising technique to assess the genotoxic potential of gamma radiation in whole-organism. Zebrafish can be a model bio-indicator of aquatic environments, capable of furnishing good measurable responses to genotoxicants and mutagenic agents. Further, positive correlation was noted between DNA damage and morphological deformities in embryo development of zebrafish. Thus, the present study reveals that the DNA damage and change in expression of *sox* genes involved in development of embryo could be the possible reasons for the morphological deformities in zebrafish larvae. Thus we may conclude that the above parameters in fishes can be used as predictive biomarkers of radioactive contaminate in the water bodies and current understanding of the potential ecotoxicological threats of gamma radiation.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2017.03.054.

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