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Original Article

Midgut cells alteration in gamma-irradiated beetles (*Blaps polycresta*, Coleoptera: Tenebrionidae)

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(With 4 figures)

Abstract

This study was conducted to examine the effect of gamma radiation on biological specimens. Thus, our concept is to clarify that exposure to accumulated dose of 0.2 Gy gamma rays (0.66 rad/Sec. dose rate) from Cs137 source induces cellular perturbations in the midgut epithelium of the F1 progeny of *Blaps polycresta*, therefore affecting nutrition and growth. Beetles were reared in laboratory conditions and the newly emerged adults were irradiated with the aforementioned dose. Histological and ultrastructure anomalies of midgut cells (digestive and regenerative cells) were observed by 72 h after radiation exposure to ensure that the cells will not return to control state. Retardation in the development of the F1 progeny was also noticed and beetles died through two weeks. In the light of these observations, biological tissue act as an indicator to the continuous exposure to environmental radiation.

Keywords: gamma irradiation, beetles, midgut cells, histological and ultrastructure anomalies.

Alteração das células do intestino médio em besouros irradiados com radiação gama (*Blaps polycresta*, Coleoptera: Tenebrionidae)

Resumo

Este estudo foi conduzido para examinar o efeito da radiação gama em espécimes biológicos. Assim, nosso conceito é esclarecer que a exposição à dose acumulada de raios gama de 0,2 Gy (0,66 rad / seg. Dose) da fonte Cs137 induz perturbações celulares no epitélio do intestino médio da progênie F1 de *Polycresta blaps*, afetando a nutrição e crescimento. Besouros foram criados em condições de laboratório, e os adultos recém-emergidos foram irradiados com a dose acima mencionada. Anomalias histológicas e ultraestruturais das células do intestino médio (células digestivas e regenerativas) foram observadas 72 horas após a exposição à radiação, para garantir que as células não retornariam ao estado de controle. Retardo no desenvolvimento da progênie F1 também foi notado, e besouros morreram por duas semanas. À luz dessas observações, os tecidos biológicos atuam como um indicador para a exposição contínua à radiação ambiental.

Palavras-chave: irradiação gama, besouros, células do intestino médio, anomalias histológicas e ultraestruturais.

1. Introduction

Environmental afflicts of ionizing radiation arose recently as a dominated research field. Natural radiation exposures enhanced from many sources such as mineral processing and use, phosphate fertilizer (production and use) and fossil fuel combustion (UNSCEAR, 1988). Radiation that has been derived exogenously from the environment known to induce cellular alterations in higher eukaryotes (Daly, 2012). Ionizing radiation removes electrons from the orbital shells in the tissues they penetrate (Borek et al., 1993). Experimental evidence of insects exposed to different doses of gamma rays has confirmed cellular radiation damage (Kheirallah, 2016; Kheirallah et al., 2017). Low doses of radiation may elicit a broad range of biochemical and tissue responses as compared to high doses (Feinendegen et al., 2004). Experimental researches assessed the effects of low doses of ionizing radiation on biological tissues as a reference to environmental radiation protection (Feinendegen et al., 2004; Kheirallah et al., 2017).

Gamma rays act as a substance capable of inducing mutation, it passes over the tissues and damage cell's nucleus (Morales-Ramirez et al., 1997; World of Microbiology and Immunology, 2003). It is evident that gamma radiation affects normal growth, development, and reproduction of insects (Hallman, 2003; Helinski et al., 2009; Kheirallah, 2016; Kheirallah et al., 2017) and may cause either sterility or mortality (Prabhakumary et al., 2011; Sengupta, 2013; Mohamed et al., 2014). A paucity of investigations reported the effect of gamma radiation on the histological and

ultrastructure of insects (Paoli et al., 2014; Kheirallah 2016; Hassan et al., 2017; Kheirallah et al., 2017).

Our previous studies cleared the effects of high and low doses of gamma radiation on the sperm ultrastructure of *B. polycresta* and *B. Sulcata* (Kheirallah, 2016; Kheirallah et al., 2017). In the current study, we are investigating cellular alterations in the midgut of a newly hatched adult of *B. polycresta* induced by accumulated dose of gamma radiation. Since the midgut is the site of digestion and absorption any disruptions to its cells will inhibit these processes and converge development retardation and mortality which have been observed in this study.

2. Materials and Methods

2.1. Sampling procedure

Beetles were collected from a non-contaminated area, the garden of the Faculty of Science Moharram Bek, Alexandria University, Alexandria, Egypt (Osman et al., 2015) during their breeding season in August. They were transported to the laboratory and sustained alive in domestic soil and plants in glass containers and held under a day/night and temperature regime that is similar to their place of origin. Specimens were then sexed to obtain the inseminated females.

2.2. Rearing procedure

Rearing procedure followed Burakowski (1993). About five inseminated females were moved to a square aquarium measuring 10 x 20 x 10 cm and covered by a glass lid to prevent crowding. A slanting layer of wet soil, 1- 4 cm thick were placed on the bottom. As the soil dries out, moistening is applied to one-third of it. Females oviposit into the wet soil and then first instar larvae appear. Larvae were then placed into a larger container 10 x 30 x 10 cm with wet soil and ant workers as a diet. Here, the larvae were fed from time to time, they pupated and adults appeared. Room temperature ranged from 27-30°C to accelerate the development. The entire development from egg to adult is 4 weeks longer. Adult development approximately takes two weeks.

3. Radiation Treatments (Gamma Radiation)

The irradiation process was performed using Gamma Cs-137 in 10/9/2018, at the National Center for Radiation Research and Technology (NCRRT, Cairo). The dose rate of the Radiation Unit was 0.66 rad/s. Fifty newly emerged adults of *B. polycresta* were divided into two groups; namely group A, group B. Beetles of group A (25 insects) were used as a control group, didn't receive radiation treatment and housed in normal environmental conditions. Beetles of group B (25 insects) were irradiated with a dose rate of 0.2 Gy. The exposure period was 8hs/day, for two weeks. At the end of the experiment, ten beetles from each group were anesthetized and dissected to remove the midgut from the alimentary canal for histological investigations. The rest of the beetles in group B maintained alive in a glass container with soil and plants to observe their development and record mortality.

4. Dissection Procedures

Beetles were dissected under a dissecting microscope in a drop of Ringer's physiological solution on a wax-fixed Petri dish. Forceps were used to open the abdominal cavity, the alimentary canal was taken out and the midgut was cutoff.

5. Histological and Ultrastructure Preparations

The histological technique followed Anderson and Gordon (1996) methods of dehydration, clearing, and paraffin embedding. The clearing agent was the xylene. Fixation of the midgut was in paraffin wax (65-60 °C) and 5µm thick sections were stained with hematoxylin and eosin.

In ultrastructure assembly, midgut was fixed in $_4F_1G$ in phosphate buffer solution (pH 7.2) at 4 °C for 3 hours and post-fixed in 2% OsO_4 in the same buffer for two hours. Samples were washed in the buffer and dehydrated at 4 °C through a series of ethanol. Specimens were submerged in Epon-Araldite mixture in labeled beam capsules. LKB ultramicrotome was used (1 µm thick) for semithin sections. Sections were mounted on a glass slide, stained with toluidine blue and examined with the light microscope. Ultrathin sections (0.06-0.07 µm thick) were cut for TEM and picked upon 200 mesh naked copper grids. Grids were stained with uranyl acetate for half an hour and lead citrate for 20-30 min.

6. Results

6.1. Histological and ultrastructure observations of the control group's midgut (group A)

Histologically, the midgut of *B. polycresta* is surrounded by muscle layers, outer longitudinal and inner circular (Figure 1a and b). Two types of cells were distinguished in the midgut epithelium: columnar digestive cells with a brush border of microvilli, facing the gut lumen and regenerative cells which occur in "nidi" (Figure 1a-c).

In the electron micrographs, the midgut cells showed normal nuclei and cytoplasmic organelles. The regenerative cells evinced with an oval nucleus, patches of heterochromatin and a well-defined nuclear envelope (Figure 2a-c). In the cytoplasm, normally distributed mitochondria, rough and smooth endoplasmic reticulum and free ribosomes were observed (Figure 2 a-c). Septate junctions, the desmosomes were found between neighboring epithelial cells (Figure 2a-d). The columnar digestive cells possess numerous mitochondria, glycogen granules, lysosomes and free ribosomes (Figure 2a, d). Their brush border has a uniformly distributed microvilli (Figure 1a, d).

6.2. Histological and ultrastructure observations of the irradiated group's midgut (group B)

Histological disruption of the midgut epithelium encompassed sever vacuolations, dense vesicles, distorted microvilli (Figure 3a-d), abnormally looking regenerative cells (Figure 3b-d) and increased numbers of lysosomal vesicles (Figure 3b).

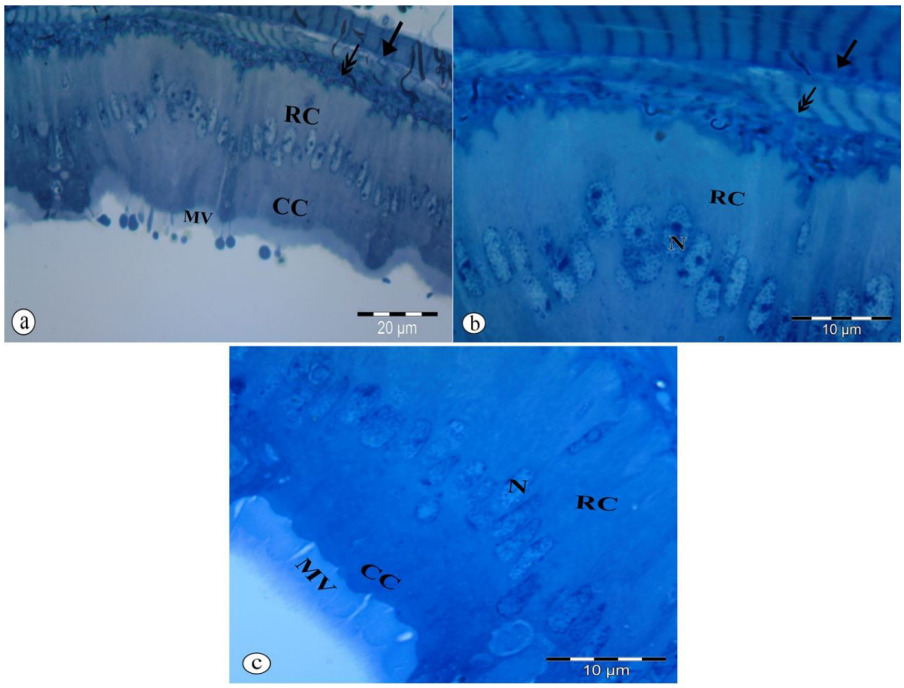

Figure 1. (a, b, c) Semithin sections of the midgut epithelium in the control group. Circular muscle fibers (arrow), longitudinal muscle fibers (double head arrow), regenerative cells (RC), columnar cells (CC), microvilli (MV), nucleus (N).

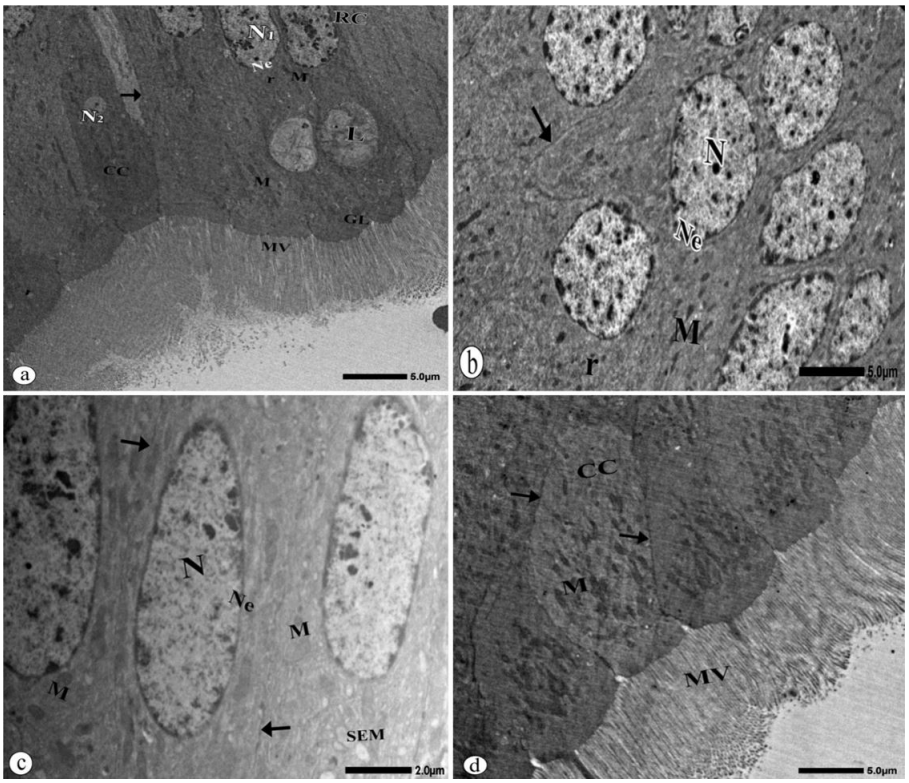

Figure 2. Electron micrographs of the midgut epithelium in the control group. (a) Regenerative cells (RC), nucleus of the regenerative cells (N_1), nuclear envelope (Ne), mitochondria (M), note: columnar cells (CC) with nucleus (N_2), numerous mitochondria (M), lysosomes (L), glycogen granules (GL), microvilli (MV). (b and c) showing normally looking regenerative cells (RC). (d) showing the apical membrane of the columnar cells (CC) with luminal border contains normally distributed microvilli (MV). Desmosomes (arrow), smooth endoplasmic reticulum (SER), free ribosomes (r).

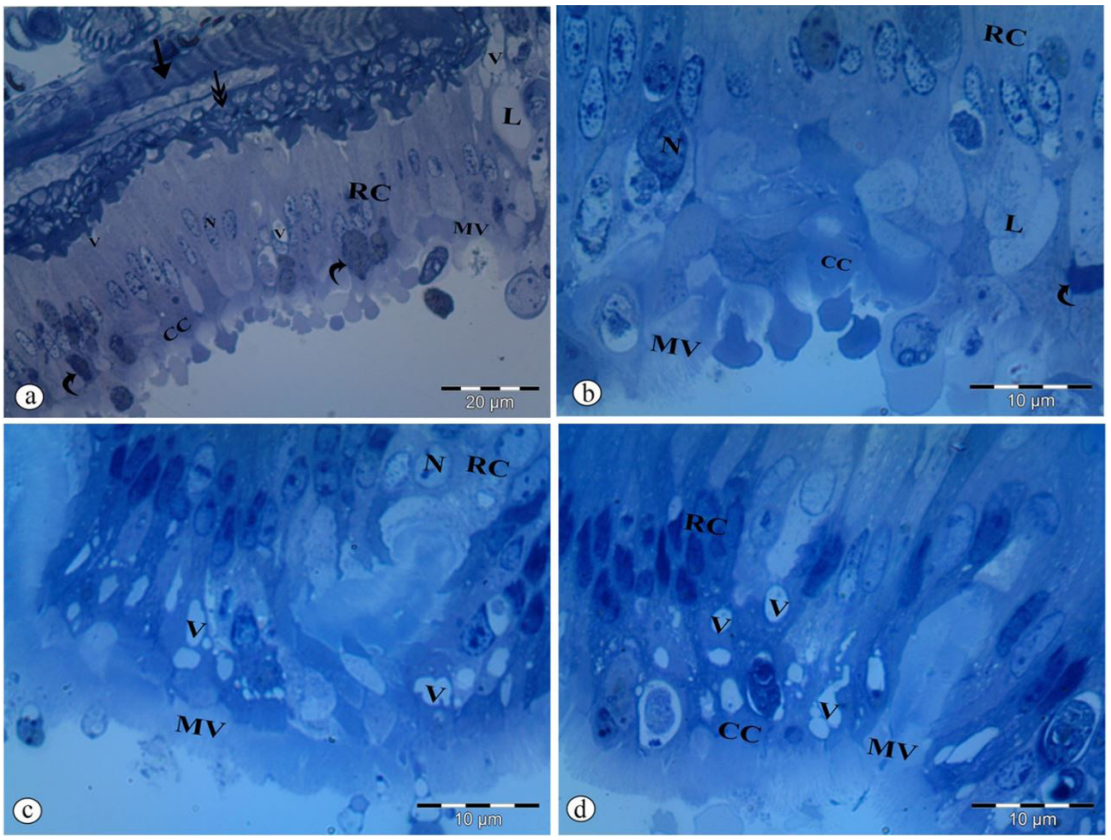

Figure 3. (a, b, c, d) Semithin sections of the midgut epithelium in gamma-irradiated group showing altered regenerative cells (RC) and columnar cells (CC), vacuoles (V), dense vesicles (curved arrow) and distorted microvilli (MV). Circular muscle fibers (arrow), longitudinal muscle fibers (double head arrow), lysosomes (L).

Electron micrographs revealed numerous anomalies in the regenerative and digestives cells. Nuclear divergent included: abnormal chromatin condensation, tubular and globular inclusions which are signs of necrosis, and intended nuclear envelope (Figures 4a-i). Apoptotic cells were also observed which resulted from nuclear and DNA fragmentations (Figures 4b, h). The cytoplasm exhibited severe vacuolation (Figures 4a, b, c, e, f, h, i) and many alterations in the cytoplasmic organelles were noted. These alterations included: swollen mitochondria (Figures 4b, c, d), dilated rough and smooth endoplasmic reticulum (Figure 4d), myelin figures (Figure 4g) electron-dense vesicles, and secretory vesicles (Figure 4c). Disruption of the brush border of the digestive cells was pronounced (Figure 4a).

6.3. Development and mortality observations in the irradiated group (group B)

Beetles in the irradiated group failed to develop further. No growing in their size was observed compared to the mature adult beetles. Non-developing insects survive for almost two weeks. 75% mortality was reported at the beginning of the third week. By the end of the third week, 100% mortality was achieved.

7. Discussion

Exposure of biological systems to different doses of ionizing radiation has been found to induce tissue and cell effects (Sugahara et al., 1992; UNSCEAR, 1994; Acad'emie des Sciences, 1995; Feinendegen, et al., 1999; Feinendegen and Neumann, 2000; Kheirallah, 2016; Kheirallah et al., 2017). These responses involve the disruption of DNA integrity and cellular damage including necrosis and apoptosis as a result of the formation of intracellular free radicals (Schmid and Schrader, 2007; Feinendegen et al., 2004; Simone et al., 2009; Faraj et al., 2011; Daly, 2012; Mohamed et al., 2016). The type and expanse of the damage vary with species, cell type, cell cycle, and metabolism (Alberts et al., 1994; Hall, 2000). This is consistent with the current results as 0.2 Gy of gamma radiation evokes midgut tissue and its cells damage. Feinendegen et al., (2004) stated that conflicting cellular response may be related to the hits of the average micro doses in an exposed micro masses as well as to different radiation qualities. It was found that 0–8 Gy of gamma irradiation induces metabolic changes in animal models, such as mice (Lanz et al., 2009; Tyburski et al., 2008; Tyburski et al., 2009), rats (Johnson et al., 2011), and nonhuman primates

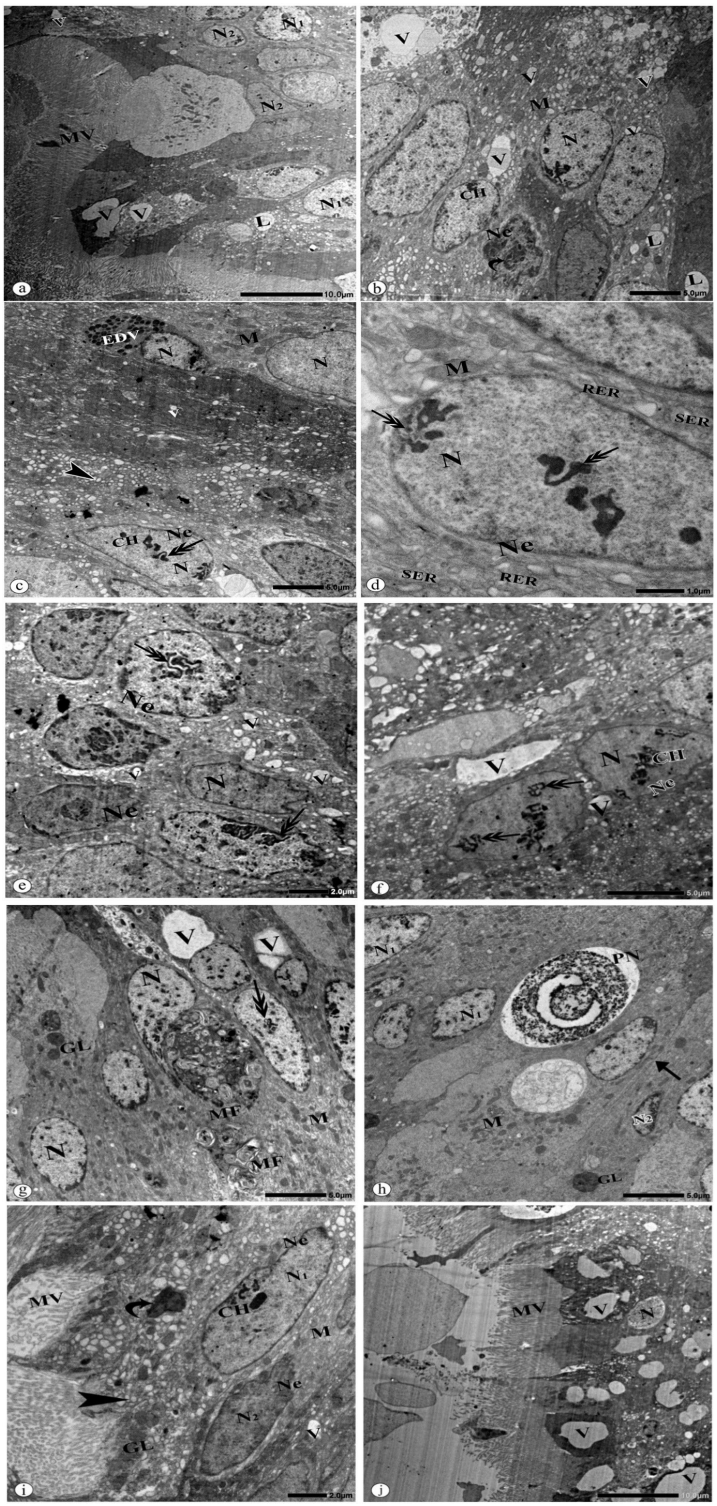

Figure 4. (a-j) Electron micrographs of the midgut epithelium in gamma-irradiated group showing abnormal nucleus of regenerative cells (N1) and columnar cells (N2) with irregular nuclear envelopes (Ne) and abnormal chromatin condensation (CH). Sever vacuolation (V) and distorted microvilli (MV) and increased lysosomal vesicles (L). Note: pyknotic nucleus (curved arrow in Figure b and arrow in Figure h), globular and tubular inclusion in the nucleoplasm (double head arrow) (Figures c, d, e, f, g). Note also: secretory vesicles (Figures c, i), swollen mitochondria (M) and dilated rough (RER) and smooth (SER) endoplasmic reticulum (Figure d), myelin figures (arrow) (Figure g), electron-dense vesicles (curved arrow) (Figure i).

(Johnson et al., 2012). Also, Faraj et al. (2011) found that low doses of Gamma Rays (0.25, 0.5, 1, 2, 4 Gy) caused DNA damage in Human Cells.

Histological and ultrastructure examination in the present study were observed after 72 h from radiation exposure to quit cell's adaptive protection against an array of exogenous attack (Kwon et al., 2014). Our major observed anomalies in the midgut epithelium of *B. polycresta* were in the nucleus of both the regenerative and the digestive cells. These anomalies include abnormal chromatin clumping, globular and tubular inclusions in the nucleoplasm, and indentation of the nuclear envelopes. Grewal and Jia (2007) suggested that chromatin clumping reduce transcription. Our results agreed with Pazir et al. (2011) who observed nuclear inclusions in the nuclei of shrimp infected with two viral diseases. They figured out that these inclusion bodies were the first sign in cellular degeneration and resulted in pyknosis. Engedal et al. (1977) stated that their formation arose by the unification of membranes in the nucleoplasm. Furthermore, the course of cell death was assigned by the indentation of the nuclear envelope (Trump et al., 1997; Pazir et al., 2011).

Another alteration which has been observed in the current study was the severe vacuolation in the cytoplasm due to the action of acid hydrolases that has been released from the lysosomes (Vandenbulcke et al., 1998; Polidori et al., 2018) which is accompanied by changes in the PH of the cytosol. Moreover, alterations in cytoplasmic organelles such as swallow of the mitochondria and dilation of the rough and smooth endoplasmic reticulum may be a result of the radiation which disrupts cytoplasmic membranes as reported earlier by Kheirallah (2016) and Kheirallah et al. (2017). Radiation induces mitochondrial malformations which lead to disruption in energy production (Coggins, 1973; Mahomud and Shoman, 2009; Kheirallah, 2016; Ibrahim et al., 2017; Kheirallah et al., 2017). The presence of myelin figures and electron dense vesicles in our electron micrographs may be attributed to the accumulation of lipoprotein macromolecules. Ibrahim et al. (2017) observed myelin bodies in the testes of the F1 progeny of irradiated parental males of cowpea weevil, *Callosobruchus maculatus* and reported that these myelin bodies are fatty insulating substance. It was obvious from our results that gamma irradiation promotes cell killing (necrosis and /or apoptosis) which is in agreement with Shinomiya et al. (2000) who reported that low-dose irradiation, 5 Gy X-ray, induces post-mitotic apoptosis in U937 cells. Additionally, the distortion of the brush border microvilli which was seen in our preparations may be responsible for the cell damages. The cessation of the cytoskeleton of the microvilli leads to the protrusion of the cytoplasmic substances into the midgut lumen (Seidman et al., 1986). These findings are in conformance with Stiles et al. (1989) worked on tsetse fly (*Glossina spp.)* populations subjected to 130 Gy gamma radiation. They reported that the first sign of damage was the deterioration of the microvillous border that resulted in cell degeneration. These compulsive changes in the cells may upset the normal physiology of insects (Rawi et al., 2011).

Our histological and ultrastructure investigations have confirmed that gamma irradiation has an inhibitory effect at cellular at subcellular levels and leads to obstruction in the development of the F1 progeny and finally to their death. Insect development is powered by hormones (Lee, 2012) which may be arrested due to the action of ionizing radiation (Mansour, 1987). Radiation disturbs the function of tissues and organs and produces anomalies (Wong et al., 2003; Moskalev, 2007). These anomalies may interrupt the developmental pathways (Møller, 2002; Natarajan, 2006). It also has an opposed effect on the development and health of the offspring (Beasley et al., 2012). Prabhakumary et al. (2011) reported that higher doses of gamma irradiation caused mortality to *Tribolium castaneum* while lower doses caused inhibition of development and sterility of the surviving insects. Aye et al. (2008) observed the inhibitory effects of increasing doses from 0.1 to 1.0 KGy gamma irradiation on the development and reproduction of *Plodia interpunctella*. Our previous recommendation (Kheirallah et al. 2017) pointed for further investigations on the effect of gamma radiation on the F1progeny. It is worth to mention that this is the first study that reported the inhibitory effect of accumulated low dose of gamma irradiation on the midgut structure, development and survival of the F1 progeny of *B. polycresta*.

8. Conclusion

The study validates the inhibitory effect of accumulated low dose of gamma irradiation on the F1 progeny of *B. polycresta*. It is also a good model to detect the ramifications of gamma radiation in biological specimens and can be utilized to speculate the consequences that may result from the exposure to environmental radiation.

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References

ACAD'EMIE DES SCIENCES. Institut De France, 1995. *Problems associated with the effects of low doses of ionizing radiations.* Lavoisier TecDoc: Paris. Rapport de l'Acad'emie des Sciences, no. 38.

ALBERTS, B., BRAY, D., LEWIS, J., RAFF, M., ROBERTS, K. and WATSON, J.D., 1994. *Molecular biology of the cell*. 3rd ed. New York: Garland Pub.

ANDERSON, G. and GORDON, K.C., 1996. Tissue processing, microtomy and paraffin sections. In: J.D. BANCROFT, and A. STEVENS, eds. *Theory and practice of histological techniques.* New York: Churchill Livingstone, pp. 47-80.

AYE, T.T., SHIM, J.K., HA, D.M., KWON, Y.J., KWON, J.H. and LEE, K.Y., 2008. Effects of gamma irradiation on the development and reproduction of *Plodia interpunctella* (Hübner) (Lepidoptera: pyralidae). *Journal of Stored Products Research*, vol. 44, no. 1, pp. 77-81. http://dx.doi.org/10.1016/j.jspr.2007.06.002.

BEASLEY, D.E., BONISOLI-ALQUATI, A., WELCH, S.M., MØLLER, A.P. and MOUSSEAU, T.A., 2012. Effects of parental radiation exposure on developmental instability in grasshoppers. *Journal of Evolutionary Biology*, vol. 25, no. 6, pp. 1149-1162. http://dx.doi.org/10.1111/j.1420-9101.2012.02502.x. PMid: 22507690.

BOREK, C., ABRAHAM, S.K. and SARMA, L., 1993. Molecular mechanisms in cancer induction and prevention protective effects of chlorogenic acid, curcumin and beta-carotene against gamma-radiation-induced in vivo chromosomal damage. *Environmental Health Perspectives*, vol. 101, suppl. 3, pp. 237-245. PMid: 8143624.

BURAKOWSKI, B., 1993. *Laboratory methods for rearing soil beetles (Coleoptera)*. Warszawa: Muzeum i Instytut Zoologii, Polska Akademia Nauk, 66 p.

COGGINS, P.B., 1973. The effect of X-radiation on spermatogenesis and the fertility of *Schistocerca gregaria* (Forsk). *Journal of Embryology and Experimental Morphology*, vol. 30, no. 1, pp. 163-177. PMid: 4354153.

DALY, M.J., 2012. Death by protein damage in irradiated cells. *DNA Repair*, vol. 11, no. 1, pp. 12-21. http://dx.doi.org/10.1016/j.dnarep.2011.10.024. PMid: 22112864.

ENGEDAL, H., JENSEN, H. and SAETERSDAL, T.S., 1977. Ultrastructure of abnormal membrane inclusions in nuclei of human myocardial cells. *British Heart Journal*, vol. 39, no. 2, pp. 145-151. http://dx.doi.org/10.1136/hrt.39.2.145. PMid: 189791.

FARAJ, K.A., ELIAS, M.M. and AL-MASHHADANI, A.H., 2011. Effect of X- and Gamma Rays on DNA in Human Cells. *European Journal of Scientific Research*, vol. 53, pp. 470-476.

FEINENDEGEN, L.E. and NEUMANN, R.D. 2000. Cellular responses to low doses of ionizing radiation. In: *Workshop of the US Department of Energy and the National Institutes of Health*, 27-30 April 1999, Washington. Washington, DC: Bethesda, MD, DOE. Report Publication, SC-047.

FEINENDEGEN, L.E., BOND, V.P., SONDHAUS, C.A. and ALTMAN, K.I., 1999. Cellular signal adaptation with damage control at low doses versus the predominance of DNA damage at high doses. *Comptes Rendus de l'Académie des Sciences. Série III, Sciences de la Vie*, vol. 322, no. 2-3, pp. 245-251. http://dx.doi.org/10.1016/S0764-4469(99)80051-1. PMid: 10196680.

FEINENDEGEN, L.E., POLLYCOVE, M. and SONDHAUS, C.A., 2004. Responses to low dose of ionizing radiation in biological systems. *Nonlinearity in Biology Toxicology and Medicine*, vol. 2, no. 3, pp. 143-171. http://dx.doi.org/10.1080/15401420490507431. PMid: 19330141.

GREWAL, S.I. and JIA, S., 2007. Heterochromatin revisited. *Nature Reviews. Genetics*, vol. 8, no. 1, pp. 35-46. http://dx.doi.org/10.1038/nrg2008. PMid: 17173056.

HALL, E.J., 2000. *Radiobiology for the Radiologist*. 5th ed. New York: Lippincott Williams & Wilkins.

HALLMAN, G.J., 2003. Ionizing irradiation quarantine treatment against plum curculio (Coleoptera: curculionidae). *Journal of Economic Entomology*, vol. 96, no. 5, pp. 1399-1404. http://dx.doi.org/10.1093/jee/96.5.1399. PMid: 14650511.

HASSAN, M., AMER, M.S., HAMMAD, K.M., GABARTY, S.T.A. and SELIM, T.A., 2017. Latent effect of gamma irradiation on reproductive potential and ultrastructure of males' testes of *Culex pipiens* (Diptera; Culicidae). *Journal of Radiation Research and Applied Sciences*, vol. 10, no. 1, pp. 44-52. http://dx.doi.org/10.1016/j.jrras.2016.11.003.

HELINSKI, M.E., PARKER, A.G. and KNOLS, B.G., 2009. Radiation biology of mosquitoes. *Malaria Journal*, vol. 8, suppl. 2, pp. 1-28. http://dx.doi.org/10.1186/1475-2875-8-S2-S6. PMid: 19917076.

IBRAHIM, H.A., FAWKI, S., ABD EL-BAR, M.M., ABDOU, M.A., MAHMOUD, D.M. and EL-GOHARY, E.E., 2017. Inherited influence of low dose gamma radiation on the reproductive potential and spermiogenesis of the cowpea weevil, *Callosobruchus maculatus* (F) (Coleoptera: chrysomelidae). *Journal of Radiation Research and Applied Sciences*, vol. 10, no. 4, pp. 338-347. http://dx.doi.org/10.1016/j.jrras.2017.09.003.

JOHNSON, C.H., PATTERSON, A.D., KRAUSZ, K.W., KALINICH, J.F., TYBURSKI, J.B., KANG, D.W., LUECKE, H., GONZALEZ, F.J., BLAKELY, W.F. and IDLE, J.R., 2012. Radiation metabolomics. 5. Identification of urinary biomarkers of ionizing radiation exposure in nonhuman primates by mass spectrometry-based metabolomics. *Radiation Research*, vol. 178, no. 4, pp. 328-340. http://dx.doi.org/10.1667/RR2950.1. PMid: 22954391.

JOHNSON, C.H., PATTERSON, A.D., KRAUSZ, K.W., LANZ, C., KANG, D.W., LUECKE, H., GONZALEZ, F.J. and IDLE, J.R., 2011. Radiation metabolomics. 4. UPLC-ESI-QTOFMS-based metabolomics for urinary biomarker discovery in gamma-irradiated rats. *Radiation Research*, vol. 175, no. 4, pp. 473-484. http://dx.doi.org/10.1667/RR2437.1. PMid: 21309707.

KHEIRALLAH, D.A., 2016. Gamma irradiation-induced spermatozoa anomalies in the Ground Beetle, *Blaps Polycresta. Journal of Cell and Tissue Research*, vol. 16, pp. 5741-5755.

KHEIRALLAH, D.A., EL-SAMAD, L.M., FAHMI, N. and OSMAN, W., 2017. Ultrastructure alterations induced by gamma irradiation in spermiogenesis of the ground beetle, *Blaps sulcata*: reference to environmental radiation protection. *Environmental Science and Pollution Research International*, vol. 24, no. 27, pp. 22102-22110. http://dx.doi.org/10.1007/s11356-017-9869-5. PMid: 28791602.

KWON, Y., HA, I.J., BAE, H.W., JANG, W.G., YUN, H.J., KIM, S.R., LEE, E.K., KANG, C.M. and HWANG, G.S., 2014. Dose-dependent metabolic alterations in human cells exposed to gamma irradiation. *PLoS One*, vol. 9, no. 11, pp. e113573. http://dx.doi.org/10.1371/journal.pone.0113573. PMid:25419661.

LANZ, C., PATTERSON, A.D., SLAVIK, J., KRAUSZ, K.W., LEDERMANN, M., GONZALEZ, F.J. and IDLE, J.R., 2009. Radiation metabolomics. 3. Biomarker discovery in the urine of gamma-irradiated rats using a simplified metabolomics protocol of gas chromatography-mass spectrometry combined with random forests machine learning algorithm. *Radiation Research*, vol. 172, no. 2, pp. 198-212. http://dx.doi.org/10.1667/RR1796.1. PMid: 19630524.

LEE, R., 2012. *Insects at low temperature*. Germany: Springer Science & Business Media, Juvenile Nonfiction, 514 p.

MAHOMUD, E.A. and SHOMAN, A.A., 2009. Studies on the radio-protective role of melatonin on the testes fine structure of medflies *Ceratitis capitata* (wied.) sterile males. *Isotope and Radiation Research*, vol. 41, pp. 1421-1441.

MANSOUR, M.Y., 1987. *The effects of ionizing radiation on the face fly, Musca autumnalis DeGeer, irradiated in nitrogen*. Iowa: Iowa State University, 80 p. PHD Thesis.

MOHAMED, H.F., EL-NAGGAR, S.E., ELBARKY, N.M., IBRAHIM, A.A. and SALAMA, M.S., 2014. The impact of each of the essential oils of marjoram and lemon grass in conjunction

with gamma irradiation against the greater wax moth, Galleria mellonella. *IOSR journal of pharmacy and biological sciences*, vol. 9, pp. 92-106.

MOHAMED, M.A., EL SAEID, A.A. and AHMED, M.A., 2016. Cellular response of blood and hepatic tissue to gamma irradiation. *Journal of Radiation Research and Applied Sciences*, vol. 9, no. 3, pp. 242-248. http://dx.doi.org/10.1016/j.jrras.2015.12.001.

MØLLER, A.P., 2002. Developmental instability and sexual selection in stag beetles from Chernobyl and a control area. *Ethology*, vol. 108, no. 3, pp. 193-204. http://dx.doi.org/10.1046/j.1439-0310.2002.00758.x.

MORALES-RAMÍREZ, P., VALLARINO-KELLY, T., ANGUIANO-OROZCO, G. and RODRIGUEZ-REYES, R., 1997. Pharmacokinetic parameters of genotoxic activity inferred from the comparison of the kinetics of MN-PCE induced by chemical agents and ionizing radiation. *Mutation Research*, vol. 391, no. 3, pp. 127-134. http://dx.doi.org/10.1016/S0165-1218(97)00034-7. PMid: 9268037.

MOSKALEV, A., 2007. Radiation-induced life span alteration of *Drosophila* lines with genotype differences. *Biogerontology*, vol. 8, no. 5, pp. 499-504. http://dx.doi.org/10.1007/s10522-007-9090-x. PMid: 17380421.

NATARAJAN, A.T., 2006. Induced transgenerational genetic effects in rodents and humans. *Journal of Radiation Research*, vol. 47, suppl. B, pp. 39-43. http://dx.doi.org/10.1269/jrr.47.B39. PMid: 17019051.

OSMAN, W., EL-SAMAD, L.M., MOKHAMER, E.H., EL-TOUHAMY, A. and SHONOUDA, M., 2015. Ecological, morphological, and histological studies on *Blaps polycresta* (Coleoptera: Tenebrionidae) as biomonitors of cadmium soil pollution. *Environmental Science and Pollution Research International*, vol. 22, no. 18, pp. 14104-14115. http://dx.doi.org/10.1007/s11356-015-4606-4. PMid: 25963070.

PAOLI, F., DALLAI, R., CRISTOFARO, M., ARNONE, S., FRANCARDI, V. and ROVERSI, P.F., 2014. Morphology of the male reproductive system, sperm ultrastructure and γ-irradiation of the red palm weevil *Rhynchophorus ferrugineus* Oliv. (Coleoptera: dryophthoridae). *Tissue & Cell*, vol. 46, no. 4, pp. 274-285. http://dx.doi.org/10.1016/j.tice.2014.06.003. PMid: 25015762.

PAZIR, M.K., AFSHARNASAB, M., JALALI JAFARI, B., SHARIFPOUR, I., MOTALEBI, A.A. and DASHTIANNASAB, A., 2011. Detection and identification of white spot syndrome virus (WSSV) and infectious hypodermal and hematopoietic necrosis virus (IHHNV) of *Litopenaus vannamei* from Bushehr and Sistan and Baloochestan provinces, Iran, during 2009-2010. *Iranian Journal of Fisheries Science*, vol. 10, pp. 708-726.

POLIDORI, C., PASTOR, A., JORGE, A. and PERTUSA, J., 2018. Ultrastructural alterations of midgut epithelium, but not greater wing fluctuating asymmetry, in paper wasps (*Polistes dominula*) from urban environments. *Microscopy and Microanalysis*, vol. 24, no. 2, pp. 183-192. http://dx.doi.org/10.1017/S1431927618000107. PMid: 29560839.

PRABHAKUMARY, C., POTTY, V.P. and SIVADASAN, R., 2011. Effectiveness of gamma radiation for the control of *Tribolium castaneum*, the pest of stored cashew kernels. *Current Science*, vol. 101, pp. 1531-1532.

RAWI, S.M., BAKRY, F.A. and AL-HAZMI, M.A., 2011. Biochemical and histopathological effect of crude extracts on *Spodoptera littoralis* larvae. *Journal of Evolutionary Biology Research*, vol. 3, pp. 67-78.

SCHMID, E. and SCHRADER, T., 2007. Different biological effectiveness of ionizing and non-ionizing radiations in mammalian cells. *Advances in Radio Science*, vol. 5, pp. 1-4. http://dx.doi.org/10.5194/ars-5-1-2007.

SEIDMAN, L.A., BERGTROM, G., GINGRICH, D.J. and REMSEN, C.C., 1986. Accumulation of cadmium by the fourth instar larva of the fly *Chironomus thummi. Tissue & Cell*, vol. 18, no. 3, pp. 395-405. http://dx.doi.org/10.1016/0040-8166(86)90059-5. PMid: 18620165.

SENGUPTA, P., 2013. Chemosterilization: spermatogenesis, steroidogenesis, reproductive functions, and behavior; from historical perspective to contemporary practice. *Journal of Basic and Clinical Reproductive Sciences*, vol. 2, no. 1, pp. 1-2. http://dx.doi.org/10.4103/2278-960X.112568.

SHINOMIYA, N., KUNO, Y., YAMAMOTO, F., FUKASAWA, M., OKUMURA, A., UEFUJI, M. and ROKUTANDA, M., 2000. Different mechanisms between premitotic apoptosis and postmitotic apoptosis in X-irradiated U937 cells. *International Journal of Radiation Oncology, Biology, Physics*, vol. 47, no. 3, pp. 767-777. http://dx.doi.org/10.1016/S0360-3016(99)00517-9. PMid: 10837963.

SIMONE, N.L., SOULE, B.P., LY, D., SALEH, A.D., SAVAGE, J.E., DEGRAFF, W., COOK, J., HARRIS, C.C., GIUS, D. and MITCHELL, J.B., 2009. Ionizing radiation-induced oxidative stress alters miRNA expression. *PLoS One*, vol. 4, no. 7, pp. e6377. http://dx.doi.org/10.1371/journal.pone.0006377. PMid: 19633716.

STILES, J.K., MOLYNEUX, D., WALLBANKS, K.R. and VAN DER VLOEDT, A.M., 1989. Effects of gamma irradiation on the midgut ultrastructure of *Glossina palpalis* subspecies. *Radiation Research*, vol. 118, no. 2, pp. 353-363. http://dx.doi.org/10.2307/3577449. PMid: 2727263.

SUGAHARA, T., SAGAN, L.A. and AOYAMA, T., 1992. *Low dose irradiation and biological defense mechanisms*. Netherlands: Excerpta Medica. pp. 255-258.

TRUMP, B.F., BEREZESKY, I.K., CHANG, S.H. and PHELPS, P.C., 1997. The pathways of cell death: oncosis, apoptosis, and necrosis. *Toxicologic Pathology*, vol. 25, no. 1, pp. 82-88. http://dx.doi.org/10.1177/019262339702500116. PMid: 9061857.

TYBURSKI, J.B., PATTERSON, A.D., KRAUSZ, K.W., SLAVIK, J., FORNACE JUNIOR, A.J., GONZALEZ, F.J. and IDLE, J.R., 2008. Radiation metabolomics. 1. Identification of minimally invasive urine biomarkers for gamma-radiation exposure in mice. *Radiation Research*, vol. 170, no. 1, pp. 1-14. http://dx.doi.org/10.1667/RR1265.1. PMid: 18582157.

TYBURSKI, J.B., PATTERSON, A.D., KRAUSZ, K.W., SLAVIK, J., FORNACE JUNIOR, A.J., GONZALEZ, F.J. and IDLE, J.R., 2009. Radiation metabolomics. 2. Dose-and time-dependent urinary excretion of deaminated purines and pyrimidines after sublethal gamma-radiation exposure in mice. *Radiation Research*, vol. 172, no. 1, pp. 42-57. http://dx.doi.org/10.1667/RR1703.1. PMid: 19580506.

UNITED NATIONS SCIENTIFIC COMMITTEE ON THE EFFECT OF ATOMIC RADIATION – UNSCEAR, 1988. *Sources, effects and risks of ionizing radiation, Report to General Assembly*. New York: United Nations.

UNITED NATIONS SCIENTIFIC COMMITTEE ON THE EFFECT OF ATOMIC RADIATION – UNSCEAR, 1994. *Adaptive responses to radiation in cells and organisms.* New York: United Nations.

VANDENBULCKE, F., GRELLE, C., FABRE, M.C. and DESCAMPS, M., 1998. Ultrastructural and auto metallographic studies of the nephrocytes of *Lithobius forficatus* (Myriapoda: Chilopoda): Role in detoxification of cadmium and lead. *International Journal of Insect Morphology & Embryology*, vol. 27, no. 2, pp. 111-120. http://dx.doi.org/10.1016/S0020-7322(98)00034-8.

WONG, K.K., MASER, R.S., BACHOO, R.M., MENON, J., CARRASCO, D.R., GU, Y.S., ALT, F.W. and DEPINHO, R.A., 2003. Telomere dysfunction and Atm deficiency compromises organ homeostasis and accelerates ageing. *Nature*, vol. 421, no. 6923, pp. 643-648. http://dx.doi.org/10.1038/nature01385. PMid: 12540856.

WORLD OF MICROBIOLOGY AND IMMUNOLOGY, 2003 [viewed 30 September 2018]. *Radiation mutagenesis*. Available from: https://www.encyclopedia.com/science/encyclopedias-almanacs-transcripts-and-maps/radiation-mutagenesis.