



Biochemical Changes Induced by Gamma Irradiation in the Ground Beetle *Blaps polycresta*

Dalia A. Kheirallah^{*1}, Lamia M. El-Samad¹

¹Department of Zoology, Faculty of Science, Alexandria University, Alexandria 21511, Egypt.

*Corresponding Author: Daliakheirallah@yahoo.com

Tel.: 002-01221775286

Fax: 002-034264455

Gamma radiation induced Biochemical Changes in the Ground Beetle

ABSTRACT

The exposure to low-dose gamma radiation from natural sources is a continuing and inescapable feature and it is common in certain occupations. The present study was designed to investigate the effects of low-dose gamma radiation on some detoxification enzymes such as; AST, ALT, ACP, ALP, GSH, GPX, SOD and CAT, as well as, its effect on total protein and DNA content in the soft tissues homogenate of male's ground beetle *Blaps polycresta*. Insects were divided into three groups (group A, B and C) of 10 beetles each. Two groups (group B and C) were irradiated with doses at a rate of 32 Gy and 64 Gy; group A was considered as the control group. The study revealed a dose-dependent effect on the tested parameters. Significant increase in the enzyme activity of AST, ALT, ACP and ALP were observed in group B and C respectively compared to the control group (group A). The findings also showed a significant inhibitions in the enzymatic activity of GSH, GST, GPX, SOD and CAT in the irradiated groups compared to the control group. Furthermore, their radiated groups reported a decrease in protein and DNA content compared to the control group. Our observations suggested that the genotoxic effects of gamma radiation induced damaging effects on enzyme molecules, protein and nucleic acid (DNA) and may explain the increase in health risk from high doses of ionizing radiation.

Keywords: Gamma radiation, Beetles, Enzymes, protein, DNA

1. INTRODUCTION

Ionizing radiation breaks down molecules, causing various effects in irradiated material [1]. For most individuals, this exposure is exceeded from all man-made sources combined. Both external and internal exposures to gamma radiation may result from naturally occurring radio nuclides released by industrial activities and many other sources such as oil and gas extraction, phosphate processing, uranium mining, emissions and building materials. Indoor exposure to gamma rays, mainly determined by the materials of construction, the highest values result from the wide use of stone or masonry materials in buildings rather than wood[2].

Insects serve as one of the promising indicators for environmental stress [3] and it is known to have a high resistance due to the high activity of detoxifying enzymes [4]. The increase or decrease in the activity of certain enzymes can be explained that the organism is under stress [5]. Enzymatic biomarkers extensively used in the biochemical studies of environmental impacts [6]. Such enzymes are: SOD that catalyzes the formation of hydrogen peroxide (H₂O₂) [7], GSH is one of the main antioxidant defense mechanisms in the living systems [8], GPx a defense system against the increase of free radicals and acts in the removal of H₂O₂ and lipid hydroperoxides, from the cell [9], GST which is a detoxification enzyme because it metabolizes a great variety of xenobiotic compounds, catalyzing their conjugation with GSH and forming substances of low toxicity [10], ACP is usually soluble in the cytosol of midgut cells in many insects and may also appear in mid gut contents or be found membrane bound in mid gut cells and ALP is a hydrolyzing enzyme responsible for removing phosphate groups from many types of molecules including nucleotides, proteins and alkaloids [11]. In addition to AST and ALT are key enzymes in the formation of non-essential amino acids, in metabolism of nitrogen waste and gluconeogenesis [12].

Irradiation induces a disturbance in biochemical metabolism resulting from inhibition of energy transfer due to the inhibition of enzymes. Gamma radiation may interrupt energy supplies and block all key enzymes which may stop normal metabolism [13]. The change in enzyme properties may lead to the conclusion that radiation adversely affects the energy production and its utilization which in turn induces slower rate of metabolism[14]. Proteins provide a chief structural element of the muscles, glands and other tissues. The balance between protein amino acids and free forms is particularly important [14]. Also irradiation induced damage to DNA and the damage is a dose-dependent effect [15].

Proteins, nucleic acids, and enzyme molecules are the essential nutritive components in the biological system in insects. Therefore, any disturbance in this component leads to the disturbance in the biological system and adult performance. The radiation effects on the molecular chemical of the living organism body will provide essential information of the radiation-induced molecular alteration that will initiate damage to the biological chain [16]. Thus this paper aims to determine the effect of gamma radiation on some essential enzymes like AST, ALT, ACP, ALP, GSH, GST, GPX, SOD and CAT, as well as, its effect on total protein and DNA content in the soft tissues homogenate of male's ground beetle *Blaps polycresta*.



2. MATERIALS AND METHODS

2.1 Specimens

Live specimens were collected from the garden of Faculty of Science Moharram Bey, Alexandria University, Alexandria, Egypt which considered as a non-polluted site [17] in September 2015. After transport to laboratory specimens were sexed, males were chosen then maintained alive in native soil and plants in glass containers until processing and held under a day/night and temperature regime that approximated their place of origin.

2.2 Radiation treatments (Gamma Radiation)

The irradiation process was performed using Gamma Cs-137, at National Center for Radiation Research and Technology (NCRRT, Cairo). In this investigation, the dose rate of the Radiation Unit was 0.708 rad/s. males of *B. polycrsta* were irradiated with two doses 32 and 64 Gy.

Thirty adult male insects were divided into three groups; namely group A, group B, and group C. Animals of group A (10 insects) were used as control group, didn't receive any treatment with radiation and housed at normal environmental conditions (the temperature inside the lab varied between 20° to 25° C, lighting condition were natural light from large windows during the day and complete darkness during the night). Insects of group B (10 insects) were irradiated with a dose rate 32 Gy and insects of group C (10 insects) were irradiated with a dose rate 64 Gy. At the end of the experiment insects were anesthetized, and dissected. Soft tissues were removed carefully for experimental investigation.

2.3 Dissection procedures

Beetles were dissected under dissecting microscope in a drop of Ringer's physiological solution on a wax-fixed Petri dish. A pair of dissecting forceps was used to open the abdominal cavity and then the soft tissues were taken out.

2.4 Biochemical studies

Enzyme activities, protein content and nucleic acid content (DNA) were determined in the soft tissues of males *Blaps polycrsta*. All experiments were carried out in triplicates.

2.5 Preparation of tissue homogenate

The specimens were dissected to isolate soft tissues. 100 mg of each tissue was homogenized in 1ml of cold potassium phosphate buffer solution (50 mM, pH 7.5 containing 1mM of ethylene diamine tetra acetic acid (EDTA). Homogenized tissues were divided into two parts. One was used to assay total protein and DNA content. The other was centrifuged at 6000 r. p. m for 15 minutes at 4°C. The resulting supernatant was separated to assay the content of GSH and the activities of AST, ALT, ACP, ALP, GPX, SOD and CAT.

2.6 Determination of Aspartate amino-transferase (AST or GOT) and Alanine aminotransferase (ALT or GPT)

This assay were carried out according to Reitman and Frankel method [18], using a commercially available kit from Diamond Colorimetric determination of AST& ALT. The oxaloacetate or pyruvate formed was measured in its derivatives from 2, 4 dinitrophenyl hydrazone at 546 nm. 0.1 ml phosphate buffer/substrate, pH 7.2 was added to 0.02 ml of the sample and incubated for 30 minutes in AST or ALT at 37°C. A 0.1 ml of colour reagent, 2, 4-dinitrophenyl hydrazine was then added. The incubation was left for 20 minutes at 20°C. Finally, 1 ml, 0.4 N sodium hydroxide was then added, mixed well and kept for 5 minutes at room temperature. The absorbance was measured at 546 using Shimadzu 160 IPC spectrophotometer. The concentration was measured in µg/100 mg tissue.

2.7 Determination of Acid phosphatase (ACP)

Acid phosphatase assay was determined according to the modified method of Hillmann [19]. Dissolve one tablet in one vial of buffer Reagent R.1. The stability of working reagent is 2 days at 2-8 °C or 6 hours at room temperature. Mix and incubate the working reagent tartrate Solution and the Sample (homogenate) for 5 minutes at 30/37 °C. Measure of the extinction will be increased per minute (for 1-3 minutes). This method is linear up to 150 U/L.

2.8 Determination of Alkaline phosphatase (ALP)

Colorimetric determination of alkaline phosphatase activity was done according to Kind and King [20]. Phenyl phosphate is hydrolysed in alkaline pH by the action of alkaline phosphatase into phenol and phosphate. The phenol liberated is measured in the presence of 4-amino antipyrine and potassium ferricyanide. The presence of sodium arsenate in the reagent stops the enzymatic reaction.

2.9 Determination of reduced glutathione (GSH) content

The method based on the reduction of 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) with GSH to produce a yellow compound. The reduced chromogen directly proportional to GSH content and its absorbance can be measured at 412nm [21].



2.10 Determination of glutathione – S –transferase (GST) activity

GST assay measures total GST activity (cytosolic and microsomal) by measuring the conjugation of 1-chloro-2, 4 dinitrobenzene (CDNB) with GSH. The conjugation is accompanied by an increased in absorbance at 340 nm (A340). The rate of increase is directly proportional to the GST activity in the sample [22].

2.11 Determination of glutathione peroxidase (GPx) activity

In GPx assay, a cell or tissue homogenate is added to a solution containing GSH, GR and NADPH. The enzyme reaction is initiated by adding the substrate H₂O₂ and A340 is recorded. The rate of decreased in A340 is directly proportional to the GPx activity in the sample [23].

2.12 Determination of superoxide dismutase (SOD) activity

This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate (PMS) mediated the reduction of nitroblue tetrazolium (NBT) dye [24].

2.13 Determination of catalase (CAT) activity

Catalase reacts with a known quantity of H₂O₂. The reaction is stopped after exactly one minute with catalase inhibitor [25].

2.14 Determination of total protein content

Protein concentration was assessed by using Biosystems kits. The procedure was carried out according to Gornall et al. [26] using the reagent (Copper (II) acetate 6 mmol/l, potassium iodide 12 mmol/l, sodium hydroxide 1.15 mmol/l, detergent). 800 µl of the reagent was pipetted with 30 µl from the sample. In another test tube, 800 µl of the reagent was pipetted with 8 µl of standard protein. The tubes were then placed in water bath for 20 minutes at 37°C. The protein in the sample reacts with copper II ion in alkaline medium forming a colored complex that can be measured spectro photometry. The absorbance of the color was measured at 450 nm using Shimadzu 160 1PC spectrophotometer.

2.15 Determination of nucleic acid (DNA) content

DNA were determined according to the method of Barron and Adelman [27], and carried out as follows: 0.5 gm of frozen insects were homogenized with polytron homogenizer at high speed for 100 second at 0°C in 5 volume of ice-cold distilled water to 2 ml homogenate, 5 ml of cold (2°C), 95% ethanol was added, blend on ice for 15 minutes and then centrifuged at 5000 x g for 15 minutes at 4°C. The pellet was washed with 5 ml of 70% ethanol, centrifuged and ethanol supernatants were discarded. 2 ml of cold (2°C) 0.6 N perchloric acid (PCA) were added. Interfering acid-soluble, low molecular weight compounds (e.g. nucleotides, amino acids) were removed in the supernatant after centrifugation at 5000 x g. RNA was extracted from the pellet using 2 ml 0.3 N KOH at 37°C for 60 minutes, cooled on ice for 30 minutes and centrifuged at 5000 x g for 15 minutes at 4°C. DNA was extracted from the pellet using 2 ml of hot (80°C) 0.6 per-chloric acid (PCA) for 15 minutes cooled on ice for 15 minutes and centrifuged at 5000 x g for 15 minutes at 4°C. DNA was quantified in the supernatant by UV absorbance at 268 nm using Shimadzu 160 1PC UV spectrophotometer. Data were expressed as average quantity of RNA or DNA per whole insect. DNA content was expressed as µg/100 mg tissue.

2.16 Data Analysis

Analysis of variance (ANOVA) was calculated according to Snedecor and Cochran [28] to test the present data.

3. RESULTS

In our present study the following observations were obtained from irradiation by 32Gy and 64 Gy doses of gamma rays in soft tissues of males *B. polycresta*.

Table 1 and Figures 1, 2 showed the specific activities of aspartate amino-transferase (AST) and alanine amino transferase (ALT). Multiple comparisons between means (ANOVA test) revealed that the activities of AST and ALT were higher in group Band C respectively compared to the control group A. Also acid phosphatase (ACP) and alkaline phosphatase (ALP) activities was significantly increased in the irradiated groups (B and C) compared to the control group A (Table 1, Figs. 3, 4).

Our findings revealed that glutathione (GSH) content and Glutathione-S-transferase (GST) activity were decreased significantly in the irradiated groups (group B and C respectively), compared to control group (Table 1, Figs. 5, 6). Also the statistical analysis of mean difference showed that there was a significant decrease in the activity Glutathione peroxidase (GPx), Superoxide dismutase (SOD) and Catalase (CAT) activity in the affected groups (B and C respectively) compared to the control group (group A) (Table 1, Figs. 7, 8, 9). The activities decreased in group B and more pronounced in group C.

The data cited in Tables 1 and Figures 10, 11 also indicate marked decline in the content of protein and nucleic acid (DNA). The data displayed significant differences amongst all groups, being highest in group A (control) and lowest in group B and C respectively.



Table 1. Activities of enzymes, total protein and nucleic acid content in soft tissues of *B. polycresta* in three studied groups according to different doses of gamma radiation

Tested parameter	Group A (Control) (n = 3)	Group B (32 (n = 3) Gy)	Group C (64 (n = 3) Gy)	p
AST	78.48 ^a ± 2.72	127.40 ^b ± 1.78	170.18 ^c ± 3.62	<0.001 [*]
ALT	66.96 ^a ± 3.43	104.10 ^b ± 3.77	143.33 ^c ± 3.34	<0.001 [*]
ACP	18.86 ^a ± 1.69	30.47 ^b ± 1.57	40.73 ^c ± 0.72	<0.001 [*]
ALP	182.27 ^a ± 2.12	263.67 ^b ± 4.51	323.0 ^c ± 5.01	<0.001 [*]
GSH	29.42 ^a ± 1.0	21.39 ^b ± 0.67	16.48 ^c ± 0.59	<0.001 [*]
GST	12.63 ^a ± 0.54	9.48 ^b ± 0.77	6.62 ^c ± 0.37	0.001 [*]
GPX	50.34 ^a ± 0.68	35.30 ^b ± 0.58	26.91 ^c ± 0.39	<0.001 [*]
SOD	84.98 ^a ± 1.95	51.28 ^b ± 0.093	41.86 ^c ± 1.55	<0.001 [*]
CAT	70.44 ^a ± 1.63	49.88 ^b ± 0.75	29.73 ^c ± 0.94	<0.001 [*]
Total protein	141.97 ^a ± 1.88	113.76 ^b ± 1.88	84.96 ^c ± 3.59	<0.001 [*]
DNA content	44.09 ^a ± 1.81	32.97 ^b ± 1.36	18.47 ^c ± 1.13	<0.001 [*]

Normally distributed data was expressed in mean ± SE and was compared using F test (ANOVA)

Different superscripts in the same row are statistically significant at p <0.05

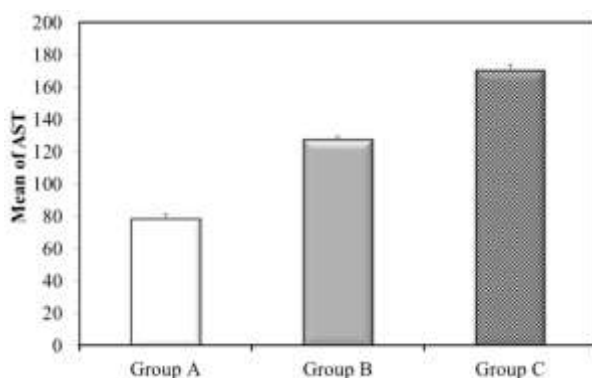


Fig 1. Activities of Aspartate amino-transferase (AST) in soft tissues of males *B. polycresta* in the three studied groups.

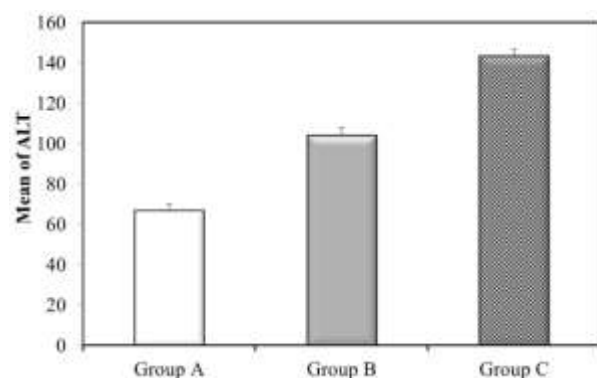


Fig 2. Activities of Alanine aminotransferase (ALT) in soft tissues of males *B. polycresta* in the three studied groups.

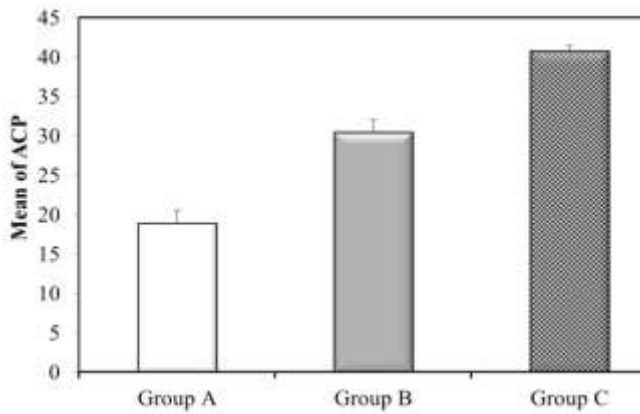


Fig 3. Activities of Acid phosphatase (ACP) in soft tissues of males *B. polycrsta* in the three studied groups.

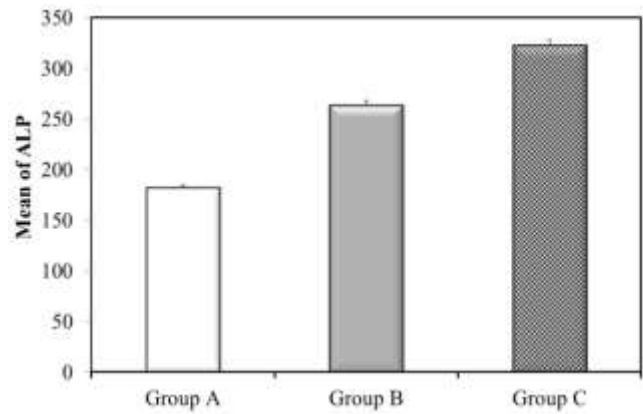


Fig 4. Activities of Alkaline phosphatase (ALP) in soft tissues of males *B. polycrsta* in the three studied groups.

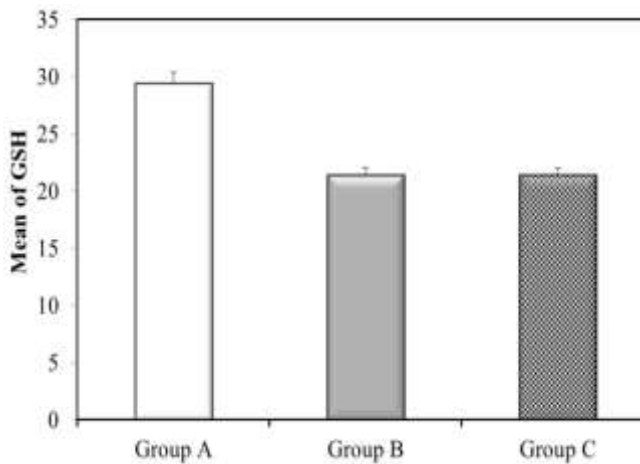


Fig 5. Activities of glutathione (GSH) content in soft tissues of males *B. polycrsta* in the three studied groups.

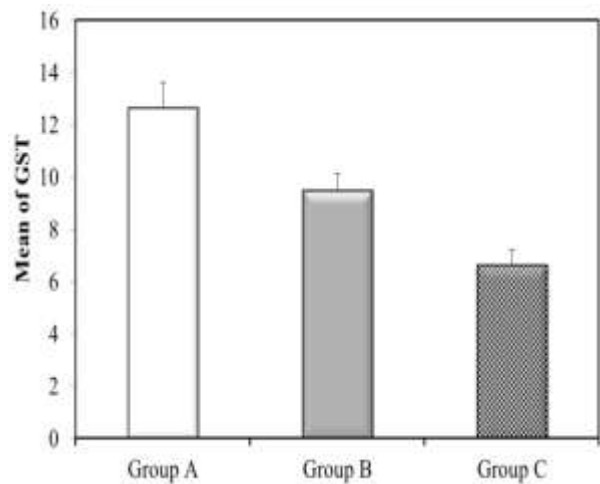


Fig 6. Activities of glutathione – S –transferase (GST) in soft tissues of males *B. polycrsta* in the three studied groups.

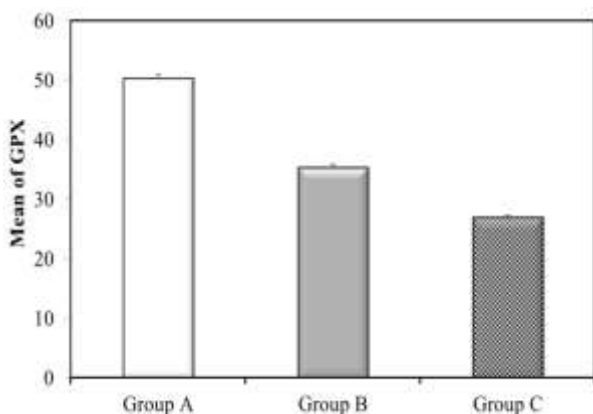


Fig 7. Activities of glutathione peroxidase (GPx) in soft tissues of males *B. polycrsta* in the three studied groups.

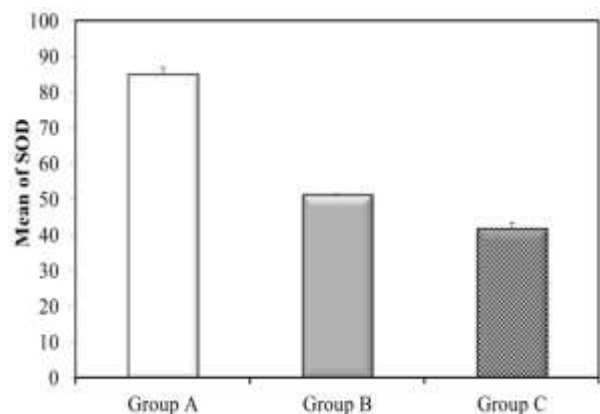


Fig 8. Activities of superoxide dismutase (SOD) in soft tissues of males *B. polycrsta* in the three studied groups.

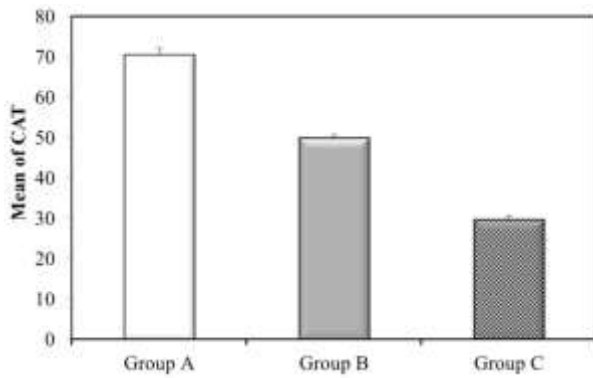


Fig 9. Activities of catalase (CAT) in soft tissues of males *B. polycrsta* in the three studied groups.

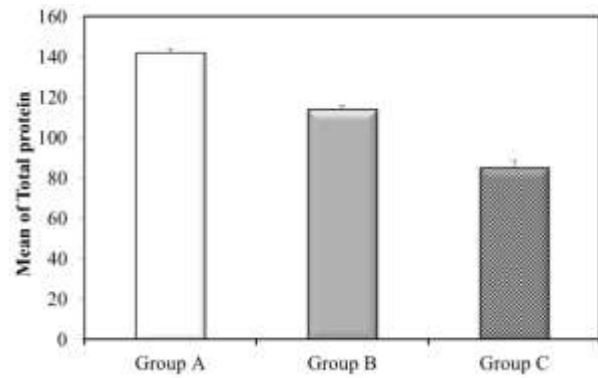


Fig 10. Total protein content in soft tissues of males *B. polycrsta* in the three studied groups.

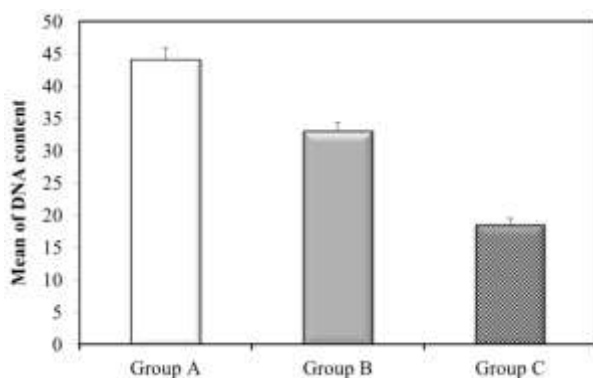


Fig 11. Nucleic acid (DNA) content in soft tissues of males *B. polycrsta* in the three studied groups.

4. DISCUSSION

Enzyme activities are liable to be influenced by radiation damage and may increase or decrease depending upon the doses of gamma radiation [29]. Detoxification enzyme in insects is generally demonstrated as the defense mechanism against foreign compounds and play a significant roles in maintaining their normal physiological functions [30]. Also the central issue in radiobiology is depression and inhibition of protein synthesis [31] and generates reactive oxygen species (ROS) that interact with cellular molecules, including DNA, lipids, and proteins which may lead to cellular damage [32]. Results of the present study, indicates that exposure of males *B. polycrsta* to gamma irradiation at a dose of 32 Gy and 64 Gy lead to biochemical disorders, manifested by elevation of the detoxifying metabolic enzymes AST, ALT, ACP and ALP, inhibition of the antioxidant enzymes GSH, GST and GPx, as well as, inhibition of total protein and DNA content.

The present results reported an elevation in AST and ALT, this may be comparable with those of the El-Shennaway et al. [33] who observed the impacts of feeding irradiated full-fat linseeds at dose levels of 2.5, 5, 7.5 and 10 kGy of gamma radiation to growing male Albino rats. They found that feeding rats with processed seeds modulated the levels of liver enzymes activities AST and ALT. Also Mohammed [34] elucidated that the exposure of albino rats to gamma irradiation at single dose (6.5 Gy) caused elevation in transaminases (AST and ALT).

The higher activities of these enzymes (AST and ALT) indicate tissue damage [35, 36]. Ionizing radiation enhanced lipid peroxidation in cell membrane which contains fatty acids and excessive production of free radicals; this in turn increases the cytoplasmic membrane permeability to organic substances and causes leakage of cytosolic enzymes such as AST, ALT [37]. The transaminase enzymes (ALT and AST) serve as a link between the carbohydrate and protein metabolism and are known to be altered during various physiological and pathological conditions [38, 39]. The increase in transaminases was the clear indication of cellular leakage and loss of functional integrity of the cell membrane [40]. ALT is a transaminase enzyme that is found in the liver in vertebrates and fat bodies in insects [41]. Elevation of ALT activity often suggest the existence of a physiological challenge in body such as microorganism infections, tissue damage, or toxic material [42].

The enhanced in the activity of Acid (ACP) and alkaline (ALP) phosphatases with the enhancement of gamma dose in the present study indicating changes of the physiological balance in the midgut. These hydrolytic enzymes are found in the intestinal epithelium of animals and the midgut has the highest ALP and ACP activity as compared to other tissues [43]. They are responsible for cytolysis of tissues during the insect development [44] and may act as hydrolases during the



final stages of digestion [45] or in gonad maturation and metamorphic moults [46]. They also remove phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids in alkaline and acidic conditions, respectively in the dephosphorylation process [47]. Increasing activities of these phosphatases denoted an increasing capability of the insect to detoxify the tested materials [48]. Our results may be comparable with those of Mohammed [34] who reported that the exposure of albino rats to gamma irradiation at single dose (6.5 Gy) caused elevation in alkaline phosphatase (ALP).

On the other hand the present results indicated a significant decrease in GSH content, GST and GPx. The higher the dose of gamma irradiation, the higher the rate of enzyme inhibition. The decrease in GSH content is an adaptive response to the increase in LPO level, indicating severe oxidative stress [49]. Furthermore, the reduction of GSH content may be due to their consumption in the scavenging free radicals [50]. Also GST in insects represents an important line of defense against free radicals [51]. The decrease of the activity of GPx indicates its reduction capacity to scavenge H₂O₂ and LOOHs [52]. This may be due to either the result of O₂⁻ production [53].

There is a close relation between GSH content in the organisms and the activity of some enzymes, such as GST activity and GPx. The changes of these enzyme activities make GSH content keep a dynamic balance in organisms [52]. Consequently, the decrease in GSH content leads to reduction in GST and GPx activities. The significant depletion in glutathione after radiation exposure might be resulted from diffusion through impaired cellular membranes and/or inhibition of GSH synthesis. Also this depletion may be due to diminished activity of glutathione reductase and the deficiency of NADPH which is necessary to change oxidized glutathione to its reduced form [54, 55]. Our data confirms the previous reports of Osman [56], El-Ghazaly and Ramadan [57], Ramadan et al. [58] and Mohammed [34]. The resultant reduction in GSH level may increase the susceptibility of the tissue to oxidative damage including lipid peroxidation [59].

Interaction of radiation with biological molecules produced toxic free radicals leading to structural and functional damage to cellular membranes. Thus a dramatic in the antioxidant enzymes leading to membrane lipid peroxidation and loss of protein thiols [60]

Our results revealed an inhibition in superoxide dismutase (SOD) and catalase (CAT) due to irradiation with the tested doses. In accordance with the present results Sachdev et al. [61] reported that SOD and CAT transcript were downregulated in the lepidopteran insect *Helicoverpa armigera* due to irradiation with gamma dose of 100 Gy. This is also consistent with previous reports by Heck et al. [62] and Polte and Tyrrell [63], showing that high doses of irradiation suppress the activity of protective enzymes, such as SOD in normal cells. Zhang et al. [64] stated that 60Co- γ irradiation resulted in decreased activities of SOD and CAT in the citrus red mite *Panonychus citri* after prolonged recovery time, accompanied by impaired antioxidant capacity and high levels of oxidative stress. Wilczek et al. [65] concluded that the decreased activity of SOD might be due to the consumption of this enzyme in converting the O₂⁻ to H₂O₂. Thus the increased H₂O₂ levels inhibited SOD activity resulting from the increase in O₂⁻ in response to radiation.

Previous studies showed that CAT can protect against oxidative stress and extend the lifespan of insects [66, 67]. Felton and Summers [68] estimated that there is a certain relationship between CAT and SOD as SOD can convert the free O₂⁻ to H₂O₂, which is then eliminated by CAT. Barbehenn et al. [69] indicated that O₂⁻ can inhibit CAT activity and the increased H₂O₂ resulting from CAT inhibition could lead finally to inhibit SOD activity. It could be concluded that the decline in the CAT activity may be due to reducing the conversion of O₂⁻ to H₂O₂

The present study investigated depletion in total content of proteins. The influence of irradiation on protein contents was more pronounced by increasing the irradiation dose. Increasing the dose was accompanied by an increase in the rate of reduction of protein contents. In this respect, Desrosier [70] stated that ionizing radiation induced denaturation in protein. Our results agreed with the findings of Abdel Megeed et al. [71], Ibrahim et al. [72], Abdel Baky et al. [73], Abdel-Bakiet al. [74], El-Bermawy et al. [75] and Al Khalaf and Abdel Baki [76]. Gabarty [16] recorded a significant decrease in the total content of hemolymph proteins in the pupae of *Agrotis ipsilon* after irradiated with 100 Gy gamma rays. Also Gabarty et al. [77] observed a reduction in the total protein tissue content in the 6th instar larvae of *Spodoptera littoralis* due to the effect of gamma irradiation (50, 100 and 150 Gy) on male pupae. Kempner [78] explained the decrease in proteins level due to gamma-irradiation can damage or inactivate proteins by two different mechanisms. First, it can rupture the covalent bonds in target protein molecules as a direct result of a photon depositing energy into the molecule and second, by acting indirectly with a water molecule, producing free radicals and other non-radical reactive oxygen species that are in turn responsible for most (99.9%) of the protein damage. Also the decrease of protein amount after irradiation may be due to its lysis by gamma radiation or it may be due to the depression of enzymes involved in the activation of amino acids and transfer ring to tRNA [79].

Proteins play a major role in the synthesis of detoxifying enzymes and help to detoxify the entered toxicants [80]. Consequently, the decrease in total protein content may reflect the decrease in the activities of some enzymes [81] this confirms our results of the inhibition in activity of the GST, GPx, SOD and CAT. Moreover, the decrease in protein content could be due to the breakdown of protein into amino acids and entrance of these amino acids to Krebs cycle as keto acid to supply energy for the insect [82].

The present study showed declines in DNA content in males of *B. polycresta*. The decline increases with increasing the dose, being higher at 64 Gy gamma rays. Sudprasert et al. [15] observed that low doses of gamma radiation (5cGy and 10cGy) induced DNA damage in human blood cells. Cell killing by irradiation is caused by misrepaired DNA double strand breakage. Much of the damage to DNA induced by ionizing radiation is considered to be caused by the hydroxyl radicals produced by the radiolysis of water in cell [83]. Gaur and Bhatia [79] stated that the decrease in DNA content after irradiation is due to an inhibition of replication in nucleus and accumulation of ribonucleotide in the cytoplasm, which is



based on the inability of irradiated cell to reduce ribonucleotide to DNA in the nucleus. Also the prolonged interphase or delayed onset of DNA synthesis after irradiation could lead to decreased content of DNA [79]).

Lower concentration of the mutagenic agent could be attributed to the increased rate of metabolism and higher secretion of enzyme. On the contrary, the lower enzyme activity could be attributed to higher concentration of the mutagenic agent when induced directly, alters the genetic structure to a high degree resulting in deleterious effect on the enzyme activity [84]. Finally, enzymes, proteins and nucleic acid (DNA) are known to be susceptible to radiation damage. The activity of enzyme may increase or decrease, depending upon the type of enzyme, exposure to mutagens and length of time elapsed between treatment and assay [85].

Consequently, the biochemical criteria of damage are the loss of the enzyme to perform its function. Several radiation-induced reactions that are reversible at low doses due to Repair Processes that occur.

5. CONCLUSION

Gamma radiation has significantly affected the enzymes activity, total protein content and DNA content in the soft tissues of males *B. polycresta* which may induce biochemical perturbations and lead to disturbance in the biology of the ground beetle. Finally *B. polycresta* may be used as a model in assessing risk of exposures to gamma radiation.

ACKNOWLEDGMENTS

The study was partially funded by Alexandria University. The authors are thankful to Zoology Department, Faculty of science and to all members of the Egyptian Atomic Energy Authority (EAEA).

REFERENCES

1. Bakri, A., Heather, N., Hendrichs, J. and Ferris, I. 2005. Fifty years of radiation biology in entomology: lessons learned from IDIDAS. *Ann. Entomol. Soc. Am.*, 98: 1– 12.
2. (UNSCEAR) United Nations Scientific Committee on the Effect of Atomic Radiation. 2000. Sources and effects of ionizing radiation, Report to General Assembly, with Scientific Annexes. United Nations, New York.
3. Zaman, K., Mac Gill, R.S., Johnson, J.E., Ahmad, S. and Pardini, R.S. 1995. An insect model for assessing oxidative stress related to arsenic toxicity. *Arch. Insect Bio chem. Physiol.*, 29:199–209.
4. Mallet, J. 1989. The Evolution of Insecticide Resistance: Have the Insects Won? *Tree*, 4: 336-340.
5. Cogo, A.J.D., Siqueira, A.F., Ramos, A.C., Cruz, Z.M.A and Silva, A.G. 2009. Utilização de enzimas do estresse oxidativo como biomarcadoras de impactos ambientais. *Nat.*, 7: 1806-7409.
6. Mishra, S., Srivastava, S., Tripathi, R.D., Kumar, R., Seth, C.S. and Gupta, D.K. 2006. Lead detoxification by coontail (*Ceratophyllum demersum* L.) involves induction of phytochelatins and antioxidant system in response to its accumulation. *Chem.*, 65: 1027-1039.
7. Nordberg, J. and Arnér, E.S. 2001. Reactive oxygen species, antioxidants and the mammalian thioredoxin system. *Free Radic. Biol. Med.*, 31: 1287-1312.
8. Valko, M., Rhodes, C.J., Moncol, J., Izakovic, M. and Mazur, M. 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem. Biol. Interact.*, 160: 1-40.
9. Rover-Junior, L., Höehr, N.F., Vellasco, A.P. and Kubota, L.T. 2001. Sistema antioxidante envolvendo o ciclo metabólico da glutatona associado a métodos eletroanalíticos na avaliação do estresse oxidativo. *Quim. Nova*, 24: 112-119.
10. Malmezat, D., Breuille, P., Capitan, P., Miranda, P. and Obled, C. 2000. Glutathione turnover is increased during the acute phase of sepsis in rats. *J. Nut.*, 130: 1239-1246.
11. Miao, Y. 2002. Studies on the activity of the alkaline phosphatase in the midgut of infected silk worm, *Bombyx mori* L. *J. Appl. Entomol.*, 38:126-142.
12. Hassan, H.A. 2002. Biological and biochemical studies on the effect of some botanical extracts on cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). M.Sc. Thesis, Fac. Sci. Ain Shams Univ., Egypt.
13. Thornburn Lurn, C.C. 1972. Isotopes and radiation in biology. Butterworth & co (LTD), London. p. 287.
14. Wigglesworth, V.B. 1972. The principles of insect physiology. Champan and Hall, seventh Edition p. 827.
15. Sudprasert, W., Navasumrit, P. and Ruchirawat, M. 2006. Effects of low-dose gamma radiation on DNA damage, chromosomal aberration and expression of repair genes in human blood cells. *Int J Hyg Environ. Health*. 209: 503-11.
16. Gabarty, A., 2008. Biological and physiological studies on the effect of some botanical oils and gamma irradiation on the greasy cut worm *Agrotis ipsilon* (Hufn). M.Sc Thesis. Faculty of Science (Girls branch). Al-Azhar University.
17. Kamal, A.A. 2013. Ecological, Histological and Biochemical Studies on the Effect of Some Environmental Pollutants on the Herbivorous Beetles, *Blaps polycresta* (Coleoptera: Tenebrionidae). PhD. Thesis. Zoology Department, Faculty of Science, Alexandria University: p, 204.



18. Reitman, S. and Frankel, S. 1957. A colourimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Amer. J. Clin. Path.*, 28: 56-60.
19. Hillmann, G. 1971. Forlaufende photometrische messung der sauren phosphatase Aktivitat. *J. Clin. Chem. Clin. Biochem.*, 3: 273-274.
20. Kind, P.R.N. and King, E.J. 1954. Estimation of plasma phosphatases by determination of hydrolysed phenol with amino anti-pyrene. *LohtaP .nilC .J*, 7: 322-326.
21. Beutler, E., Duron, O. and Kelly, M.B. 1963. Improved method for the determination of blood glutathione. *J. Lab. Clin. Med.*, 61: 882.
22. Habig, W.H., Pabst, M.J. and Jacoby, W.B. 1974. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. *J. Biol. Chem.*, 249: 7130-7139.
23. Paglia, D.E. and Valentine, W.N. 1967. Studies on the quantitative and qualitative characterization of erythrocytes peroxidase. *J. Lab. Clin. Med.*, 70: 158-169.
24. Nishikimi, M., Roa, N.A. and Yogi, K. 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem. Biophysiol. Res. Common.*, 46: 849-854.
25. Aebi, H. 1984. Catalase in vitro. *Methods Enzymol.*, 105: 121-126.
26. Gornall, A.G., Bardawill, C.S. and David, M.M. 1949. Determination of serum proteins by means of Biuret reaction. *J. Biol. Chem.*, 177: 751-766.
27. Barron, M.G. and Adelman, I.R. 1984. Nucleic acid, protein content and growth of larval fish sublethally exposed to various toxicants. *Can. J. Fish. Aquat. Sci.*, 41: 141-150.
28. Snedecor, G.W. and Cochran, W.G. 1969. *Statistical Methods*, 6th ed. The Iowa State Unvers. Press. Anes., Iowa, USA.
29. Gabarty, A. and Mahmoud, E.A. 2015. Biochemical changes produced by gamma irradiation in the alimentary canal of males *Rhynchophorus ferrugineus* (Olivier). *J. Entomol. Zool. Stud.*, 3: 290-294.
30. Li, X.Z. and Liu, Y.H. 2007. Diet influences the detoxification enzyme activity of *Bactrocera tau* (Walker) (Diptera: Tephritidae). *Acta. Entomol. Sin.*, 50: 989-995.
31. El-Naggar, M.A. 2009. *Medical Radiation Biology*, Edn 1, Al Tobgy Press: Cairo, Egypt.
32. Tominaga, H., Kodama, S., Matsuda, N., Suzuki, K. and Watanabe, M. 2004. Involvement of reactive oxygen species (ROS) in the induction of genetic instability by radiation. *J. Rad. Res.*, 45: 181-8.
33. El-Shenna way, H.M., El-Niely, H.F. and Hamaza, R.G. 2010. Physiological and Biochemical Impacts of Radiation Processed Full-Fat Linseed. *J. Radiat. Res. Appl. Sci.*, 42: 965-986.
34. Mohammed, M.M. 2010. The Possible Protective Role of *Foeniculum vulgare* Mill. Against Radiation-Induced Certain Biochemical Changes in Albino Rats. M.Sc. thesis. Zoology Department, Faculty of Science, Beni-Suif University: p, 182.
35. Ali, N.S., Ali, S.S. and Shakoori, A. 2013. Effect of sublethal doses of Malathion on biochemical components of Malathion resistant and susceptible adults of *Rhyzopertha dominica*. *Pak. J. Zool.*, 45: 203-212.
36. Upadhyay, R.K. 2013. *Capparidaceae* extracts induced toxicity, Biochemical and Enzymatic Alteration in *Rhyzopertha dominica* (Fabr.) (Coleoptera: Bostrichidae). *World J. of Zool.*, 8: 256-266.
37. Weiss, J.F. and Lander, M.R. 2003. Protection against ionizing radiation by antioxidant nutrients and phytochemicals. *Toxicol.*, 189(1-2): 1-20.
38. Etebari, K., Mirohoesieni, S.Z. and Matindoost, L. 2005. Study on inter specific Biodiversity of eight groups of silkworm (*Bombyx mori*) by biochemical markers, *Insect Sci.*, 12: 87-94.
39. Ender, I., Ferah, A., Kemal, B. and Ahmet, G. 2005. Biochemical stress indicators of greater wax moth, *Galleria mellonella* exposure to organo phosphorus insecticides. *J. Economic Entomol.*, 98, 358-366.
40. Saraswat, B., Visen, P.K., Patnaik, G.K. and Dhawan, B.N. 1993. Anticholestatic effect of picroliv, active hepato-protective principles or principle of *Picrohiza kurrooa*, against carbon tetra chloride induced cholestasis. *Ind. J. Exp. Boil.*, 3: 316-8.
41. Thomas, L. 1998. *Clinical Laboratory Diagnostic*. 1st ed. TH Books Leaving society, Frankfurt, pp. 89-94.
42. Giboney, P.T. 2005. Mildly Elevated Liver Transaminase Levels in the Asymptomatic Patient. *Am. Fam. Physician*, 71: 1105-1110.
43. Sakharov, I.Y., Makarova, I.E. and Ermolin, G.A. 1989. Chemical modification and composition of tetrameric isozyme K of alkaline phosphatase from harp seal intestinal mucosa. *Comp. Biochem. Physiol. Part B*, 92: 119-122.
44. Dadd, R.H. 1970. Arthropod nutrition. In: "Chemical Zoology" (Florkin M. and Scheer, B.T., eds.). Vol. 5. Academic Press New York, pp. 35-95.



45. Cheung, W.W.K. and Low, K.W. 1975. Ultrastructural and functional differentiation of the midgut of the sugar cane beetle *Protaetia acuminata* (F.) (Coleoptera: Cetoniidae). *Int. J. Insect Morphol. Entomol.*, 4: 349-361.
46. Tsumuki, H. and Kanehisa, K. 1984. Phosphatases in the rice stem borer, *Chilo suppressalis* Walker (Lepidoptera: Pyralidae): Some properties and changes of the activities during hibernation. *Cytobiology*, 21: 177-182.
47. Zibae, A., Zibae, I. and Sendi, J.J. 2011. A juvenile hormone analog, pyriproxyfen, affects some biochemical components in the hemolymph and fat bodies of *Eurygaster integriceps* (Puton) (Hemiptera: Scutelleridae). *Pest. Biochem. Physiol.*, 100: 289-298.
48. Sharifi, M., Kosari, A.A., Zibae, A. and Sendi, J.J. 2013. Effects of Pyriproxyfen on detoxifying and intermediary enzymes of *Ephesia kuehniella* Zeller (Lepidoptera: Pyralidae). *Plant Pests Res.*, 3: 35-44.
49. Kavitha, P. and Rao, V.J. 2009. Sub-lethal effects of profenofos on tissue-specific antioxidative responses in a euryhaline fish, *Oreochromis mossambicus*. *Ecotoxicol. Environ. Saf.*, 72: 1727-33.
50. Ben Amara, I., Soudani, N., Troudi, A., Bouaziz, H., Boudawara, T. and Zeghal, N. 2011. Antioxidant effect of vitamin E and selenium on hepatotoxicity induced by dimethoate in female adult rats. *Ecotoxicol. Environ. Saf.*, 74: 811-819.
51. Perić-Mataruga, V., Blagojević, D., Spasić, M.B., Ivanović, J. and Janković-Hladni, M. 1997. Effect of the host plant on the antioxidative defence in the midgut of *Lymantria dispar* L. caterpillars of different population origins. *J. Insect Physiol.*, 43:101-106.
52. Wu, H., Zhang, R., Liu, J., Guo, Y. and Maa, E. 2011b. Effects of malathion and chlorpyrifos on acetylcholinesterase and antioxidant defense system in *Oxya chinensis* (Thunberg) (Orthoptera: Acrididae). *Chem.*, 83: 599-604.
53. Bagnasco, M., Camoirano, A., De Flora, S., Melodia, F. and Arillo, A. 1991. Enhanced liver metabolism of mutagens and carcinogens in fish living in polluted seawater. *Mutat. Res.*, 262: 129-137.
54. Pulpanova, J., Kovarova, H. and Ledvina, M. 1982. Change in the reduced Glutathione concentration and the activity of glutathione reductase in some tissues of the rats gamma-irradiated after administration of cystamine. *Radiobiol. Radiother.*, 23: 50-6.
55. Said, U.Z., Rezk, R.G. and Saada, H.N. 2005. Role of calcium pantothenate in recovery of radiation induced injury to mammalian organs. *Egypt. J. Rad. Sci. Applic.*, 18: 17.
56. Osman, S.A. 1996. Melatonin boosting antioxidant capacity for effective radiation protection in albino rat. *Egypt. J. Med. Sci.*, 17: 281-295.
57. El-Ghazaly, M.A. and Ramadan, L.A. 1996. Alterations in some metabolic functions in albino rats after irradiation and possible role of thiola. *J. Egypt. Soc. Toxicol.*, 16: 105-9.
58. Ramadan, L.A., Shouman, S.A., Sayed-Ahmed, M.M. and El-Habit, O.H. 2001. Modulation of radiation-induced organs toxicity by cremophor-el in experimental animals. *Pharmacol. Res.*, 43:18591.
59. Moldeus, P. and Quanguan, J. 1987. Importance of GSH cycle in drug metabolism. *Pharmacol. Therapeutics*. 33: 37-40.
60. Devi, P.U. and Ganasoundari, A. 1999. Modulation of glutathione and antioxidant enzymes by *Ocimum sanctum* and its role in protection against radiation injury. *Ind. J. Exp. Biol.*, 37: 262-8.
61. Sachdev, B., Zarin, M., Khan, Z., Malhotra, P., Seth, R.K. and Bhatnagar, R.K. 2014. Effect of gamma radiation on Phenoloxidase pathway, antioxidant defense mechanism in *Helicoverpa armigera* (Lepidoptera: Noctuidae) and its implication in inherited sterility towards pest suppression. *Int J Radiat Biol.*, 90:7-19.
62. Heck, D.E., Vetrano, A.M., Mariano, T.M. and Laskin, J.D. 2003. UVB light stimulates production of reactive oxygen species: Unexpected role for catalase. *J. Biol. Chem.*, 278: 22432-22436.
63. Polte, T. and Tyrrell, R.M. 2004. Involvement of lipid peroxidation and organic peroxides in UVA-induced matrix metalloproteinase-1 expression. *Free Rad. Biol. Med.*, 36: 1566-1574.
64. Zhang, K., Li, Z., Zhu, S. and Weng, Q. 2014. 60Co-γ Irradiation Affects the Enzymatic Antioxidant System of the Citrus Red Mite *Panonychus citri* (Acari: Tetranychidae). *Molecules*, 19: 6382-6392.
65. Wilczek, G., Babczynska, A., Augustyniak, M. and Migula, P. 2004. Relations between metals (Zn, Pb, Cd and Cu) and glutathione-dependent detoxifying enzymes in spiders from a heavy metal pollution gradient. *Environ. Pollut.*, 132: 453-461.
66. Orr, W.C. and Sohal, R.S. 1994. Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster*. *Sci.*, 263: 1128-1130.
67. Melov, S., Ravenscroft, J., Malik, S., Gill, M.S., Walker, D.W., Clayton, P.E., Wallace, D.C., Malfroy, B., Doctrow, S.R. and Lithgow, G.J. 2000. Extension of life-span with superoxide dismutase/catalase mimetics. *Sci.*, 289: 1567-1569.
68. Felton, G.W. and Summers, C.B. 1995. Antioxidant systems in insects. *Arch. Insect Biochem. Physiol.*, 29: 187-197.



69. Barbehenn, R.V., Bumgarner, S.L., Roosen, E.F. and Martin, M.M. 2001. Antioxidant defenses in caterpillars: role of ascorbate-recycling system in the midgut lumen. *J. Insect Physiol.*, 47: 349–357.
70. Desrosier, N.W. 1970. *The technology of food preservation* Avl publishing Company. Inc. London. p. 313.
71. Abdel-Megeed, M.I., EL-Banby, M.A., Sobeiha, A.K. and Abdel-Baky, S.M. 1987b. Amino Acid Contents of *Ephestia kuehniella* (Zell) as affected by gamma irradiation. *Bull. Ent. Soc. Egypt. Econ. Ser.*, 16:157-164
72. Ibrahim, S.M. and Abdel-Baky, S.M. 1989. Protein and free amino acids contents in the progeny of gamma irradiated black cutworm *Agrotis ipsilon* (HUFN). Male parents. *Proc. Ist .Int. Conf. Econ. Ent.*, 11:431-436.
73. Abdel-Baky, S.M.Z., Hassaballa, A. and Darwish, Y.A. 1991. Biological and chemical studies on the irradiated rice meal moth *Corcyra cephalonica* (Staint). *Ass. J. Agric. Sci.*, 22: 203-214.
74. Abdel-Baki, S.M., Ahmed, S.M. and Abdel Rahman, H.R.2000. Determination of some biochemical components in gammairradiated pupae of *Lasioderma serricorne* (F). *J. Agric. Sci.*, 25:8223-8228.
75. El-Bermawy, S.M., Ahmed, Z.A. and Ali. F. 2000. Changes in Total proteins in larvae of rice moth *Corcyra cephalonica* due to irradiation and heat stress. *Aral. J. Nucl. Sci. Appl.*, 33(2).
76. Al khalaf, A.A. and Abdel Baki, S.M. 2013. Gamma irradiation effects on larvae of the rice moth, *Corcyra cephalonica* (Staint) (Lepidoptera-Pyralidae). *J. Entomol. Nematol.*, 5: 45-49.
77. Gabarty, A., El-Sonbaty, S.M. and Ibrahim, A.A. 2013. Synergistic effect of gamma radiation and entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* on the humoral immune enzyme response in cotton leaf worm *Spodoptera littoralis* (Boisd). *Acad. J. of Bio. Sci.*, 6:1–10.
78. Kempner, E. S., 2001, Effects of high-energy electrons and gamma rays directly on protein molecules. *J. Pharm. Sci.*, 90: 1637-1646.
79. Gaur, A. and Bhatia, A.L. 2009. Effects of Radiation on the Protein and DNA Content in Genistein Treated Swiss Albino Mice. *Iran. J. Pharm. Res.*, 5: 239-248.
80. Wikinson, C.F. 1976. *Insecicide biochemistry and physiology*. Plenum, New York, 768 p.
81. Abdel-Aziz, H.S., Mansy H.S., Desuky, W.M. and Elyassaki, S.M. 2007. Toxicity evaluation and biochemical impacts of some *Bacillus thuringiensis* formulation on *Spodoptera littoralis* larvae. *J. Egypt Acad. Soc. Environ. Develop.*, 8: 1-10.
82. Bizhannia, A.R., Etebari, K. and Sorati, R. 2005. The effects of juvenile hormone analogue, Admiral, application on protein metabolism of silkworm larvae. *Iran J. Entomol. Soc.*, 25: 43–56.
83. Sato, M. and Bremner, I. 1993. Oxygen free radicals and metallothionein. *Free Radicals Biol. Med.*, 14: 325-337.
84. Lokesh, G., Ananthanarayana, S.R. and Yogananda Murthy, V.N. 2012. Changes in the Activity of Digestive Enzymes in Response to Chemical Mutagen Diethyl Sulfate in the Silkworm *Bombyx mori* L. (Lepidoptera: Bombycidae). *Asian j. appl. sci.*, 5: 431-437.
85. Kumari, L., Ananthanarayana, S.R. and Jayaprakash, B. 1997. Effect of radiation on the activity of digestive enzymes in the silkworm, *Bombyx mori* L. *Sericologia*, 37: 221-228.



Dalia A. kheirallah
Lecturer of Entomology
Department of Zoology
Faculty of Science,
Alexandria University Egypt



This work is licensed under a Creative Commons Attribution 4.0 International License.