Research Article

A Wee Study on Behavioural, Organ Somatic Index and Histological Alterations of the Fresh Water Fish *Pangasius sutchi* in Response to Protection Studies, Exposed to Gamma Radiation Perceived by Genotoxic Assays

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Received: 09-03-2018; **Revised:** 02-05-2018; **Accepted:** 18-05-2018.

ABSTRACT

The aim of the present study was to evaluate radio-protective effect of *Gymnema sylvestre* and its active compound gymnemagenin against gamma radiation (60Co) in *Pangasius sutchi*. The fishes were treated with lowest concentration of amifostine (83.3mg/kg of b.wt.), *G. sylvestre* plant extract (25mg/kg of b.wt.), and gymnemagenin (0.3mg/kg of b.wt.) as intramuscular injection one hour prior to exposure of gamma radiation. The result revealed destructive histological alteration of irradiated fishes compared to pre-treatment group of amifostine, G. sylvestre, and gymnemagenin. Similarly, behavioural changes and organ somatic index also showed variations among the irradiated and pre-treatment groups which might reflect metabolic and physiologic disturbances under the effect of radiation. The pre-treatment of G. sylvestre showed similar result as compared to amifostine which indicates the existence of radio-protective properties in the plant.

Keywords: Gamma radiation, Amifostine, *Gymnema sylvestre* and Gymnemagenin

INTRODUCTION

Radiological protections of environment become most vital environmental safety concern, because of atomic power energy source for the future human development[1]. Contamination of water bodies occurs either from discharge of industrial effluent or from nuclear power accidents, due to which the radioactive materials became major water pollutants and are toxic to aquatic biota. These disasters are indication of negative changes in environment and also enlightening the importance of radiation protection and radiation safety for human as well as the environment[2]. Fish erythrocytes are sensitive to DNA damage which is the major site for ROS production due to their role in the oxygen transport via haemoglobin[3]. So it required systematic study using sensitive biomarkers to evaluate the DNA damage in non-human biota[4].

The genotoxic assays has gained popularity in aquatic toxicity research due to its sensitivity, simplicity and reliability for detecting cytogenetic DNA damage[5-8]. Histopathology is also one of the effective tool to visualize the stress-induced structural changes in cells and tissues, and has been widely used as biomarkers in the evaluation of various stressors such as microbial pathogens, toxic compounds, radiation, nutritional and adverse environmental conditions[9] both in the laboratory[10, 11, 12] and field studies[13, 14]. The histological changes were reported for different organs of fish including: kidneys[16, 17, 18], gills [17, 19, 20], brain[9], liver[21, 27, 20]. Study of changes in biological functions of aquatic animals under the effect of radiation allows determining the health condition of economic aquatic products which also reflect the state of pollution in the environment[15]. So, the substances that are able to protect organisms from ionizing radiation are of major concern for radiation protection studies.

Amifostine is most effective compound studied for its radio-protective efficacy[22]. Jacqueline *et al.* (2012)[23] stated that administration of amifostine can be act as prodrug where, amifostine converts by alkaline phosphatase into an active thiol through dephosphorylation[24]. The protection of cells by amifostine is based on the differences between the physiological environments of normal and damaged cells because damaged cells are hypovascular, have low pH, and lower expression of alkaline phosphatase than normal cells[25]. Hence, amifostine acts better on normal cells, rather than damaged or tumor cells. Once the amifostine gets activated, it accumulates within the cells and acts as a scavenger by eliminating free radicals, maintaining membrane integrity, and preventing DNA damage.

Similarly, on the other hand, use of natural products developed from the plant material is beneficial for protecting organisms against the radiation-induced damage, as they are less toxic compared to the synthetic compounds at their optimum protective dose levels [26]. *G. sylvestre* is a conventionally used medicinal plant with described as a medication for diabetes mellitus, diuretic problems, stomachic and plant extract is also used in ayurvedic, and homeopathic systems of medicine[27]. Gymnemagenin is the simplest form of Gymnemic acid which is the major active compounds of *G. sylvestre* which plays a key role in detoxification of drugs and toxic compounds. So, the purpose of the present study was to evaluate the effects of amifostine, *G. sylvestre* and gymnemagenin against gamma radiation based on behavioural, organ somatic index and histological changes in *Pangasius sutchi.*

MATERIALS AND METHODS

Test Species

Pangasius sutchi is one of the fast growing catfishes, cultured in many places due to its market demand and commonly known as freshwater shark belongs to the family pangasidae[4] was collected from local fisheries of Chennai, Tamil Nadu and maintained in laboratory condition.

Determination of median lethal dose ($LD_{50/30}$) by gamma radiation

Fishes (n=70) were used for the $LD_{50/30}$ determination which were segregated into 7 groups. Each of 10 fishes, were exposed to gamma radiation [gamma radiation source: cobalt 60 (^{60}Co)] at doses of 2.5Gy, 5Gy, 7.5Gy, 10Gy, 15Gy and 20Gy respectively at Indira Gandhi Centre for Atomic Research (IGCAR), Kalpakkam, Tamil Nadu India. Both control and treated groups were maintained for 30 days and the numbers of dead fishes were recorded once every 12 h. Apart from the mortality of fishes, their lethality-related behaviour, as well as morphological manifestations of radiation effects were also observed and recorded[8]. The data was tabulated for determination of 50% of mortality (LD_{50}) by Prohibit method by using Stat-direct version3 software. The $LD_{50/30}$ value was found to be 10.2Gy. After the 30th day, fish blood samples were collected from different treatment groups for genotoxic assays except 20Gy, because of its 100% mortality on 16th day.

Determination of Lowest observed effect level (LOEL) of amifostine, *Gymnema sylvestre* and gymnemagenin by genotoxic assays

Fishes (n=144) were segregated into 18 groups, each of 8 fishes. The intra muscular injection (I.M) of different concentrations of amifostine: 83.3mg/kg - 500 mg/kg of b.wt, *G. sylvestre* plant extract: 25mg/kg - 200mg/kg of b.wt, and Gymnemagenin: 0.3mg/kg - 10mg/kg of b.wt were given to fishes as per Gulgun *et al.,* (2016)[28] and observed for 4 days. Blood samples were collected from each treatment group for genotoxic assays.

Alkaline comet assay

The alkaline comet assay was done as described by Arunachalam *et al.,* (2013)[29] with slight modification. The slides were air-dried and stained with 100 µl of EtBr (20 µg/ml) and examined by the fluorescence microscope (NIKON Eclipse 400). The comet images were analysed for the percentage of tail DNA damage using CASP Software[30, 32].

Micronucleus (MN) assay

The assay was carried out as mentioned by Arunachalam *et al.,* (2013)[29] Minimum of 1000 erythrocytes were counted and scored under 400 X magnification with a Carl Zeiss microscope. Erythrocytes abnormalities were categorized using a method proposed by Ayllon and Garcia-Vazquez, (2000)[31, 32].

Determination of radio-protective effects of amifostine, *Gymnema sylvestre* and gymnemagenin on *Pangasius sutchi*

For the radio-protective study, fishes (n= 30) were divided into five groups each containing 6 individuals, the group I- Control, group II- LD_{50} (10.2Gy), group III- LD_{50} + amifostine (Ami), group IV- LD_{50}+*G. sylvestre* plant extract (GS) and group V- LD_{50}+gymnemagenin (GG). The optimum concentration of amifostine, *G. sylvestre* plant extract and gymnemagenin were injected to the fishes one hour prior to the irradiation and observed for 32 days and different organs such as liver, gills, brain, kidney and muscles samples from treatment groups were collected at different interval of time for further analysis.

Behavioural changes

The behavioural changes in each group were observed and the following parameters like hyperactivity, loss of balance, rate of swimming, convulsions, rate of opercular activity were taken into the consideration and categorized as mild (+, <25% of no. of fishes/group), moderate (++, 25-50% of no. of fishes/ group) and Severe (+++, >50% of no. fishes/ group)[33].

Organs Somatic Index

The organs somatic index is the ratio of individual organs to the total weight of the body which determines the health condition of the organism and reflects the state of pollution in the environment. The calculation of the organ somatic Index such as Hepatosomatic (HSI), Gills-somatic (GSI), Neurosomatic (NSI), Renalsomatic (RSI) and Muscles-somatic (MSI) index was done by random selection of three individual fishes from each treatment group and total weight of individual were noted. The organs of the fishes like liver, gills, brain, kidney and muscles were removed carefully and weighed in an electronic weight machine, after removing moisture by blotting paper. Organs Somatic Index was calculated using the formula for the 4th and 32nd day of observation[34].

$$\text{OSI} = \frac{\text{Organ weight}}{\text{Total fish weight}} \times 100$$

Histology

Tissues were prepared at the end of exposure time of 4th and 32nd day. Randomly three fish from each group were sacrificed and dissected out organs such as liver, gill, brain, kidney and muscles from control and treated groups. After the dissection, samples were carefully fixed in 5% of formaline solution for 24 h and then were processed for paraffin (m.p. 62 ◦C) embedding. Paraffin blocks of all the organs were cut at 6 m thickness and stretched on sterilized glass slides. After deparaffinization, sections were stained with Haematoxylin–Eosine and observed under light microscopy with 100X magnification. The histopathological alterations in the tissues were examined in triplicates and randomly selected sections of fishes from each treated group[33].

Statistical analysis

Statistical analysis was performed by using with PRISM, STATISTICA 6.0 software packages, the results were analysed by one- way ANOVA. The correlations, if any, between induction of Mn and comet were determined by Pearson correlation analysis with level of significance set at 95% (α=0.05).

RESULTS AND DISCUSSION

Determination of LD50/30 in *Pangasius sutchi*

The relationship between the gamma radiation doses and the mortality rate of *Pangasius sutchi* was determined according to Finney's Probit Analysis. The LD_{50} value of gamma radiation was 10.2Gy which shown in Fig 1[36]. There was no mortality in control, 2.5Gy and 5Gy groups, because cells itself have repair mechanism due to which it reduce the mortality rate but 100 % mortality rate was observed on 16th day at 20Gy treated group. Similar work has been also reported by Anbumani *et al.,* (2012)[35]. The data showed that the toxicity and the mortality of fishes were directly proportional to the doses of gamma radiation with time.

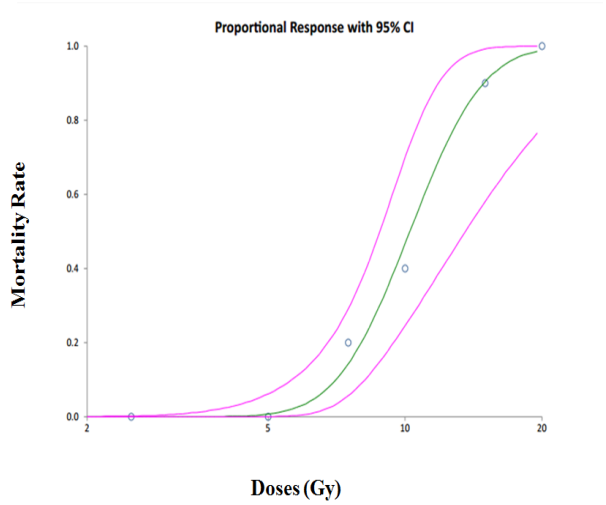


Figure 1: Finney probit graphs for determining $LD_{50/30}$ of gamma radiation in *Pangasius sutchi.*

Genotoxic effects of gamma radiation on 30th day

The DNA damage observed in the erythrocytes of *P. sutchi* at the end of the 30th day and percentage of tail DNA damage were calculated for each treatment group using Comet Assay Software Project (CASP) software[40]. The highest percentage of DNA damage was 58.95% and lowest percentage was 19.47% for 15Gy and 2.5Gy respectively on the 30th day shown in Fig.2. It is mention that the decrease in genetic damage during the post irradiation time may indicate either repair of damaged DNA or loss of heavily damaged cells (apoptotic, cell turnover and dilution by cell replication) or both[37, 38, 39].

Determination of LOEL of Amifostine, *Gymnema sylvestre* and Gymnemagenin Genotoxic effects on 4th day

Similar to gamma radiation, percentage of tail DNA damage were observed for treated groups of amifostine: 83.3mg/kg - 500mg/kg b.wt.; *Gymnema sylvestre* plant extract - 25mg/kg - 200mg/kg b.wt.; and Gymnemagenin - 0.3mg/kg - 10mg/kg b.wt. The highest percentage of tail DNA damage were 48.33% , 42.51% 36.11% and the lowest percentage were 3.14%, 9.151%, and 5.22% on 4th day which shown in Fig. 3, 4,5, and 6 . Report stated that there were some comets of the apoptotic type found in each groups after one hr. of exposure of 5Gy, which showed very high percentage of tail DNA ranging from 50 - 80% by Sowmithra *et al.,* (2015)[41]. Whereas; for this study, the percentage of tail DNA damaged for all the treated and control groups varied from 2.50 – 58.95%. Based on the lowest percentage of tail DNA damage, the optimum concentration of amifostine, *G. sylvestre* plant extract and gymnemagenin were taken for radio-protective study.

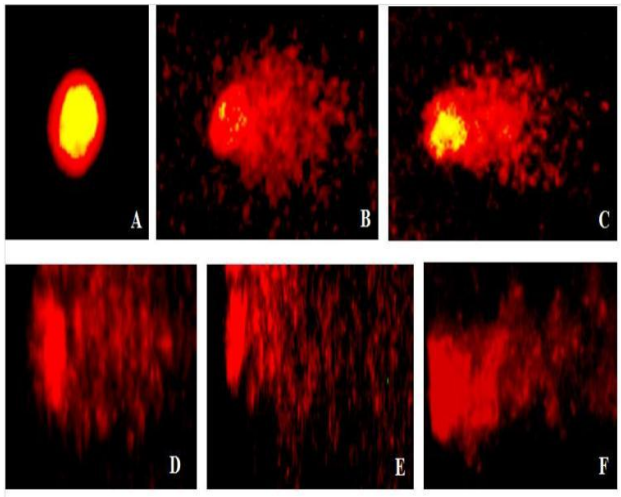


Figure 2: Comet image showing the DNA damage due to Gamma radiation (30th day): (A) control; (B) 2.5Gy; (C) 5Gy; (D) 7.5Gy; (E) 10Gy; (F) 15Gy.

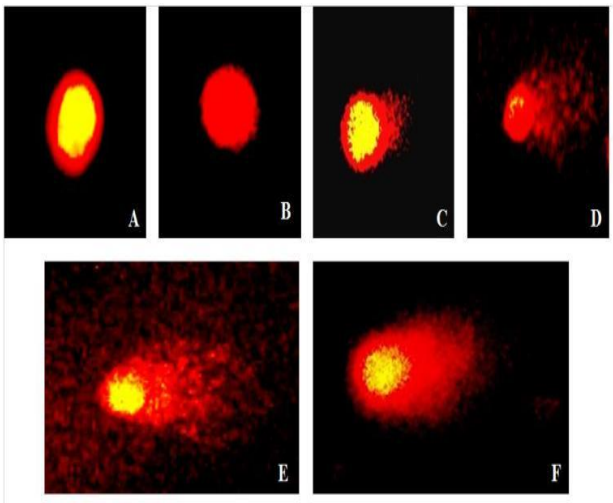


Figure 3: Comet image showing the DNA damage due to Amifostine injection- IM (4th day): (A) control; (B) 83.3mg/kg; (C) 166.6mg/kg; (D) 250mg/kg; (E) 333.3mg/kg; (F) 146.6mg/kg.

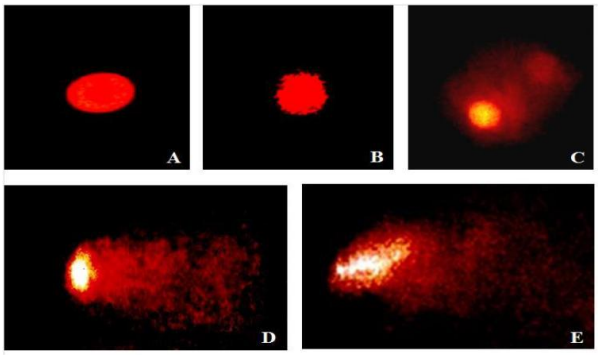


Figure 4: Comet image showing the DNA damage due to *Gymnema sylvestre* injection- IM (4th day): (A) control; (B) 25mg/kg; (C) 50mg/kg; (D) 100mg/kg; (E) 200mg/kg.

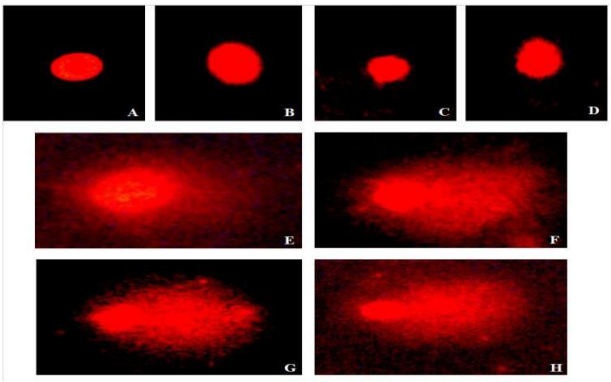


Figure 5: Comet image showing the DNA damage due to Gymnemagenin injection-IM (4th day): (A) control; (B) 0.3mg/kg; (C) 0.5mg/kg; (D) 1mg/kg; (E) 2.5mg/kg; (F) 5mg/kg; (G) 7.5mg/kg; (H) 10mg/kg.

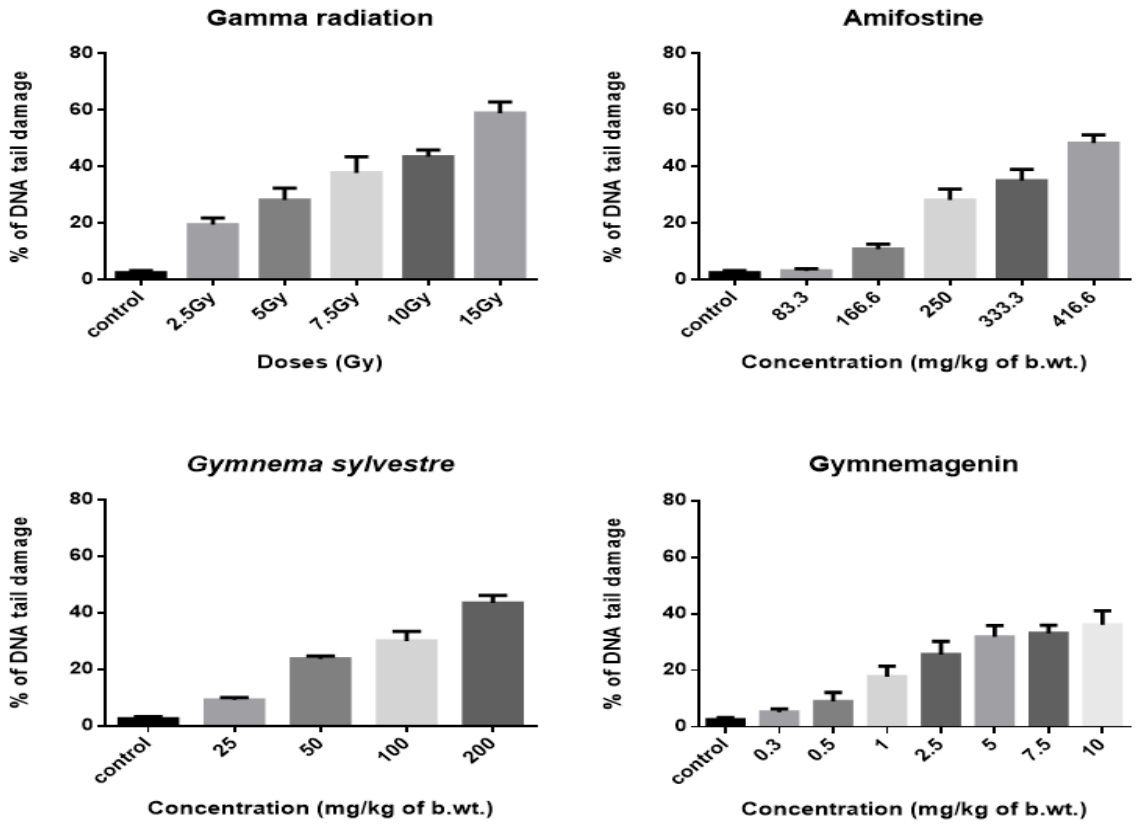


Figure 6: Percentage of DNA damage of fishes exposed to Gamma radiation (30th day); Amifostine, *Gymnema sylvestre* and Gymnemagenin (4th day).

Erythrocyte Micronuclei (MN) assay

The cytogenetic techniques used to detect nuclear abnormalities such as micronuclei, binuclei, trinuclei, multinucleated cells considered as indicators of cytotoxicity and genotoxicity. Micronuclei are cytoplasmic chromatin masses with the appearance of small nuclei that arise from chromosomes fragments or intact whole chromosomes lagging behind in the anaphase stage of cell division. The presence of micronuclei, binuclei, trinuclei bud formation, abnormal cell division are the indication of structural or numerical chromosomal aberrations arising during mitosis and also due to blocking of cytokinesis which leads to genetic imbalance in the cells and involved in carcinogenesis[42]. Echinocytes or spine like projections on the cytoplasmic membrane were noted in fishes which due to the dehydration of red blood cells or expansion of the outer membrane leaflet leading to cellular energy depletion (ATP) which in turn inhibits the ATP-dependent sodium/potassium pump, eventually resulting in echinocyte formation[43]. The inclination of micronucleated, binucleated, trinucleated, multinucleated, vaculated cells observed in all the four treated groups. Formation of bud, elliptocytes observed

only in gamma radiation exposed group. Cell wall damage, cytoplamic bridge formation, enuclus and apoptotic cells were seen in treated group of gamma radiation, *G. sylvester,* gymnemagenin as compare to amifostine and control group. A trend was observed for doses of gamma radiation and concentration of amifostine, *G. Sylvester* plant extract and gymnemagenin (P= 0.0087 < 0.05, F= 4.398, R square= 0.4334) which showed strong statistical significant between the control and treatment groups which reveal that the inductions of erythrocytes abnormality were dose and concentration - dependent. The highest number of erythrocytes abnormality showed by the dose of gamma radiation (15Gy) after 30th day was 18.50% and for the amifostine, *G. sylvester* plant extract and gymnemagenin treated groups were 13.19%, 17.67% and 18.19% respectively at the end of the 4th day. 1.06% abnormalities were seen in control group which given in Fig. 7 and 8.

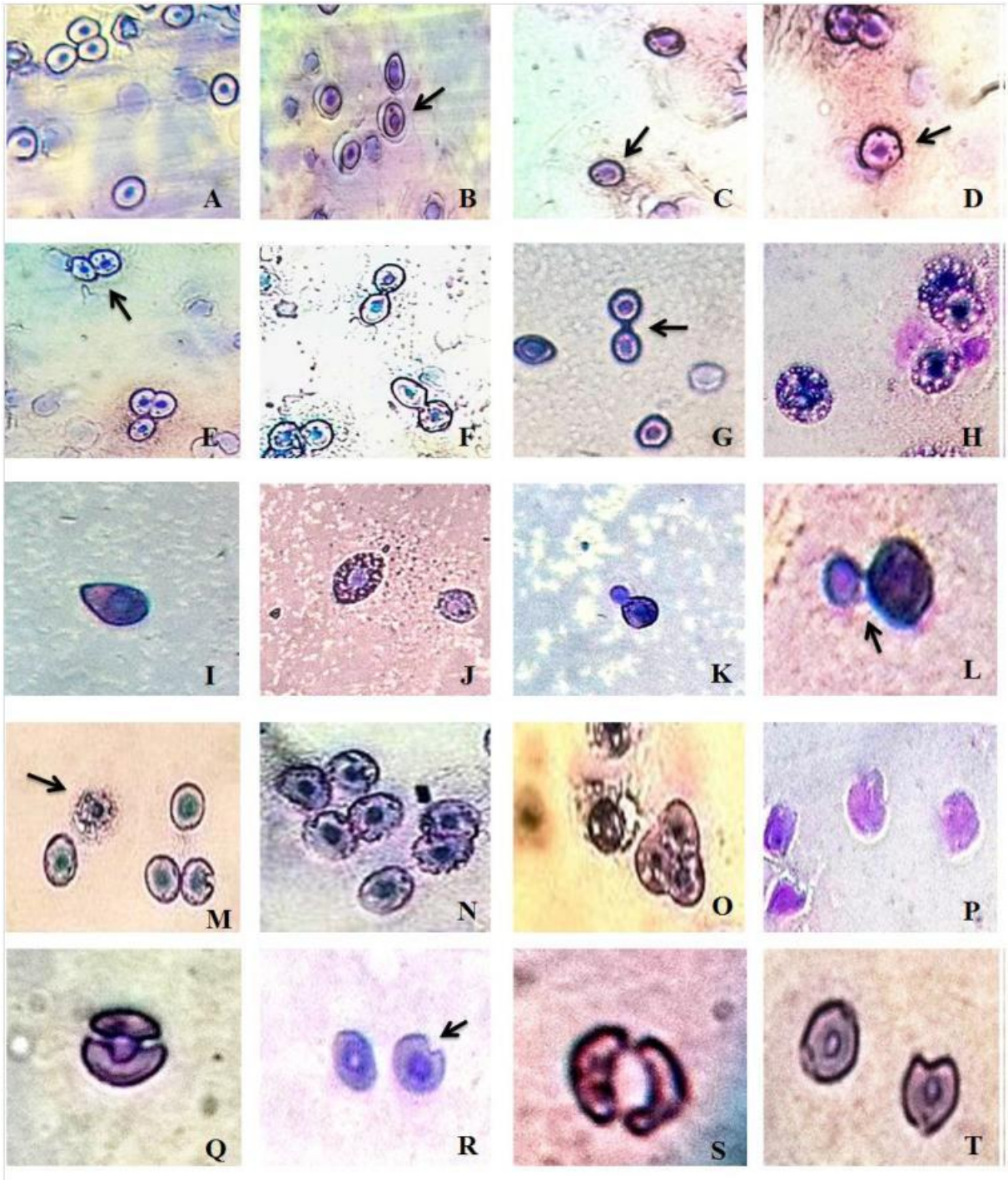


Figure 7: Erythrocytes abnormalities in fish observed at 400X magnification: (A) Normal cells; (B) Micronuclei; (C) Binucleated cell; (D) Trinucleated cell, (E) Multinucleated cell; (F) Abnormal cell division; (G) Cell contain cytoplasmic bridge; (H) Vacuolated cells; (I) Echinocytes; (J) Cell-wall and cytoplamic damaged cell (K) Budding cell; (L) Budding cell and loop formation; (M) Apoptotic cell; (N) Cell wall damage; (O) Lobed cell; (P) Enucleus cell (Q-S) Other abnormality(Unknown).

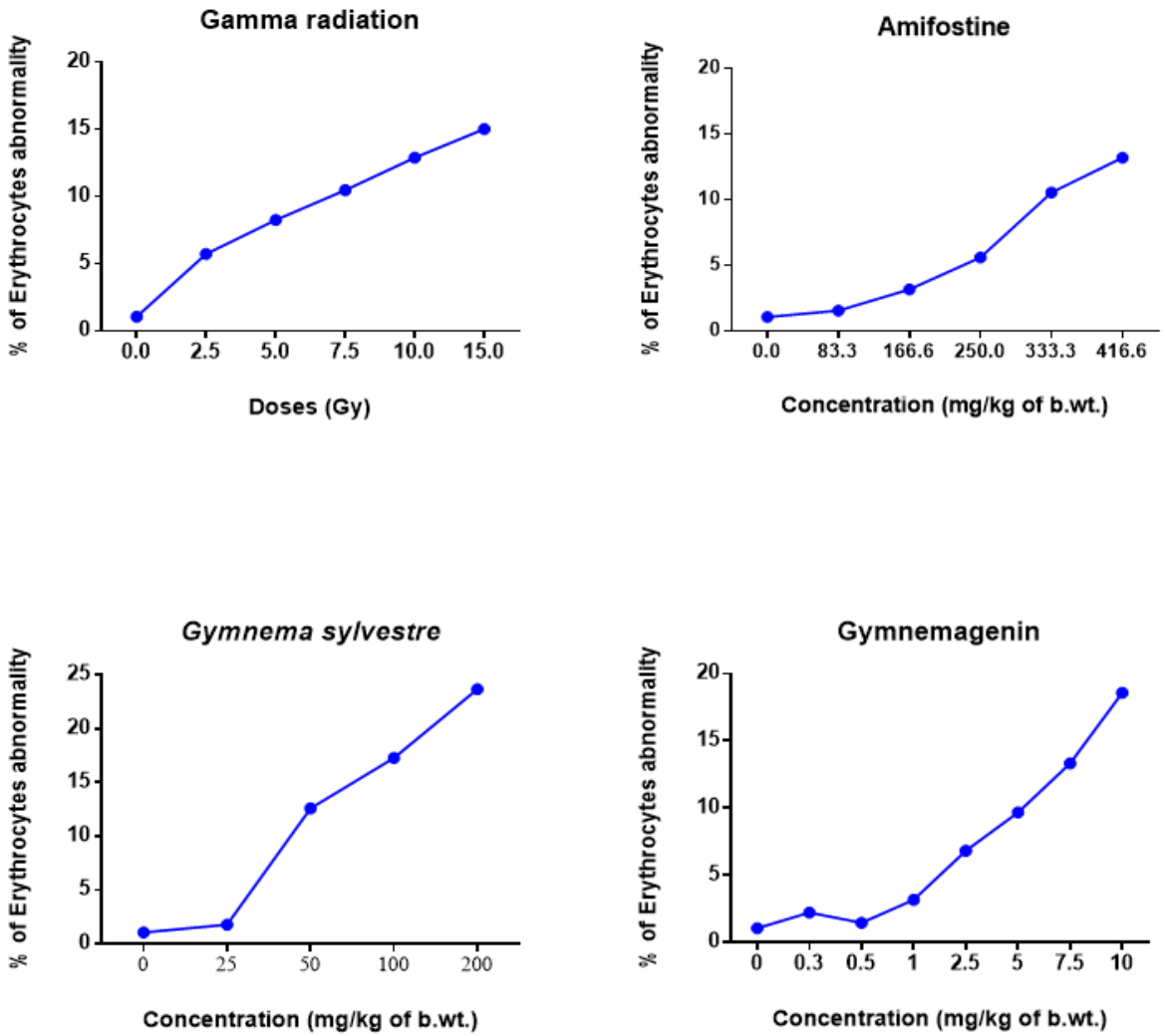
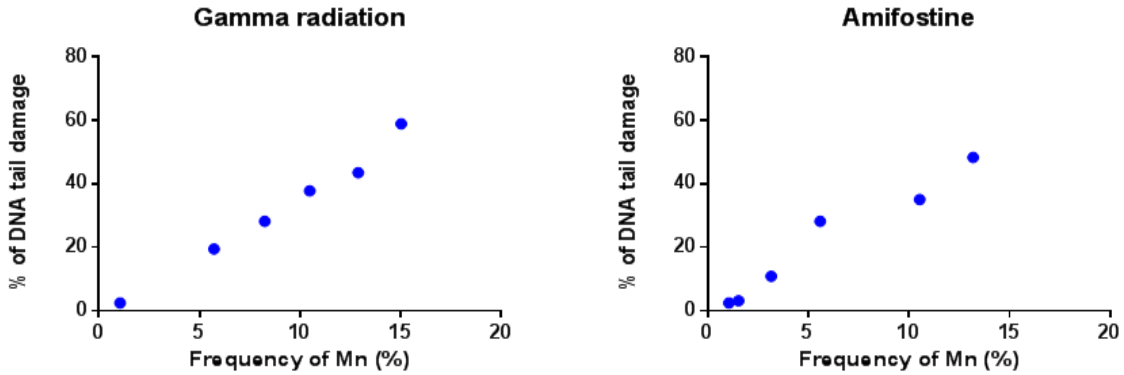


Figure 8: Percentage of erythrocytes abnormalitie of fishes exposed to Gamma radiation (30th day); amifostine, *Gymnema sylvestre* and Gymnemagenin (4th day).

Correlation between Mn and Comet assay

The pearson correlation test indicated significant positive correlation between induction of micronuclei and DNA damage represented in Fig. 9.The comet test confirms DNA strand breaking activity and the Mn test is related to the induction of genetic damaged and genomic instability and indicates genotoxic effect. A combined use of Mn test and comet assay confirms the possible single and double strand breaks induced by gamma radiation(r=0.9928, p<0.0001), amifostine (r=0.9767, p=0.0008), *Gymnema Sylvester* plant extract (r=0.9914, p=0.0010) and gymnemagenin (r=0.9113, p=0.0016) which were statistical significant between comet and micronuclei assays.



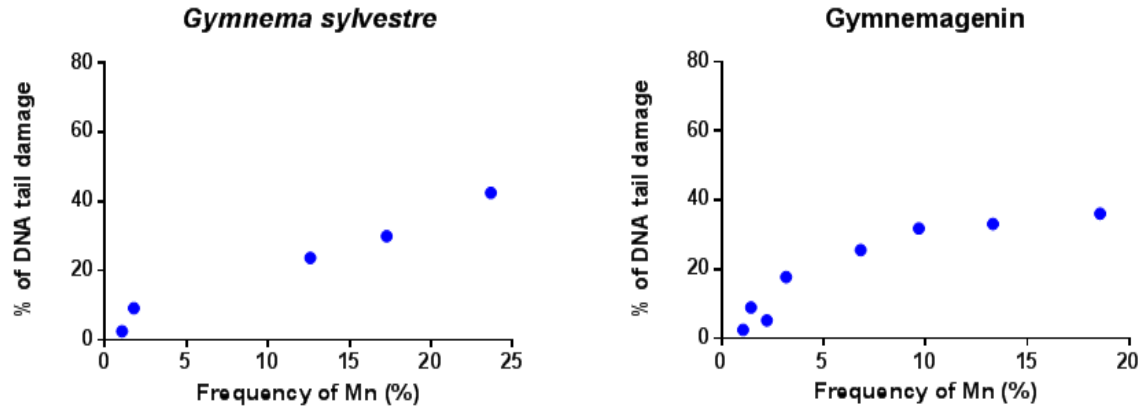


Figure 9: Correlation between of Mn and comet assays in pangasius sutchi exposed to gamma radiation, amifostine, *Gymnema sylvester* and Gymnemagenin. Level of significant of pearson correlation set as 95%(α=0.05)

Radio-protective effect of amifostine, *G. sylvestre* and gemnemagenin in *Pangasius sutchi*

Behavioural changes

The behaviour of fishes was observed for the 4th and 32nd day. Fishes showed the highest behavioural changes in treated group II- LD50 and group V- LD_{50}+GG as compare to group I-Control, group III- LD_{50}+Ami and group IV- LD_{50}+GS. The variation such as hyperactivity, loss of balance, the rate of swimming, the rate of opercular activity and convulsions observed on the 4th day compare to 32nd day. The Group III- LD_{50} + Ami showed similar observation as of group I- control on 32nd day. Group II- LD_{50} and IV- LD_{50} + GS group showed mild changes in rate of swimming which is given in Table.1. Similar studies have been done on *Channa punctatus* which was exposed to chromium concentration of 20mg/l and 40mg/l. as a result, acute chromium toxicity severely affects the vital organs and normal behaviour of the fish which may be deleterious for fish populations[33].

Table 1: Behavioural changes of *Pangasius sutchi* on 4th and 32nd day.

Parameters	Control		LD_{50}		LD_{50} + Ami		LD_{50} + GS		LD_{50} + GG	
Days.	4th Day	32nd Day	4th Day	32nd Day	4th Day	32nd Day	4th Day	32nd Day	4th Day	32nd Day
Hyperactivity	_	_	++	+	+	_	+	_	++	_
Loss of Balance	_	_	++	+	+	_	+	_	++	+
Rate of swimming	_	_	++	++	+	_	++	+	++	+
Rate of opercular activity	_	_	++	+	+	_	++	_	++	_
Convulsions	_	_	++	+	+	_	+	_	++	_

Note: Parameter was categorized (-, None) as mild (+,<25% of no. of fishes/group), moderate (++, 25-50% of no. of fishes/group) and Severe (+++, >50% of no. of fishes/group).

Organ- Somatic Index

Organ somatic index reflects the health condition of organisms. For the present study, it decreased in group II- LD_{50} and increased in all the pre-treated groups. There was not much variation seen in the muscles-somatic index of all the groups as compare to other organs. The HSI of group I- control after 32nd days was 3.53, group II- LD_{50} was 2.21, group III- LD_{50} + Ami was 2.87, group IV- LD_{50} + GS was 2.30 and Group V- LD_{50} + GG was 2.31; similarly for GSI was 5.45, 3.44, 4.74, 4.28 and 3.38; NSI was 1.72, 1.25, 1.90, 1.81 and 1.57; RSI was 2.36, 1.46, 1.94. 1.75 and 1.65; MSI was 7.86, 7.11, 7.58, 7.48 and 7.57 for respective groups which showed in Fig. 10. Similar experiment was done by Nuray *el al*. (2015)[21] and stated that HIS and GSI of *Oreochromis niloticus* reflect metabolic and physiologic disturbances under the effect of Cd (1.6 ppm) and Zn (6ppm). Any physical or chemical alterations occurring in the environment cause stress in aquatic organisms as a result there is metabolic, physiologic, biochemical, and behavioural changes which have negative effects on growth, development and reproduction[50].

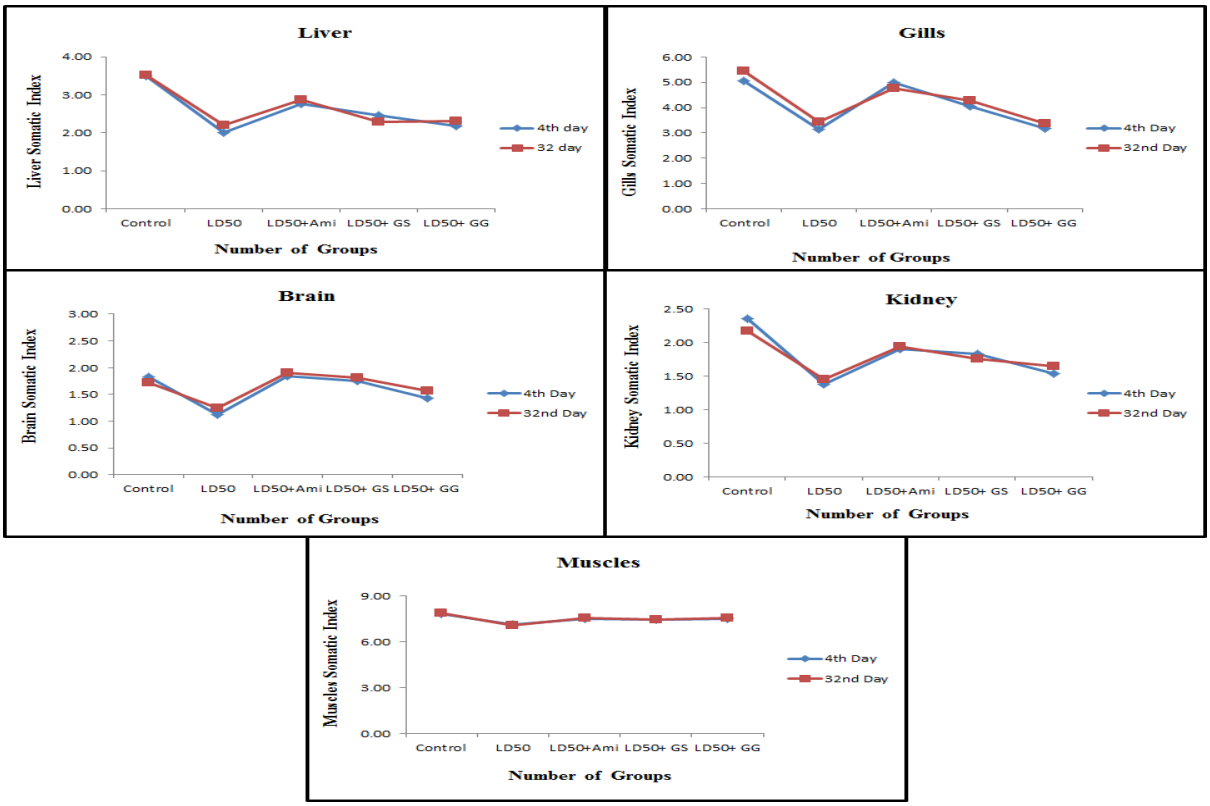


Figure 10: Organ Somatic Index (Liver, Gills, Brain, Kidney and Muscle) of *Pangasius sutchi* on 4th and 32nd Day.

Abbreviations: BS- Blood sinusoids, H- Hepatocytes, LP- Lipid droplet, ND- Nuclear degeneration, HSHC- Hydropic swelling of hepatocytes, Cog- Congested with blood, CV- Central vein, CyV- Cytoplasmic vaculation, FC- fusion of cells, BC- Blood congestion, DS- Dilation of sinusoids, NP- Nuclear Pykonesis.

Histological alteration

Histopathological alterations can be used as an indicator for the various toxic substances which affects the organisms and can indicate the reflection of the overall health of the population in the ecosystem. In the present study, histopathological changes were observed in the different organs of *Pangasius sutchi*.

Liver

The microscopic section of control fish's liver showed hepatocytes with polyedric, a round nucleus at the centre of the cell whereas the hepatocytes in the irradiated fish had stellar form and the nuclei were displaced eccentrically. Typically, lipid vacuoles, lipid droplet, hydropic swelling of hypatocytes and diffuse sinusoidal dilatation were observed in the liver of the irradiated (LD_{50}) fishes. These changes recognized due to effects of radiation on hepatocytes. In LD_{50}+Ami and LD_{50}+GS treated group, Hydropic swelling of hepatocytes, cytoplasmic vacuolization, fusion of cells and nucleus pykonesis were observed. Congestion of portal vein, dilution of sinusoids and nuclear degeneration were observed in LD_{50}+GG treated group which showed in Fig. 11. Bukhari *et al.* (2012)[44] mentioned that the histological variations in liver of freshwater fish *Oreochromis mossambicus* occurred due to exposure of (^{60}Co) gamma irradiation; the alterations included mild congestion of blood vessels, structural alteration, cellular swelling, vacuolation and necrotic liver cells. Maharajan *et al.* (2016)[45] stated that *Lates calcarifer* showed similar histopathological variation in liver due to the exposure of copper.

Gills

The pathological changes in the gill structure such as a swollen tip of the primary and secondary gill lamellae, degeneration of the epithelium in the inter lamellar region and swelling of the secondary gill lamellae tip, distortion in the lamella, degeneration of the epithelium of the inter-lamellar region and the primary gill lamellae, congested gill apex, lamellar tangiectasis and filament cartilage were observed more on LD_{50} dose of gamma radiation on 4th day, whereas, gills section of LD_{50}+Ami group showed mild changes such as swelling of primary gills lamellae, lamellar tangiectasis and filament cartilage. LD_{50}+GS and LD_{50}+GG groups showed severe hyperplasia, degeneration changes, moderate mucoid metaplasia and mild degeneration of base epithelial on 4th day compare to 32nd day. Variation was showed in Fig. 12. Similar changes were also observed when *P. parrah* exposed to 0.03-0.1 mg/L of copper and 0.6-1.8 mg/L of zinc [46]. Tamil *et al.* (2012)[47] mentioned that histopathological changes such as lamellar telangiectasis (clubbed appearance) along with oedema and mucoid metaplasia have been seen in gill tissue of Catla catla, when it exposed to Sublethal Concentration of Pesticide Methyl Parathion and Ferous Sulpha.

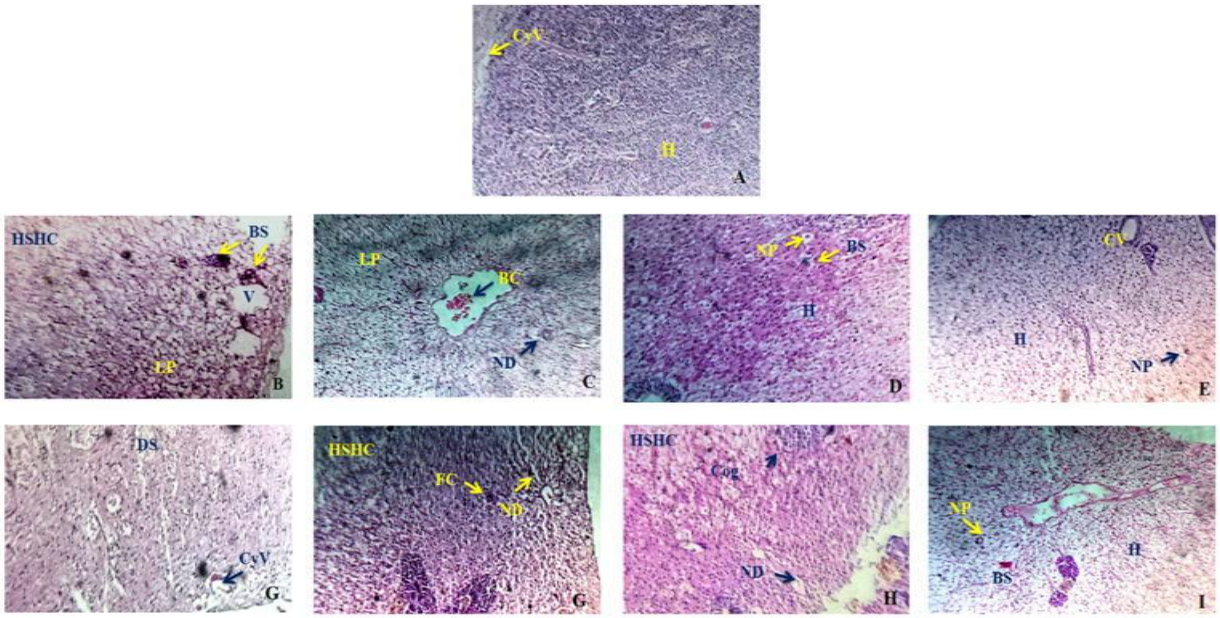


Figure 11: Histological alteration in liver exposed to Gamma radiation and pre-treated group of Amifostine, *Gymnema sylvestre* and Gymnemagenin on the 4th and 32nd day, observed with 100X magnification. (A) Control, (B) LD_{50} 4th day (C) LD_{50} 32nd day, (D) LD_{50+} Ami 4th day, (E) LD_{50+} Ami 32nd day, (F) LD_{50+} GS 4th day, (G) LD_{50+} GS 32nd day, (H) LD_{50+} GG 4th day, (I) LD_{50+} GG 32nd day.

Abbreviations: ABA- Afferent branchial artery, EBA- Efferent branchial artery, AT- Adipose tissue, SM- Submucosa, I- Inter secondary lamellae space, S- secondary lamellae, P- Primary lamellae, EC- epithelial cells, SPL- Swelling of primary lamellae, DL- Distortion of lamellae, CG- Congested gill apex, DBE- Degeneration of base-epithilial, DC- Degenerative changes. HP- Hyperplasia, LT- Lamellar tangiectasis, Mm- Mucoid metaplasia, FC- Filament cartilage.

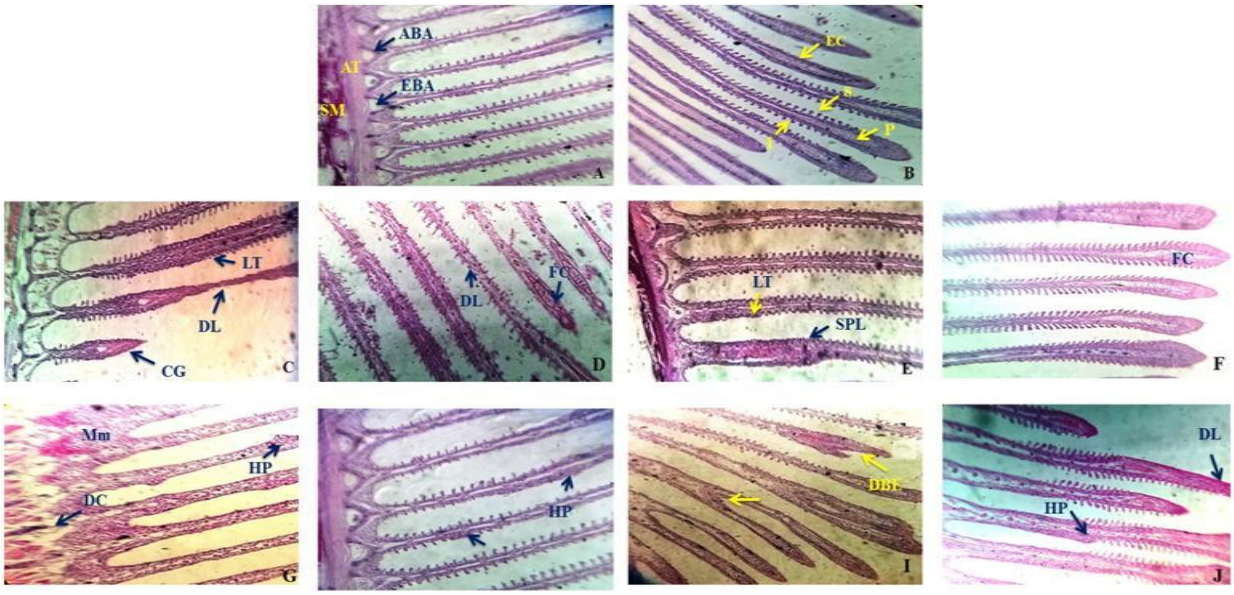


Figure 12: Histological alteration in gills exposed to Gamma radiation and pre-treated groups of Amifostine, *Gymnema sylvestre* and Gymnemagenin on the 4th and 32nd day, observed with 100X magnification. (A) and (B) Control, (C) LD_{50} 4th day (D) LD_{50} 32nd day, (E) LD_{50+} Ami 4th day, (F) LD_{50+} Ami 32nd day, (G) LD_{50+} GS 4th day, (H) LD_{50+} GS 32nd day, (I) LD_{50+} GG 4th day, (J) LD_{50+} GG 32nd day.

Abbreviations: ABA- Afferent branchial artery, EBA- Efferent branchial artery, AT- Adipose tissue, SM- Submucosa, I- Inter secondary lamellae space, S- secondary lamellae, P- Primary lamellae, EC- epithelial cells, SPL- Swelling of primary lamellae, DL- Distortion of lamellae, CG- Congested gill apex, DBE- Degeneration of base-epithilial, DC- Degenerative changes. HP- Hyperplasia, LT- Lamellar tangiectasis, Mm- Mucoid metaplasia, FC- Filament cartilage.

Brain

Numerous histo-pathological changes were observed in the different layers of optic tectum in brain of *P. sutchi* due to the LD_{50} dose of gamma radiation and the different concentration of amifostine, *G. sylvestre* and gymnemagenin in response to protection of fishes. The

severities of the damage were found to be excess in group II- LD_{50} dose and group V- LD_{50} +GG. In control fish, outer surface were found intact with each other and there were no detachment, or displacement of granular cells such as Stratum (Str.) periventriculare (SPV) in the inner region, the Str. album centrale(SAC), followed by the Str. griseum centrale (SGC), the Str. fibro-sum et grisium superficiale (SFGS), the Str. opticum (SO), and the Str.marginale (SM). In the LD_{50} group, there were severe detachment in the SO and SM layers, SFG showed gaps with degeneration of neurons, because of this they were reduced in size, structural degeneration, necrosis and moderate vacuoles formation found on 4th day and slightly moderate on 32nd day. In case of group III- LD_{50}+Ami, group IV- LD_{50}+GS group there were no such changes only moderate vacuoles formation and structural degeneration after 4th day and reduced on 32nd day. In group V- LD_{50}+GG group showed severe detachment of neural cells of SO and SM lining and moderate structural degeneration and vacuoles formation 4th day which showed in Fig. 13. Similar histological variation on *Channa punctatus* has been observed after exposed them to pesticide Chlorpyrifos[9] and increase in necrosis of neurons, cytoplasmic vacuolization and lesions in the brain observed on 15th and 30th day on brain of *Cyprinus carpio*, after exposed to organophosphorus insecticide phorate[48].

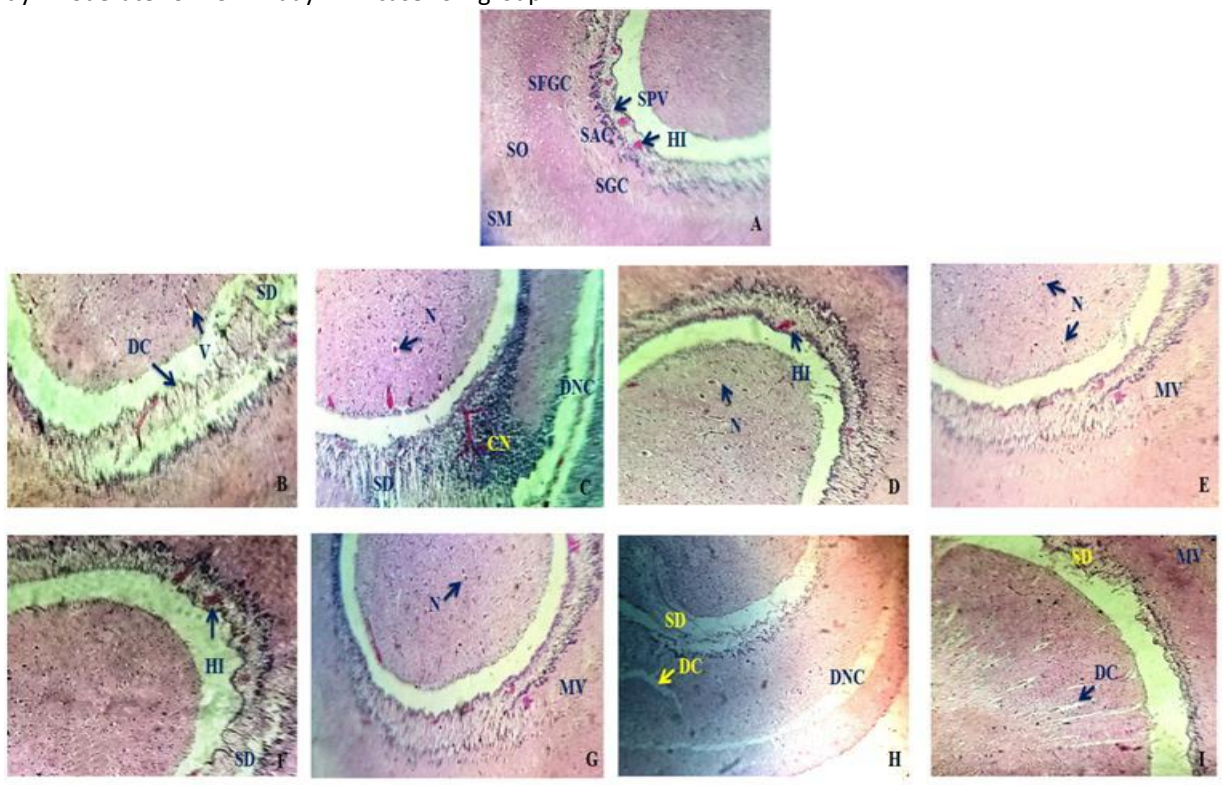


Figure 13: Histological alteration in brain exposed to Gamma radiation and Amifostine, *Gymnema sylvestre* and Gymnemagenin on the 4th and 32nd day, observed with 100X magnification. (A) Control, (B) LD_{50} 4th day (C) LD_{50} 32nd day, (D) LD_{50} + Ami 4th day, (E) LD_{50} + Ami 32nd day, (F) LD_{50} + GS 4th day, (G) LD_{50} + GS 32nd day, (H) LD_{50} + GG 4th day, (I) LD_{50} + GG 32nd day.

Abbreviations: SFGC- Stratum fibrosum grisium superficiale, SAC- Str. Album central, SO- Str. opticum, SM- Str. marginale, SGC-Str. griseum central, SPV- Str. periventriculare, HI- Hippocampus, N- Nuclei, V- Vacuoles, DNC-Detachment of neuronal cells SO and SM lining, SD-Structural degeneration, DC- Degenerative changes, CN-congestion and necrosis of granular cells, MV- Minor vacuoles.

Kidney

Examination of kidney section after 4th and 32nd day, group II- LD_{50} dose of gamma radiation showed severe pyktosis of nuclei, hypertrophies of glomeruli, rupture of renal corpuscles, distortion of renal tubules and moderate necrosis, edema and vacuoles formation, in case of group III- LD_{50}+Ami and group IV- LD_{50}+GS, moderate to mild changes observation at the end of 32nd day as compare to group I- control and in group V- LD_{50}+GG group, severe pyktosis of nuclei, hypertrophies of glomeruli, rupture of renal corpuscles observed after 4th day and mild vacuole formation, hypertrophies of glomeruli and pyktosis were seen after 32nd day as shown in Fig. 14. Similarly, Imam *et al,* (2013)[49] investigated the potential protective effects of tomato and Vit. E against the impacts of Cd toxicity on *Oreochromis niloticus* exposed for 15th and 30th day showed swelling and hypertrophy of tubules with nuclear deteriotration and pyktosis. The toxicities of copper and zinc on *Puntius parrah* were observed by histological changes with the sublethal concentrations of 0.05 mg/L of Cu and 0.9 mg/L of Zn for 28 days of exposure which showed similar changes with the present studies[46].

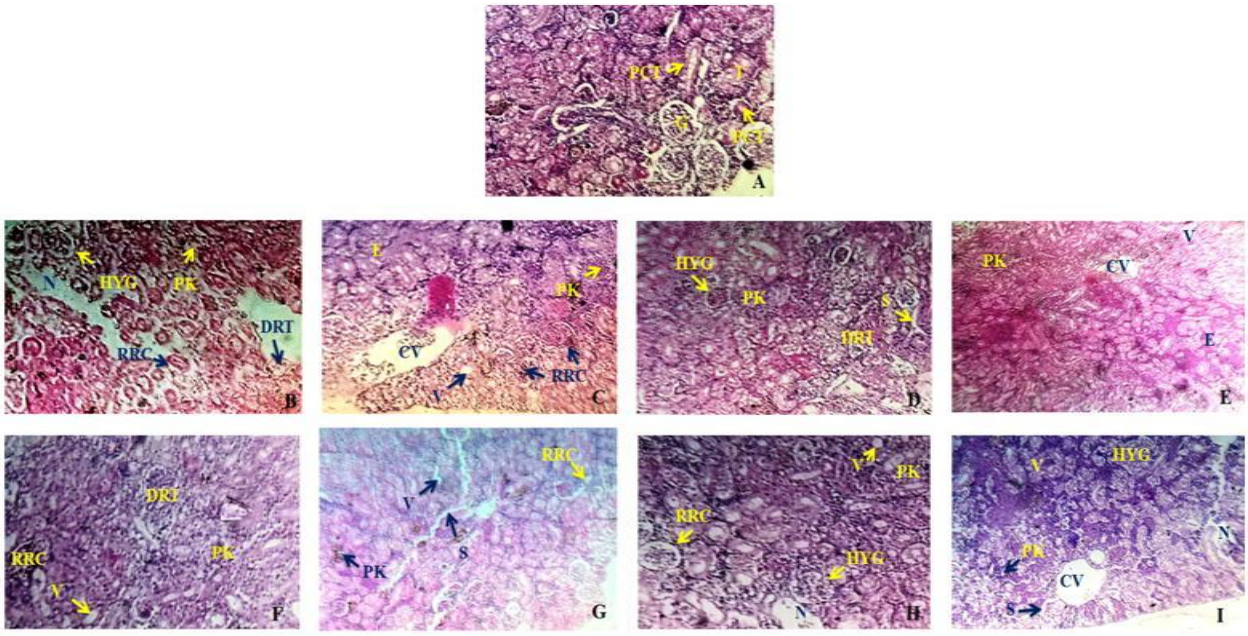


Figure 14: Histological alteration in kidney exposed to Gamma radiation, Amifostine, *Gymnema sylvestre* and Gymnemagenin on the 4th and 32nd day, observed with 100X magnification. (A) Control, (B) LD_{50} 4th day (C) LD_{50} 32nd day, (D) LD_{50} + Ami 4th day, (E) LD_{50} + Ami 32nd day, (F) LD_{50} + GS 4th day, (G) LD_{50} + GS 32nd day, (H) LD_{50} + GG 4th day, (I) LD_{50} + GG 32nd day.

Abbreviations: G- Glomerules, T- Tubules, DCT- Convoluted tubules(Distal), PCT- Convoluted tubules(Proximal), CV- Central vein, N- Necrosis, V- Vacuoles, HYG-Hypertrophies of glomeruli, RRC- Rupture of renal corpuscles, DRT- Distortion of renal tubular, PK- Pyktosis of nuclei, S-Blood sinusoid, E- Edema,

Muscles

In group II- LD_{50}, muscle section showed moderate inter-myofibrillar space, oedema and severe disintegrated myofibrils, interstitial materials, gap formation in myofibrils and mild vacuolar degeneration in muscle bundles on 4th day. In group III- LD_{50}+Ami, group IV- LD_{50}+GS and group V- LD_{50}+GG severe changes of inter myofibrilar space, disintegrated myofibrils and gap formation occurred on 4th day as compare to 32nd day which showed in Fig. 15. Histological changes reported in muscle of *L. calcarifer* showed similar alteration when it exposed to Cu for 28day[45].

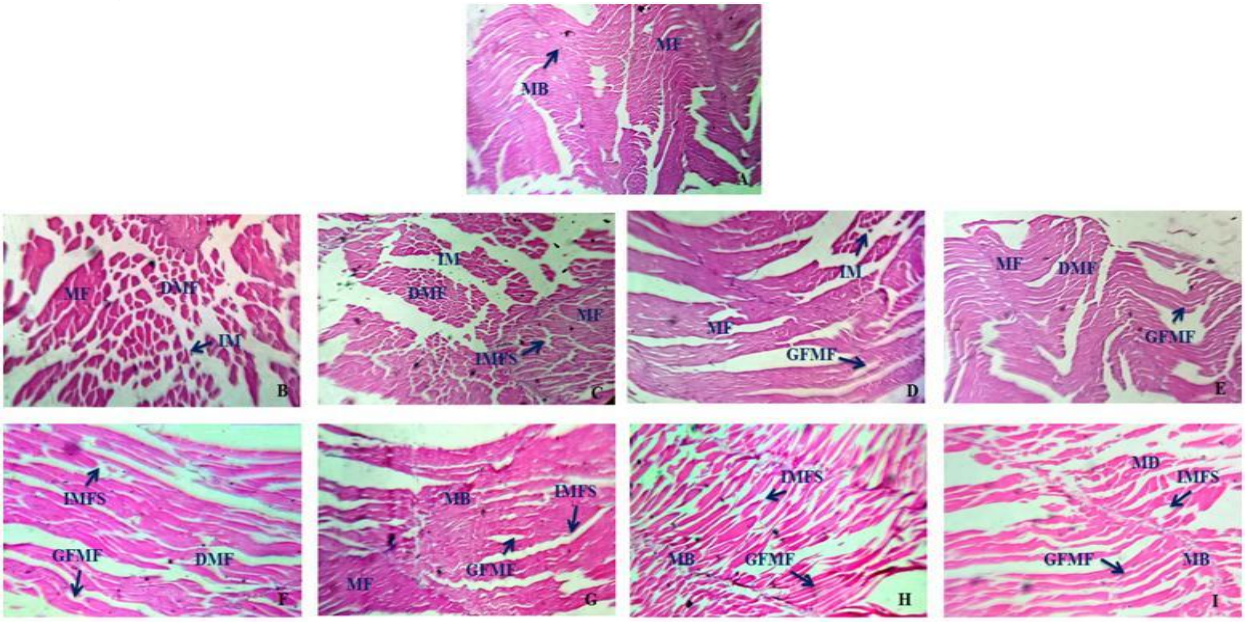


Figure 15: Histological alteration in Muscle exposed to Gamma radiation, Amifostine, *Gymnema sylvestre* and Gymnemagenin on the 4th and 32nd day, observed with 100X magnification. (A) Control, (B) LD_{50} 4th day (C) LD_{50} 32nd day, (D) LD_{50} + Ami 4th day, (E) LD_{50} + Ami 32nd day, (F) LD_{50} + GS 4th day, (G) LD_{50} + GS 32nd day, (H) LD_{50} + GG 4th day, (I) LD_{50} + GG 32nd day.

Abbreviations: MF- Myofibrils, MB- Myofibrillar bundles, IM- Interstitial materials, DMF- Disintegrated myofibrils, IMFS- Inter myofibrillar space. GFMF- Gap formation in myofibrils.

CONCLUSION

In the present study, administration of (1hr. prior) intramuscular injection of amifostine and *G. sylvestre* and gymnemagenin to the irradiated fishes indicate remarkable protective effect against gamma radiation. Data indicates that histological lesion of organs, behavioural and organ somatic index changed due to oxidative stress induced by gamma radiation and reduced due to the pre- treatment of amifostine, *G. sylvestre* and gymnemagenin. Previous report revealed that amifostine have ability to modify the level of gamma irradiation-induced chromosomal aberration on human peripheral blood lymphocytes[22] and the natural bioactive molecules gymnemagenin of *G.sylvestre* plant have ability to protect the liver from radiation damage[26], which resemblances and supports the existing data. The protective efficacy of *G. sylvestre* plant indicates the existence of radio-protective properties against the radiation hazards and can be used as radio protector.

Acknowledgments: Authors wish to thank IGCAR, kalpakkam and management of SRMIST, Kattankulathur, Tamil Nadu, India, for providing necessary facilities and support for the completion of this work.

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Source of Support: Nil, **Conflict of Interest:** None.