Impact of gamma irradiation on tissues of the mud crab, Scylla serrata (Forskål, 1775) (Decapoda, Portunidae) — electron microscopic study and DNA comet assay

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CRUSTACEANA

IMPACT OF GAMMA IRRADIATION ON TISSUES OF THE MUD CRAB, *SCYLLA SERRATA* (FORSKÅL, 1775) (DECAPODA, PORTUNIDAE) — ELECTRON MICROSCOPIC STUDY AND DNA COMET ASSAY

BY

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ABSTRACT

Radiopreservation using gamma radiation is widely in use as a safe method for extending the shelf life of shellfish. This study explored the consequences of different doses of gamma radiation (0.5 kGy, 1.0 kGy and 2.0 kGy) on various tissues of *Scylla serrata* at cellular and nuclear level, with the aid of electron microscopy and DNA comet assay. The highly radio exposed (2.0 kGy) pyloric muscles showed a reduction in sarcomere length, disordered organization with expanded gap between adjacent myofibrils, ruptured sarcotubular system, mitochondrial swelling with crushed cristae, significant increase in nucleus size coupled with less dense nucleoplasm, etc. Comet assay on tissues such as muscle, hepatopancreas and testis irradiated with 2.0 kGy radiation also revealed a significant degree of nuclear damage by gamma irradiation in a dose-dependent manner. The tail length of the comet showed a tissue-specific tolerance level. The present study clarified the precise dose of irradiation as 1.0 kGy and the results can be relevant for commercial purposes to qualitatively categorize the irradiated crabs.

Key words. — Pyloric muscle, pentad, swollen mitochondria, DNA content, food irradiation

RÉSUMÉ

La radio préservation par les rayons gamma est largement utilisée comme une méthode sûre pour prolonger la vie des produits de la mer. Cette étude explore les conséquences de différentes doses de rayons gamma (0,5 kGy, 1,0 kGy et 2,0 kGy) sur différents tissus de *Scylla serrata* au niveau cellulaire et nucléaire, à l'aide de la microscopie électronique et du test AND des comètes.

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Les muscles pyloriques exposés à une forte radiation (2,0 kGy) ont montré une réduction de la longueur des sarcomères, une organisation désordonnée avec un élargissement de l'espace entre des myofibrilles adjacentes, un système sarcotubulaire rompu, des mitochondries enflées avec des crêtes écrasées, une augmentation significative de la taille des noyaux couplée à un cytoplasme moins dense, etc. Le test comète sur les tissues tels que muscles, hépatopancréas et testicules irradiés avec une radiation de 2,0 kGy à aussi révélé un degré significatif et dose dépendent de détérioration nucléaire par cette irradiation gamma. La longueur de la queue de comète a montré un niveau de tolérance spécifique à chaque tissu. Cette étude clarifie la dose précise d'irradiation à 1,0 kGy et les résultats peuvent être utiles pour classer qualitativement les crabes irradiés à des fins commerciales.

Mots clés. — Muscle pylorique, mitochondries enflées, contenu en DNA, irradiation des aliments

INTRODUCTION

Irradiation appears to be the one of the modern potential tools for improving the shelf life of food products including those derived from aquatic resources (Javanmard et al., 2006; Farkas & Mohácsi-Farkas, 2011; Zhang et al., 2014; Arshad et al., 2015a, b). However, to determine an optimum dose for irradiation without compromising changes in structural and functional properties, and thereby thus maintaining the nutritional quality of a food product, is a crucial phase in the process of food irradiation (Miller, 2005; Miyagusku et al., 2007). The most obvious changes have frequently been reported in structural and functional properties due to irradiation in biological systems (Somosy, 2000; Yooket et al., 2001; Yoon, 2003). In bovine muscle, for instance, gamma irradiation caused a dose-dependent increase in the gap between myofibrils coupled with the disappearance of the A-band and M-line and an increase in sarcomere length (Yooket et al., 2001). Yoon (2003) also observed shrinkage and disruption of myofibril units and distortions in the arrangement of the sarcomere units in chicken breast meat after gamma irradiation with doses ranging from 0.5-5.0 kGy. Gamma irradiation up to the dose of 50 Gy caused the condensation of myofibrils and reduction in the amount of sarcoplasmic reticulum in the smooth muscle layer of the guinea pig ileum (Claro et al., 2008). In many cells, outer and inner membranes of mitochondria are also prone to irradiation-induced damage, resulting in vacuolization and disruption (Jordan, 1967; René & Evans, 1970; Schwartz et al., 1994).

Yet, despite the fact that the increased aquaculture production of seafood products demands improved techniques, like irradiation, for their proper preservation and prolonged shelf life, serious research on this line still appears to be fragmentary. Gamma irradiation carried out for the purpose of long shelf life induces structural damages in the structural muscle proteins like actin and myosin, by producing crosslinks and scission of peptide bonds (Perng & Yang, 1990; Mahtoet et al., 2015). Though no distinct histological changes have been reported in the muscles of the freshwater prawn, *Macrobrachium rosenbergii* (De Man, 1879), in response

to gamma irradiation up to 10 kGy, changes like an increased gap between the muscle fibres and detachment of the perimysium were clearly noticed after 20 kGy irradiation (Mahtoet et al., 2015). The aforesaid reports signify the importance of a comprehensive study on the impact of dose-dependent gamma radiation on different tissues at the structural and functional level, irrespective of the type of seafood, before suggesting an optimum dose for their improved shelf life.

Mud crabs, which represent the most promising coastal aquaculture shellfish and, among the edible species, *Scylla serrata* (Forskål, 1775), is commercially important. This is for various reasons, i.e., because of its size, meat quality, and high price, and it consequently has an immense market demand all over the world, particularly in South-East Asian countries. India earns foreign exchange up to 18 million US$ by exporting live mud crabs captured from the lowlying coastal belts across the country (Laxmappa, 2016). Recent studies from our laboratory on this shellfish (*S. serrata*) show, that gamma radiation in the range of 1.0-2.0 kGy can reduce the microbial load to its lowest level at a low temperature of 4°C for a maximum of 7 days (Arshad et al., 2015b). The present paper depicts the dose-dependent gamma irradiation-induced changes (0.5, 1.0 and 2.0 kGy) in the architecture of tissues derived from *S. serrata*, through histological and ultrastructural studies on pyloric muscle, as well as by DNA comet assay on tissues such as muscle, hepatopancreas and testis.

MATERIAL AND METHODS

Sampling

Live adult mud crabs, *Scylla serrata*, with an average carapace width of 8.00 cm and an average body weight of 200 g, were collected from the Cherukunnu estuary in Kerala, India (12°0′N 75°18′E). Crabs were immediately brought to the laboratory, examined thoroughly, and samples having visible signs of injury were culled (Arshad et al., 2015). The moult stage of the crabs was characterized through microscopic observation of the setogenesis of the maxilliped (Sudha & Anilkumar, 2007; Sudhaet et al., 2012; Supriya, 2016); only healthy intermoult male crabs were selected. A total of 12 crabs were selected and divided into 3 sets, thus each containing four crabs. Subsequently, the body surface of each crab was rinsed with sterile distilled water, and crabs were packed individually in presterilized polyethylene bags (with a thickness of 3.6 μm), and these were sealed using an electric sealing machine. The packed crabs were subsequently subjected to a gamma irradiation treatment. The samples taken were processed separately for electron microscopic study and for the DNA comet assay.

Gamma irradiation

Three crabs from each set were subjected to dose-dependent gamma irradiation (Cobalt 60 (Co-60)) at the dosages of 0.5, 1.0 and 2.0 kGy with a dose rate of 3.8 kGy per hour using the gamma irradiation facility (GC-5000, BRIT, Mumbai, India) available at the Meat Technology Unit, Kerala Agricultural University; the remaining one crab (from each set) was treated as a non-irradiated fresh (control) sample (Arshad et al., 2015a, b). In order to nullify the dosage uncertainties, the exposure times were controlled as 7 minutes 54 seconds, 15 min. 47 s and 31 min. 35 s for 0.5, 1.0 and 2.0 kGy irradiation, respectively.

Light and electron microscopic studies of pyloric muscle of *Scylla serrata*

The pyloric muscle both from fresh, non-irradiated crabs, and from crabs (*S. serrata*) immediately after dose-dependent irradiation (0.5, 1.0 and 2.0 kGy), was fixed in Karnovisky's fixative (4% paraformaldhyde and 3% glutaraldehyde at pH 7.2) for 24 hours. The tissue was processed according to the procedure described by Williams & Carter (2009). Briefly, the fixed tissue was washed with phosphate buffer (pH 7.4) for approximately 15 minutes and post-fixed in 1% osmium tetroxide (OsO_4). The specimen was again washed with buffer for half an hour and subsequently dehydrated in ethanol grades followed by enblock staining (2% uranyl acetate in 95% ethyl alcohol) and dehydration. Clearing and embedding was carried out in propylene oxide and araldite, respectively. Semithin sections (1.0 μm) stained with methylene blue were subjected to light microscopic observation (Leica research microscope DM-750). Ultrathin sections (0.5 μm) stained with uranyl acetate and lead citrate were examined under the Transmission Electron Microscope (TEM; Tecnai G2), a facility extended from the Electron Microscopic Laboratory at the National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore.

Single cell gel electrophoresis (DNA comet assay)

In order to assess the irradiation-induced nuclear damage, if any, the tissues such as muscle, hepatopancreas and testis from both fresh, non-irradiated crabs and from crabs (*S. serrata*) immediately after dose-dependent irradiation (0.5, 1.0 and 2.0 kGy), were subjected to single cell gel electrophoresis (DNA comet assay) (after Singh et al., 1988). For this purpose, the mixture of cell suspension (prepared in phosphate buffered saline (PBS)) of the tissues and 1% low melting agarose in the ratio of 1 : 5 (20 μl : 100 μl) was poured over the slides containing 1% high melting agarose. The slides were then kept in lyses buffer (2.5 M NaCl + 0.01 M TrisHCl + 0.1 M EDTA (pH 10.0) + 1% sodium sarcosinate + 1%

Triton X 100) for one hour at 4°C. After transfer to the electrophoresis buffer (0.3 mMNaOH + 1.0 mM EDTA + 0.2% DMSO) and being kept there for 10 minutes, single cell gel electrophoresis was carried out for 30 minutes at 4°C. This was followed by washing with neutralizing buffer (0.4 mMTris-HCl, pH 7.4) and staining with syber green. The stained slides were observed under the fluorescence microscope (200×) (H600, Hund Wetzlar, Germany). The extent of the DNA migration (DNA comet) was determined with the image analysis system, "CASP" (CASP1.2.3 beta 1, CASP Lab comet assay software, KrazysztofKonca, CaspLab.com) (Koncaet et al., 2003). Based on the tail length of the comet as the criterion, the DNA migration/damage was quantified. Head DNA content, tail DNA content, and tail moment were also measured using "CASP", with a view to derive a better conclusion.

Statistical analysis

Statistical analyses were performed using standard statistical software, GraphpadInstat™ (GraphPad Software, Inc., La Jolla, CA; 1990-1993 Graphpad Software. V2 00, Uchitel, UC Irvine 921687S) and the data were expressed as Mean ± SD. Student's *t*-test (one tail) was performed to analyse any significant difference between two groups. One-way ANOVA was performed to analyse the significance of dose-dependent gamma irradiation. Tukey's test was performed in samples showing statistically significant difference ($P<0.05$) to separate means. Interaction effects of different tissues and dose-dependent gamma irradiation was analysed by two-way ANOVA. P values less than 0.05 were considered statistically significant. The correlation between the comet parameters and the doses of irradiation was also analysed.

RESULTS

Pyloric muscle from fresh, non-irradiated *Scylla serrata*

Light microscopy.— Under the light microscope, the methylene blue stained semithin sections of the fresh, non-irradiated pyloric muscle of *S. serrata* appears with compactly arranged bundles of spindle-shaped muscle fibres, the diameter of which ranged between 32 μm and 85 μm (fig. 1). Each muscle fibre shows the presence of a distinct, basophilic sarcolemma and regular striations with well-marked continuous Z-lines (fig. 1b). Large and round/oval shaped nuclei are either sub-sarcolemmal or inter-myofibrillar in position (fig. 1b-f). Myofibrils and a narrow inter-myofibrillar space are also distinctly visible (fig. 1c-f). Each junctional region of two or more muscle fibres shows the presence of a basophilic

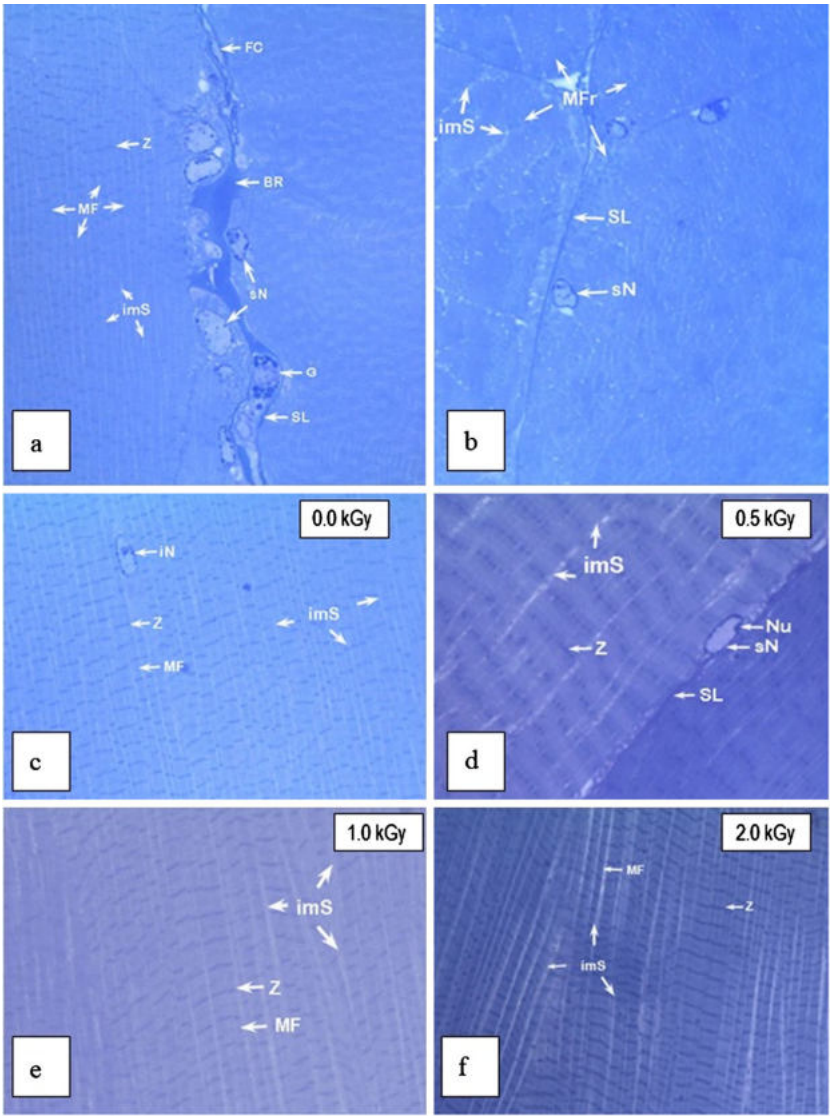

Fig. 1. Light microscopic views of pyloric muscle fibres of *Scylla serrata* (Forskål, 1775): a, longitudinal section (LS), 1000×; b, cross section (CS), 1000×; showing: basophilic region (BR) between muscle fibres, sarcolemma (SL), fibroblast cell (FC), Z-band (Z), myofibrils (MF), inter-myofibrillar space (imS), sub-sarcolemmal nucleus (sN), granulocyte-like cell (G), and compactly arranged muscle fibres (MFr); c-f, muscle fibres derived from untreated (0.0 kGy) and gamma-irradiated (0.5, 1.0 and 2.0 kGy) material (all LS, 1000×): showing inter-myofibrillar nucleus (iNd), sub-sarcolemmal nucleus (sN), nucleolus (Nu), Z-band (Z), myofibril (MF), inter-myofibrillar space (imS), and sarcolemma (SL).

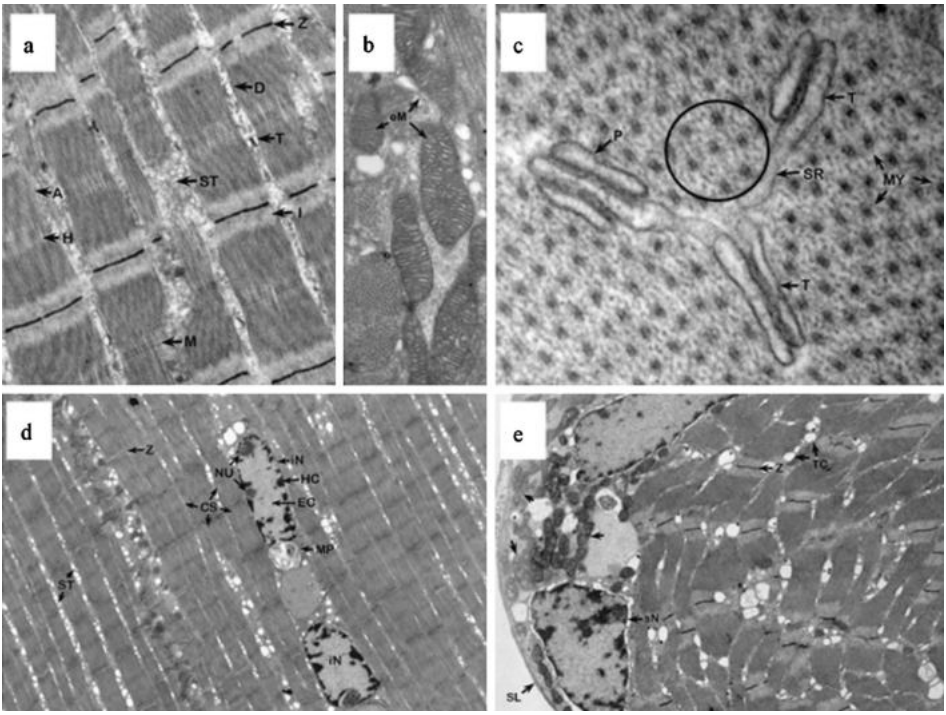

Fig. 2. Electron micrographs of the portion of the pyloric muscle fibres of *Scylla serrata* (Forskål, 1775): a, showing sarcotubular system (ST), diad (D), triad (T), and sarcomeres with Z-band (Z), A-band (A), I-band (I), H-zone (H), and M-line (M) (longitudinal section (LS), 4800×); b, electron dense mitochondria (eM) (cross section (CS), 11 000×); c, myosin (MY), hexagonal arrangement of actin and myosin (in circle), sarcoplasmic reticulum (SR), pentad (P), and triad (T) (CS, 68 000×); d, pyloric muscle fibres in contracted state, showing contracted sarcomeres (CS), Z-line (Z), sarcotubular system (ST), inter-myofibrillar nucleus (iN), nucleolus (NU), euchromatin (EC), heterochromatin (HC), and membrane processes (MP) (LS, 2900×); e, electron dense mitochondria aggregated (arrow mark) near the sub-sarcolemmal nucleus (sN), sarcolemma (SL), Z-line (Z), and terminal cisternae (TC) (LS, 4800×).

mass (fig. 1a and b). The inter-fibre space also exhibits the presence of fibroblast cells and granulocyte-like cells (fig. 1). No visible light microscopic changes could be observed in the pyloric muscle derived from the dose-dependent (0.5, 1.0 and 2.0 kGy) gamma-irradiated crabs (*S. serrata*), when compared to that of normal, non-irradiated crabs (fig. 1c-f).

Electron microscopy.— Under the electron microscope, though the pyloric muscle fibres of *S. serrata* are closely packed, each inter-fibre region shows the presence of both fibroblast cells and connective tissue (fig. 2a). The average diameter of the myofibrils is 1.16 ± 0.22 μm and they are limited by the sarcotubular system (figs. 2a and 3a) The distinct sarcomere in its resting (fig. 2a) and contracted (fig. 2d) states, was measured at 3.88 ± 0.51 and 3.42 ± 0.14 μm,

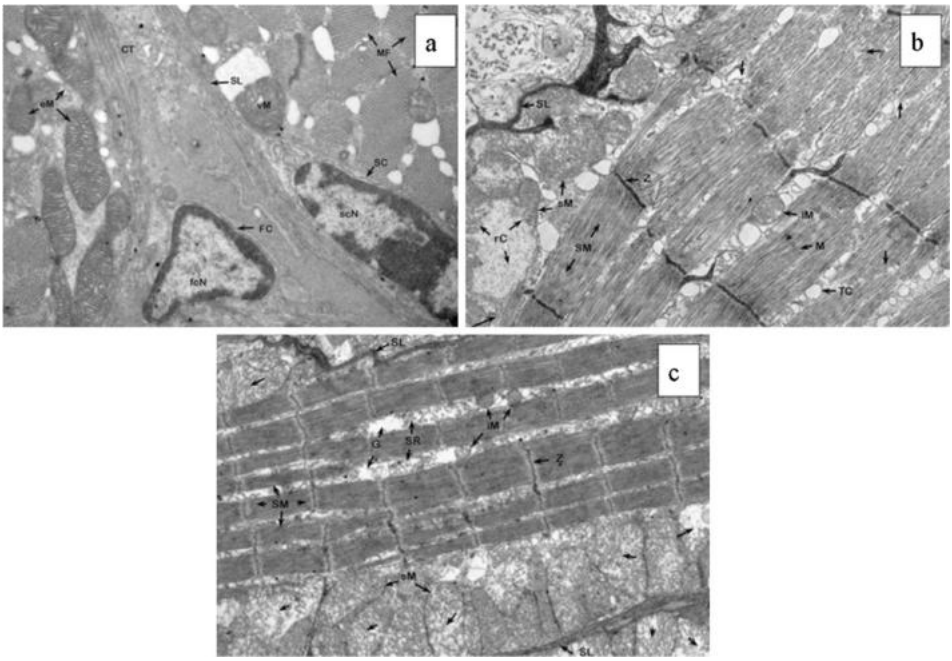

Fig. 3. Electron micrographs of portions of the pyloric muscle fibres of gamma-irradiated *Scylla serrata* (Forskål, 1775). a, 0.5 kGy irradiation, showing the inter-fibre connective tissue (CT), fibroblast cell (FC) with nucleus (fcN), satellite cells (SC) with nucleus (scN), electron dense mitochondria (eM), vesicular mitochondria (vM), myofibrils (MF), and sarcolemma (SL) (cross section (CS), 11 000×); b, 1.0 kGy irradiation, showing the damaged myofibrils (arrow mark), contracted sarcomere (SM), with M-line (M), and Z-line (Z), sarcolemma (SL), subsarcolemmal mitochondria (sM) with ruptured cristae (rC), inter-myofibrillar mitochondria (iM), and terminal cisternae (TC) (longitudinal section (LS), 6800×); c, 2.0 kGy irradiation, showing the gap (G) between the myofibrils, damaged sarcoplasmic reticulum (SR), sub-sarcolemmal mitochondria (sM) with ruptured cristae (arrow marks), inter-myofibrillar mitochondria (iM), sarcomeres (SM), with Z-line (Z), and sarcolemma (SL) (LS, 4800×).

respectively. The sarcomere also shows the presence of an I-band, A-band, and H-zone with M-line, with lengths of 1.0 ± 0.11 μm, 2.99 ± 0.34 μm, and 0.92 ± 0.18 μm, respectively (fig. 2a). The diameter of the myosin filament and the space between adjacent myosins is 177.65 ± 6.1 Å and 692.82 ± 41.88 Å, respectively. Each myosin is surrounded by 6-7 actin filaments and together with these forms a hexagonal lattice within the myofibril (fig. 2c). The sarcotubular system shows the presence of distinct T-tubules and single/double layered sarcoplasmic reticulum invading the interstices of the myofibril and forms the boundary for each myofibril (fig. 2a and d). In the cross section of the muscle fibre, a large number of well-developed terminal cisternae of sarcoplasmic reticulum surrounding each myofibril is also evident (fig. 2d). An association of T-tubules with one or two terminal cisternae of sarcoplasmic reticulum in the form of a diad or triad, respectively,

is also frequently seen between the A-band and the I-band (fig. 2a-c). Though only seldom, the pentad (occasional forking of the T-tubule together with the associated cisternae) is also present (fig. 2c). Supporting the present light microscopic observation, the nucleus is either present at the sub-sarcolemmal (fig. 2e) or inter-myofibrillar region (fig. 2d), with an average length and width of 6.70 ± 1.18 μm and 2.85 ± 0.24 μm, respectively. The presence of one or two eccentrically placed nucleoli (diameter 0.9 μm) and euchromatin, as well as a membrane adhered by electron dense heterochromatin (0.25-1.00 μm) are also common in the nucleus (fig. 2d). Both sub-sarcolemmal and inter-myofibrillar regions are populated with the diversely shaped electron dense or light mitochondria (figs. 2b-e and 3b), of which the elongated or oval shaped electron dense ones (2.01 ± 0.72 μm) show the presence of numerous closely arranged cristae, traversing the entire length of the organelle (fig. 2b) and many of these are aggregated (with 10-20 in number) near the sub-sarcolemmal nucleus (fig. 2e). Closely packed electron light spheroidal mitochondria (with an average diameter of 0.42 ± 0.09 μm) with vesicular cristae are also housed beneath the sarcolemma (fig. 3a-b).

Pyloric muscle from dose-dependent gamma-irradiated *Scylla serrata*

Electron microscopic observations.— Gamma irradiation caused significant changes in the ultrastructural architecture of pyloric muscle tissue, with an increased intensity concomitant with the increased radiation dose, as evidenced by the present electron microscopic observations. The closely packed myofibrils were de-organized, with the appearance of a wide gap between adjacent myofibrils (figs. 3b-c, 4). Also, a statistically significant ($P<0.05$) dose-dependent reduction in sarcomere length was evident (3.21 ± 0.29 μm, 2.91 ± 0.26 μm, and 2.65 ± 0.19 μm in 0.5, 1.0, and 2.0 kGy irradiated muscles, respectively) when compared to that of non-irradiated ones. The sarcomere width also showed a considerable reduction (1.61 ± 0.56 μm, 1.63 ± 0.85 μm, and 1.67 ± 0.53 μm in 0.5, 1.0, and 2.0 kGy irradiated muscles, respectively), though statistically not significant ($P>0.05$), as compared to that of non-irradiated sarcomeres. Interestingly, the diameter of the myosin filament of irradiated muscle showed a sharp, statistically significant ($P<0.05$), increase, measuring 309.33 ± 14.69 Å, 331.17 ± 47.05 Å, and 297.62 ± 53.77 Å at the irradiation doses 0.5, 1.0 and 2.0 kGy, respectively, compared to that of non-irradiated ones. A considerable level of distortion was also noted in the characteristic sarcomere organization, inasmuch as I-band, A-band and H-zone lost their distinctive appearance irrespective of the irradiation doses to which the tissues were exposed; an M-line was barely seen in some of the sarcomeres of 0.5 kGy irradiated samples (fig. 3b, c). The sarcotubular system was ruptured and the level of damage was found be increased concomitant with

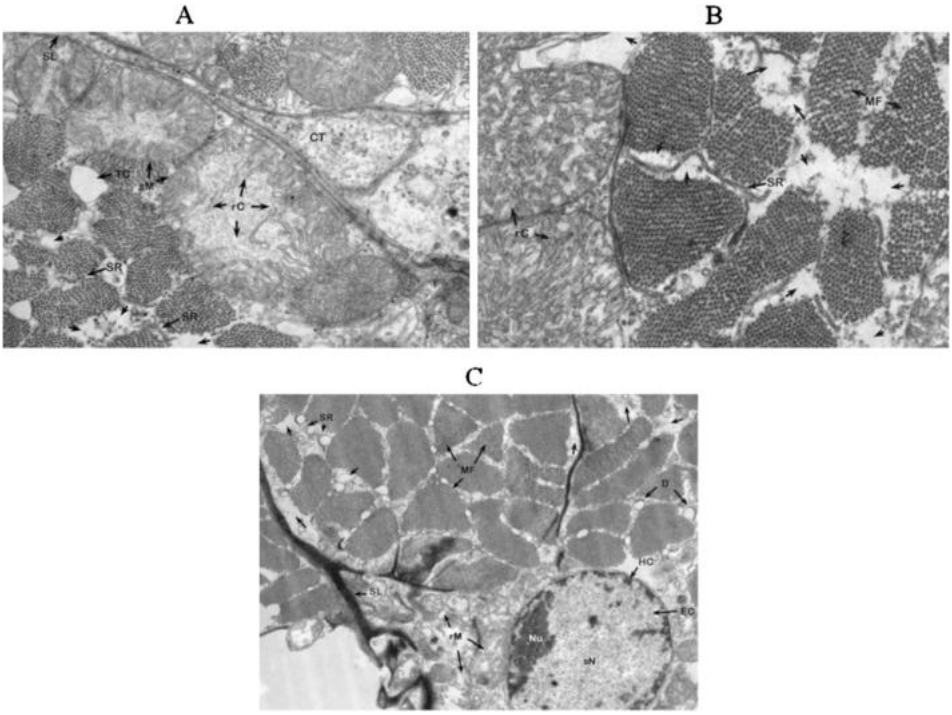

Fig. 4. Electron micrographs of pyloric muscle fibres from gamma-irradiated *Scylla serrata* (Forskål, 1775): A, 0.5 kGy, showing the wide gap (arrow mark) between the myofibrils (MF) and the appearance of subsarcolemmal nucleus (sN) with nucleolus (Nu), euchromatin (EC) and heterochromatin (HC), sarcoplasmic reticulum (SR), diad (D), mitochondria with ruptured cristae (rM), and sarcolemma (SL) (cross section (CS), 6800×); B, 1.0 kGy, showing the increased gap (arrow mark) between the myofibrils, disorganized myofilaments in the myofibrils (MF), damaged sarcoplasmic reticulum (SR), and mitochondria with ruptured cristae (rC) (CS, 4800×); C, 2.0 kGy gamma irradiated *S. serrata*, showing the increased gap (arrow marks) between the myofibrils, damaged sarcoplasmic reticulum (SR), sub-sarcolemmal mitochondria (sM) with ruptured cristae (rC), tubular cisternae (TC), connective tissue (CT), vesicular mitochondria (vM), and sarcolemma (SL) (CS, 11 000×).

the increase in the irradiation dose (figs. 3b-c, 4). The presence of Diads and Triads, the characteristic features of normal muscle fibre, were also totally lost in the irradiated muscle fibre. Though the nuclear membrane was intact without any visible disruption, the size of the nucleus showed a significant ($P<0.05$) increase coupled with the changes, such as a decreased size of the heterochromatin, highly dispersed euchromatin, and less dense nucleoplasm, compared to that of non-irradiated normal muscle fibre (figs. 5 and 4c); the average nucleus diameter in the 0.5, 1.0 and 2.0 kGy irradiated muscle fibre being 5.88 ± 0.43 μm, 5.19 ± 0.66 μm, and 5.20 ± 1.62 μm, respectively. Due to gamma irradiation, the vesicular (electron light) mitochondria were also damaged as evidenced by their

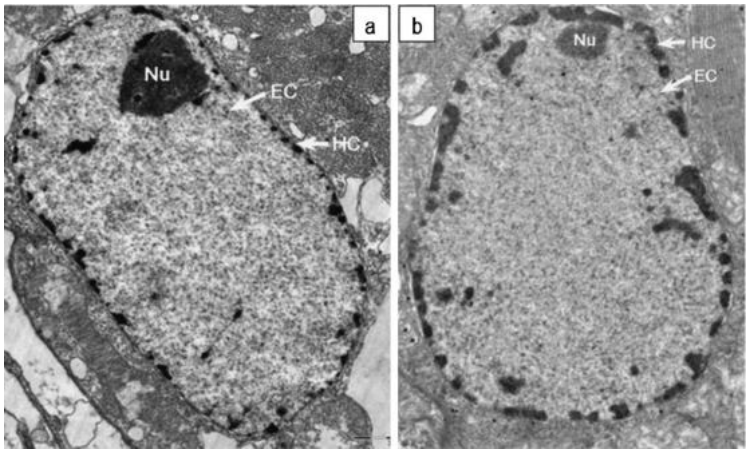

Fig. 5. Electron micrographs of a nucleus in the gamma irradiated pyloric muscle fibres of *Scylla serrata* (Forskål, 1775), showing the appearance of the nucleolus (Nu), euchromatin (EC), and heterochromatin (HC), after: a, 0.5 kGy irradiation (cross section (CS), 6800×); and, b, 1.0 kGy irradiation (longitudinal section (LS), 11 000×).

overall swelling, reaching a diameter of 1.13 ± 0.34 μm, 1.72 ± 0.25 μm, and 1.41 ± 0.31 μm in 0.5, 1.0, and 2.0 kGy irradiated samples, respectively, as well as crushed cristae (figs. 3b-c and 4). Significantly, electron dense mitochondria, normally present in non-irradiated muscles, could not be observed in any of the post-irradiation samples, irrespective of the irradiation doses.

Single cell gel electrophoresis (DNA comet assay).— The DNA comet assay revealed the presence of significant nuclear damage in the tissues (muscle, hepatopancreas and testis) of *S. serrata* upon their exposure to dose-dependent (0.5, 1.0 and 2.0 kGy) gamma irradiation, and in all analysed tissues the intensity of the damage was increased concomitant with the increase in the irradiation dose. The DNA migration (tail length) in muscle cell in response to radiation doses 0.5, 1.0 and 2.0 kGy was 65.44, 76.17 and 141.27, respectively, which was significantly higher ($P = 0.0001$) than in the non-irradiated sample, in which it was measured to be only 3.44 (table I, fig. 6a). Testis also showed a similar pattern in the tail length after exposure to gamma irradiation in a dose-dependent manner (table I). The hepatopancreas, after receiving 0.5 and 1.0 kGy, on the other hand, showed statistically significant reduction in tail length ($P = 2.51 \times 10^{-13}$) compared to that of muscle and testis, which received the similar doses of irradiation. Generally, all the observed tissues [muscle (fig. 6a), hepatopancreas (fig. 6b) and testis (fig. 6c)] responded more or less in a similar pattern, showing maximum comet length towards the radiation dose of 2.0 kGy. The tail length of the comet varies significantly ($P = 3.84 \times 10^{-6}$) among the tissues at a given dose of gamma irradiation, indicating that each tissue has a dose-dependent specific tolerance level (table I). The

TABLE I

Comet parameters in the muscle, hepatopancreas and testis of fresh, non-irradiated and dose-dependent (0.5, 1.0 and 2.0 kGy) gamma irradiated mud crab, *Scylla serrata* (Forskål, 1775), immediately after irradiation (Mean ± SD) (number of samples = 25)

Comet parameter	Tissue	Non-irradiated (0.0 kGy)	0.5 kGy	1.0 kGy	2.0 kGy
Tail length	Muscle	$^{ax}3.44 \pm 1.03$	$^{bx}65.44 \pm 10.99$	$^{bx}76.17 \pm 7.70$	$^{cx}141.27 \pm 21.10$
	Hepatopancreas	$^{ax}3.44 \pm 0.89$	$^{ay}3.07 \pm 0.27$	$^{by}39.31 \pm 10.49$	$^{cx}151.00 \pm 24.02$
	Testes	$^{ax}3.31 \pm 0.60$	$^{bx}68.63 \pm 5.78$	$^{bx}75.56 \pm 8.54$	$^{cy}188.33 \pm 26.30$
Head DNA	Muscle	$^{ax}99.39 \pm 0.94$	$^{bx}77.53 \pm 6.64$	$^{cx}68.66 \pm 5.96$	$^{dx}43.00 \pm 4.99$
	Hepatopancreas	$^{ax}99.60 \pm 0.56$	$^{ay}98.05 \pm 3.94$	$^{by}81.34 \pm 7.36$	$^{cx}43.21 \pm 8.31$
	Testes	$^{ax}99.51 \pm 0.59$	$^{bz}82.28 \pm 3.10$	$^{cx}71.89 \pm 3.77$	$^{dx}45.12 \pm 3.72$
Tail DNA	Muscle	$^{ax}0.61 \pm 0.94$	$^{bx}22.46 \pm 6.64$	$^{cx}31.34 \pm 5.96$	$^{dx}57.00 \pm 4.99$
	Hepatopancreas	$^{ax}0.40 \pm 0.57$	$^{ay}1.95 \pm 3.94$	$^{by}18.66 \pm 7.36$	$^{cx}56.80 \pm 8.31$
	Testes	$^{ax}0.49 \pm 0.59$	$^{bz}17.72 \pm 3.10$	$^{cx}28.11 \pm 3.77$	$^{dx}54.88 \pm 3.72$
Tail moment	Muscle	$^{ax}0.02 \pm 0.03$	$^{ax}12.55 \pm 6.00$	$^{bx}30.03 \pm 9.91$	$^{cx}129.01 \pm 41.10$
	Hepatopancreas	$^{ax}0.02 \pm 0.03$	$^{ay}1.64 \pm 4.50$	$^{ay}14.66 \pm 12.79$	$^{by}178.83 \pm 73.90$
	Testes	$^{ax}0.02 \pm 0.03$	$^{ax}9.74 \pm 5.31$	$^{bx}30.81 \pm 9.08$	$^{cx}100.79 \pm 27.70$

$^{a\text{-}d}$ Mean ± SD with different superscript letters within the same row differ significantly ($P < 0.05$). $^{x\text{-}z}$ Mean ± SD with different superscript letters within the same column for a comet parameter differ significantly ($P < 0.05$).

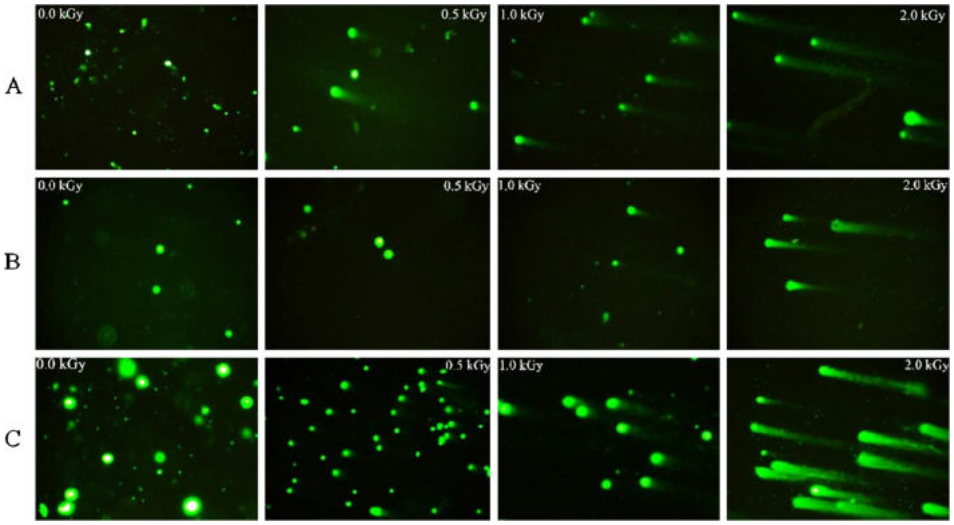

Fig. 6. Fluorescence microscopic image of DNA comet assay in: A, muscle; B, hepatopancreas; and, C, testis; of untreated (0.0 kGy) and gamma irradiated (0.5, 1.0, and 2.0 kGy), *Scylla serrata* (Forskål, 1775).

results of one way ANOVA also revealed that dose-dependent gamma irradiation has a statistically significant effect for increasing the tail length of the comet in a progressive manner in all the tissues [muscle ($P = 1.87 \times 10^{-35}$; F = 327.17), hepatopancreas ($P = 6.09 \times 10^{-42}$; F = 482.51) and testis ($P = 1.06 \times 10^{-41}$; F = 473.29)] observed during the present study. The interaction effect of different irradiation doses (0.5, 1.0 and 2.0 kGy) and tissues (muscle, hepatopancreas and testis) analysed by two-way ANOVA, was also found statistically significant ($P = 9.03 \times 10^{-14}$; F = 25.55).

The present study also gave evidence of a gradual and statistically significant increase in the tail DNA coupled with the decrease in the head DNA in response to increased doses of gamma irradiation in all analysed tissues [muscle ($P = 1.94 \times 10^{-37}$; F = 335.56), hepatopancreas ($P = 6.41 \times 10^{-37}$; F = 321.65) and testis ($P = 1.91 \times 10^{-49}$; F = 874.12)]; the amount was found at its maximum (more than 50%) in 2.0 kGy irradiated sample (table I). At the same time, the tail DNA in the non-irradiated control sample was 0.40 to 0.61%. This increase in the tail DNA concomitant with the increase in irradiation dose was relatively high in muscle compared to those of hepatopancreas and testis. A statistically significant interaction effect by different gamma irradiation doses and tissue types (muscle, hepatopancreas and testis) on the increasing tail DNA or decreasing head DNA ($P = 7.73 \times 10^{-18}$; F = 19.98) was also observed in the present study. The tail moment, another parameter considered in this study, also showed a similar pattern in all three tissues against dose-dependent gamma irradiation (0.5. 1.0 and 2.0 kGy)

TABLE II

The regression equations, correlation coefficient and R^2 value of comet parameters against irradiation doses in the muscle, hepatopancreas and testis of *Scylla serrata* (Forskål, 1775), immediately after irradiation

Comet parameter	Tissue	Regression equation	Correlation coefficient	R^2
Tail length	Muscle	$y = 65.13x + 15.03$	0.89	0.946
	Hepatopancreas	$y = 78.00x - 19.05$	0.87	0.933
	Testes	$y = 88.07x + 6.89$	0.92	0.956
Head DNA	Muscle	$y = 27.00x + 95.78$	−0.96	0.922
	Hepatopancreas	$y = 29.81x + 106.6$	−0.94	0.883
	Testes	$y = 26.59x + 97.97$	−0.98	0.972
Tail DNA	Muscle	$y = 27.00x + 4.21$	0.96	0.922
	Hepatopancreas	$y = 29.81x + 6.68$	0.94	0.883
	Testes	$y = 26.59x + 2.03$	0.99	0.972
Tail moment	Muscle	$y = 65.9x - 14.76$	0.89	0.790
	Hepatopancreas	$y = 92.55x - 32.24$	0.82	0.669
	Testes	$y = 51.92x - 10.08$	0.92	0.838

and it was found to range from less than 0.1 in the non-irradiated sample to greater than 100 in a 2.0 kGy irradiated sample (table I). Correlation-regression analysis showed a significant, negative correlation between head DNA and irradiation dose and a positive correlation between irradiation dose and tail DNA, tail moment, and tail length (table II).

DISCUSSION

Microscopic view of pyloric muscle of *Scylla serrata*

A comprehensive study on the ultrastructure of pyloric muscle of *Scylla serrata* using light and electron microscopy was preliminarily conducted for the first time and the results revealed structural resemblances with the fast muscle structure described in the crab, *Cancer magister* Dana, 1852, in several respects. For instance, the diameter of myofibrils ranges between 0.81 and 1.62 μm, which is parallel with the average myofibrillar diameter of 1.5 μm, reported for the fast muscle of *C. magister* (cf. Fahrenbach, 1967). The length of the sarcomere is measured as 3.88 ± 0.51 μm at resting state and 3.42 ± 0.14 μm in contracted state, which is comparable to the reports on the distal fibres of distal accessory flexor muscle of *C. magister* (cf. Fahrenbach, 1967). Shorter and longer sarcomeres have been shown to associate with the muscles involved in rapid and slower movements, respectively (North, 1963; Fahrenbach, 1967; Read et al., 1994; Longo & Díaz, 2013). In *S. serrata*, each sarcomere showed the presence of a well-marked I-band, A-band, and H-zone with M-line and Z-line,

analogous to the reports on other crustacean muscle (Fahrenbach, 1963; Endo, 1964; Rosenbluth, 1969; Jahromi & Charlton, 1979; Mieguelet et al., 1992; Read et al., 1994), insect flight muscle and vertebrate skeletal muscle (Paniaguaet et al., 1996). In addition, the diameter of myosin filaments and the inter-myofilamentar space between adjacent myosin filaments is measured to be 177.65 ± 6.1 Å and 692.82 ± 41.88 Å, respectively, akin to the previous study reported by Fahrenbach (1967) on *C. magister*, in which inter-myofilamentar space and myosin diameter of fast fibre had been measured as 190 Å and 580 Å, respectively. In the slow fibres of crabs such as *C. magister* and *Callinectes* sp., the myosin filament diameter is reported to be 750-900 Å and 23-45 nm, respectively (Fahrenbach, 1967). In crustaceans, the number of actin filament surrounding the myosin filaments ranges between 6 and 12, according to its functional properties (Paniaguaet et al., 1996). The myosin-to-actin filament ratio in each hexagonal lattice in the pyloric muscle from *S. serrata* is around 1 : 6. In crabs, 6-8 and 10-12 myosin and actin filaments are usually seen associated with fast and slow muscles, respectively (Fahrenbach, 1967; Eastwood et al., 1978). Some unusually fast acting muscles, such as those found in the antenna of lobsters, the ratio is further reduced to 1 : 3 (Rosenbluth, 1969).

In *S. serrata*, pyloric muscle displays a well developed sarcotubular system composed of T-tubules and single or double layered sarcoplasmic reticulum (SR) along with well-marked terminal cisternae, a characteristic feature of crustacean fast muscle. In crustacean fast muscle fibre and sarcolemma, clefts together with sarcoplasmic reticulum were shown to play an important role in modulating the cytosolic Ca^{2+} concentration, required for the muscle contraction and relaxation (Ushio & Watabe, 1993). Rosenbluth (1969) found a more profuse sarcoplasmic reticulum in the unusually fast acting, synchronous "remotor" muscle of the second antenna of lobsters, indicating its significant role in exceedingly reducing the duration of the contraction-relaxation cycle. A highly developed sarcoplasmic reticulum as the characteristic feature of fast fibres has also been reported in the leg muscle of lobsters (Jahromi & Atwood, 1971). Frequent observation of diads and triads in the pyloric muscle of *S. serrata* further confirmed its fast acting nature as reported by previous investigators on other invertebrates, including crustaceans (stomatopods, amphipods and decapods) and vertebrates (Selverston, 1967; Paniaguaet et al., 1996; Rosenbluth et al., 2010). In lobster leg muscle, the number of diads shows variation according to the type of muscle fibre; slow fibre possesses few numbers, on the other hand, a profuse number of diads is the characteristic feature of fast fibres (Jahromi & Atwood, 1971). Though rarely, the present study could also detect the presence of the pentad, supporting the previous reports on muscles of shellfish (Fahrenbach, 1963, 1967) and in finfish (Suzuki, 2003), birds (Takekuraet et al., 1993) and

rat (Revel, 1962; Takekura et al., 2001). The uncoordinated development of T-tubules in the absence of morphogenetic influence of the nerve in muscle fibres is believed to induce the formation of pentads (Ishikawa, 1968). However, the exact mechanism of their formation and physiological significance is yet to be explored.

The characteristic features of elongated electron dense and spheroid electron light mitochondria present at both sub-sarcolemmal and inter-myofibrillar regions of the pyloric muscle fibres of *S. serrata* showed much resemblance to that reported in the claw closer muscles of two estuarine crabs, *Cyrtograpsus angulatus* Dana, 1851 and *Neohelice granulata* (Dana, 1851) by Longo & Díaz (2013). The spheroid electron light sub-sarcolemmal/inter-myofibrillar mitochondria with vesicular or tubular cristae are also reported in the myotomal musculature of teleost fishes (Devincenti et al., 2000a, b). In ostracods (*Acetabulostoma* sp.), acarids (*Halacarellus thomasi* (Newell, 1984)) (Royuelaet al., 2000b) and in annelids (*Eisenia foetida* Savigny, 1826) (Paniaguaet al., 1996), the mitochondria are reported to be restricted only to the inter-myofibrillar regions, whereas in primitive crustaceans like cephalocarids, sub-sarcolemmal mitochondria are most common (Read et al., 1994). The profuse presence of large-sized mitochondria observed during the present study, indicates the fatigue resistance capacity of the pyloric muscle for rapid movement as reported in other fast muscles of crabs and other invertebrates (Paniaguaet al., 1996; Royuelaet al., 2000a; Johnson et al., 2004; Longo & Díaz, 2013).

Dose-dependent gamma irradiation in the pyloric muscle of *Scylla serrata*

After thoroughly analysing the structural features of pyloric muscle in *S. serrata*, we aimed to explore the effect of radio preservation in shellfish by proposing *S. serrata* as a model organism.

Muscle fibre.— Dose-dependent gamma irradiation in *S. serrata* unfolds some interesting facts on how the architecture of pyloric muscle fibre is affected. Gamma irradiation significantly altered the microstructure of pyloric muscle of *S. serrata*; muscle fibre that received irradiation at doses of 0.5, 1.0 and 2.0 kGy showed the presence of length-reduced but width-increased sarcomeres, compared to that of non-irradiated normal muscle fibre. Significant differences in the size of sarcomeres in terms of length and width and disruption of myofibrils in gamma-irradiated samples has also been reported in chicken breast muscle by Yoon (2003). However, in irradiated bovine muscle, Yook et al. (2001) had a different observation; they found remarkable swelling of the sarcomeres in irradiated muscle, suggesting the disruption of the bonds between myosin and actin. Gamma irradiation induced denaturation of the myosin head part, which has

also been reported in bovine skeletal muscle (Lee et al., 2000). The diameter of the myosin filament in the irradiated pyloric muscle fibre of *S. serrata* (present study) showed a significant increase ($P < 0.05$) compared to that of non-irradiated muscle, apparently due to the irradiation induced disruption of the myosin filaments. Our further observation on the disorganized sarcomere coupled with the missing of I-band, A-band, and H-zone in the gamma irradiated pyloric muscle of *S. serrata*, appears akin to the reports in shrimp (Perng & Yang, 1990) and bovine muscle (Yook et al., 2001), suggesting the possibility of an irradiation-induced de-polymerization of myosin. Gamma irradiation induces denaturation of fibrous proteins, and thereby the destruction of muscle fibres has also been reported (Horowitset al., 1986). The characteristic changes like increase in the space between the myofibrils in the electron micrograph of gamma irradiated muscle of *S. serrata* observed in the present study, agree with the observation made by Yook et al. (2001) in bovine muscle, in which more gaps were formed in between the myofibrils as a result of gamma irradiation at the dose range of 3.0-5.0 kGy. According to them, gamma irradiation induced damage to the costamers and the desmins apparently increase the gap between the myofibrils.

Sarcotubular system.— In the irradiated muscle of *S. serrata*, the sarcotubular system lost its continuity and the ruptured system showed no sign for the presence of triads or pentads as reported for the irradiated skeletal muscles of grass shrimp (Perng & Yang, 1990) and of vertebrates (Yook et al., 2001; Yoon, 2003). Though the nuclear membrane disruption was not clear, the gamma irradiation caused a significant swelling of the nucleus in the muscle of *S. serrata*, akin to the reports in a wide variety of cells as a result of low dose irradiation (Anderson et al., 1975; Gasperin et al., 1992). Heterochromatin, euchromatin and nucleoplasm of irradiated muscle fibre of *S. serrata* have also shown slight variation in their appearance compared to that of non-irradiated ones. Previous investigators have also reported the marginal condensation of chromatin to the nuclear lamina and the formation of large, dense chromatin clumps and ring-like chromatin nuclear bodies in low dose irradiated cell nuclei of vertebrate cells (Jordan, 1967; Barham & Walters, 1978; Gasperin et al., 1992).

Mitochondria.— Mitochondria are reported to be another major target of ionizing radiation, which increases the mitochondrial oxidative stress, and thereby inducing apoptosis (Hosokiet al., 2012; Kam & Banati, 2013). Characteristic changes, including the elongation, branching, reversible increase of their size, and the development of giant forms, are reported in the fine structure of mitochondria after ionizing radiation (Betzoldet al., 1992). Observations on the disappearance of electron dense mitochondria in the irradiated muscle of *S. serrata* during the present study appear to be significant and demand further study. Swelling of the mitochondria as a result of irradiation has been reported even at lower doses, like

50 Gy in somatic cells (Wi et al., 2005). Though the swelling and disruption of transverse cristae were usual in the vesicular (electron light) mitochondria of irradiated pyloric muscle of *S. serrata*, the intensity of the damage is more visible in the 1.0 and 2.0 kGy irradiated tissue. In mouse hepatocytes, loss of the mitochondrial matrix has been reported after 5.3 Gy mixed neutron-gamma irradiation (René & Evans, 1970). Though disruption of outer and inner membranes, vacuolization, and loss of matrix have previously been reported in the mitochondria of a wide variety of irradiated cells (Schwartz et al., 1994), the present study could not detect such a characteristic change in the gamma irradiated pyloric muscle of *S. serrata*.

DNA.— The generation of hydroxyl radicals is considered as the major cause of DNA damage in vitro and in vivo due to irradiation (Kiefer, 1990). The radiation sensitivity of different organisms/organs/tissues can well be understood directly from the estimation of the DNA damage through DNA comet assay (Ueno et al., 2007; McKenna et al., 2008; Jayakumar et al., 2012; Sowmithraet al., 2015). The percentage of DNA in the tail and the tail length are reported as the most reliable parameters of the comet assay for assessing DNA damage in vivo and in vitro, respectively (Collins, 2004; Ueno et al., 2007). Ostling & Johanson (1984), in their in vitro experiments using cultured cells, detected ionizing radiation-induced DNA damage at doses ranging from 1 to 3 Gy. The high coefficient of determination ($r^2 = 0.9977$) between the absorbed dose and the % DNA in the tail, was reported from the lung of mice in the range of 0-30 Gy of X-ray in vivo (Risomet al., 2003). Further, a relatively high correlation has also been reported between the radiation dose and both tail length and % DNA in the tail in almost all organs of mice (Ueno et al., 2007). The present DNA comet assay in the gamma- irradiated tissues (muscle, hepatopancreas and testis) of *S. serrata*, also revealed the actual incidence of a significant level of nuclear damage, and the extent of that damage appears to be dose-dependent, akin to the results published in previous reports (Singh et al., 1988; Collins et al., 1996). In earthworms, though the DNA damage shows a dose-dependent increase, in the course of time this damage has a tendency for a gradual decrease, thereby suggesting the possibility for repair the damaged DNA and/or loss of heavily damaged cells by apoptosis, cell turnover, and dilution as a result of cell replication (Salehaet al., 2001; Revankar & Shyama, 2009; Hertel-Aas et al., 2011; Sowmithraet al., 2015). A recent study on the blood cells of the freshwater fish, *Cyprinus carpio* Linnaeus, 1758, also reports a gradual recovery from dose-dependent DNA damage, while keeping the species in a radiation-free environment, and such increasing with the duration of post-irradiation time (Praveen Kumar et al., 2015). Gagnaire et al. (2015), made similar observations in early-life stages of the zebrafish, *Danio rerio* (Hamilton, 1822), induced by external gamma irradiation.

A tissue-dependent variation in the radiation sensitivity of *S. serrata* is also evident from the present study; the extent of DNA migration in terms of % DNA in the tail of the comet is relatively more in muscle tissue compared to that of testis and hepatopancreas. The variations in the sensitivity/tolerance to irradiation in response to the type of tissue and also its origin, have been reported upon previously (Hasan & Khan, 1998). The comet assay has revealed the difference in the DNA damage in the meat derived from chicken, pork, and beef irradiated at the dose of 2.0 kGy, with chicken being the sample damaged to a greater extent than the others (Miyahara et al., 2002). Ueno et al. (2007) also reported a significant, three-fold higher difference of comet parameters in the glandular stomach compared to other organs in mice exposed to X-rays. Contrary to these results, the comet assay in two species of bivalves, *Paphia malabarica* (Dillwyn, 1817) and *Meretrix casta* (Gmelin, 1791), indicated an almost identical sensitivity to gamma radiation in both species, as measured by DNA damage (Praveen Kumar et al., 2014).

CONCLUSION

A precise description of ultrastructuaral features of pyloric muscle tissue of *Scylla serrata* is presented, based on light- and electron-microscopic analysis. Subsequent experiments were focused on the cellular and genotoxic impact of gamma irradiation in this crab's tissues. In the pyloric muscle, the study determines gamma irradiation as low as 1.0 kGy is able to vigorously damage the nucleus, mitochondria and sarcotubular system, the major cellular sites. Further, the DNA comet assay revealed that different tissues show different tolerance levels for a particular dose of irradiation, as evidenced by a statistically significant interaction effect of irradiation doses and tissues on the progressive increase of the comet tail length, and the tail moment. Overall, the present study appears to be truly relevant to qualitatively categorize the irradiated crabs for commercial purposes. Also, the significant difference in comet measurements elucidates the nuclear damage-evoking dose of gamma radiation, which in turn helps to calculate the accurate dose of irradiation for the preservation of this crab and its commercial products.

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REFERENCES

ANDERSON, R. E., G. B. OLSON, J. L. HOWARTH, G. L. WIED & P. H. BARTELS, 1975. Computer analysis of defined populations of lymphocytes irradiated in vitro. II. Analysis of thymus-dependent versus bone marrow-dependent cells. American Journal of Pathology, **80**(1): 21-32.

ARAÚJO, M. M., N. S. MARÍN-HUACHACA, J. MANCINI-FILHO, H. DELINCÉE & A. L. C. H. ILLAVICENCIO, 2004. Identification of irradiated refrigerated pork with the DNA comet assay. Radiation Physics and Chemistry, **71**(1-2): 185-187.

ARSHAD, K., K. SUDHA, A. A. M. HATHA, P. T. ANEESH, A. K. HELNA & G. ANILKUMAR, 2015b. Effect by gamma irradiation and low-temperature storage on bacteriological profile of edible estuarine crab *Scylla serrata*. Journal of food processing and preservation, **39**(6): 2473-2484.

ARSHAD, K., K. SUDHA, A. A. M. HATHA & G. ANILKUMAR, 2015a. Combined effect of gamma irradiation and cold temperature storage on the sensory properties of edible estuarine crab *Scylla serrata*. International Food Research Journal, **22**(6): 2253.

ATTIA, A. M. M., G. M. NABIL, D. FRANKENBERG & M. FRANKENBERG-SCHWAGER, 2011. Measurement of X-ray-induced DNA double-strand breaks at various stages of the cell cycle using the total fluorescence as a comet assay parameter. Radiation Physics and Chemistry, **80**(11): 1178-1185.

BARHAM, S. S. & R. A. WALTERS, 1978. X-irradiation-induced nuclear lesions in cultured mammalian cells: an ultrastructural analysis. Radiation Research, **76**(1): 105-126.

BETZOLD, J. M., W. SAEGER & D. K. LÜDECKE, 1992. Ultrastructural-morphometric effects of radiotherapy on pituitary adenomas in acromegaly. Experimental and Clinical Endocrinology, **100**(3): 106-111.

BLANK, K. R., M. S. RUDOLTZ, G. D. KAO, R. J. MUSCHEL & W. G. MCKENNA, 1997. The molecular regulation of apoptosis and implication for radiation oncology. International Journal of Radiation Biology, **71**: 455-466.

CLARO, S., M. E. M. OSHIRO, E. FREYMULLER, E. KATCHBURIAN, E. G. KALLAS, P. S. CERRI & A. T. FERREIRA, 2008. Gamma-radiation induces apoptosis via sarcoplasmatic reticulum in guinea pig ileum smooth muscle cells. European Journal of Pharmacology, **590**(1-3): 20-28.

COLLINS, A. R., 2004. The comet assay for DNA damage and repair: principles, applications, and limitations. Molecular Biotechnology, **26**(3): 249-261.

COLLINS, A. R., M. DUSINSKÁ, C. M. GEDIK & R. STĚTINA, 1996. Oxidative damage to DNA: do we have a reliable biomarker? Environmental Health Perspectives, **104**(Suppl.): 465-469.

COQUERELLE, T. & U. HAGEN, 1978. Radiation effects on the biological function of DNA. In: J. HÜTTERMANN, W. KÖHLEIN, R. TEOULE & A. J. BERTINCHAMPS (eds.), Effects of ionizing radiation on DNA, physical, chemical and biological aspects: 261-303.

DEVINCENTI, C. V., A. O. DIAZ & A. L. GOLDEMBERG, 2000a. Lateral musculature in the whitemouth croaker (*Micropogonias furnieri*): its characterization with respect to different gonadal conditions. Anatomia, Histologia, Embryologia, **29**(2): 65-72.

DEVINCENTI, C. V., A. O. DÍAZ & A. L. GOLDEMBERG, 2000b. Characterization of the swimming muscle of the anchovy *Engraulis anchoita* (Hubbs and Martini, 1935). Anatomia, Histologia, Embryologia, **29**(4): 197-202.

EASTWOOD, A. B., D. S. WOOD & J. P. REUBEN, 1978. Unusual thick and thin filamentpacking in a crustacean muscle. Journal of Cell Biology, **77**: 48-58.

ENDO, M., 1964. Entry of a dye into the sarcotubular system of muscle. Nature, **202**(4937): 1115-1116.

EREL, Y., N. YAZICI, S. ÖZVATAN, D. ERCIN & N. CETINKAYA, 2009. Detection of irradiated quail meat by using DNA comet assay and evaluation of comets by image analysis. Radiation Physics and Chemistry, **78**(9): 776-781.

FAHRENBACH, W. H., 1963. The sarcoplasmic reticulum of striated muscle of a cyclopoid copepod. Journal of Cell Biology, **17**: 629-640.

FAHRENBACH, W. H., 1967. The fine structure of fast and slow crustacean muscles. Journal of Cell Biology, **351**: 69-79.

GAGNAIRE, B., I. CAVALIÉ, S. PEREIRA, M. FLORIANI, N. DUBOURG, V. CAMILLERI & C. ADAM-GUILLERMIN, 2015. External gamma irradiation-induced effects in early-life stages of zebrafish, *Danio rerio*. Aquatic Toxicology, Amsterdam, **169**: 69-78.

GASPERIN, P., M. GOZY, O. PAUWELS, J. FROHLING, P. VAN HOUTTE, J. L. PASTEELS & R. KISS, 1992. Monitoring of radiotherapy-induced morphonuclear modifications in the mxt mouse mammary carcinoma by means of digital cell image analysis. International Journal of Radiation Oncology Biology Physics, **22**(5): 979-987.

HASAN, M. & A. R. KHAN, 1998. Control of stored-product pests by irradiation. Integrated Pest Management Reviews, **3**(1): 15-29.

HERTEL-AAS, T., D. H. OUGHTON, A. JAWORSKA & G. BRUNBORG, 2011. Induction and repair of DNA strand breaks and oxidised bases in somatic and spermatogenic cells from the earthworm *Eisenia fetida* after exposure to ionising radiation. Mutagenesis, **26**(6): 783-793.

HOROWITS, R., E. S. KEMPNER, M. E. BISHER & R. J. PODOLSKY, 1986. A physiological role for titin and nebulin in skeletal muscle. Nature, **323**(6084): 160-164.

HOSOKI, A., S. I. YONEKURA, Q. L. ZHAO, Z. L. WEI, I. TAKASAKI, Y. TABUCHI, L. L. WANG, S. HASUIKE, T. NOMURA, A. TACHIBANA, K. HASHIGUCHI, S. YONEI, T. KONDO & Q. M. ZHANG-AKIYAMA, 2012. Mitochondria-targeted superoxide dismutase (SOD2) regulates radiation resistance and radiation stress response in HeLa cells. Journal of Radiation Research, **53**: 58-71.

ISHIKAWA, H., 1968. Formation of elaborate networks of t-system tubules in cultured skeletal muscle with special reference to the t-system formation. Journal of Cell Biology, **38**(1): 51-66.

JAHROMI, S. S. & H. L. ATWOOD, 1971. Structural and contractile properties of lobster leg-muscle fibers. Journal of Experimental Zoology, **176**(4): 475-486.

JAHROMI, S. S. & M. P. CHARLTON, 1979. Transverse sarcomere splitting — a possible means of longitudinal growth in crab muscles. Journal of Cell Biology, **80**: 736-742.

JAYAKUMAR, S., H. N. BHILWADE, B. N. PANDEY, S. K. SANDUR & R. C. CHAUBEY, 2012. The potential value of the neutral comet assay and the expression of genes associated with DNA damage in assessing the radiosensitivity of tumor cells. Mutation Research, **748**(1-2): 52-59.

JOHNSON, L. K., R. M. DILLAMAN, D. M. GAY, J. E. BLUM & S. T. KINSEY, 2004. Metabolic influences of fiber size in aerobic and anaerobic locomotor muscles of the blue crab, *Callinectes sapidus*. Journal of Experimental Biology, **207**: 4045-4056.

JORDAN, S. W., 1967. Ultrastructural studies of spleen after whole body irradiation of mice. Experimental and Molecular Pathology, **6**(2): 156-171.

KAM, W. W. Y. & R. B. BANATI, 2013. Effects of ionizing radiation on mitochondria. Free Radical Biology & Medicine, **65**: 607-619.

KIEFER, J., 1990. Biological radiation effects. (Springer-Verlag, New York).

KOŃCA, K., A. LANKOFF, A. BANASIK, H. LISOWSKA, T. KUSZEWSKI, S. GÓŹDŹ, Z. KOZA & A. WOJCIK, 2003. A cross-platform public domain PC image-analysis program for the comet assay. Mutation Research / Genetic Toxicology and Environmental Mutagenesis, **534**(1-2): 15-20.

LAXMAPPA, B., 2016. Fisheries development, Telengana, India. International Aquafeed Magazine, **19**(3): 24-27.

LEE, J. W., H. S. YOOK, K. H. LEE, J. H. KIM, W. J. KIM & M. W. BYUN, 2000. Conformational changes of myosin by gamma irradiation. Radiation Physics and Chemistry, **58**(3): 271-277.

LONGO, M. V. & A. O. DÍAZ, 2013. Morphology of the claw closer muscle in two estuarine crab species (Crustacea, Varunidae): an ultrastructural study. Zoological Science, **30**(8): 663-669.

LUCIA, A., C. H. VILLAVICENCIO, J. MANCINI-FILHO & H. DELINCE, 2000. Application of a rapid screening method to detect irradiated meat in Brazil. Radiation Physics and Chemistry, **57**: 295-298.

MAHTO, R., S. GHOSH, M. K. DAS & M. DAS, 2015. Effect of gamma irradiation and frozen storage on the quality of fresh water prawn (*Macrobrachium rosenbergii*) and tiger prawn (*Penaeus monodon*). LWT — Food Science and Technology, **61**(2): 573-582.

MANGIACOTTI, M., G. MARCHESANI, F. FLORIDI, G. SIRAGUSA & A. E. CHIARAVALLE, 2013. Official checks by an accredited laboratory on irradiated foods at an Italian market. Food Control, **33**(2): 307-312.

MARÍN-HUACHACA, N. S., M. T. LAMY-FREUND, J. MANCINI-FILHO, H. DELINC & C. H. VILLAVICENCIO, 2002. Detection of irradiated fresh fruits treated by e-beam or gamma rays. Radiation Physics and Chemistry, **63**: 419-422.

MCKENNA, D. J., S. R. MCKEOWN & V. J. MCKELVEY-MARTIN, 2008. Potential use of the comet assay in the clinical management of cancer. Mutagenesis, **23**(3): 183-190.

MIEGUEL, A., T. KOBAYASHI & Y. MAEDA, 1992. Isolation, purification and characterization of tropomyosin and troponin subunits from the lobster tail muscle. Journal of Muscle Research and Cell Motility, **32**: 608-618.

MIYAHARA, M., A. SAITO, H. ITO & M. TOYODA, 2002. Identification of low level gamma-irradiation of meats by high sensitivity comet assay. Radiation Physics and Chemistry, **63**(3-6): 451-454.

NORTH, R. J., 1963. The fine structure of the myofibers in the heart of the snail *Helix aspersa*. Journal of Ultrastructure Research, **8**(3-4): 206-218.

OLIVE, P. L., 1999. DNA damage and repair in individual cells: applications of the comet assay in radiobiology. International Journal of Radiation Biology, **75**: 395-405.

OSTLING, O. & K. J. JOHANSON, 1984. Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. Biochemical and Biophysical Research Communications, **123**(1): 291-298.

PANIAGUA, R., M. ROYUELA & B. FRAILE, 1996. Ultrastructure of invertebrate muscle cell types. Histology and Histopathology, **11**: 181-201.

PERNG, F. S. & J. S. YANG, 1990. Ultrastructural effect of gamma radiation on grass shrimps (*Penaeus monodon* Fabricius). Radiation Physics and Chemistry, **35**(1-3): 258-262.

PRAVEEN KUMAR, M. K., S. K. SHYAMA, B. S. SONAYE, U. R. NAIK, S. B. KADAM, P. D. BIPIN & R. C. CHAUBEY, 2014. Evaluation of γ-radiation-induced DNA damage in two species of bivalves and their relative sensitivity using comet assay. Aquatic Toxicology, Amsterdam, **150**: 1-8.

PRAVEEN KUMAR, M. K., K. S. SOORAMBAIL, S. BHAGATSINGH HARISINGH, A. D'COSTA & C. RAMESH CHANDRA, 2015. The effect of gamma radiation on the common carp (*Cyprinus carpio*): in vivo genotoxicity assessment with the micronucleus and comet assays. Mutation Research / Genetic Toxicology and Environmental Mutagenesis, **792**: 19-25.

READ, A. T., R. R. HESSLER & C. K. GOVINDL, 1994. Muscle and nerve terminal fine structure of a primitive crustacean, the cephalocarid *Hutchinsoniella macracantha.* Biol. Bull., Woods Hole, **187**(1): 16-22.

RENÉ, A. A. & A. S. EVANS, 1970. Correlation of radiation-induced ultrastructural changes in mouse hepatocytes with alterations in plasma concentration of protein-bound neutral hexoses. Radiation Research, **44**(1): 224-236.

REVANKAR, P. R. & S. K. SHYAMA, 2009. Genotoxic effects of monocrotophos, an organophosphorous pesticide, on an estuarine bivalve, *Meretrix ovum*. Food and Chemical Toxicology, **47**(7): 1618-1623.

REVEL, J. P., 1962. The sarcoplasmic reticulum of the bat cricothyroid muscle. Journal of Cell Biology, **12**(3): 571-588.

RISOM, L., P. MØLLER, P. E. G. KRISTJANSEN, S. LOFT & U. VOGEL, 2003. X-ray-induced oxidative stress: DNA damage and gene expression of HO-1, ERCC1 and OGG1 in mouse lung. Free Radical Research, **37**(9): 957-966.

ROSENBLUTH, J., 1969. Sarcoplasmic reticulum of an unusually fast-acting crustacean muscle. Journal of Cell Biology, **42**(2): 534-547.

ROSENBLUTH, J., A. G. SZENT-GYÖRGYI & J. T. THOMPSON, 2010. The ultrastructure and contractile properties of a fast acting, obliquely striated, myosin-regulated muscle: the funnel retractor of squids. Journal of Experimental Biology, **213**(14): 2430-2443.

ROYUELA, M., V. B. MEYER-ROCHOW, B. FRAILE & R. PANIAGUA, 2000. Muscle cells in the tiny marine Antarctic mite *Halacarellus thomasis*: an ultrastructural and immunocytochemical study. Polar Biology, **23**(11): 759-765.

SALEHA BANU, B., K. DANADEVI, M. F. RAHMAN, Y. R. AHUJA & J. KAISER, 2001. Genotoxic effect of monocrotophos to sentinel species using comet assay. Food and Chemical Toxicology, **39**(4): 361-366.

SCHWARTZ, I., C. S. KIM & S. O. SHIN, 1994. Ultrastructural changes in the cochlea of the Guinea pig after fast neutron irradiation. Otolaryngology — Head and Neck Surgery, **110**(4): 419-427.

SELVERSTON, A., 1967. Structure and function of the transverse tubular system in crustacean muscle fibers. American Zoologist, **7**(3): 515-525.

SINGH, N. P., M. T. MCCOY, R. R. TICE & E. L. SCHNEIDER, 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Experimental Cell Research, **175**(1): 184-191.

SOMOSY, Z., 2000. Radiation response of cell organelles. Micron, **31**(2): 165-181.

SOWMITHRA, K., N. J. SHETTY, S. K. JHA & R. C. CHAUBEY, 2015. Evaluation of genotoxicity of the acute gamma radiation on earthworm *Eisenia fetida* using single cell gel electrophoresis technique (comet assay). Mutation Research / Genetic Toxicology and Environmental Mutagenesis, **794**: 52-56.

SUDHA, K. & G. ANILKUMAR, 2007. Elevated ecdysteroid titer and precocious molt and vitellogenesis induced by eyestalk ablation in the estuarine crab, *Metopograpsus messor* (Brachyura: Decapoda). Journal of Crustacean Biology, **27**(2): 304-308.

SUDHA, K., N. T. SUPRIYA, V. KRISHNAKUMAR, A. GOPINATHAN & E. S. CHANG, 2012. Hemolymph ecdysteroid titers in a brachyuran crab. Zoological Studies, **51**(7): 966-976.

SUPRIYA, N. T., K. SUDHA, V. KRISHNAKUMAR & G. ANILKUMAR, 2016. Molt — reproduction enhancement and hemolymph ecdysteroid elevation under eyestalk ablation in the female fiddler crab, *Uca triangularis* (Brachyura: Decapoda). Chinese Journal of Oceanology and Limnology, **35**(3): 645-657.

SUZUKI, S., 2003. Ultrastructural organization of the transverse tubules and the sarcoplasmic reticulum in a fish sound-producing muscle. Journal of Electron Microscopy, **52**(3): 337-347.

TAKEKURA, H., N. FUJINAMI, T. NISHIZAWA, H. OGASAWARA & N. KASUGA, 2001. Eccentric exercise-induced morphological changes in the membrane systems involved in excitation-contraction coupling in rat skeletal muscle. Journal of Physiology, **533**(2): 571-583.

TAKEKURA, H., H. SHUMAN & C. FRANZINI-ARMSTRONG, 1993. Differentiation of membrane systems during development of slow and fast skeletal muscle fibres in chicken. Journal of Muscle Research and Cell Motility, **14**(6): 633-645.

UENO, S., T. KASHIMOTO, N. SUSA, H. NATSUME, M. TOYA, N. ITO, S. TAKEDA-HOMMA, Y. NISHIMURA, Y. F. SASAKI & M. SUGIYAMA, 2007. Assessment of DNA damage in multiple organs of mice after whole body X-irradiation using the comet assay. Mutation Research, **634**(1-2): 135-145.

USHIO, H. & S. WATABE, 1993. Ultrastructural and biochemical analysis of the sarcoplasmic reticulum from crayfish fast and slow striated muscles. Journal of Experimental Zoology, **267**(1): 9-18.

WI, S. G., B. Y. CHUNG, J. H. KIM, M. H. BAEK, D. H. YANG, J. W. LEE & J. S. KIM, 2005. Ultrastructural changes of cell organelles in *Arabidopsis* stems after gamma irradation. Journal of Plant Biology, **48**(2): 195-200.

WILLIAMS, D. B. & C. B. CARTER, 2009. The transmission electron microscope. In: D. B. WILLIAMS & C. B. CARTER (eds.), Transmission electron microscopy — a textbook for materials science: 3-17. (Springer, Boston, MA).

YOOK, H. S., J. W. LEE, K. H. LEE, M. K. KIM, C. W. SONG & M. W. BYUN, 2001. Effect of gamma irradiation on the microstructure and post-mortem anaerobic metabolism of bovine muscle. Radiation Physics and Chemistry, **61**(2): 163-169.

YOON, K. S., 2003. Effect of gamma irradiation on the texture and microstructure of chicken breast meat. Meat Science, **63**(2): 273-277.

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