



Studying the Ameliorative Effect of Bee Venom Against Damage and Inflammation Induced in Gamma-Irradiated Rats

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The aim of the present study is investigating the possible protective effect of Bee Venom (BV) against gamma radiation induced damage and inflammation in male rats. Gamma-irradiation (6 Gy) resulted in a significant elevation in the level of tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), creatinine kinase-MB (CK-MB) and cardiac troponin I (cTnI), lactate dehydrogenase (LDH), creatine phosphokinase (CPK), serum aspartate transaminase (AST) and alanine transaminase (ALT), serum glucose, malondialdehyde (MDA) concentration and xanthine oxidase activity associated with remarkable decrease in insulin level, glutathione content (GSH) and the activity of xanthine dehydrogenase (XDH), superoxide dismutase (SOD) and catalase (CAT) in heart and liver tissues compared to control group. Injection of BV (5ml/kg b. wt. / day/6weeks) to γ -irradiated rats was found to offer protection against γ -irradiation induced oxidative stress and significantly ameliorated the changes occurred in the above investigated biochemical parameters. It could be concluded that Bee venom clarified a modulatory role against gamma radiation induced oxidative damage and inflammation in the heart and liver tissues.

Keywords: Bee venom/ Ionizing radiation/ Inflammation/ Antioxidants

Introduction

Ionizing radiation (IR) can cause changes in the chemical balance of cell and affects humans by depositing energy in body tissue [1]. Exposure to ionizing radiation increase the production of reactive oxygen species (ROS) such as superoxide ($O_2^{\cdot -}$), hydroxyl radical (OH^{\cdot}) and hydrogen peroxide (H_2O_2) and cause lipid peroxidation in cell membrane and damage to cellular activities leading to physiological disorders and dysfunction of cells and tissues [2]. Furthermore, IR induces impairment of the immune response as well as a persistent inflammatory status through the deregulation of cytokine production and also induces pro-inflammatory processes in which tumor necrosis factor (TNF- α), interferon (IFN- γ) and interleukin levels are altered, eventually

leading to inflammatory disorders [3]. Use of antioxidants from natural sources may provide protection against irradiation as antioxidants are capable to scavenge free radicals from the radiolysis of water and can protect cells from damage [4].

Bee venom therapy is a treatment modality that may be thousands of years old and involves the application of live bee stings to the patient's skin or, in more recent years, the injection of bee venom into the skin with a hypodermic needle [5]. The venom of honey Bee (*Apis mellifera*) is a natural toxin produced by the honey bee and it has a prime role of defense for the bee colony [6]. It consists of complex mixtures of biologically-active proteins and peptides, such as phospholipases, hyaluronidase, phosphatase, α -glucosidase,

serotonin, histamine, dopamine, noradrenaline, and adrenaline. Additionally, it contains melittin, apamin, and mast cell degranulating peptide in BV [7]. Bee venom (BV) has been used as a traditional medicine as a potential anti-bacterial agent against inflammatory skin disease [7]. Furthermore, the anti-inflammatory effects of bee venom and its individual compounds might be attributed to their capacity to reduce pro-inflammatory cytokines such as tumor necrosis factor (TNF- α) and interleukins (IL 1 β , or IL-6) [8]. The present investigation is carried out to investigate the biological effects of bee venom on biochemical alteration and inflammation induced by gamma-rays in rats.

Material and Methods

Lyophilized *Apis Mellifera* purified Bee venom (BV) was obtained from VACSERA, Egypt (1mg/vial) It was reconstituted using 1 ml sterile distilled water and then injected into rats subcutaneously at a dose level (5 μ g/kg) according to **Nahed and Amany** [9]. Chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Radiation Facility

Whole body gamma irradiation of Wistar rats at a dose level of (6 Gy) was performed using a Canadian Gamma Cell-40, (^{137}Cs) (Atomic Energy of Canada Ltd, Ottawa, Ontario, Canada), located at the National Center for Radiation Research and Technology (NCRRT), in Nasr City, Cairo, Egypt. The dose rate of the irradiation process was 0.43 Gy/min at the time of the experiment calculated according to the dosimeter department in the NCRRT.

Experimental Animals

Male Wistar rats (10 \pm 2 weeks old; 120 \pm 20 g) were purchased from the Egyptian Holding Company for Biological Products and Vaccines (Cairo, Egypt) and used for the different investigations carried out in the present study. Wistar rats were acclimated to controlled laboratory conditions for two weeks. Wistar rats were maintained on stock rodent diet and tap water that were allowed ad libitum. All animal procedures were carried out in accordance with the Ethics Committee of the National Research Centre conformed to the Guide for the Care and Use of Laboratory Animals, published by the US National

Institutes of Health (NIH publication No. 85 – 23, 1996).

Experimental Design

The animals (28 rats) were randomly divided into 4 groups (seven Wistar rats in each group) as follows; Control group: rats fed on a balanced diet for 6 weeks, BV group: Rats were injected intraperitoneal with BV at dose (5ml/kg b. wt./day) [9] for 6weeks, IRR group: rats were irradiated with one shot dose (6Gy) at the 1st week of the experimental period and injected intraperitoneal (i.p.) with 0.9% isotonic saline solution at a dose (10 ml/kg b.wt) [10], and Group (IRR & BV group): irradiated animals were daily injected intraperitoneal (i.p.) with BV (5 ml/kg b.wt)

At the end of the experiment, the rats were fasted for 8 hours and anesthetized with diethyl ether. Blood samples were collected through heart puncture and allowed to coagulate and centrifuged for to obtain serum for biochemical analysis. Also, liver and heart were removed for biochemical investigation.

Biochemical Analysis

Serum levels of tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6), creatinine kinase-MB (CK-MB) and cardiac troponin I (cTnI) were performed by ELISA technique (BioSource International, Camarillo, CA, USA) according to the manufacturer's instructions. The levels of lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) were determined using the method of **King** [11], aspartate transaminase (AST) and alanine transaminase (ALT) were determined according to **Reitman and Frankel** [12]. Serum samples were analyzed for glucose [13] and insulin hormone was determined by radioimmunoassay kit supplied by Diasari, Italy. Liver and heart were dissected, thoroughly washed with ice-cold 0.9% NaCl, weighed, minced and homogenized (10% w/v) using 66 mmol/L chilled phosphate buffer (pH 7.0). The tissue homogenates were centrifuged at 6000 rpm for 15 min and the supernatants were used to estimate the level of malondialdehyde (MDA) [14], the activity of xanthine oxidase (XO) and xanthine dehydrogenase (XDH) [15], glutathione content (GSH) [16] and the activity of superoxide dismutase (SOD) [17] and catalase (CAT) [18].

Statistical Analysis

The results were presented as mean \pm SE (n = 7). Experimental data were analyzed using one way analysis of variance (ANOVA). Duncan's multiple range test was used to determine significant differences between means. The statistical analyses were performed using the computer program, Statistical Packages for Social Science (SPSS) [19]. Differences between means were considered significant at $P < 0.05$.

Results

The results, in Table (1), show that there were significant elevation in the level of TNF- α and IL-6 of gamma-irradiated group compared to BV and control groups. Supplementation of γ -irradiated Wistar rats with BV showed significant decrease in the level of TNF- α and IL-6 relative to γ -irradiated group.

The results, in Table (2), indicate that exposure of the whole body to a single dose of gamma radiation induced a highly significant increase in serum levels of ALT, AST, ALP, LDH and CPK, CK-MB and cardiac troponin I (cTnI) as compared with the corresponding normal values. Treatment of gamma-irradiated rats with BV resulted in a significant reduction in the levels of

the above mentioned parameters when compared to gamma-irradiated group.

The results, in Table (3), record a remarkable increase in the level of glucose with a significant decrease in insulin level as a result of exposure to gamma-rays relative to the control group. Injection of gamma-irradiated rats with BV induced significant reduction in glucose concentration and elevation of insulin level compared to gamma-irradiated rats.

Gamma-irradiation resulted in significant increases in hepatic and cardiac MDA and XO activity and decreases in GSH level and the activity of XDH, SOD and CAT compared to control rats. Treatment with BV showed a high significant decrease in MDA level and XO activity with marked significant increase in GSH level, XDH, CAT and SOD activities comparing with gamma-irradiated rats (Table4).

Table (1): Effect of BV injection on the level of TNF- α and IL-6 of γ -irradiated rats

Parameters	Control	BV	IRR	IRR+ BV
TNF- α (pg/mL)	682.23 \pm 50.84 ^b	675.81 \pm 49.18 ^b	911.52 \pm 58.63 ^a	693.56 \pm 60.52 ^b
IL-6 (pg/mL)	339.81 \pm 27.27 ^c	332.63 \pm 25.38 ^c	488.95 \pm 34.67 ^a	397.12 \pm 23.75 ^b

Values are expressed as means \pm S.E. (n=7).

Values in the same row with different superscript are differing significantly at $P < 0.05$

BV: Bee venom group

IRR: irradiated group

Table (2): Effect of BV injection on the serum LDH, CPK, ALT and AST activities of γ -irradiated rats

Parameters	Control	BV	IRR	IRR+ BV
ALT (U/L)	25.56 \pm 0.78 ^c	25.39 \pm 0.82 ^c	40.12 \pm 0.84 ^a	30.64 \pm 0.83 ^b
AST (U/L)	36.85 \pm 1.86 ^c	35.56 \pm 1.72 ^c	61.51 \pm 2.23 ^a	44.11 \pm 1.62 ^b
LDH (U/ml)	226.96 \pm 13.25 ^c	224.35 \pm 12.52 ^c	447.25 \pm 20.12 ^a	335.82 \pm 17.42 ^b
CPK (U/L)	273.46 \pm 8.71 ^c	268.27 \pm 8.32 ^c	483.18 \pm 11.59 ^a	334.45 \pm 12.66 ^b
CK-MB (ng/mL)	3.28 \pm 0.68 ^c	2.92 \pm 0.71 ^c	7.85 \pm 0.93 ^a	5.96 \pm 0.83 ^b
cTnI (ng/mL)	26.18 \pm 1.16 ^c	25.48 \pm 1.25 ^c	74.08 \pm 2.56 ^a	45.80 \pm 1.93 ^b

Values are expressed as means \pm S.E. (n=7).

Values in the same row with different superscript are differing significantly at $P < 0.05$

BV: Bee venom group

IRR: irradiated group

Table (3): Effect of BV injection on the serum glucose and insulin levels of γ -irradiated rats

Parameters	C	BV	IRR	IRR+BV
Glucose(mg/dl)	120.10 \pm 3.36 ^c	112.63 \pm 3.57 ^c	156.88 \pm 5.42 ^a	130 \pm 3.83 ^b
Insulin(μ U/ml)	34.66 \pm 2.63 ^c	35.23 \pm 2.62 ^c	20.76 \pm 2.85 ^a	28.77 \pm 2.46 ^b

Values are expressed as means \pm S.E. (n=7).

Values in the same row with different superscript are differing significantly at $P < 0.05$

BV: Bee venom group

IRR: irradiated group

Table (4): Effect of BV injection on the oxidants and antioxidant levels in the heart and liver γ -irradiated rats

Parameters		Control	BV	IRR	IRR+ BV
MDA (n mol/g tissue)	Liver	160.91 \pm 3.54 ^c	154.28 \pm 3.76 ^c	256.32 \pm 5.47 ^a	191.58 \pm 4.48 ^b
	Heart	78.12 \pm 0.82 ^c	75.23 \pm 1.22 ^c	129.36 \pm 2.18 ^a	88.52 \pm 2.33 ^b
XO (mU/mg protein)	Liver	2.57 \pm 0.13 ^c	2.48 \pm 0.14 ^c	3.90 \pm 0.17 ^a	2.92 \pm 0.15 ^b
	Heart	5.11. \pm 0.25 ^c	5.02. \pm 0.22 ^c	6.72 \pm 0.35 ^a	5.50 \pm 0.27 ^b
XDH (mU/mg protein)	Liver	3.46 \pm 0.14 ^a	3.55 \pm 0.15 ^a	1.67 \pm 0.12 ^c	2.98 \pm 0.13 ^b
	Heart	13.84 \pm 0.42 ^a	13.87 \pm 0.46 ^a	9.62 \pm 0.41 ^b	13.23 \pm 0.56 ^a
GSH (mg/g tissue)	Liver	28.36 \pm 1.17 ^a	28.65 \pm 1.05 ^a	16.19 \pm 0.65 ^b	26.78 \pm 0.88 ^a
	Heart	15.87 \pm 0.58 ^a	16.18 \pm 0.56 ^a	10.38 \pm 0.49 ^c	13.79 \pm 0.52 ^b
SOD (U/mg protein)	Liver	45.34 \pm 1.25 ^a	45.81 \pm 1.32 ^a	29.17 \pm 0.90 ^c	39.25 \pm 0.86 ^b
	Heart	27.95 \pm 1.32 ^a	28.12 \pm 1.27 ^a	22.53 \pm 1.18 ^c	25.10 \pm 1.29 ^b
CAT (U/mg protein)	Liver	32.17 \pm 1.11 ^a	32.56 \pm 1.13 ^a	17.54 \pm 0.76 ^c	28.45 \pm 0.88 ^b
	Heart	39.57 \pm 1.15 ^a	40.08 \pm 1.03 ^a	23.83 \pm 1.02 ^c	33.64 \pm 1.25 ^b

Values are expressed as means \pm S.E. (n=7).

Values in the same row with different superscript are differing significantly at $P < 0.05$

BV: Bee venom group

IRR: irradiated group

Discussion

The present study is designed to address the effect of using BV as a natural antioxidant in the amelioration of damage effects resulting from gamma-radiation exposure. Gamma-irradiation of rats, in the present work, resulted in increased serum TNF- α and IL-6 indicating the role of these cytokines in irradiation-induced toxicity. Similarly, the study of **Shah** [20] found that the production of TNF- α and IL-6 was elevated significantly after radiation exposure. Exposure of Wistar rats to γ -radiation induced production of ROS that mediate the activation of transcription factor NF- κ B. The activation of NF- κ B up-regulates the expression of genes of the pro-inflammatory cytokines [IL-1 β , TNF- α and IL-6] [21]. On the other hand, treatment with BV post gamma-irradiation showed a significant reduction in both TNF- α and IL-6 levels, as compared to gamma-irradiated non-treated group. These results are in agreement with other studies that confirmed the potent anti-inflammatory action of BV through the direct

inhibition of NF- κ B transcription factor [22] and key inflammatory mediators, such as TNF- α [23]. In the present study, a single dose (6Gy) of whole body gamma irradiation induced a marked increase in serum enzymes (CPK and LDH activity), in addition to the increase in CK-MB and cardiac troponin 1 (cTnI). The mechanism of radiation-induced toxicity has been reported to be through the formation of superoxide anions and their derivatives, particularly highly reactive and damaging hydroxyl radicals, which induces peroxidation of cell membrane lipid [4]. LDH is released during tissue injury and the increase in its reported value usually indicate cellular death and leakage of the enzyme from the cell [24]. This study reported also that the Wistar rats exposed to 6 Gy of γ -radiation have highly significant increase in the activities of the serum AST and ALT and that could be referred to the drastic dysfunction of the liver cells induced by radiation interaction with cellular membranes and also related to an extensive breakdown of liver parenchyma [25].

The present study found that treatment of gamma-irradiated Wistar rats with BV had significantly decreased the elevation of serum ALT, AST, LDH, CK-MB and cardiac troponin 1 (cTnI) levels, indicating the protective effect of BV, which might be explained by the reduction of elevated hepatic nuclear factor kappa B (NF- κ B) expression in liver [26]. These results are consistent with other studies that showed the potent protective effect of BV by inhibiting the secretion of pro-inflammatory cytokines, and decreasing the elevated serum amino-transferase enzymes in different models of induced hepatic injury [27].

A significant elevation in serum glucose level with noticeable reduction in insulin concentration was observed in the group of gamma-irradiated rats relative to control rats. The recorded hyperglycemia in the present results could be attributed to endocrine abnormalities induced by irradiation, that promote the secretion of peptide which has relation to carbohydrate metabolism, by increasing gluconeogenesis in liver [28]. Hyperglycemia may result from accelerated gluconeogenesis [29] and diminished utilization of glucose by irradiated tissues [30]. The lowering effect of γ -irradiation exposure on insulin level could be attributed to the production of free radicals that induced oxidative stress, resulted in reduction in insulin secretion and DNA damage [31]. Serum glucose level decreased and insulin level increased following treatment of gamma-irradiated Wistar rats with bee venom. This may be attributed to substances like mellitin and phospholipase A2 contained in the venom. They may play a role in diminishing inflammation of Islets of Langerhans and thus elevating insulin level. With regard to the fact that insulin regulates blood glucose level, bee venom could decrease glucose content via increasing insulin secretion [32].

The present results demonstrated a significant reduction in hepatic and cardiac GSH concentration and the activity of XDH, SOD and CAT as well as elevation in MDA and the activity of XO by γ -irradiation. This could be attributed to enhanced utilization of the antioxidant system in an attempt to detoxify radiation generated free radicals [33]. The decrease in the activity of antioxidant enzymes might result from radiation-induced cell membrane damage and alterations in dynamic permeability of membranes due to peroxidation, which is followed by the release of

intracellular enzymes to the blood stream [34]. Xanthine oxidoreductase system includes two intra-convertible enzymatic activities XO and XDH. Gamma-irradiation may have caused the conversion of XDH to XO resulting in an increase in XO-specific activity in both time intervals [35]. GSH reduction has often been considered to be indicative of increased oxidative stress and thus result in tissue damage [36]. The decrease in the level of GSH and the activities of SOD and CAT might be due to their utilization by the enhanced production of ROS, which interacts with the enzyme molecules causing their denaturation and partial inactivation [37]. **Abdel-Magied and Ahmed** [38] reported that the decrease in SOD activity may result in an increased flux of super oxide in cellular compartments which may be the reason for the increased in MDA.

The present results recorded that injection of BV post gamma-irradiation induced a significant decrease of in tissue MDA and the activity of XO, associated with a significant increase in serum GSH concentration and the activity of XDH, SOD and CAT compared to gamma-irradiated non treated group. **Stanley et al.**, [39] found that Bee venom inhibits the production of superoxide anion by human neutrophils. **Rain** [40] and **Hegazi** [41] stated that BV therapy is a potent antioxidant leading to a decrease in the levels of reactive oxygen species (ROS), which may be associated with the observations of BV affecting glutathion, superoxide dismutase (SOD) and catalase.

It could be concluded that exposure to gamma radiation, led to biochemical disorders, inflammation and oxidative damage. Moreover, the use of Bee venom as an antioxidant drug results in suppression of adverse effects caused by gamma radiation and acts as a potent scavenger of free radicals to prevent or ameliorates the toxic effects of ionizing radiation as shown in this study, and BV might provide substantial protection against radiation induced inflammatory damages.

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