EFFECTS OF GAMMA IRRADIATION ON LIPID PEROXIDATION LEVELS OF *Apis mellifera* FORAGERS

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ABSTRACT *Apis mellifera foragers were irradiated with 1, 5, 10, 20, 30, 40, 50 and 60 Gy doses of gamma radiation and 6, 12, 24, 48 and 72 h post irradiation samples were subjected to lipid peroxidation (LPO) assay. Significant increase in malondialdehyde (MDA) level was observed at 20 Gy (6 h); 30 and 40 Gy (6, 12 and 24 h); 50 and 60 Gy (6, 12, 24 and 48 h) sample when compared to control. There were no significant differences in the MDA level of 72 h post irradiation sample. The present study showed a dose dependent increase and post irradiation time dependent decrease in LPO level of forager bees for the studied doses.*

INTRODUCTION

All living creatures are frequently exposed to ionizing radiation (Little, 2003); these include radiation from natural and artificial radionuclides. The upsurge in ionizing radiation applications, particularly nuclear, has been followed by the growth of public anxiety for the prospective associated risks (Amaral, 2005). The protection of the environment from the effects of ionizing radiations has become a major concern for various international organizations in the field of radiation protection (Keum et al., 2010). To develop a framework for the evaluation of the environmental influence of radiation, it is essential to establish the relationship between exposure and the effects that may be induced in plants and animals (Real et al., 2004).

The honey bee, *Apis mellifera* is generally documented as a beneficial insect of agronomic, ecological, and scientific importance. Since the bees are in contact with numerous pollutants during foraging, it has been considered as an environmental sentinel of high sensitivity (Dai et al., 2010). Census of pollinating insects in the Chernobyl disaster area (after 25 years of nuclear accident) showed that *A. mellifera* are now extremely rare in the Chernobyl Exclusion Zone, leaving insect pollination to species of wild bees, bumblebees, and butterflies (Moller et al., 2012).

Gamma rays are uncharged ionizing particles and are the most penetrating radiation from natural and man-made sources (IAEA-TECDOC-1363, 2003). Gamma ray exposure was reported to induce oxidative stress with overproduction of reactive oxygen species (ROS), which react quickly with almost all structural and functional organic molecules, including proteins, lipids and nucleic acids triggering disturbance of cellular metabolism (Al-Rumaih and Al-Rumaih, 2008). Lipids are vulnerable targets of oxidation due to their molecular structure, rich with reactive double bonds (Ho et al., 2013). Overproduction of ROS can lead to increased lipid peroxidation (LPO) and the process of LPO results in the formation of malondialdehyde (MDA) (Jia et al., 2011). MDA levels in insects have been shown to change under oxidative stress and this may serve as a biomarker (Kayis et al., 2015). In light of the above information, this study aims to determine the effects of gamma irradiation on LPO levels of the *A. mellifera* foragers.

MATERIALS AND METHODS
Bees
Frames with capped worker broods (near of adult emergence)

were collected from *A. mellifera* colonies and kept in an incubator and maintained at 32°C, 60% relative humidity under 24 h dark conditions. Newly emerged bees were collected within 12 h of emergence and marked on the thorax. Marked bees were released back into the source hives. On the 24th day these marked foraging bees were collected at the hive entrance and brought back into the laboratory. Each 10 bees were placed in a plastic cage (11 X 6.2 X 5 cm) and maintained in an incubator as above mentioned conditions. Flies were fed ad libitum with 50% (weight/volume) sucrose solution and multifloral pollen paste.

Irradiation and lipid peroxidation assay of bees

Twenty five days old forager bees were irradiated with 1, 5, 10, 20, 30, 40, 50 and 60 Gy doses of Co-60 gamma radiation with a dose rate of 303.13 cGy/min. Irradiated bees were maintained in an incubator. Ten replicates were exposed for each dose and each replicates includes 8 bees. Further 6, 12, 24, 48 and 72 h post irradiation samples were collected and same age non-irradiated bees were used as control. The bees were starved for 2 h before the sampling of the LPO assay so that all bees were equal in terms of their gut contents.

Flies were homogenized in chilled phosphate-buffered saline (pH - 7.0) and the homogenate was centrifuged at 3000xg for 12 min at 4°C. Supernatant was collected and LPO was determined by measuring MDA formed as per Buege and Aust (1978). The values were expressed as nanomoles of MDA formed/mg wet weight of tissue.

Statistical analysis

Data obtained were statistically analyzed by using SPSS v17 software and the values were expressed in mean ± standard error (SE). To test the difference in the mean MDA level of irradiated and control bees, one-way analysis of variance (ANOVA) was conducted. Significant differences were determined by Tukey's honestly significant difference (HSD) post hoc test at $P \leq 0.05$.

RESULTS

Figures 1-5 represents the mean LPO level (MDA formed) of irradiated and non-irradiated foragers during 6, 12, 24, 48 and 72 h post irradiation time respectively. MDA level was elevated in irradiated flies and it was highest at 6 h post irradiation samples. Data revealed that there were no significant changes ($P > 0.05$) in the LPO level of 1, 5 and 10 Gy irradiated bees when compared to their respective controls. During 6 h post irradiation

time 20, 30, 40, 50 and 60 Gy doses showed a significant increase (F = 151.118; df = 8, 81; P < 0.05) in the LPO level. Exposure to 30, 40, 50 and 60 Gy doses showed significant increase in the MDA level during 12 h (F = 79.772; df = 8, 81; P < 0.05) and 24 h (F = 39.521; df = 8, 81; P < 0.05) post irradiation time. 50 and 60 Gy dose irradiation caused a significant increase (F = 10.374; df = 8, 81; P < 0.05) in the 48 h LPO post irradiation sample. There were no significant differences in the MDA level of 72 h post irradiation sample with their corresponding control (F = 2.644; df = 8, 81; P > 0.05).

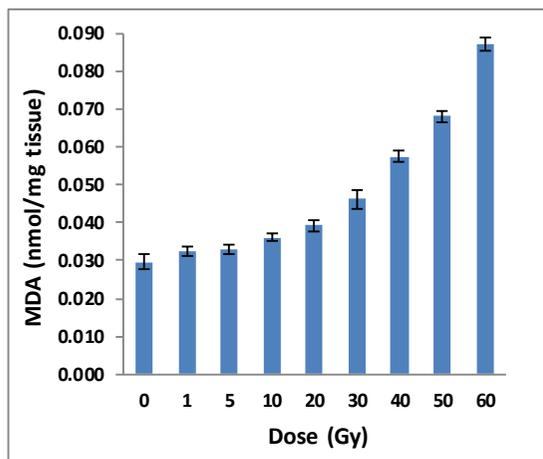**Figure 1: Mean ± SE MDA level of irradiated and control foragers during 6 h post irradiation time.**
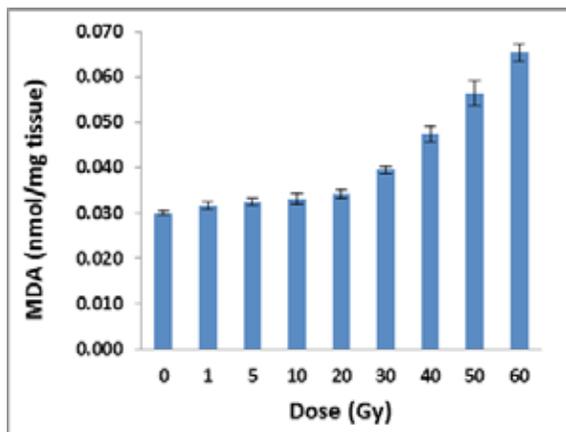

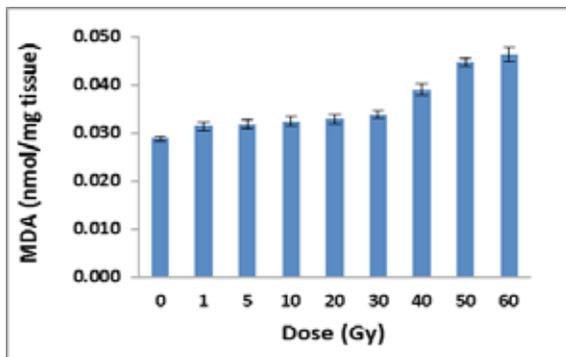**Figure 2: Mean ± SE MDA level of irradiated and control foragers during 12 h post irradiation time.**

Figure 3: Mean ± SE MDA level of irradiated and control foragers during 24 h post irradiation time.
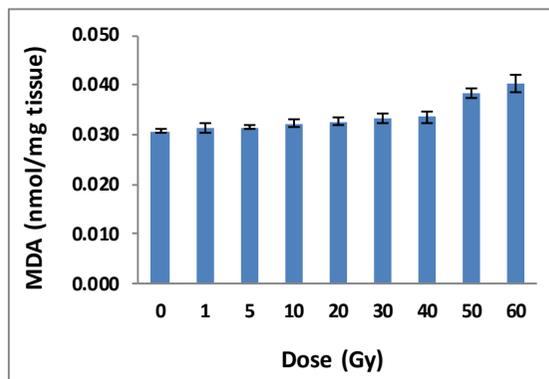

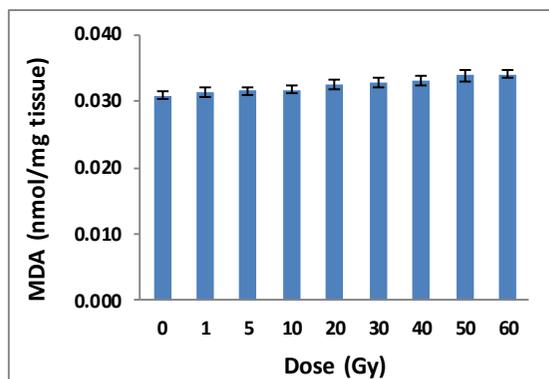**Figure 4: Mean ± SE MDA level of irradiated and control foragers during 48 h post irradiation time.**

Figure 5: Mean ± SE MDA level of irradiated and control foragers during 72 h post irradiation time.

DISCUSSION

Gamma radiation has been shown to elevate LPO in a variety of biological systems (Kiang et al., 2012). In the present study irradiated foragers showed raised MDA level. Agrawal and Kale (2001) reviewed that biological systems consist of about 60-80% water, predominantly the biological effects of ionizing radiation are mediated through radiolysis of water and ˙OH radical is considered to be accountable for the initiation of radiation - induced lipid peroxidation. Lipid peroxidation reactions can happen at both the cell membrane and mitochondria membranes and it can successively prompt cell death through apoptosis and/or autophagy (Kiang et al., 2012; Choi et al., 2007). Even though many LPO products induce cyctotoxicity, sublethal concentrations of LPO products, persuade cellular adaptive responses and improve tolerance against subsequent oxidative stress through upregulation of antioxidant compounds and enzymes (Niki, 2009).

The degree of radiation - induced LPO depends on the dose and dose rate of ionizing radiation (Agrawal and Kale, 2001). In accordance with this, our data showed that in irradiated bees increase in dose resulted in an increase in MDA level. Different kinds of antioxidants with diverse functions impede LPO and the harmful effects caused by its products. Antioxidant defenses may be divided into four categories: prevention of the formation of active oxidants, scavenging, quenching and elimination of active oxidants, healing of damage and elimination of lethal oxidation products, and adaptive responses. Free radical-mediated LPO may be inhibited by the inhibition of chain initiation and chain propagation and/ or acceleration of chain termination (Niki et al., 2005). In the present study as the post irradiation time proceeded the LPO level was reduced and this may be due to the action of antioxidants.

An insight into the impact of dose in correlation with ionizing radiation for non-human biota will provide us to draw radiological protection baselines of ecosystems (Nakamori et al., 2009; Shameer et al., 2015). The present study gives an idea about the doses that causes significant increase in LPO level. The data collected from this study showed a dose dependent increase and post irradiation time dependent decrease in LPO level of foragers. This indicates that oxidative stress takes place by the action of irradiation and the assessments of LPO can serve us with useful index of biological impairment caused by gamma irradiation.

ACKNOWLEDGMENTS

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