Egypt. J. Rad. Sci. Applic. **Vol. 33,** No.2, pp. 77- 88 (2020)

Egyptian Journal of Radiation Sciences and Applications
http://ejrsa.journals.ekb.eg/

EGYPTIAN ATOMIC ENERGY AUTHORITY

The Efficacy of Royal Jelly in the Restoration of Liver Injury in Irradiated Rats

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RADIATION therapy is associated with a risk of long-term adverse effects. Royal jelly (RJ), a natural product extracted from honey bees is rich in compounds with many biological activities. This study aims at evaluating the role of RJ against gamma-radiation induced liver injury. Twenty-four male albino rats were divided into four groups. Control, IRR: whole-body exposed to gamma radiation (3 Gy every 3 days up to 9 Gy), RJ: received RJ by gavages (250 mg/kg BW) during 15 days, RJ+IRR: received RJ before the 1st radiation fraction and the treatment continued for 14 days. Animals were sacrificed one day post the last RJ dose. The results revealed that RJ significantly reduced the radiation-induced increases in liver malondialdehyde MDA, protein carbonyl CO, and 8-hydroxy-2-deoxyguanosine (8-OHdG), markers of lipid, protein and DNA oxidation, accompanied by significant elevations of glutathione content (GSH) and superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities. A significant reduction in the elevation of liver DNA fragmentation and caspase-3 activity was also recorded. In the serum, the level of the inflammatory markers, tumor necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β) and the level of the fibrotic markers collagen IV and laminin were significantly lower than their corresponding values in irradiated rats. The amelioration in the extent of liver injury obtained by RJ treatment was substantiated by significant improvement of liver functions verified by lowering the activity levels of liver function enzymes. It could be concluded that RJ by suppressing oxidative stress and decreasing inflammatory, apoptotic and fibrotic markers may alleviate the progression of radiation-induced liver injury.

Keywords: Apoptosis, Fibrosis, Inflammation, Liver, Radiation, Rats, Royal Jelly.

Introduction

Liver is an active metabolic organ that is easily influenced by many environmental stresses including chemicals, UV and ionizing radiation and organism's endogenous processes such as replication stress and metabolism. Liver is mostly exposed to radiation during radiotherapy or total body irradiation. Exposure to ionizing radiation can induce functional and structural changes in the liver (Kim & Jung, 2017) mainly caused by the excess of free radicals and oxidative stress.

Oxidative stress is a crucial factor in liver damage (Jadeja et al., 2017). Hepatocyte's lipids, proteins, and nucleic acids are among the cellular structures to be affected primarily by reactive oxygen species (ROS). It is well documented that lipid peroxidation disrupts the normal membrane structure (Niki, 2009), proteins oxidation affect signal transduction and DNA repair enzymes (Dalle-Donne et al., 2003; Munoz-Rugeles et al., 2018; Gegotek & Skrzydlewska, 2019)Moreover, DNA oxidation leads to the formation of 8-hydroxy-deoxyguanosine (8-OHdG) in addition to mutation in the DNA strands (Voulgaridou et

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Received 27/6/2020; Accepted 11/10/2020
DOI: 10.21608/ejrsa.2020.33962.1099

al., 2011). These processes result in structural and functional abnormalities in the liver (Cichoż-Lach & Michalak, 2014). The effects of ROS are counteracted by antioxidants including glutathione (GSH), the most important antioxidant molecule, superoxide dismutase and GSH peroxidase (Valko et al., 2007).

Compelling evidence confirmed that in response to liver injury, hepatocytes undergo apoptosis (Guicciardi et al., 2013). Apoptosis implicates the activation of a group of cysteine proteases called "caspases" and a complex cascade of events that link the initiating stimuli to the final demise of the cell. Caspase-3 (Casp-3) is considered to be the most important factor responsible for chromatin condensation and DNA fragmentation (Elmore, 2007).

Apoptosis is a prominent feature in liver fibrosis (Wang et al., 2013), which results from an imbalance between extracellular matrix deposition and degradation, and ultimately alterations of tissue architecture and function (Iredale et al., 2013). Evidence suggests that a key step in the fibrogenic mechanisms is the activation of hepatic stellate cells (HSC) that migrate to the site of injury to engulf the apoptotic bodies (Eulenberg & Lidbury, 2018). This engulfment promotes the activation of HSC to hepatic myofibroblasts. Upon activation, they produce excessive fibrillar collagens, pro-inflammatory cytokines, and inhibitors of matrix proteases. This results in an excessive deposition of extracellular matrix protein (Friedman, 2008) in particular, collagen, and laminin (LN) (Baiocchini et al., 2016). Moreover, the activation of HSC is associated with increased synthesis of hyaluronic acid (HA) (George et al., 2004), an important component of the extracellular matrix. Liver fibrosis is also characterized by elevated level of hydroxyproline (HYP), an important constituent of collagen (Srivastavaa et al., 2016).

The evidence has shown that the supplementation of natural compounds has the ability to alleviate radiation hazards**.** Interest in Royal Jelly has increased after publications on its antioxidant and free radical scavenging capacity (Almeer et al., 2018). Royal Jelly (RJ) is safe as a supplement agent and the oral administration of 10 g/kg RJ showed no acute toxicity in a mouse model (Kobayashi et al., 2001). Royal Jelly is rich in amino acids, proteins, sugars, fatty acids, minerals, and vitamins, and was reported to possess anti-inflammatory (Kohno et al., 2004) and radio-protective activities (Yasemin & Kemal 2014; Elkady & Ibrahim, 2014). RJ was reported to modulate the alcohol-induced liver injury, and to improve the liver function (Changchun et al., 2011).

In view of these considerations, the objective of this study is to evaluate the role of Royal Jelly in radiation-induced liver damage by measuring the variations of hepatic malondialdehyde (MDA), protein carbonyl (CO) and 8-OHdG markers of lipids, protein and DNA oxidation, respectively, as well as the content of glutathione (GSH), the activity of SOD and GSH-Px as markers of oxidative stress, tumor necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1 β) to evaluate hepatic inflammatory markers, Casp-3 activity and DNA fragmentation as an indicator of apoptosis. Collagen IV and laminin (LN), as factors of fibrosis and alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) are important parameters to check liver function.

Materials and Methods

Male albino rats Sprague Dawley (150±20g; 4±1month old) obtained from the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt, were used as experimental animals. Rats were housed in specially designed cages and maintained under standard conditions of ventilation, temperature (25°C ± 5°C), humidity (60%), and illumination (light/dark cycle 13 h: 11 h). Animals received standard food pellets diet and *water ad libitum*. All the experiments were conducted under national research center guidelines for the use and care for laboratory animals and were approved by an independent ethics committee of the National Center for Radiation Research and technology (NCRRT).

Whole-body gamma-irradiation of animals was performed at National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt, using Gamma cell-40 (137Caesium), a biological irradiator manufactured by Canada Ltd. Ottawa, Ontario, Canada. Animals were placed in a plastic sample tray with a lid and support provided for use in the sample cavity. The unit had ventilation

holes which align with ventilation parts through the main shield to provide a means for uniform irradiation for small animals. Rats were exposed to 3 Gy every 3 days up to 9 Gy, given at a dose rate of 0.68 Gy/min.

Royal Jelly obtained from Sigma-Aldrich, St Louis, MO, USA was dissolved in distilled water and given to the rats orally by gavages at a dose of 250 mg/kg body-weight/day for 15 days (Elkady & Ibrahim, 2014).

Animal groups

The experimental animals were divided into 4 groups (6 rats each). Control: received distilled water via gavages for 15 days; RJ: received Royal Jelly via gavages (250mg/Kg body-weight/day) for 15 days; IRR: whole body γ-irradiated were given distilled water before the 1st irradiation fraction and continued during 14 days, IRR+RJ: whole body γ-irradiated were given Royal Jelly before the 1st irradiation fraction and continued during 14 days. All chemicals and kits were obtained from Sigma-Aldrich, St Louis, MO, USA. Measurement of absorbance was conducted using a T60 UV/VIS spectrophotometer (PG instruments, London, UK).

Animals in all groups were sacrificed by decapitation 1 day post the last dose of Royal Jelly. Blood was collected and serum was obtained. The liver was quickly removed and a homogenate (10% weight/volume) was prepared in normal 0.9% saline using Teflon-glass homogenizer (Glass-Col, Terre Haute, Ind., USA) and then the homogenates were centrifuged at 10 000 xg for 15 minutes in a refrigerated centrifuge (K3 Centurion Scientific Ltd, London, UK) and the supernatants were used for further analysis

Assessment of liver oxidative stress and apoptotic markers

Lipid peroxidation was assayed according to Ohkawa et al. (1979). Protein oxidation was evaluated by measuring protein carbonyl (CO) content as described by Levine et al. (1990). Glutathione (GSH) content, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities were estimated as described by Beutler et al. (1963), Minami & Yoshikawa (1979) and Paglia & Valentine (1967), respectively. DNA oxidation was assessed using a competitive enzyme-linked immunosorbent assay ELISA kit Cat. No. KA0444 from Abnova Co., Taiwan as described by the manufacturer's prescripts. DNA fragmentation was measured as described by Peradones et al. (1993). The percentage of fragmentation was calculated as the ratio of DNA in the supernatant to the total amount of DNA in pellet and supernatant. The activity of the apoptotic marker, caspase-3 was estimated according to the manufacture of R&D Systems kits (Catalog Number: BF3100).

Determination of serum inflammatory and fibrotic markers

Tumor necrosis factor-alpha (TNF-α) and IL-1β were quantified using their respective ELISA kit Bio Source International, Camarillo, CA, USA, according to the manufacturer's instruction. Laminin (LN) and Collagen IV were assayed using their respective ELISA Kit Cat. No. CSB-E04646r, and Cat. No. CSB-E08883, from CUSABIO, as described by the manufacturer's prescripts.

Liver function was determined by measuring the serum activity of gamma glutamyl transferase (GGT) using GGT Activity Colorimetric Assay Kit Cat. No. MAK089, according to Rosalki (1975), aspartate amino transferase (AST) and alanine amino transferase (ALT) using Assay Kits (Biodiagnostic, Egypt) as described by Reitman & Frankel (1957), and alkaline phosphatase activity (ALP) using assay Kit (Biodiagnostic, Egypt) as described by Belfield & Goldberg (1971).

Statistical analysis

Data is expressed as Mean ± Standard deviation (SD). Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by LSD test as a Post Hoc test to determine the significance between groups. The differences were considered significant at the level of $P< 0.05$.

Results

The effects of irradiation, royal jelly and their combination on some biochemical parameters in rats are shown in Tables (1, 2, 3 and 4). The administration of Royal Jelly (250mg/Kg body-weight/day) for 15 days, to normal rats, has not significantly affected the level of liver malondialdehyde (MDA), protein carbonyl (CO), GSH content and the activity of the antioxidant enzymes SOD and GSH-Px in the liver when compared to their respective values in rats not

supplemented with Royal Jelly (Table 1). Also, the mean values of 8-hydroxydeoxyguanosine (8-OHdG), DNA fragmentation and the activity of caspase-3 in the liver showed normal values (Table 2). In the serum, the level of the inflammatory markers, tumor necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1 β) in addition to fibrotic markers collagen IV and laminin showed normal values (Table 3). The results also revealed normal liver function demonstrated by normal levels in the activity of ALT, AST, GGT and ALP (Table 4).

The exposure of rats to γ-radiation (3 Gy/

TABLE 1. The effect of Royal Jelly (RJ) on malondialdehyde (MDA), protein carbonyl (CO), Glutathione (GSH), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in the liver of rats in different animal groups

	Groups			
	Control	**RJ**	**IRR**	**IRR + RJ**
MDA (nmoles/g tissue)	100 ± 15 [a]	92 ± 20 [a]	160 ± 20 [b]	125 ± 20 [c]
CO (nmoles/g tissue)	190 ± 30 [a]	187 ± 25 [a]	300 ± 40 [b]	242 ± 30 [c]
GSH (mg/g tissue)	8.13 ± 0.43[a]	8.50 ± 0.40[a]	2.43 ± 0.20 [b]	6.29 ± 0.53 [c]
SOD (U/mg protein)	82.30 ± 0.45 [a]	83.20 ± 0.43 [a]	62.1 ± 0.28 [b]	77.6 ± 0.35 [c]
GSH-Px (U/mg protein)	56.23 ± 0.05 [a]	57.23 ± 0.05 [a]	38.2 ± 0.05 [b]	49.2 ± 0.05 [c]

- Each value represents the mean ± standard deviation.
- Different superscripts a, b, c in the same row indicate significant differences between groups (n = 6). P < 0.05.
- IRR: irradiated rats.

TABLE 2. The effect of Royal Jelly (RJ)on 8-hydroxydeoxyguanosine (8-OGdG), apoptotic markers (DNA fragmentation and caspase-3) in the liver of rats in different animal groups

	Groups			
	Control	**RJ**	**IRR**	**IRR+ RJ**
8-OHdG (ng/g tissue)	2.00 ± 0.25 [a]	1.86 ± 0.35 [a]	3.10 ± 0.20 [b]	2.50 ± 0.30 [c]
DNA fragmentation %	10.0 ± 1.55 [a]	9.10 ± 2.00 [a]	14.00 ± 2.50 [b]	12.50 ± 3.00 [c]
Caspase-3 (ng/ g tissue)	3.00 ±0.65 [a]	2.70 ± 0.50 [a]	6.00 ± 0.70 [b]	3.80 ± 0.50 [c]

- Each value represents the mean ± standard deviation.
- Different superscripts a, b, c in the same row indicate significant differences between groups (n = 6). P < 0.05.
- IRR: irradiated rats.

TABLE 3. Effect of Royal Jelly (RJ) on the inflammatory markers, tumor necrosis factor-alpha (TNF-α) , interleukin-1β (IL-1β), fibrotic markers, Collagen IV and Laminin in serum of rats in different animal groups

	Groups			
	Control	**RJ**	**IRR**	**IRR+ RJ**
TNF- α (pg/ml)	30.00 ± 5.10 [a]	31.00 ± 7.50 [a]	60.00 ± 10 [b]	45.30 ± 9 [c]
IL-1β(pg/ml)	33.00 ± 5.90 [a]	32.00 ± 6.50 [a]	53.00 ± 11 [b]	43.89 ± 10 [c]
Collagen IV (ng/ml)	35.00 ± 6.10 [a]	31.90 ± 7.00 [a]	52.50 ± 11 [b]	45.20 ± 10 [c]
Laminin (pg/ ml)	200 ± 33 [a]	180 ± 40 [a]	294 ± 50 [b]	260 ± 30 [c]

- Each value represents the mean ± standard deviation.
- Different superscripts a, b, c in the same row indicate significant differences between groups (n = 6). P < 0.05.
- IRR: irradiated rats.

TABLE 4. Effects of Royal Jelly (RJ) on the activity of alanine and aspartate amino transferase (ALT&AST), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP) and AST/ ALT ratio in serum of rats in different animal groups

	Groups			
	Control	**RJ**	**IRR**	**IRR+ RJ**
ALT (U/L)	16.78 ± 0.20[a]	17.03 ± 0.20 [a]	52.80 ± 1.05[b]	30.80 ± 1.60 [c]
AST (U/L)	12.50 ± 0.20 [a]	12.9 ± 0.30 [a]	76.07 ± 1.40 [b]	29.00 ± 1.10[c]
GGT (U/L)	32 ± 3.0 [a]	30.0 ± 3.5 [a]	47.3 ± 4.0 [b]	40.2 ± 5.0 [c]
ALP (U/L)	100 ± 10.0 [a]	96.0 ± 15 [a]	150 ± 13.0 [b]	123 ± 12.0 [c]
AST/ ALT ratio	0.74	0.76	1.44	0.94

- Each value represents the mean ± standard deviation.
- Different superscripts a, b, c in the same row indicate significant differences between groups (n = 6). $P < 0.05$.
- IRR: irradiated rats.

every 3 days to a total dose of 9Gy) has induced significant increases in the content of MDA and CO associated with significant decreases in GSH level and antioxidant enzymes SOD, GSH-Px activity compared with their respective values in the liver of control rats (Table 1). Also, γ-radiation induced significant increases in DNA fragmentation, 8-OHdG and the activity of caspase-3 (Table 2). Irradiation has also elevated significantly the level of TNF-α, IL-1β, collagen IV and laminin in the serum (Table 3) compared with their respective values in the liver of control rats. These fluctuations in the oxidative, inflammatory, apoptotic and fibrotic markers were associated with impaired liver function denoted by significant increases in ALT, AST, GGT and ALP activities compared with their respective values in the liver of control rats (Table 4).

In contrast, the administration of RJ during the irradiation-period has significantly attenuated the severity of radiation-induced oxidative stress. The contents of MDA and CO were significantly reduced while the content of GSH as well as the activity of the antioxidant enzymes SOD and GSH-Px activity were significantly increased (Table 1.) The content of 8-OHdG, DNA fragmentation and the activity of the apoptotic enzyme Caspase-3 were also significantly decreased (Table 2). Royal Jelly treatment induced a significant improvement in the fibrotic markers (Table 3), which was accompanied by a significant amelioration of liver function in comparison to irradiated rats not receiving RJ treatment (Table 4).

Discussion

Liver is one of the most frequently injured organs during radiation therapy performed for cancers of the abdominal region with a potential risk of unexpected liver damage as a result of O_2 generation. Natural products as beneficial for health are more or less easily obtained and they are fairly safe. Royal jelly, a creamy substance, produced by the hypopharyngeal and mandibular glands of worker honeybees is a mixture of natural products and is commonly used as a dietary supplement (Xue et al., 2017; Kunugi & Ali, 2019).

Exposure of mammals to ionizing radiations (IR) leads to the development of a complex, dose-dependent series of changes, including injury to different organs, which cause changes in the structure and function of cellular components. Exposure to IR triggers oxidative stress in different tissues (Saada et al., 2009; Said et al., 2012). Oxidative stress with the subsequent production of ROS was postulated as one of the mechanisms of radiation damage resulting in cellular damage and cell death (Panganiban et al., 2013).

In the current investigation, γ-irradiation of rats with fractionated dose of gamma radiation (3 Gy every 3 days up to a total dose of 9 Gy), has induced a significant elevation in the level of MDA, CO, and 8-OHdG associated with a significant reduction in the activity of SOD, GSH-Px and GSH content which are indicators of liver damage.

The major forms of cellular damage induced by radiation are increased lipid, protein and DNA oxidation products denoted by significant increase of MDA, CO and 8-OHdG respectively, which may be ascribed to the interaction of hydroxyl radicals (•OH); generated in the body through

radiolysis of water molecules upon ionizing radiation, with unsaturated fatty acids present in the phospholipids portion of cellular membranes, amino acids, and guanine (is one of the four main nucleobases found in the DNA), respectively (Singh et al., 2019). Depletion in GSH causes elevation in hydroxyl radicals which in turn attack on lipid membranes and results in lipid peroxidation (Bhartiya et al., 2008). In the same way, the increase of CO level might be attributed to the interaction of proteins with ROS (Meaney et al., 2008). However, excessive ROS production by IR causes antioxidant imbalance and leads to lipid peroxidation and antioxidant depletion (Weijl et al., 1998).

Glutathione, a non-enzymatic antioxidant produced in body plays a pivotal role in the maintenance of the balance of cellular redox status. It directly quenches reactive hydroxyl free radicals and protects the cells and tissues from lipid peroxidation. Depletion of GSH content in liver tissues after whole body irradiation of animals might be due to the oxidation of GSH with the free radicals resulting in the formation of thiyl radicals that react to produce oxidized glutathione (GSSG) which is toxic to the cells (Rossi et al., 2005).

Royal jelly contains many important compounds with biological activity such as amino acids, aspartic acid, cysteine and cystine, which take part in the synthesis of GSH, an effective cellular antioxidant. GSH breaks down reactive oxygen species and detoxifies carcinogens, both directly and by antioxidant enzymes with which it reacts (Zimmermann, 2002).

A major cellular defense against ROS is provided by SOD and GSH-Px, to reduce oxidized lipids and protein targets of ROS. Radiation exposure caused a severe damage to liver tissue most likely by ROS generation as apparent by perturbation in the antioxidant enzymes (SOD and GSH-Px) that lead to increased lipid peroxidation (Cayir et al., 2009). Radiation exposure stimulates ROS production by damaged mitochondria, which increase free radical production and decrease antioxidant production (Kawai et al., 2006). The decrease of SOD, GSH-Px activities and GSH content may contribute to the consequence of cellular membrane damages (Saada et al., 2003) or might be due to their increased utilization in scavenging free radicals induced by the ionizing radiation radicals, thus causing irreversible inhibition in their activities (Ihechiluru et al., 2015).

Royal jelly administrated to irradiated-rats group, showed comparable results to the control regarding the oxidative stress indicator parameters. RJ prevented oxidative stress which could be related to the fact that it contains hepatocyte-stimulating substance and glutathione precursor cystine and cysteine having an important role in the liver detoxication system as well as free amino acids such as glycine, aspartic acid. As indicated by previous researchers, the antioxidant effect of RJ may be related to its free amino acid content (Tamura et al., 2009).

8-hydroxy-2-deoxyguanosine is believed to be one of the predominant DNA lesions, resulting from free radical-induced oxidative stress in nuclear and mitochondrial DNA, and is widely used as a sensitive biomarker of DNA oxidative damage (Valavanidis et al., 2009). The increase of 8-OHdG might be attributed to the increase in the lipid peroxidation chain reaction which activates oxygen radicals that attack the eighth carbon atom of the guanine base in the DNA molecule to produce 8-hydroxydeoxyguanosine (8-OHdG) (Seki et al., 2005). Feeding mice with RJ protected the DNA tissue from oxidative damage which reduced the oxidative stress marker levels (8-OHdG) in kidney DNA and serum (Silici et al., 2011).

In the current investigation, gamma-irradiated rats with fractionated dose of gamma radiation (3 Gy every 3 days up to a total dose of 9 Gy) increases the activities of serum ALT, AST, ALP and GGT. Similar findings were previously reported by Said & Hanafy (2006). In the present study, when compared to the control group, the elevation in liver enzymes (ALT, AST, ALP and GGT) activities in irradiation group indicated necrosis or hepatocellular injury. The rise in serum AST, ALT and GGT has been attributed to the damaged structural integrity of the liver, because these are normally located in the cytoplasm and are released into the circulation after hepatic damage (Sener et al., 2006). Hepatic ALP is present on the canalicular and luminal domain of the bile duct epithelium and its levels rise because of increased synthesis and consequent release into the circulation due to biliary obstruction (Limdi & Hyde, 2003).

The AST/ALT ratio increases in liver

functional impairment. The normal AST/ALT ratio is approximately 0.8. Also, AST/ALT ratios greater than 1 are found in 70–80% of patients with hepatitis fibrosis advances and cirrhosis (Nyblom et al., 2007). The increases AST/ALT ratio in the present study might be due to relative changes in their release, synthesis, or metabolic distribution and clearance. A frequent explanation given for the increase in serum AST/ALT ratios in liver disease is a change in the differential release of these two enzymes from the liver. ROS causes injury to hepatocyte mitochondria, and mitochondria are relatively enriched in AST activity (as compared with the hepatocyte cytosol) (Nyblom et al., 2006). A second explanation given for the increase in the AST/ALT ratio is that there is a relative decrease in the synthesis of ALT activity by the liver injury. Nyblom et al. (2004) have reported changes in the relative amounts of ALT and AST in liver cells of various liver diseases.

Co-treatment of irradiation and RJ resulted in a significant improvement in liver enzymes towards the normal values of the control. Uzbekova et al. (1998) have reported that RJ displayed a significant decrease in heapato-toxicity and improved liver enzymes on thyroxin-induced liver damage and that may be attributed to their naturally high antioxidant potential and its ability to act as a free radical scavenger, thereby protecting membrane permeability (Ashry & Elkady, 2014).

There is a convincing evidence that oxidative stress (Kurundkar & Thannickal, 2016), inflammation (Yu et al., 2016), and apoptosis (Niska et al., 2015) induces hepatic fibrosis (Kurundkar & Thannickal, 2016). In the current investigation, gamma-irradiation at fractionated dose of (3 Gy every 3 days up to 9 Gy total doses) increases TNF-α and IL-1β, in blood serum. In this study, the whole body exposure of rats to gamma-radiation has induced oxidative stress triggered the inflammatory response through a significant elevation in the level of TNF-α and IL-1β, thereby initiating apoptosis which is accompanied by an increase in the activity of Caspase-3 and the fibrotic markers LN, and Col IV.

The results demonstrate that exposure to ionizing radiation generates an excess of free radicals that contribute to the increase of fibrotic factors. Caspase-3 enzyme regulates inflammation and apoptosis signaling networks and it is a mediator of the mitochondrial apoptotic pathway and is known to be an indicator of oxidative stress-induced necrosis (Baskaran et al., 2018). The increase in caspase-3 activity might be correlated with radiation-induced apoptosis, where oxidative stress induces the release of mitochondrial cytochrome c into the cytosol, where it can activate caspases and lead to apoptosis (Orrenius & Zhivotovsky, 2005).

Increase inflammatory-cytokines such as interleukin-1β (IL-1 β) and tumor necrosis factor-alpha (TNF- α) might be correlated with the mechanism of γ-radiation induced liver injury (Criswell et al., 2003). ROS participate in the activation of pro inflammatory NF-κB pathway, which in turn leads to the production of tumor necrosis factor alpha (TNF-α) and subsequent generation of inflammatory and angiogenic mediators such as cytokines (interleukin) (Wilkinson-Berka et al., 2014).

Fibrotic markers are one of the main glycoproteins of the basement membrane and mainly synthesized by hepatocytes. LN and Col IV are important biomarkers of hepatic fibrogenesis that can indirectly reflect the degree of hepatic fibrosis and the highest serum LN and Col IV content was observed in active cirrhosis and chronic active hepatitis (Castera et al., 2000). In this study, the serum levels of IL-1, Col IV, MDA and LN, and the liver content of 8-OHdG were significantly higher in the irradiated group than in the normal control group, suggesting the liver injury after radiation exposure. The increase in the fibrotic markers LN, and Col IV might be correlated mechanism of γ-radiation induced liver injury, stimulates collagen synthesis and consequentially hepatic fibrogenesis (Krętowski et al., 2017).

In this study, royal jelly treatment has significantly attenuated the severity of oxidative stress, decreased the activity of the apoptotic enzyme Caspase-3 and induced a significant improvement in the fibrotic markers. These changes were accompanied by a significant amelioration of liver function in comparison to the irradiated rats not receiving royal jelly treatment.

In the present study, irradiated rats treated with RJ showed a significant decrease in the level of MDA content, with concomitant significant increase in the activity of SOD , GSH-Px, and

in the content of GSH supporting the role of RJ as a potential antioxidant (Petelin et al., 2019), which may be attributed to the presence of vitamin C and vitamin E, in addition to quercetin and cinnamic flavonoids (Kocot et al., 2018); all of which are powerful natural antioxidants that increase antioxidant enzymes, and decrease MDA concentration. Moreover, RJ contains many trace elements including Cu, Mn, Zn, Se and Fe (Stocker et al., 2005), Zn stabilizes the structure of SOD. Selenium (Se) is an important element of GSH-Px, which regulates lipid metabolism, prevents fatty liver formation, and improves antioxidant ability in rats

Considering the antioxidant activity, very important ingredients of the royal jelly are flavonoids and phenolic compounds (Nabas et al., 2014) and there is a vital relationship between polyphenols and royal jelly antioxidant capacity (Alzahrani et al., 2012). RJ reduced MDA levels and induced significant elevation in the activities of GSH-Px, SOD and CAT of hepatic tissue in radiation-induced oxidative stress in rats (Cihan et al., 2013). The researchers attributed the antioxidant properties of royal jelly to the presence of substances such as 10-hydroxy-2-decenoic acid (Silici et al., 2011; Inoue et al., 2018; Kunugi & Ali, 2019) and free amino acids including proline (which is suggested to act as an antioxidant due to hydroxyl radical-scavenging activity) as well as cystine and cysteine (participating in the synthesis of effective cellular antioxidant glutathione) (Silici et al., 2009; 2011; Kanbur et al., 2009a).

In addition, some researchers reported other therapeutic effects of royal jelly such as hepatoprotective (Cemek et al., 2010; Ahmed et al., 2014), cardioprotective (Malekinejad et al., 2016), or anti-inflammatory (Manzo et al., 2015). The postulated hypotheses explaining the antioxidant effect are the restoration of ascorbic acid availability by royal jelly, regulation of retinol loss (Cemek et al., 2010), antioxidant effect of some free amino acids (Ahmed et al., 2014), or radical-scavenging activities of RJ and its components (Nejati et al., 2016).

It has been demonstrated that RJ prevents carbon tetrachloride- (Cemek et al., 2010), cadmium- (Cavuşoğlu et al., 2009), and paracetamol- (Kanbur et al., 2009b) induced liver toxicity genotoxicity and nephrotoxicity, respectively. Furthermore, RJ inhibited oxidative stress, inflammation, and hepatic tissue injury; normalized enzymatic and non-enzymatic antioxidant molecules and the anti-apoptotic protein Bcl-2. Hence, RJ can be used as a hepatoprotective agent against the toxic effects of cadmium chloride-induced hepatotoxicity (Almeer et al., 2018).

Conclusion

According to the results obtained in this study, royal jelly attenuates oxidative stress, enhances liver functions, reduces the increase of inflammatory, apoptotic, and fibrotic markers and thus may exert a beneficial impact on radiation-induced liver damage. However, further investigations are needed to determine the mechanism of Royal Jelly actions.

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