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REVIEW

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# New Findings on Biological Actions and Clinical Applications of Royal Jelly: A Review

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**ABSTRACT**

Royal jelly (RJ) is a natural bee product with great potential for use in medicine. The chemical composition of RJ indicates the presence of various bioactive substances including 10-hydroxydecanoic acid and 24-methylenecholesterol. In addition, a number of biological and pharmacological activities of RJ have been documented. The aim of this study was to review the biological and medical effects of RJ. The search was conducted in articles from electronic and scientific literature databases such as Pub Med, Science Direct, Scopus, Medline, and ISI Web of Science published from 1990 to 2017 using keywords of pharmacological, biological, and clinical effects and royal jelly. Data were chosen after the primary survey of all abstracts and selected full articles. Comparison among related data was done by the authors. Literature has shown that RJ possesses many beneficial effects on biological systems. For example, the therapeutic uses of RJ have been reported in several diseases such as hypercholesterolemia, diabetes, hypertension, and cancers. It was also found to possess neurotrophic, hypotensive, immunomodulatory, antimicrobial, antioxidant, antidiabetic, antihypercholesterolemic, antitumor, and anti-inflammatory effects. Owing to the broad spectrum of biological effects and valuable clinical trials, evaluating the beneficial pharmaceutical effects of RJ in animal and human models seems to be important.

**KEYWORDS**

antidiabetic; antioxidant; biological effects; royal jelly

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## Introduction

Royal jelly (RJ) is a yellowish white, creamy liquid secreted by the mandibular and hypopharyngeal glands of the nurse bees (*Apis mellifera* L.) for the nutrition of young larvae in the colony and the queen bee (Viuda-Martos et al., 2008). Queen larvae consume RJ throughout their lifetime and ultimately become queen bees. Many advantages could be considered for queen bees, including large size (double that of worker honeybees), functioning sexual organs, long lifespan (approximately 5–6 years, while that of the nurse bees is only 35–40 days) (Isidorov et al., 2012; Izuta et al., 2009).

RJ is composed mainly of important compounds with biological and health-promoting activities such as proteins, lipids, sugars, vitamins, minerals, and free amino acids (Nakajima et al., 2009). RJ contains vitamins such as riboflavin, thiamine, niacin, folic acid, biotin,

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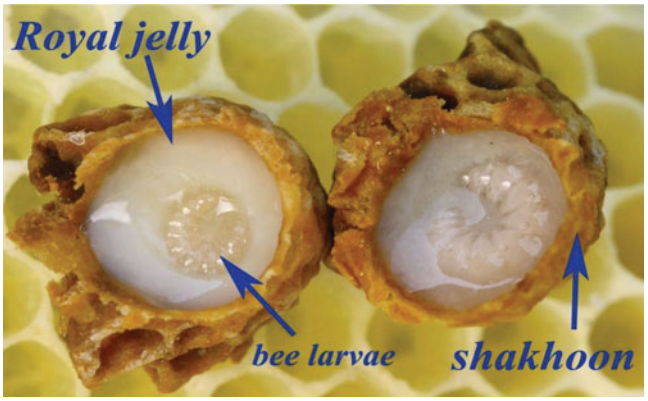


**Figure 1.** Developing queen larvae surrounded by royal jelly in open queen cells.

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and pyridoxine and smaller amounts of vitamins C, D, A, and E (Nagai and Inoue, 2004). Moreover, calcium, sodium, potassium, copper, iron, zinc, and manganese are the main minerals in RJ (Ramadan and Al-Ghamdi, 2012). One of the main bioactive compounds of RJ is 10-hydroxy-trans-2-decenoic acid (10HDA), an unsaturated fatty acid that is only found in RJ in nature. The different biological activities of RJ depend on the type of cell (Fujii et al., 1990; Oka et al., 2001); for example, its HDA has important anticancer activity (Yang et al., 2010).

Shakhoon (queen cell), a frame made of beeswax and filled with RJ, is the place of the honeybee larva. RJ plays a key role in larva feeding and development into a longevous and fertile mature honeybee queen (Leung et al., 1997) (Figure 1). Royalactin is a monomeric protein of RJ that activated p70 S6 kinase, which was responsible for the enhancement of body size. It also shortened developmental time and increased the juvenile hormone titre, which was an essential hormone for ovary development in queens (Kamakura, 2011). RJ is now used in cosmetics, health foods, and therapeutic products and possesses a wide repertoire of biological processes beneficial for human health, such as hypotensive activity (Tokunaga et al., 2004), antioxidant activities (Fujii et al., 1990), antihypercholesterolemic effects (Hang et al., 2007), anti-inflammatory activities (Kohno et al., 2004), antitumor effects (Bincoletto et al., 2005; Shirzad et al., 2013), antimicrobial effects (Lees and Aliabadi, 2002), hypoglycemic effects (Münstedt et al., 2009), and immune activity (Šver et al., 1996), that can serve remedial purposes to promote human health. The aim of this study was to review the literature regarding biological activities of RJ (Table 1).

This study was performed by a comprehensive search of the biological and pharmacological properties of RJ, including antimicrobial, antioxidant, antidiabetic, antihypercholesterolemic, antitumor, anti-inflammatory, neurotrophic, hypotensive, and immunomodulatory properties, in Science Direct, PubMed, Medline, and Scopus databases from 1990 to 2016. Data were selected after the primary survey of all abstracts, and the most relevant articles were chosen to be read in full.

## Biological properties of RJ proteins

Over the past 20 years, RJ has been critically studied so that several proteins have been identified to play a pivotal role in the biological process. As an important ingredient, the family of major royal jelly protein, or apalbumins, encompasses about 83%–90% of the total proteins of RJ with the important role of feeding the queen bee. In addition, the significant effect of apalbumins seems to be production of honeybee products such

**Table 1.** The biological activity of royal jelly.

| Cell/system | Effect | Preparation | Reference |
|---|---|---|---|
| Microrganism | Antibacterial | 10HDA | Genç and Aslan (1999); Kitahara et al. (1995) |
| | Antibacterial | RJ | Bincoletto et al. (2005); Manfredi and Chiodo (2000) |
| | Antibacterial | RJ | Bărnuţiu et al. (2011); Bílikova et al. (2015); Boukraâ et al. (2008); Fontana et al. (2004) |
| Microrganism | Antifungal | Royalisin | Moselhy et al. (2013) |
| Wounds | Antimicrobial | Royalisin | Bărnuţiu et al. (2011) |
| Foot ulcers | Wound-healing activity | Ointment (RJ and panthenol) | Abdelatif et al. (2008) |
| In vitro | Antioxidant activity | RJ proteins (RJPs) | Guo et al. (2009) |
| Kidney | Decrease MDA levels and increase GPx, CAT, GSR, and SOD | RJ | Inoue et al. (2003); Karadeniz et al. (2011); Silici et al. (2009) |
| Erythrocyte | Antioxidant activity | RJ supplementations | Pourmoradian et al. (2014) |
| Human breast cancer cells (MCF-7) | Anticancer activity | RJ | Nakaya et al. (2007) |
| Human umbilical vein endothelial cells (HUVEC) | Inhibition of cell migration and proliferation | 10-Hydroxy-2-decenoic acid | Izuta et al. (2009) |
| Blood | Antidiabetic activity | RJ | Kimura (2008); Shirzad et al. (2013) |
| Blood | Decrease insulin resistance | RJ | Zamani et al. (2012) |
| Colon | Anti-inflammatory | RJ | Karaca et al. (2012) |
| Peripheral blood mononuclear cells | Inhibit proliferation | 3, 10-dihydroxy-decanoic acid (3, 10-DDA) | Mihajlovic et al. (2014) |
| Blood serum | Decrease cholesterol and total lipids levels | RJ | Kamakura (2011); Abdelhafiz and Muhamad (2008); Vittek (1995) |
| Blood serum | Serum cholesterol-lowering activity | Major RJ protein 1 (MRJP1) | Kashima et al. (2014) |
| Reproductive system | Asthenozoospermia treatment | RJ plus bee honey | Abdelhafiz and Muhamad (2008) |
| Blood pressure | Antihypertension activity | MRJPs | Leung et al. (1997) |
| | | RJ | Feng et al. (2015) |
| Reproductive system of male | Effects on fertility | RJ | Amirshahi et al. (2014); Ghanbari et al. (2015); Gimenez-Diaz et al. (2012) |
| Reproductive system of female | Inhibition of hormonal disorders | RJ | Elnagar (2010); Eshtiyaghi et al. (2016); Kohguchi et al. (2007); Kridli and Al-Khetib (2006) |
| Hormone | Increases secretion of testosterone, progesterone, and luteinizing hormone | 10% solution of RJ | Hang et al. (2008). |
| Liver | Hepatoprotective activity | RJ | Cihan et al. (2013); El-Nekeety et al. (2007); Kanbur et al. (2009); Yildirim et al. (2012) |
| Bone | Prevents osteoporosis | RJ | Hidaka et al. (2006) |
| Other | Decreases postmenopausal osteoporosis | RJ | Nagai and Inoue (2004); Kim et al. (2010); Park et al. (2012) |

RJ = royal jelly; MDA = malondialdehyde; GPx = glutathione peroxidase; CAT = catalase; GSR = glutathione reductase; SOD = superoxide dismutase.

as pollen-bread and pollen-pellet as well as the differentiation of queen from worker bees. Two isoforms of the apalbumins family, MRJP1 and MRJP2, are known to act as allergens that lead to induction of mouse macrophages to produce tumor necrosis factor $\alpha$ (TNF-$\alpha$) (Rosmilah et al., 2008). Other RJ proteins include royalisin, jelleines, royalactina, and aspimin; jelleines and royalisin have a known antibacterial effect (Fratini et al., 2016).

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Royalactina has the same effect as MRJP1 in differentiation of queen from worker bees (Bilikova et al., 2002). Apolyporphin III–like protein as primarily identified protein in RJ is a lipid-associated protein that leads to transfer of lipids in aqueous environments as a protein-lipid complex (Kim and Jin, 2015). In addition to its antibacterial facet, apolyporphin III–like protein is involved in the development of immunological reactions in bee larvae (Fujita et al., 2012).

Research has shown that glucose oxidase as a component of RJ catalyzed the oxidation of glucose to its peroxide form with an antibacterial activity. According to previous studies, the hypocholesterolemic effect of dietary proteins and peptides is related to their potential to bind bile acids and Their metabolisms in the gastric system. It has been reported that bile acid–binding proteins in RJ possesses a hypocholesterolemia activity. The proposed mechanisms of lipid-attenuating effects of RJ have been attributed to the binding of RJ proteins to bile acids in the intestine and suppressing the uptake of them to increase the secretion of bile acids and cholesterol (Kashima et al., 2014).

Research has shown that the average dose of RJ (0.1%–0.3%) in frozen extender is associated with enhancement of sperm motility. The increased motility of sperm after freezing may be due to the aggressive effect of proteins and high antioxidant capacity of RJ. On the other hand, the membrane-protective effect of RJ during sperm freezing seems to be associated with the appearance of active biological amino acids such as aspartic acid, cysteine, glycine, cysteine, tyrosine, leucine, lysine, isoleucine, and valine. It is anticipated that proline amino acid in protein structure is able to protect cellular membranes against stress conditions. On the other hand, cysteine as a potent antioxidant neutralizes free radicals and modulates the synthesis of glutathione in the freezing period (Shahzad et al., 2016).

MRJP1 and MRJP2 were identified in the preliminary analyses of RJ proteins using SDS-PAGE. Daily administration of nine capsules of RJ for three months led to increased concentration of dehydroepiandrostenedione and dramatic reduction of serum total cholesterol and low-density lipoprotein cholesterol (LDL-C). Therefore, RJ is able to act as a cholesterol-reducing agent to attenuate the development of cardiovascular diseases. The exact mechanism of hypocholesterolemic activity of RJ, especially MRJP1, needs to be clarified (Chiu et al., 2017). The physiological function of RJ proteins has shown that MRJPs stimulate cell proliferation. In addition, it has been reported that some RJ proteins prevent the proliferation of human breast cancer cells induced by bisphenol A (Ramadan and Al-Ghamdi, 2012).

## Antimicrobial activity

The medical importance of RJ has been known since ancient times; aqueous solution of pure RJ has been used as a strong antibacterial agent against a wide variety of bacteria (Lees Aliabadi, 2002; Manfredi and Chiodo, 2000). Many researchers have reported that the major antibacterial property of RJ is attributed to a special ten-carbon molecule of 10HDA, the most important fatty acid of RJ (Table 1) (Genç and Aslan, 1999; Kitahara et al., 1995).

In addition, several antimicrobial peptides have been found in RJ containing apisimin, apalbumin $\alpha$, jeleines I, II, III, and IV, and royalactin (Bărnuțiu et al., 2011) (Table 2). In vitro studies have shown the effectiveness of royalactin isolated from RJ in a number of gram-positive and garm-negative bacteria, including methicillin-resistant *Staphylococcus aureus* (Boukraâ et al., 2008; Fontana et al., 2004; Sheridan et al., 1993) (Table 2). Interestingly, royalisin is one of the functional proteins in RJ with extensive sequence homology to other antibacterial peptides. It has effective inhibitory effect on the growth of gram-negative and gram-positive bacteria and fungi (Bílikova et al., 2015). The growth inhibitory effect of royalisin has been observed against a variety of gram-negative bacteria, fungi (Tseng et al.,

**Table 2.** Isolated compounds of royal jelly and their reported effects.

| Compound name | Biological activity | Reference |
|---|---|---|
| Royalactin | Antibacterial | Bărnuţiu et al. (2011); Bílikova et al. (2015); Boukraâ et al. (2008); Fontana et al. (2004); Sheridan et al. (1993) |
| Royalisin | Antimicrobial | Tseng et al. (2011) |
| 10-Hydroxy-2-decenoic Acid (10HDA) | Anticancer, antiangiogenesis, and immunomodulatory | Yang et al. (2010); Izuta et al. (2009) |
| Trans- 2-decenoic acid (2DEA) | Estrogenic effect | Suzuki et al. (2008) |
| Adenosine monophosphate (AMP) N1-oxide | Neurotrophic effects | Hattori et al. (2007a) |
| Royal jelly peptides (RJPS) | Antioxidant activity | Guo et al. (2009) |
| 24-methylenecholesterol (24MET) | Estrogenic effect | Suzuki et al. (2008) |
| MRJP1 | Antihyperlipidemic activity | Kashima et al. (2014) |

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2011), and *Paenibacillus larvae* (Table 2) (Bachanová et al., 2002; Bíliková et al., 2001; Kim and Jin, 2015).

The antibacterial effect of major royal jelly protein 2 (MRJP2) on *Paenibacillus larvae* seems to be due to the presence of apidaecin and hymenoptaecin peptides in the RJ (Sagona et al., 2015; Chan et al., 2009). The high concentrations of RJ play a crucial role in defending against *Pseudomonas aeruginosa* (García et al., 2010). The antibacterial activity of RJ against *P. aeruginosa* may be of importance in the development of ointments for wound treatment (Table 1) (Abdelatif et al., 2008).

RJ exhibited a wide spectrum of antifungal activities against *Aspergillus fumigants, Aspergillus niger, Candida albicans*, and *Syncephalastrum racemosum* (Moselhy et al., 2013) (Table 1). On the other hand, the ether-soluble fraction of RJ is also effective against bacteria such as *Staphylococcus aureus, Escherichia coli, Streptomyces griseus*, and three unclassified strains of *Streptomyces* (Eshraghi and Seifollahi, 2003).

With the purpose of analyzing the effect of phosphorylation modification of proteins in RJ, Han et al. (2014) synthesized native Jelleine-II and phosphorylated Jelleine-II one residue as Jelleine–II phosphothreonine and studied their antibacterial activities against *Paenibacillus larvae, Staphylococcus aureus, Bacilus subtilis, Pseudomonas aeruginosa*, and *Escherichia coli*. The results showed that native Jelleine-II has antibacterial activities against these species, whereas the Jelleine-II showed an apparent decrease in antibiosis activity with no antibacterial activity against *P. aeruginosa* and *S. aureus*, even at the concentration of 320 $\mu$g/mL, while it inhibited *E. coli* (Han et al., 2014).

Therefore, phosphorylation of threonine (Thr) residues in jelleines provides a negative charge in the peptide, causing a significantly decreased antibacterial function. Therefore, the net charge of the jelleines likely has an important role in their antibacterial property. Hence, phosphorylated Thr residues have a strong negative effect on the reaction between the membrane of microorganisms and Jelleine-II peptides, which influences their antibacterial activity.

## Wound-healing activity

Pedyphar ointment contains RJ and panthenol in an ointment base and can cause an alkaline environment in the wound that obliterates infection and ameliorates diabetic foot ulcers (Abdelatif et al., 2008) (Table 1). In fact, antimicrobial peptide royalisin isolated from RJ plays an important role in protecting wounds against infection (Table 1) (Bărnuţiu et al., 2011).

Some reports showed that RJ possesses an anti-inflammatory action by reducing exudation and collagen formation in granulation tissue and augmented wound healing in

streptozotocin-induced diabetic rats. RJ also significantly decreased the healing period of desquamated skin lesions (Fujii et al., 1990). A clinical study has shown that RJ improves wound healing in patients with diabetic leg ulcers, and it is more useful when used along with other standard treatments (Siavash et al., 2011). In hamsters, the topical application of RJ in a dose-dependent manner has healing action on severe oral mucositis induced by chemotherapy (Suemaru et al., 2008).

## Antioxidant activity

RJ has recently received particular attention as a highly efficient antioxidant and a potent free-radical scavenger (Cemek et al., 2010; Silici et al., 2009). In a recent study, 29 antioxidative peptides were separated from RJ hydrolysate through a membrane anion-exchange chromatography, gel-filtration chromatography, reverse-phase high-performance liquid chromatography, and ultrafiltration. The examination of the antioxidative effects of 12 small peptides with 2–4 amino acid residues (Phe-Arg, Ala-Leu, Lys-Phe, Ile-Arg, Phe-Lys, Arg-Tyr, Tyr-Asp, Lys-Leu, Lys-Tyr, Lys-Asn-Tyr-Pro and Tyr-Tyr, Leu-Asp-Arg) showed the activity of radical scavenging by hydroxyl groups of amino acids of these peptides. For example, three tyrosyldipeptides (Tyr-Tyr, Lys-Tyr, and Arg-Tyr) in RJ have free-radical-scavenging activity by rapid donation of a hydrogen atom from their phenolic hydroxyl group of free radicals. The antioxidant effects of these peptides were reported to be due to a combination of their abilities to scavenge reactive oxygen species (ROS) of antioxidative systems (Tables 1 and 2) (Guo et al., 2009).

As reported by Silici et al. (2009), the administration of RJ to cisplatin-treated rats increased the activities of superoxide dismutase, catalase, and glutathione-peroxidase and decreased levels of malondialdehyde in the testicular tissue. Another study reported that RJ remarkably decreased oxidative stress caused by fumonisin and certainly increased antioxidant status (El-Nekeety et al., 2007). In a previous study, we explained the main mechanism of RJ's antioxidant effect involves enhancement of total antioxidant capacity, and catalase (CAT) activity, and reduction of malondialdehyde (MDA) levels (Ghanbari et al., 2015).

Data have shown that RJ is able to scavenge free radicals such as superoxide radicals, hydroxyl radicals, and 1, 1-diphenyl-2-picrylhydrazyl hydrate (DPPH) radicals (Watanabe et al., 2013). A previous report suggested that RJ remarkably decreased the levels of 8-hydroxy-2-deoxyguanosine, a marker of oxidative stress, in serum and kidney tissue of mice (Inoue et al., 2003). A study showed that administration of RJ caused a significant protective effect against cadmium-induced oxidative damage and genotoxicity through inhibition of malondialdehyde production and up-regulation of the levels of reduced glutathione (Çavuşoğlu et al., 2009).

In addition, RJ showed a dominant protective role against oxidative stress in liver and renal tissues exposed to carbon tetrachloride and cisplatin; this effect is associated with declined MDA production and significant increased activities of the main cellular antioxidant enzymes such as glutathione reductase (GR), glutathione peroxidase ($GP_X$), superoxide dismutase (SOD), and CAT (Karadeniz et al., 2011; Silici et al., 2009). Clinical studies have shown that RJ supplementation reduced oxidative stress via improved levels of MDA and activities of GPx and SOD in erythrocytes of diabetic patients (Table 1) (Pourmoradian et al., 2014).

## Anticancer activity

RJ has a remarkable anticancer property in mice and humans (Kimura, 2008). While intraperitoneal or subcutaneous administration of RJ 7 and 14 days before or 2 and 9 days after tumor

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cell inoculation had no effect on formation of mammary metastases, RJ remarkably inhibited the formation of metastases in the lungs of mice when administered synchronously with tumor cells (Orsolic et al., 2005). It has been reported that crude extraction of RJ protein inhibits Bisphenol A– (BPA-) induced proliferation of human breast cancer cells (MCF-7) (Nakaya et al., 2007).

Vascular endothelial growth factor (VEGF) is one of the pro-angiogenic proteins well characterized as an inducer of cancer neovascularization (Pan et al., 2013). Treatment with 10HDA suppressed VEGF-induced tube formation and inhibited migration and proliferation in human umbilical vein endothelial cells (HUVECs); therefore, this compound inhibits in vitro HUVECs angiogenesis (Tables 1 and 2) (Izuta et al., 2009).

It has been suggested that N-acetylation process catalyzed by cytosolic N-acetyl transferase (NAT), a key enzyme in the metabolism and biotransformation of different drugs and compounds, seems to play an important role in the etiology of colorectal, bladder, and breast cancers (Premratanachai and Chanchao, 2014). However, it is not clear whether RJ can inhibit cytochrome P450 activity in the human hepatocellular carcinoma derived J5 cell line, while it declined the N-acetylation of 2-aminofluorene cells and inhibited N-acetyltransferase activity in tumor cells (Zamani et al., 2012).

## Antidiabetic activity

The results of 8 weeks RJ (1,000 mg) administration to type 2 diabetes patients showed that RJ significantly decreased fasting blood glucose levels and elevated levels of serum insulin (Pourmoradian et al., 2014) (Table 1). Clinical studies have shown that RJ administration (20 gr) remarkably decreased serum glucose levels in healthy individuals (Münstedt et al., 2009).

Insulin resistance is associated with changes in the levels of oxidative stress (Kumashiro et al., 2008; Rösen and Osmers, 2006). Moreover, a study reported that RJ has antioxidant capacity (El-Nekeety et al., 2007). These findings imply that RJ may decrease insulin resistance via anti-oxidative properties. In addition, RJ may be effective in hypertension associated with insulin resistance. Study results showed that treatment with RJ (100 and 300 mg/kg doses) for eight weeks ameliorated insulin resistance in fructose-drinking rats (Zamani et al., 2012).

## Immunomodulatory and anti-inflammatory activity

Researchers believe that the anti-inflammatory functions of RJ are expressed through the inhibitory properties on the capillary permeability in the acute phase of inflammation and by the decreased granulation of tissue in the chronic phase of inflammation in streptozotocin-induced diabetic rats (Fujiwara et al., 1990).

Conversely, RJ inhibited the production of some proinflammatory cytokines such as interleukin- (IL-) 1, TNF-$\alpha$, and IL-6 without having cytotoxic properties of macrophages (Kohno et al., 2004). Oral dose of RJ (150 mL/kg for four weeks) in rats with colitis induced by acetic acid caused a significant decrease in the number of mast cells and the area of colonic erosion (Karaca et al., 2010).

A report showed that RJ has anticolitogenic functions and increased mucin content of the colonic mucosa in rats with ulcerative colitis; these actions may be attributed to the improvement of the antioxidant defense status of these rats (Nieto et al., 2002). The proportion of colonic CD68+ macrophages and T cells (CD3+, CD5+, CD45+) were reduced in colitised

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rats treated with RJ, and decline in number of CD68+ cells is attributed to the decrease in mucosal inflammation (Karaca et al., 2012).

The report showed that RJ might be beneficial for the treatment of autoimmune diseases (Mannoor et al., 2009). Recent studies have revealed that RJ fatty acids such as 3, 10-dihydroxy-decanoic acid (3, 10-DDA) and 10-HDA at the concentration of 500 μM using an in vitro model of phytohaemagglutinin-activated peripheral blood mononuclear cells (PBMCs) inhibited the proliferation of PBMCs, suppressed immune responses of Th1 and Th2, and modulated the production of cytokine tumor necrosis factor-$\alpha$ (TNF-$\alpha$) and IL-1$\beta$. While 3, 10-DDA at the concentration of 500 μM had no effect on the levels of proinflammatory cytokines TNF-$\alpha$ and IL-1$\beta$, the same dose of 10-HDA suppressed the production of these cytokines by stimulated PBMCs. Therefore, this compound in RJ may be an effective immunosuppressive factor (Table 1) (Mihajlovic et al., 2014).

According to the study by Oka et al. (2001), RJ suppresses allergic reactions in association with the improvement of Th1/Th2 cell responses and the restoration of macrophage function in mice (Oka et al., 2001). In addition, one of the salutary activities of RJ supplement is improvement in the quality of life of individuals with autoimmune disorders such as inflammatory bowel disease and rheumatoid arthritis (Kohno et al., 2004). In mice, RJ stimulates immune-competent cell proliferation and antibody production; in another animal model, it has immunomodulatory activities via depressing humoral immune functions (Šver et al., 1996).

As stated, RJ increased the production of interferon-gamma (IFN-$\gamma$) and induced the proliferation of healthy lymphocytes. RJ therapy in patients with autoimmune Graves' disease changed the ratio of Th1/Th2 cytokines toward the Th1. This suggests that RJ may be potentially effective in Graves' disease as an immunomodulatory factor and antithyroid drug treatment (Erem et al., 2006).

Moreover, MRJP3 glycoproteins in RJ have an immunosuppressive activity in the mammalian immune system (Okamoto et al., 2003). Antiallergic factors in RJ reduced the production of IL-4 by anti-CD3–stimulated lymph node cells from ovalbumin (OVA)/alum-immunized mice. Using a series of column chromatographies, researchers purified major RJ protein 3 (MRJP3), a 70 kDa glycoprotein, that suppressed the production of IL-4, IL-2, and IFN-$\gamma$ by T cells accompanied with inhibition of proliferation. The MRJP3-mediated inhibition of the production of IL-4 was clear when lymph node cells were stimulated with OVA plus antigen-presenting cells (APCs) in mice immunized with OVA/alum. In addition, the effect of the purified suppressive agent on OVA/alum-induced allergic responses in mice showed that MRJP3 administration is clearly inhibited in immunized serum levels of anti-OVA IgG1 and IgE. Moreover, heat-treated MRJP3 had markedly reduced antigenicity but retained its inhibitory activity on antibody responses to OVA. In in vitro and in vivo experiments, findings revealed that MRJP3 could show strong immunoregulatory activities. In addition, immunomodulatory activity of MRJP3 can be of clinical importance in designing MRJP3-isolated antiallergic polypeptides through diagnosis of the associated peptide sites (Okamoto et al., 2003).

Many bioactive compounds of RJ have immunomodulatory effects; however, the mechanism of its function on the immune system has not yet been completely revealed. On the other hand, researchers have stated the role of 3, 10-dihydroxydecanoic acid (3, 10-DDA) on maturation of human monocyte-isolated dendritic cells (MoDCs). They reported that 3, 10-DDA induced maturation of MoDCs through up-regulating the gene expression of CD1a, CD40, CD54, and CD86 and enhanced their allostimulatory capability with allogenic CD4 +T cells in co-culture; 3, 10-DDA-treated MoDCs increased IL-18 and IL-12 production and induced interferon-c production with allogeneic CD4 +T cells while downregulating the level of IL-10

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in co-culture as compared to control MoDCs. In vitro, the findings showed that 3, 10-DDA induced Th1 polarizing potential of human MoDCs and maturation, which may be advantageous for antiviral and antitumor immune responses (Dzopalic et al., 2011).

## Antihyperlipidemic activity

Clinical studies have suggested that RJ consumption decreased levels of serum cholesterol and total lipids in humans with atherosclerosis (Vittek, 1995). A recent human study revealed RJ ameliorates hypercholesterolemia in human participants (Hang et al., 2007).

Major RJ protein 1 (MRJP1), a peptide derived from RJ, showed cholesterol-lowering activity and decreased the cholesterol absorption in Caco-2 cells. Hence, MRJP1 might also inhibit the micellar solubility of cholesterol in these cells, thus lowering levels of cholesterol. This compound binds to bile acids and induces a tendency to enhance the excretion of fecal cholesterol, and increases cholesterol catabolism in the liver (Kashima et al., 2014). Moreover, a recent study demonstrated that administration of RJ delayed the formation of atheroma in the aorta of rabbits with atherosclerosis (Abdelhafiz and Muhamad, 2008).

The administrations of RJ for three months in healthy mild hypercholesterolemic individuals could considerably increase the level of dehydroepiandrosterone sulfate and thereby significantly decrease the serum levels of total cholesterol and low-density lipoprotein cholesterol. Accordingly, RJ could act as an excellent hypocholesterolemic agent and impressively reduce the risk of cardiovascular disease (Chiu et al., 2017).

## Antihypertension activity

Hypertension has become a serious risk factor that may result in heart failure, acute myocardial infarction, and cerebral stroke in humans (Takaki-Doi et al., 2009). Recently, it has been reported that gastrointestinal enzyme hydrolyses of RJ may be responsible for reducing high blood pressure in humans (Kajimoto et al., 2005). A scientific report showed that MRJPs have a potential functional effect in resisting hypertension (Leung et al., 1997) (Table 1), although the functional ingredient is not yet evident. In in vitro experiments, the vascular smooth muscle cell (VSMC) is best cell model used for the assay of vascular diseases (Devlin et al., 2000).

The abnormal migration and contraction of VSMCs are important stimulating agents of several vascular diseases such as vascular stenosis after vascular remodeling, atherosclerosis, and hypertension (Chu et al., 2011; Kim et al., 2008). Researchers stated that Ang II has an important biological function in the migration and contraction of VSMCs (Daemen et al., 1991), and scratch wound test is a beneficial method to evaluate the ability of cell migration of VSMCs (Kotha et al., 2009). Here, the expression level of anti–$\alpha$-smooth-muscle actin ($\alpha$-SMA) remarkably reduced and inhibited cell migration of Ang II–induced VSMCs. It has been suggested that glycosylated MRJP1 is an important agent in RJ for the regulation of blood pressure after identification of glycosylated MRJP1. The glycosylated MRJP1 isolated from eastern bees has a stronger effect on blood pressure regulation than that of western bees. Because their regulatory mechanism remains unclear, this offers new hope for the treatment of hypertensive disease using glycosylated MRJP1.

N-glycosylated MRJP1 in two samples of RJ revealed antihypertensive properties and showed a stronger effect in *Apis cerana cerana* (Acc), indicating that modification and specific RJ protein are functionally important in the therapy of high blood pressure disease in humans. Feng et al. (2015) reported that the two RJ samples have evolved a species-specific

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glycosylation form to fine tune maximum molecular activity of protein as immune factors effect on human health care (Table 1).

## Effects on fertility

A clinical trial reported that intravaginal administration of RJ plus bee honey may be an effective treatment for infertility caused by asthenozoospermia (Abdelhafiz and Muhamad, 2008). The authors reported that treatment with RJ for 48 days led to increased sperm parameters such as progressive motility of sperm, sperm count, and viability as well as testosterone concentration in bleomycin-treated rats (Amirshahi et al., 2014). Diabetes mellitus had significant harmful effects on reproductive parameters such as testosterone concentration, sperm count, sperm viability, and motility of the sperm. RJ ameliorated the diabetes-induced decreases in weight of testis, DNA integrity, sperm viability, and sperm motility and increased testosterone concentration (Ghanbari et al., 2016a; Ghanbari et al., 2015) (Table 1).

Recent reports suggested that a single RJ (500 mg) injection increased reproductive performance of seasonal sheep breeds in the anestrus season (Gimenez-Diaz et al., 2012). Administration of RJ to ewes increased lambing and pregnancy rates, but it was not effective in increasing estrus during the transition season of breeding to nonbreeding (Kridli and Al-Khetib, 2006). However, it has been shown that RJ remarkably improved their physiological status and increased the seminal fructose, ejaculate volume, number of sperm output, sperm motility, and serum testosterone concentration in bucks (Elnagar, 2010) (Table 1).

Kohguchi et al. (2007) reported that hamsters treated with RJ showed a significant elevation in total sperm counts relative to the control group. Eshtiyaghi et al. (2016) declared that treatment of ovine oocytes with RJ (10 mg/mL) during in vitro maturation (IVM) increased oocyte and nuclear maturation rate, fertilization rate, and blastocyst formation, which might be due to increased activity of antioxidant enzymes in both oocyte and cumulus cells (Table 1).

In vivo study showed that oral administrations of RJ to adult mice treated with oxymetholone at 100 mg/kg body weight for 30 days led to a significant increase in percentage of blastocysts and fertilization rate as well as a marked decrease in the percentage of arrested embryos (Zahmatkesh et al., 2015). Oral or intraperitoneal administration of RJ (10% solution) to male rats stimulated the production of hormones such as testosterone, progesterone, and luteinizing hormone (Hang et al., 2008). Previous studies reported that diabetes mellitus induced a testicular apoptotic cell death (caspase-3–positive cells). Daily treatment with 400 mg/kg of RJ in diabetic male rats for 28 days caused a reduction in the mean number of caspase-3–positive cells (Karaca et al., 2015).

## Estrogenic effects

RJ has shown estrogenic effects in vitro and in vivo that were mediated via interaction with estrogen receptors (ERs) followed by alterations in the cell proliferation and gene expression (Mishima, Miyata, et al., 2005; Mishima, Suzuki, et al., 2005). Suzuki et al. (2008) stated that four bioactive substances isolated from RJ—10-hydroxy-trans-2-decenoic acid (10H2DA), 10-hydroxydecanoic acid (10HDA), trans- 2-decenoic acid (2DEA), and 24-methylenecholesterol (24MET) (Figure 2)—showed estrogen receptor $\beta$– (ER $\beta$–) binding effect and inhibited the binding of 17 $\beta$-estradiol (E2) to the ER $\beta$ in vivo (Table 2). Another study showed that royal jelly supplementation in frozen extender can improve post-thaw quality and in vitro fertilizing capacity of Nili-Ravi buffalo bull sperm (Shahzad et al., 2016).

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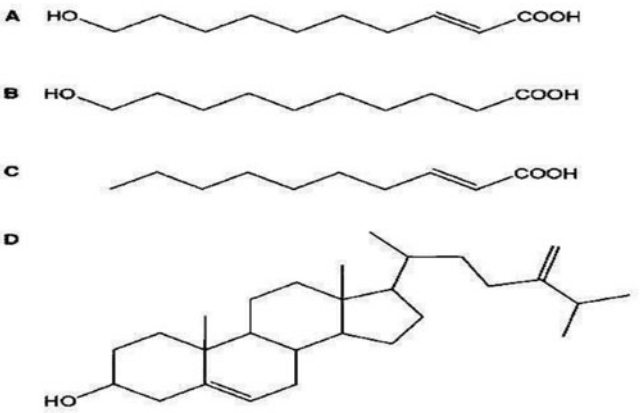


**Figure 2.** Isolated bioactive components from royal jelly. (A) 10-Hydroxy-2-decenoic acid (10HDA), (B) 10-hydroxy-trans-2-decenoic acid (10H2DA), (C) trans-2-decenoic acid and (2DEA), (D) 24-methylenecholesterol (24MET). (Suzuki et al., 2008).

## Hepato-renal protective activity

RJ ameliorates the cisplatin-induced serum level of alanine aminotransferase and histological changes in the hepatic tissue via its antioxidant properties, free-radical scavenging, and antiapoptotic effects (Yildirim et al., 2012) (Table 1). The ability of RJ to induce the regeneration of liver and kidney tissues in fumonisin intoxication may be due to hepatoprotective activity and the free-radical scavenging of RJ (Table 1) (El-Nekeety et al., 2007).

Other researchers have reported protective properties of RJ on irradiation-induced injury to liver tissue. This protection may be attributed to the amelioration of antioxidant defense system, the inhibition of MDA production, and the free-radical-scavenging activity of RJ (Cihan et al., 2013) (Table 1). Acute liver damage induced by paracetamol has been reported to be ameliorated in female mice fed with a dietary supplement of RJ at a dose of 200 mg/kg body weight for seven days (Table 1) (Kanbur et al., 2009).

The protective effect of RJ against liver and kidney damage caused by diabetes mellitus was examined. RJ consumption (100 mg/kg for 6 weeks) led to significant improvement in urine parameters such as creatinine, urea, albumin, and uric acid and histopathological changes of kidney and liver tissue in diabetic rats (Ghanbari, Nejati, and Azadbakht, 2015; Ghanbari et al., 2016b). Silici et al. (2011) have reported significant enhancement in the levels of serum urea nitrogen, total protein, uric acid, and bilirubin in rats with cisplatin nephrotoxicity. In addition, treatment with RJ before and after cisplatin administration remarkably improved the levels of these parameters. They also reported that RJ may have protective activity against harmful effects of cisplatin.

## Neurotrophic effects

In vivo studies showed that treatment of trimethyltin-intoxicated mice with RJ caused a significant elevation in the number of Nissl-stained cells in damaged dentate gyrus (Hattori et al., 2010). On the other hand, administration of RJ stimulates neurite outgrowth of cultured pheochromocytoma PC12 cells (Hattori et al., 2007b; Hattori et al., 2006) through adenosine A2a receptors, and elevates the phosphorylation of both cAMP-response element-binding protein (CREB) (Hattori et al., 2007c) and extracellular signal-regulated kinase 1/2 (ERK1/2)

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in cultured PC12 cell lines (Hattori et al., 2007b; Hattori et al., 2010) (Table 2) neural progenitors or neural stem cells (NPC/NSs).

Different studies have also reported that RJ facilitates the differentiation to various types of brain cells such as neurons from cultured NPC/NSs. Hence, it is conceivable that RJ may induce neurogenesis in the hippocampal dentate gyrus in vivo model. Adenosine monophosphate (AMP) N1-oxide as one of the ingredients of RJ (Hattori et al., 2007b) facilitates the generation of astrocytes of NPC/NSs (Hattori et al., 2007a) and induces neuronal differentiation of PC12 cells (Hattori et al., 2006).

In fact, 10-HDA is a compound found only in RJ; it reduces the generation of astrocytes and oligodendrocytes and enhances differentiation of neurons from neural stem cells (NSCs) (Hattori et al., 2007c).

## Other effects

Hidaka et al. (2006) showed that both RJ and protease-treated RJ (pRJ) have a stimulatory effect on bone formation and may prevent osteoporosis induced in ovariectomy rats through increasing intestinal calcium absorption, but not via antagonizing the action of parathyroid hormone (PTH), while these compounds do not have an inhibitory effect on bone resorption (Table 1).

A few studies reported that postmenopausal osteoporosis was caused by decreased production of estrogen hormone, and RJ has estrogenic effects to ameliorate postmenopausal osteoporosis. Furthermore, RJ is effective on bone metabolism and collagen biosynthesis and enhances collagen production (Fujii et al., 1990; Kim et al., 2010; Park et al., 2012) (Table 1).

Researchers have suggested that treatment with RJ stimulated gene expression of collagen crosslinking and encoding enzymes of collagen-modifying *in vitro*. Judging from these data, RJ may ameliorate the quality of bone through regulating the posttranslational modifications of type I collagen (Kaku et al., 2014).

RJ enhances collagen production by suppressing matrix metalloproteinase (MMP) activity via the inhibition of JNK-AP-1 and p38 signaling pathways and inhibits the production of transforming growth factor-b1 (TGF-b1) (Yang et al., 2010; Satomi et al., 2004). RJ does not have considerable side effects, and treatment of rats with ovariectomy-induced estrogen deficiency with RJ indicated protective effects against skin aging via increased production of collagen (Satomi et al., 2004).

## New findings of royal jelly

RJ promoted the reduction of dietary-related obesity and glucose intolerance mediating reinforcement of thermogenesis in mouse brown adipose tissue. Therefore, RJ may be a novel promising dietary supplement to fight obesity and metabolic disorders (Yoneshiro et al., 2017). RJ led to reduction of oxidative stress induced by cyclophosphamide through reduction of MDA and accession of total antioxidant capacity and glutathione peroxidase activity. The antioxidant activity of RJ seems to be a novel and safe process to attenuate cyclophosphamide-mediated prostate damage (Abdel-Hafez et al., 2017).

The RJ treatment of children with systemic lupus erythematosus for three months improved several immunological markers such as reduction of apoptotic T lymphocytes, enhancement of the percentage of CD4+, CD25+, and T cells regulating CD4+, and increase of CD4+ T cells (Zahran et al., 2016). However, the study was conducted with a small number

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of patients, and it is necessary to conduct an extensive study to determine the alleviating effect of RJ in treatment of systemic lupus erythematosus.

RJ has been used since ancient times to promote wound healing, and the antiviral and wound-healing effect of RJ to control dermal infection induced by *Staphylococcus aureus* resistant to methicillin has been demonstrated repeatedly (El-Gayar et al., 2016). Administration of RJ (150 mg) for three months has been shown to lead to dramatic improvement of serum lipid factors (reduction of total cholesterol and LDL and increase of HDL) of postmenopausal women (Lambrinoudaki et al., 2016). Ovariectomy mice exposed to RJ show improvement in mental impairment and depression-like behavior. RJ reduced the promotion of cancer through activation of estrogen receptor $\alpha$ and (slightly) estrogen receptor $\beta$. It seems that RJ can be used as a dispensable factor instead of hormones to treat neurological symptoms of menopause complications (Minami et al., 2016).

Recent research has shown that RJ as an efficient food is useful for patients with type 2 diabetes. It is associated with a desirable effect on glucose and apolipoprotein A-I that attenuates cardiovascular attack in patients with type 2 diabetes. However, further investigation is required to survey the effect of RJ on modulation of lipoproteins (Khoshpey et al., 2016).

## Conclusions

RJ contains various active compounds (such as 10HDA, AMP N1-oxide, and RJPs) that have been shown to possess remarkable therapeutic properties for different diseases. RJ and its bioactive compounds exhibit broad medicinal uses due to its antioxidant effects and antibacterial, antidiabetic, anticancer, anti-inflammatory, antihyperlipidemic, and immunomodulatory properties. It also plays an important key role in liver and kidney protection, wound healing, and in the reproductive system. In diabetic patients, it showed hypoglycemic effects, decreased lipid peroxidation levels, and elevated the activities of antioxidant enzymes such as CAT, GSH-PX, and SOD. It is necessary to be aware of the side effects of RJ such as allergic responses. Regarding the several biological properties of RJ, more clinical trials are required to prove the effectiveness of RJ in human models and to find effective compounds of RJ that may lead to the synthesis of new medications.

## Declaration of interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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