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Effects of Acute Gamma Irradiation on Reduced Glutathione (GSH) Levels of *Apis mellifera* Foragers

KEYWORDS: Antioxidant, Bee, Ionizing radiation, Oxidative stress

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ABSTRACT *Apis mellifera foragers were exposed to various doses of gamma radiation, i.e., 1, 5, 10, 20, 30, 40, 50 and 60 Gy and 6, 12, 24, 48 and 72 h post irradiation samples were subjected to reduced glutathione (GSH) level estimation. Significant increases in the GSH levels were observed at 5 Gy (6 h); 10 Gy (6, 12 and 24 h); 20 and 30 Gy (6, 12, 24 and 48 h) and 40, 50 and 60 Gy (6, 12, 24, 48 and 72 h) samples with respect to their controls. There were no significant changes in the GSH level of 1 Gy irradiated flies when compared to their respective controls. The present study shows that the elevated GSH level in irradiated foragers might have elicited an adaptive response to neutralize the irradiation-induced oxidative stress.*

INTRODUCTION

The interests in nuclear power as a low carbon emission energy source, together with anxiety regarding past and possible nuclear accidents reveals the impact of radionuclides on the environment, dictates as an international issue (Fuller et al., 2015). The substantial release of ionizing radiation to zones near nuclear power plants may cause major permanent shifts in ecosystems and severe impairment to human health. Understanding the dangers and measuring the outcomes of nuclear accidents is of worldwide concern (Steen and Mousseau, 2014). Any system for assessing the impact of a contaminant on the environment requires an analysis of the possible effects of these on the organisms and ecosystems are on high concern (Copplestone et al., 2008).

Radioactive contamination can deleteriously affect the abundance of living beings through the radiation and chemical lethal effects of radionuclides or the effects of mutation accumulation over time (Moller et al., 2013). A survey of pollinating insects in the Chernobyl disaster area (after 25 years of nuclear accident) showed that *Apis mellifera* are currently very rare in the Chernobyl Exclusion Zone, leaving insect pollination to species of wild bees, bumblebees, and butterflies (Moller et al., 2012). Because of its morphology and ethological features (such as extensive area of patrol and foraging activity), the honey bee can be considered an admirable bioindicator (Porrini et al., 2003). Bee-farming products, which reflect work results of the bee family by a collection of nectar, pollen and plant gum in a radius of about 2 km, can serve as appropriate biological indicators of soil radioactive pollution (Pilipchuk and Arkhipov, 2001).

Gamma rays are uncharged ionizing particles and are the most penetrating radiation from natural and artificial sources (IAEA-TECDOC-1363, 2003). Ionizing radiation is composed of particles able to release electrons from atoms or molecules and thus generates partially reduced chemical species, the most common being reactive oxygen species (ROS), which are involved in chain reactions that are potentially detrimental to cells (Galvan et al., 2014; Riley, 1994). Several antioxidant enzymes (Superoxide dismutase, Catalase, Peroxidases, Glutathione S-transferase) and a network of low molecular weight antioxidants (ascorbate, glutathione, tocopherol and phenolic compounds) are identified to guard the cells from radiation-induced ROS (Suman et al., 2009). Glutathione, a vital thiol tripeptide (γ-glutamylcysteinylglycine) antioxidant present in cells, exists in two forms: in a reduced form as GSH and another in oxidized form as glutathione disulfide (GSSG) (Ates et al., 2009; Meister and Anderson, 1983; Rousar, 2012). Reduced glutathione (GSH) is the most important intracellular antioxidant, one of the antioxidants most liable to radiation and its redox status (GSH/GSSG) represents a significant index of cellular oxidative stress (Galvan et al., 2014). Antioxidant levels and the activity of antioxidative enzymes, which preclude cell injury instigated by oxidation, are key factors determining the good health of an organism (Clarkson and Thompson, 2000; Farjan et al., 2014). In view of the above information an attempt has been made to expose the forager bees to various doses of gamma radiation to determine the effect of radiation on GSH level.

MATERIALS AND METHODS

Foragers

Foragers for the irradiation studies were obtained according to the protocol of Shameer et al. (2016). Briefly, frames with sealed worker broods were collected from *A. mellifera* colonies and kept in an incubator. Newly emerged worker bees were collected, marked on the thorax and released back into the mother hives. On the 24th day, these marked foraging bees were collected at the hive entrance and brought back into the laboratory. Each 10 foragers were placed in a plastic cage (11 X 6.2 X 5 cm) and kept in an incubator maintained at 32°C, 60% relative humidity under 24 h dark condition. Bees were fed ad libitum with 50% sucrose solution and multifloral pollen paste.

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Irradiation and reduced glutathione estimation of foragers

Twenty five days old foragers were exposed with 1, 5, 10, 20, 30, 40, 50 and 60 Gray (Gy) doses of gamma radiation. Co-60 gamma radiation (Theratron 780-C Telecobalt unit) with a dose rate of 166.59 centiGray (cGy/min) was used as the radiation source. Exposure periods of 36 s, 3 min, 6 min, 12 min, 18 min, 24 min, 30 min and 36 min were used for 1, 5, 10, 20, 30, 40, 50 and 60 Gy doses, respectively. Dosimetry confirmed that the doses provided were within the 5% error range. Irradiated bees were maintained in an incubator. Ten replicates were irradiated for each dose and each replicates includes 8 bees. Further 6, 12, 24, 48 and 72 h post exposure samples were collected and same age non-irradiated flies were used as control. The bees were starved for 2 h before the sampling of the GSH estimation so that all bees were equal in terms of their gut contents (Shameer et al., 2016).

The bees were sacrificed for the experiment by keeping in the freezer, then were rinsed with phosphate-buffered saline (PBS), cautiously dried using a filter paper and weighed. Flies were homogenized using chilled PBS (pH - 7.0) and the homogenate was centrifuged at 3000xg for 12 min at 4°C. Supernatant was collected and GSH content was determined by Ellman (1959) method. The values were expressed as micrograms of GSH/100 mg wet weight of tissue.

Statistical analysis

Data collected were statistically analyzed by using SPSS v17 software and the values were expressed in mean ± standard error (SE). To test the change in the mean GSH level of irradiated and control foragers, 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 GSH level of irradiated and non-irradiated bees during 6, 12, 24, 48 and 72 h post irradiation time correspondingly. Reduced glutathione level was raised in irradiated foragers. Data showed that there were no significant changes ($P > 0.05$) in the GSH level of 1 Gy irradiated flies when compared to their respective controls. During 6 h post irradiation time 5, 10, 20, 30, 40, 50 and 60 Gy doses showed a significant surge in the GSH level ($F = 47.56$; df = 8, 81; $P < 0.05$). Irradiation of 10, 20, 30, 40, 50 and 60 Gy doses showed a significant rise in the GSH level at 12 h ($F = 82.21$; df = 8, 81; $P < 0.05$) and 24 h ($F = 119.07$; df = 8, 81; $P < 0.05$) post irradiation time. 20, 30, 40, 50 and 60 Gy dose γ- radiation exposure caused a significant elevation ($F = 44.70$; df = 8, 81; $P < 0.05$) in the 48 h GSH level of post irradiation sample. During 72 h post irradiation time 40, 50 and 60 Gy doses showed a significant increase ($F = 32.75$; df = 8, 81; $P < 0.05$) in the GSH level.

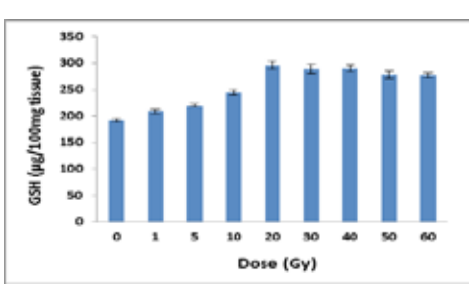

Figure 1: Mean ± SE GSH level of irradiated and control foragers during 6 h post irradiation time.

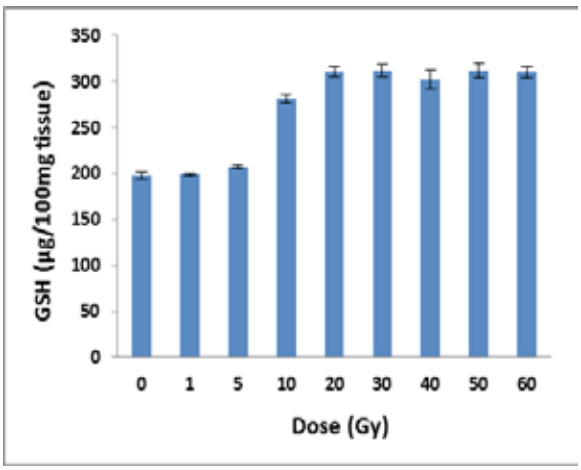

Figure 2: Mean ± SE GSH level of irradiated and control foragers during 12 h post irradiation time.

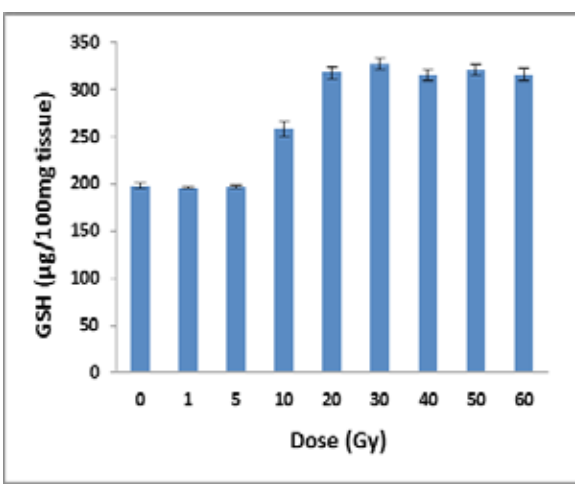

Figure 3: Mean ± SE GSH level of irradiated and control foragers during 24 h post irradiation time.

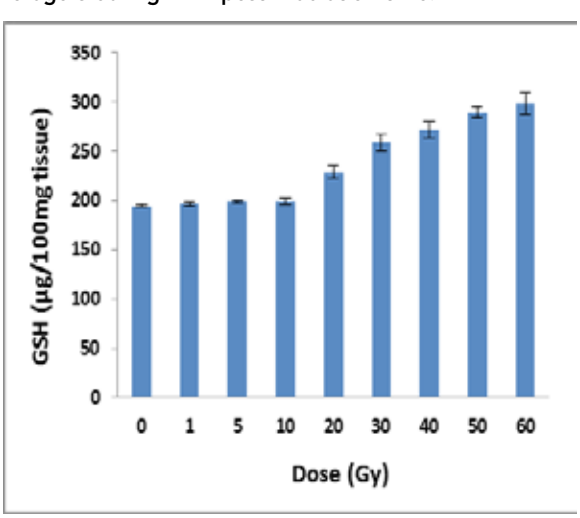

Figure 4: Mean ± SE GSH level of irradiated and control foragers during 48 h post irradiation time.

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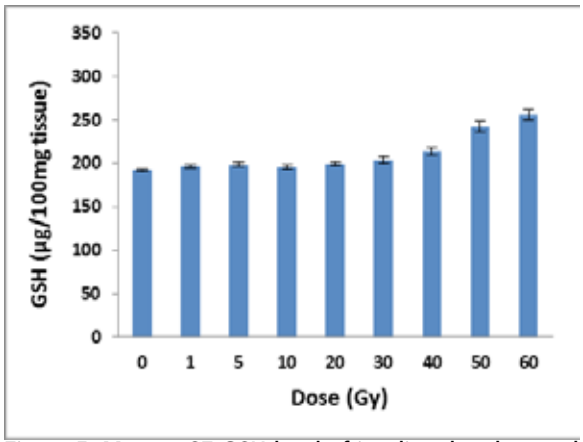

Figure 5: Mean ± SE GSH level of irradiated and control foragers during 72 h post irradiation time.

DISCUSSION

Thiol-group (SH-group) containing compounds are the imperative components, maintaining redox homeostasis in cells, tissues and biological fluids in an organism. Reversible modification of SH-groups is considered as a nonspecific defense mechanism of an organism in response to the extreme conditions. SH-group containing compounds are the subject of oxidative stress in the first place and deliver the first line of defense through hunting of hydroxyl radicals. Thiol's autooxidation, leading to cellular and tissue hypoxia, is considered as the defensive reaction of thiol-containing compounds against irradiation and its accompanying oxidative stress (Sapojnikova et al., 2012). GSH is the most important and ubiquitous low molecular weight thiol compound (Gaur, 2010). Cellular radiosensitivity has been shown to be inversely correlated to the endogenous level of reduced glutathione (Chatterjee, 2013).

Galvan et al. (2014) reported that chronic exposure to low-dose radiation at Chernobyl favours adaptation to oxidative stress (GSH levels and body condition increased, and oxidative stress and DNA damage decreased) in birds with increasing background radiation. Gamma irradiation studies in Sf9 cells (originally derived from the ovaries of lepidopteran insect *Spodoptera frugiperda*) and BMG-1(Human brain malignant glioma) showed that significantly fewer radiation-induced reactive oxygen/nitrogen species generation, protein carbonylation and growth inhibition in Sf9 cells as compared to mammalian cells. Sf9 cells have elevated basal ascorbate peroxidase (~4-fold), catalase (~1.7- fold), superoxide dismutase (~1.3-fold) activity and GSH level (~2.2-fold) when compared to mammalian cells. This implies the stronger antioxidant system of Sf9 cells which protects against radiation-induced macromolecular damage, growth inhibition and cell death (Suman et al., 2015). In the present study irradiated bees showed an elevated GSH level. Gamma irradiation studies in RAW 264.7 cells (mouse macrophage-like cell line) showed that low dose gamma irradiation induced increase in glutathione level is mediated by transcriptional regulation of the γ-*GCS* gene, mainly through the AP-1 binding site in its promoter (Kawakita et al., 2003).

Evaluating the biological effect of ionizing radiation on non-human biota has been recognized as an essential approach towards protecting and mitigating the impacts of future radioactive releases to the environment by a number of international directives (e.g., ERICA and PROTECT [Howard et al., 2010; Larsson, 2008; Fuller et al., 2015]). Our earlier study showed that gamma irradiation of forager bees caused a dose (1-60 Gy) dependent increase and post irradiation time (6-72 h) dependent decrease in lipid peroxidation (LPO) level. This indicated that oxidative stress takes place by the action of irradiation and the post irradiation time decrease in LPO levels might be due to the action of antioxidants (Shameer et al., 2016). Ionizing radiation produces oxidative stress, but organisms can acclimatize to their exposure with physiological adaptive responses (Galvan et al., 2014). Increase in GSH contents of gamma ray irradiated foragers in the present study may be an adaptive response to neutralize the irradiation-induced oxidative stress.

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