

■ Review

Royal jelly: a natural therapeutic?

Arı sütü: doğal bir terapötik mi?

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ABSTRACT

Royal jelly is a secretion of honeybees that is actually for feeding of bee larvae. It contains many bioactive substances such as jelleins, royalisin, major royal jelly proteins and 10-hydroxy-2-decenoic acid. Recently, many studies were published investigating activities of royal jelly. In overall, royal jelly found to have antioxidant, neurotrophic, antidiabetic, hypocholesterolemic, regulatory on blood pressure, antimicrobial, immunomodulatory, anti-tumor and tissue-protective effects. These activities are strongly related to each other and cannot be evaluated separately. Researchers claim that royal jelly can be an excellent therapeutic agent (or an additional agent), especially in treatments of cancer and metabolic syndrome. However, there are huge gaps due to limited number of in vivo studies and there are standardization issues both for usage and investigations. Furthermore, wide variability of contents in royal jelly due to geographic locations, climate, etc also causes a problem in choice of exact royal jelly. Currently it seems to be safe and effective nutritious agent for healthy people, but it is hard to gain an overall perspective in usage as a therapeutic, since there is a necessity of wider studies with more different types of royal jelly.

Keywords: royal jelly, apitherapy, honeybee products, biopharmaceuticals, complementary medicine, integrative medicine

ÖZ

Arı sütü aslen arı larvalarının beslenmesi için bal arıları tarafından üretilen bir sekresyondur. Jelleinler, royalizin, ana arı sütü proteinleri ve 10-hidroksi-2-desenoik asit gibi çok sayıda biyoaktif madde içermektedir. Son yıllarda arı sütü konusunda çok sayıda makale yayımlanmıştır. Genel olarak, arı sütü, antioksidan, nörotropik, antidiyabetik, hipokolesterolemik, kan basıncı düzenleyici, antimikrobiyal, immünomodülatör, anti-tümör ve doku koruyucu etkileri göstermektedir. Bu etkiler aslen bir bütündür ve mekanizmaları ayrı ayrı değerlendirmek mümkün değildir. Araştırmacılar, özellikle kanser ve metabolik sendrom tedavisinde iyi bir tedavi ajanı (veya tedaviye ek bir ajan) olabileceğini iddia etmektedir. Öte yandan, in vivo çalışmaların sayısı son derece sınırlıdır ve hem araştırma hem kullanım alanlarında standardizasyon sorunu vardır. Ayrıca, coğrafi konum, iklim gibi koşullara bağlı olarak arı sütü içeriğinin ciddi farklılıklar göstermesi, arı sütünün seçiminde sorunlar yaratmaktadır. Şu an için, sağlıklı insanlarda güvenli ve etkin bir besleyici gıda olarak gözükmemekte, fakat terapötik kullanımı konusunda genel bir görüş elde etmek mümkün gözükmemektedir. Farklı türlerde arı sütleri üzerinde daha geniş çaplı araştırmalar yapılmalıdır.

Anahtar kelimeler: arı sütü, apiterapi, arı ürünleri, biyofarmasötik ajanlar, geleneksel ve tamamlayıcı tıp

INTRODUCTION

Royal jelly (RJ) is a secretion of honeybees that is actually for feeding of bee larvae. Although there are many ancient medical records of its usage, it has been widely used and consumed especially in Asia. Recently, it has been reported that RJ has various actions with the potential of improving human health, especially focusing on reproductive health, neurologic diseases, wound healing, anti-aging and anti-cancer via several mechanisms [1,2]. Antioxidant, neurotrophic, hypocholesterolemic, tissue-protective, regulatory on blood pressure, antimicrobial, antiinflammatory, immunomodulatory and antitumor effects were observed in many scientific studies. These actions mainly depend on bioactive substances such as jelleins, royalisin, major royal jelly proteins (MRJPs) and 10-hydroxy-2-decenoic acid (10-HDA). These substances have strong pharmaceutical actions on human physiology [3-5].

POTENTIAL ACTIONS

It is very difficult to line strong borders in order to separate modes of actions from each other. However, studies on RJ have mainly focused singularly on particular actions or some of particular substances. Despite of these issues, it is possible to make a general vision and explain potential actions in separated headings. In overall, RJ has antioxidant, neurotrophic, antidiabetic, hypocholesterolemic, regulatory on blood pressure, antimicrobial, immunomodulatory, anti-tumor and tissue-protective effects.

Antioxidant Activity and Tissue-protective Effect

Although the effect seems to be time-dependent, activity against oxidative stress and DNA damage was shown in studies on rats [4,6]. Narita et al. [7] studied on

endocrinologic alterations on mice and reported that RJ had a strong regulatory effect against aging. Another study stated that radiation induced apopytosis can significantly be prevented with RJ [8]. El-Nekeety et al. [9] reported strong histopathologically antioxidative activity on the livers and kidneys of RJ-consuming mice during experimental oxidative damage by fumonisin. Similar effects of RJ against cisplatin-induced toxicity were studied by Silici et al. [10], and they reported significant improvements on testosterone levels, normal sperm ratios and on histopathologic damage of testicle, prostate and epididymis tissues in RJ group. Anbara et al. [11] and Manas et al. [12] were also stated endocrinologic regulation and decrease on testicular damage with RJ. Another study on female rats indicated that RJ had the potential of being therapeutic agent regarding female infertility via same antioxidant activities [13]. Abdel-Hafez et al. [14] investigated cyclophosphamide-induced prostate damage, and observed that RJ significantly reduced eNOS and Bax expression, regulated biochemical markers and decreased histopathological damage. Pajovic et al. [15] also reported similar effects of RJ as a potential therapeutic agent in benign prostatic hyperplasia.

In many studies, RJ also found protective via antioxidant mechanisms in other body parts. Studies with cisplatin and oxymetholone strongly indicated hepatoprotective and nephroprotective effects [16-20]. Malekinejad et al. [21] also reported cardioprotective activity dose dependently. In a recent study, researchers found particular improvements on diabetic markers and liver function panel results by regular consuming of RJ in streptozotocin-induced diabetic rats. Regarding these findings, they claimed a direct relationship with antioxidant activity of RJ [22]. Similarly, Kanbur et al.

[23] studied on paracetamol-induced hepatotoxicity, and they stated that they could not find any difference between RJ and control group, with significant hepatotoxicity in single-parasetamol group. Additionally, Kamakura et al. [24] published an in vitro study indicating that RJ proteins can stimulate proliferation of rat hepatocytes. Interestingly, oral consuming of RJ was also found to have an effect against oxidative stress in the dry eye by increasing lacrimal tear secretions [25].

A few studies focused on bone tissues. Araki et al. [26] compared mice tibia cells between RJ and control groups, and they stated that RJ had positive impact on bone formation such as increasing the extracellular matrix. The effect mechanism is actually still under shadows, but Hidaka et al. [27] reported an indirect pathway indicating that RJ actually stimulates gastrointestinal calcium absorption, but it does not affect by blocking parathyroid hormone mechanisms to prevent osteoporosis.

Although there are several studies on tissue damages, data about molecular interactions of RJ is very limited. There are articles indicating that RJ somehow provokes activities of glutathione peroxidase and superoxide dismutase [10,28]. In an example study of Makino et al. [29], it was found that RJ substances had a direct regulatory effect on expression of superoxide dismutase. In another study on central nervous system, significant positive activities of RJ on glutathione mechanisms were stated [30]. However, there are also reports, which cause suspicion about these specialties of RJ [31]. Of course, it should be noted that chemical compositions of bee products are strongly related with geographical and climate conditions, and chemical diversity is commonly observed. Regarding to this, potential effects may change from one RJ to another. Additionally, there isn't standardization on studies of bee products, so different results may be caused. Thus, in vivo studies from different locations and with different RJs have a great importance.

Neurotrophic Effects

Antioxidant activities of RJ are actually expected to have similar effects on neuron tissue, but neurotrophic effects have become also a center of focus. As an example, in the study of Hattori et al. [32] 10-HDA was found to stimulate neurogenesis and differentiation of various types of nervous system cells such as neurons, astrocytes and oligodendrocytes. In another study of Hattori et al. [33], RJ was found to be successful to regain cognitive abilities in hippocampal dentate gyrus damaged mice. In an experimental Alzheimer's disease model, RJ consumption significantly improved spatial memory in mice [34]. In the

same model, RJ reduced neurodegeneration and resulted particular improving effects against amyloid-beta toxicity [35]. A recent study indicated that RJ consumption increased cognitive functions and improved spatial memory by effecting on sistein and taurin metabolisms in elder mice [36]. Hashimoto et al. [37] investigated RJ-induced potential alterations on mRNA expressions of neutrophic factors, their receptors and neural cell markers to observe neuroprotective effects and variations of neurogenesis. They found that mRNA expressions of glial cell line-derived neurotrophic factor (a potent neurotrophic factor), neurofilament H (a specific marker for neural axons in hippocampus) were increased by RJ. Morita et al. [38] compared healthy human control and RJ groups and they reported RJ group showed statistically significant superiorities about mental health, handling mental stress and social functionality. Additionally, in an interesting study, Minami et al. [39] stated that RJ consumption reduced postmenopausal neurologic symptoms in rat models. As understood, studies are mostly on animal models, and there is a very limited data, but it seems RJ provides a huge potential especially for neurodegenerative diseases.

Antidiabetic, Hypocholesterolemic, Regulatory on Blood Pressure

RJ can decrease serum glucose level by its insulin-like peptides and other contents (sulphur, Vit B3, Vit H, etc) [3]. Morita et al. [38] stated that RJ did not make any change in HbA1c levels, but they observed serious improvements in insulinogenic index and fasting plasma glucose (FPG) ratios, that indicates stimulation of insulin secretion. They also claimed that RJ caused an increase in testosterone levels, which resulted with arised glucose tolerance and eritropoiesis. Despite of these data, Pourmoradian et al. [28] found decreasing HbA1c levels, but for FPG ratios, there was not any alteration. This conflict may be a result of sample size or dose-dependency, since researchers also reported arised insulin levels and accordingly, decreased serum glucose. Zamami et al. [40] stimulated hypertriglyceridemia and hyperinsulinemia in rats, and in the RJ group, there was significantly lower triglyceride and insulin levels without any particular difference in serum glucose levels comparing to control group. They commented that these results indicated an effect of RJ against insulin resistance. In another study, RJ was found as a serious regulatory agent for diabetic biomarkers [22]. RJ also seems to improve fertilization skills in diabetic mice [41]. Münstedt et al. [42] studied on serum glucose levels during oral glucose tolerance tests in human volunteers and levels were found significantly lower in RJ group, which indicated RJ to have insulin-like activity.

Yoshida et al. [43] focused on potential molecular changes caused by long-term RJ consumption. Many alterations were found such as decreasing of G6Pase expression, increasing of *AdipoQ* ve *AdipoR1* mRNA expression. In another study, alterations were also reported in apolipoprotein levels [44].

As understood, RJ consumption does not only affect serum glucose, but also triglyceride (TG) and cholesterol levels. In the study of Guo et al. [45] on healthy human volunteers, RJ provided significant decrease in total cholesterol and LDL, despite there was no change in HDL and TG levels. Similar decreases on LDL and total cholesterol, and stable conditions of TG and HDL were also observed by Chui et al. [46]. In another study on postmenopausal volunteers, same alterations were found for total cholesterol and LDL, but significant increase in HDL levels was also noted [47]. On the other hand, arised HDL, decreased LDL and unchanging TG levels were reported with RJ consumption in a study on hypercholesterolemic patients [48]. In a recent meta-analysis study, long-term consumption of RJ was pointed as the critical key for the particular decrease in total cholesterol and LDL [49]. RJ seems to have more complex activities also in adipose tissue. Yoneshiro et al. [50] stated that RJ had a regulatory effect on hepatic TG levels and provided changes in white adipose tissue in a positive way. They also observed molecular regulations in brown adipose tissue.

There are only a few studies about effects of RJ on blood pressure. Takaki-Doi et al. [51] reported that RJ contains protein fractions, which shows anti-hypertensive effect by inhibiting angiotensin-converting enzyme-1. In a recent study of Fan et al. [52], inhibitory activity of MJP-1 on vascular smooth muscle contraction and muscle cell proliferation was found.

In overall, RJ seems to have huge potential and strong utilities in fighting against so-called "novel epidemic" obesity, hypertension, diabetes mellitus and their combination named metabolic syndrome. However, molecular mechanisms have not been totally understood yet and the number of human studies is very few. So, RJ is very promising and deserves the focus of researchers to adapt it into routine treatment models.

Anti-Tumor Effects

Of course, anti-cancer activity is strongly related with antioxidant mechanisms. However, RJ has been investigated for primarily anti-tumoral actions, too. Townsend et al. [53] studied on 10-HDA against four different tumor tissues and stated that both RJ as a whole and 10-HDA itself had anti-tumoral activities. Shirzad et al. [54] observed that

fibrosarcoma tissues in mice showed significant difference in tumor growth in favor of RJ group. They claimed that RJ provided this action by its immunomodulatory effect, and they could observe the difference in long-term therapies. They also commented that this effect may be more apparently provided by consumption of RJ before tumoral formation occurrence. Zhang et al. [55] supported these data, since they also found inhibitory effect of RJ on tumor growth, furthermore they noted that this effect depended on especially cytokine regulation. On the other hand, the study of Izuta et al. [56] indicated notably direct mechanisms. They observed that RJ inhibited cell proliferation and cell migration, and it also corrupted tumor vascularization by inhibiting vascular endothelial growth factor. It seems RJ has anti-cancer effects especially with long-term consumption, and its immunomodulatory activity is an additional utility, but it provides also a potential for novel chemotherapeutics.

Immunologic Activities

Immunologic effects of RJ are strictly connected with antioxidant and anti-tumor effects. However, immunologic activities caused by RJ have a wider perspective. Immunomodulatory, cytokine regulatory and other actions including cell-level alterations were reported. Sver et al. [57] reported immunomodulatory effect dose-dependently. In cytokine studies, RJ was found as an inhibitor for proinflammatory cytokine release, reducer for IL-2 release, inhibitor for expression of IL-2 cytokine receptors [58,59]. Despite of these, RJ stimulated IL-10 production, which is also known as human cytokine synthesis inhibitory factor and acts as an anti-inflammatory cytokine [59,60]. Kohno et al. [58] also reported that fatty acids of RJ downregulated splenic T cells and expression of MHC-II and CD86, and additionally inhibited IL-12 synthesis. Gasic et al. [61] also studied on RJ fatty acids, they showed immunomodulatory effects on T cells and significant decrease of IL-2 and nitric oxide in vitro. In a study on immunodeficient mice, 1% RJ consumption resulted with significant regulation of cytokine release and T, B cell responses [62]. An interesting recent study indicated that long-term RJ consumption caused strong reduction in pulmonary fibrosis and suppression in proinflammatory cytokines and IFN γ [63]. These immunoregulatory activities caused a questionnaire whether RJ may be a solution to autoinflammatory diseases. Mannoor et al. [64] studied on experimental systemic lupus erythematosus (SLE) mice model comparing RJ and control groups. RJ significantly delayed emerging of symptoms, decreased proteinuria, and reduced anti-dsDNA antibody, anti-ssDNA antibody, anti-erythrocyte antibody and splenic autoreactive B cells. In another study on pediatric SLE

patients, three-month RJ consumption resulted with a particular increase in CD4+ regulatory T cell levels and decrease in apoptotic CD4+ T cell cell levels [65]. Erem et al. [66] studied T cell culture for potential effects of RJ on healthy human and patients with Graves disease. RJ inclined the Th1/Th2 ratio to Th1 side, increased IFN γ levels, decreased TNF α , IL-4, IL-10 and so, suppressed humoral immunity. Due to strong relations between Graves disease and thyroid-stimulating hormone receptor antibody, they claimed that suppression of humoral immunity could be a solution to this pathology. As understood, RJ has various actions, even seems to be opposite in different conditions, which means the mechanism is actually seriously complex. On the other hand, immunomodulatory effects are obvious, so RJ and its contents should be a topic of many human and animal studies.

Antimicrobial Effects

Antimicrobial effects of RJ have relatively less studied regarding other actions. RJ contents such as royalisin, 10-DHA, MJPs and jelleins were actually investigated for antibacterial activities [67,68]. MRJPs and jelleins were found effective especially against bacterial cell wall and bactericidal activities were observed on common gram positive and negative pathogens [69]. In an interesting study comparing RJ and 2% chlorhexidine solution, RJ was reported to have serious potential for dental procedures as a disinfectant [70]. Dinkov et al. [71] stated significant inhibitory activity against methicillin-resistant *Staphylococcus aureus*, which is a problem for hospital-acquired infections. Similar results were found against *Aeromonas hydrophila* [72]. Mierzejewski [73] performed a wider study with honey, propolis and RJ against several bacterial agents, comparing with some antibiotics. Although each bee product had antibacterial activity, potency seemed to be different according to pathogen agent and the product. Interestingly, RJ did not show any activity against *Pseudomonas aeruginosa*, but it reduced adhesion ability of the bacteria [74]. So, in vitro antibacterial activity of RJ may not be the same as in vivo, which needs further studies for actual evaluation. Antifungal activity of RJ is controversial and there is very limited data about this topic [75]. In overall, consideration of bee products as a whole (or combined) seems to be more appropriate according to the current data. On the other hand, antimicrobial molecules provide potential of being future therapeutics. Recently, antimicrobial activities of bee products are routinely used in especially wound care [76].

CONCLUSION

Therapeutic effects of bee products and their bioactive substances are major topics of many studies. RJ comes forward with its strong antioxidant and immunomodulatory activities, but it has the potential of having other effects. Generally, researchers are divided into two groups, claiming that these bee products cannot be evaluated as different biopharmaceuticals or investigating each product (even each substance) singularly. For now this remains unclear, both opinions seem to be true, that valuable research studies with wide future perspectives are published. However, RJ and other bee products show wide variability according to climate, ecosystem and geographic conditions. Because, generally, studies are localized, it is hard to gain an overall perspective, which results with the necessity of wider studies with more different types of RJ. Furthermore, there are only a few human studies, which were performed by focusing on only specific topics and with limited sample sizes. In addition, standardization is also a problem for researches, since extraction protocols affect study results, in vitro efficiency and potency [61].

More questions come forward in case of RJ usage as a therapeutic. There is no standard dosage recommendation to gain desired effects. Potential intoxication and side effects remain unclear. Although RJ, as in combination, provides a potential to avoid adverse effects of chemotherapeutics, comorbidities of patients and unknown effects of RJ remain as a question for these patient groups. Because it contains many immunogenic substances, allergic reactions including anaphylaxis must also be considered [77].

In conclusion, RJ provides many effects that can be beneficial as a therapeutic or a substance of a combination therapy. There are gaps worth to investigate in order to put RJ in routine applications, but currently it seems to be safe and effective nutritious agent. More in vitro and in vivo studies are needed for therapeutic usage, but also for better understanding its medicinal potential.

DECLARATION OF CONFLICT OF INTEREST

The Authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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