Royal Jelly Attenuates Ionizing Radiation Induced Cardiac Toxicity in Male Albino Rats

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Abstract

The aim of this study was focused on investigating the possible protective effect of Royal Jelly (RJ) against gamma radiation induced cardiotoxicity and inflammation in male albino rats. Twenty four albino rats were divided into four equal groups as follows: Control group: Without radiation or treatment, irradiated group: Animals subjected to whole body gamma irradiation at a dose level of 6Gy, treated group: Rats were given orally RJ at a dose of 250mg/kg/day for 15 days by oral tube and treated irradiated group: Animals were treated orally with RJ at a dose of 250mg/kg/day for 15 days by oral tube, then exposed to whole body gamma irradiation at a dose of 6Gy. The obtained results revealed that the administration of RJ to irradiated rats significantly ameliorated the changes occurred in the investigated biochemical parameters. The histopathological results showed distinctive pattern of myocardial injuries in irradiated group, while in treated-irradiated group the myocardial tissues showed minimum injury with or without congested blood vessels or edema. In conclusion, RJ acts as a potent scavenger of free radicals to prevent or ameliorates the toxic effects of gamma irradiation. Also, RJ might provide substantial protection against radiation-induced inflammatory damages.

Key Words: Antioxidant – Radiation – Royal jelly – Male rats.

Introduction

IONIZING radiation (IR) has attracted a lot of attention due to its beneficial as well as possible harmful effects to human population [1]. Ionizing radiation is known to induce oxidative stress through generation of reactive oxygen species (ROS) resulting in imbalance of the pro-oxidant and antioxidant in the cells, which is suggested to culminate in cell death [2]. The deleterious effects of the free radicals are kept under check by a delicate balance between the rate of their production and the rate of their elimination by body’s defense systems. When there is an excessive addition of free radicals from exogenous sources added to the endogenous production, the available tissue defense system becomes overwhelmed resulting in oxidative damage to the tissues [3]. Radiation exposure attenuates endogenous antioxidant enzymes, which are considered to function as a part of the first line defense mechanism to maintain redox balance and normal biochemical processes. Thus, supplementation of antioxidants to improve the efficacy of radiotherapy is a current proposed strategy as antioxidants are capable to scavenge free radicals from the radiolysis of water and to protect cells from damage [4].

Recently, royal jelly (RJ) has received particular attention because of studies that have reported that it is a highly efficient antioxidant and has free radical scavenging capacity [5,6]. RJ is a secretion produced by the hypopharyngeal and mandibular glands of worker honeybees (Apis mellifera). It contains many important compounds with biological activity such as free amino acids, proteins, sugars, fatty acids, minerals, and vitamins [7]. So far, RJ has been demonstrated to possess several physiological activities in experimental animals, including vasodilative and hypotensive activities [8], the induction of RJ decreases serum cholesterol levels [9], antimicrobial [10], antiallergic [11], anti-inflammatory [12], immunomodulatory [6,13] and antioxidant properties [7]. In addition [14], revealed the protective effect of RJ against paracetamol induced liver damage in mice. Apoptosis is a gene-regulated event related to special morphological changes such as shrinkage of cell, chromatin condensation, and DNA damages [15]. Thus, the aim of the present study was to investigate the protective effect of RJ in gamma radiation-induced oxidative damages of rat heart by biochemical and histopathological methods.
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Material and Methods

Animals:
Male Swiss Albino rats (135-150g) were obtained from the Egyptian organization for biological product and vaccines Giza, from Jan. 2013 – Aug. 2013 Egypt. Animal were kept under good ventilation and illumination condition and received standard diet and had free excess to water.

Chemicals and drugs:
RJ was obtained from Sigma Company (Cairo).

Preparation of royal jelly:
Doses of 250mg/kg/day RJ were dissolved in distilled water and aliquot of different concentrations were given orally to animals with a gavages needle.

Radiation processing:
It was performed by using gamma cell-40 (Cesium-137) located at the National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt. Animals were irradiated at an acute single dose level of 6Gy delivered at a dose rate of 0.46Gy/min. at the time of experimentation. Animals were not anesthetized before irradiation.

Experimental design:
Twenty four rats were divided into four groups (n=6). Group I (control group), included rats neither treated nor irradiated. Rats of this group received orally an equivalent volume of distilled water (vehicle of RJ) during the period of RJ administration. Rats in group II (irradiated group) were exposed to 6Gy whole body gamma radiations as a single dose shot and like control group received orally an equivalent volume of distilled water. In group III (treated group), rats were administrated by RJ (250mg/kg/day) by stomach tube for 15 days. Group IV (treated irradiated group) included rats that were administrated by RJ (250mg/kg/day) by stomach tube for 15 days before exposure to whole body gamma irradiation of 6Gy as a single dose shot. Rats were sacrificed on the 3rd day post radiation exposure or RJ administration and subjected to serum and heart biochemical and histopathological investigations.

Samples collection:
After an overnight fasting, rats were anesthetized with ether and then sacrificed. Blood samples from each rat were collected by retro-orbital puncture using blood capillary tubes. Serum was obtained immediately by centrifugation of blood samples at 3000 rpm for 10min. Heart was directly separated after sacrificing, washed in ice-cold saline then homogenized in distilled water (10% W/V) using homogenizer. The cell debris was removed by centrifugation at 3000 rpm for 10min. The homogenates supernatant were subjected to biochemical analysis. Tissue specimens from heart were collected and fixed in 10% buffered formalin solution and fixed tissues were dehydrated, cleared and embedded in natural paraffin. Another sample of the heart animals were isolated and prepared for paraffin blocks. Section of 5-micron thickness were prepared and stained routinely with haematoxylin and eosin [16] and examined microscopically.

Estimation of biochemical parameters:
Creatine phosphokinase (CPK) level was estimated according to the method of [17] and lactate dehydrogenase (LDH) was assayed depending on the method of [18]. Moreover, serum total cholesterol (TC) concentration was estimated as described by [19]. High density lipoprotein-cholesterol (HDL-C) and low density lipoprotein-cholesterol (LDL-C) were determined according to the methods described by Demacker et al., [20] and Marchal [21] respectively. The activity of asparatate amino transferase (AST) was determined according to the method of Reitman and Frankel [22]. Serum levels of 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. CAT activity was assayed using the method of Sinha [23]. SOD was determined according to the method described by Kakkar and Vishwanath [24] and MDA level was estimated following the method reported by Buege and Aust [25].

Statistical analysis:
Data were analyzed using one way analysis of variance (ANOVA). The results obtained were expressed as mean±standard deviation (SD) of the mean. Differences were considered significant at p<0.05 [26].

Results
As presented in Table (1), whole body gamma irradiation induced a significant increase in the activity of serum CPK, LDH, cholesterol and LDL-C while a significant decrease in HDL-C concentration was noticed compared to control group. Pretreatment with RJ prior to gamma irradiation was found to significantly abolish these radiation-induced elevations in the levels of serum CPK, LDH, cholesterol and LDL-C and also maintained
the level of HDL-C near the normal level. Serum levels of creatine kinase-MB (CK-MB) and cardiac troponin 1 (cTnI) were a significant increase in irradiated group. Animal group treated with RJ showed non-significant changes in the concentration of serum CPK, LDH, cholesterol, LDL-C, HDL-C, cTnI and (CK-MB) compared to those of control group.

The effects of gamma radiation on endogenous antioxidant status are shown in Table (2). Gamma-irradiation induced a significant decrease in the activity of heart CAT and SOD as well as a significant increase in the level of MDA compared to control group. Administration of RJ prior to gamma irradiation of rats restored the reduced CAT and SOD activity while it decreased MDA level compared to irradiated group. Animal group treated with RJ showed insignificant changes in the activity of heart CAT, SOD and MDA compared to those of control group.

Light microscopic examination of cardiac muscle of group I (controls) showed the appear of cardiac muscle cells normal with one or two large oval nuclei occupying a central position. The perinuclear sarcoplasm region is distinct. The intercalated disks are irregular and wider than the normal cross-striations, and represent specialized junctions between cardiac muscle fibers (Fig. 1). In group II (the irradiated group), the myocardial muscles were necrotic with effacement of their structural details of their and replaced by spindle cells (fibroblasts) in some cases. In other cases the necrotic muscles fibers were middle infiltrated with leucocytes associated with loss of striation and disappearance of nuclei and intercalated disks (Fig. 2). Moreover, in few cases, edema and hyalinization of myocardial muscles were remarked (Fig. 3). In group III (RJ treated rats), the cardiac muscle showed normal structure (Fig. 4). On the other hand, most cases of group IV (treated irradiated group) myocardial muscle showed slightly preserved architecture without necrosis, degenerative changes or hyalinization. In few cases preserved myocardial muscle were seen with dilatation of coronary blood vessels and edema (Fig. 5).
Discussion

The exposure to ionizing radiation is known to induce oxidative stress. Oxidative modification of DNA, proteins, lipids and small cellular molecules by reactive oxygen species (ROS) which plays a role in a wide range of common diseases and age-related degenerative conditions [27,28]. These include cardiovascular disease [29], inflammatory conditions, and neurodegenerative diseases such as Alzheimer’s disease [30]. Oxidant damage by ROS is linked to photoaging, radiation toxicity, cataract formation and macular degeneration; it is implicated in ischemia/reperfusion tissue injury and also play a role in decreased function of some immune cells. Antioxidants including those in RJ, which protect against oxidative damage lower the risk of injury to vital molecules and to varying degrees may help or prevent the onset and progression of diseases [28,31].

A single dose (6Gy) of whole body gamma irradiation induced a marked acute cardiotoxicity in rats. This was demonstrated by increased cardiac serum enzymes (CPK and LDH activity), in addition to the increase in total cholesterol, LDL-C, HDL-C, (CK-MB) and cardiac troponin 1 (cTnI). A significance decrease in HDL, with the disturbance in antioxidant status (Tables 1,2) had been noticed. It is well known that the magnitude of CK
and LDH activities in blood after myocardial injury reflects the extent of damage in its musculature [32]. The mechanism of radiation-induced cardiotoxicity has been reported to be through formation of superoxide anions and their derivatives, particularly highly reactive and damaging hydroxyl radicals, which induces peroxidation of cell membrane lipid [33].

The cardiotoxicity occurs following exposure of rats to gamma radiation was significantly ameliorated by RJ treatment (Tables 1,2). It has been shown that chronic intake of RJ enhanced superoxide dismutase and catalase activities in heart tissue which offers protection against oxidative stress associated with ischemic reperfusion injury [34,35]. Moreover, all biochemical parameters are significant attenuated to normal levels. Khaled et al., [36] reported that the biochemical, hemato- logical and histological amelioration observed in royal jelly treated irradiated rats might be due to the antioxidant capacity of royal jelly active constituents. Silici et al., [5] and Cemek et al., [6] suggested that royal jelly has received particular attention because of studies that have reported that it is a highly efficient antioxidant and has free radical scavenging capacity.

The histopathological results of the present study indicated that irradiated group (2) revealed the myocardial muscles were necrotic with effacement of their details and replaced by spindle cells (fibroblasts) in some cases. In other cases the necrotic muscles fibers were highly infiltrated with leucocytes associated with loss of striation and disappearance of nuclei and intercalated disks Moreover, in few cases, edema and hyalinization of myocardial muscles were remarked. While in treated irradiated group (group 4) the structure of myocardial muscles were showed slightly preserved architecture without necrosis, degenerative changes or hyalinization. In few cases preserved myocardial muscle were seen with dilatation of coronary blood vessels and edema. Such results are agreement with the results reported by Khaled et al., (2011) who recorded that the histopathology of heart of irradiated rats were sticky, ill-defined shape and severely damaged cardiac muscle fibres, sever hyaline degeneration, dilated congested vein and congested blood capillary. They added that The supplementation of RJ to rats before the exposure to the irradiation exposure ameliorate the structure of cardiac muscles, improve nuclei and ameliorating features of capillaries and veins in endomysium were noticed.

In conclusion, RJ acts in the cardiac muscle as a potent scavenger of free radicals to prevent or ameliorates the toxic effects of ionizing radiation as shown in the biochemical and histopathological study and also royal jelly might provide substantial protection against radiation induced inflammatory damages.

References
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