

Biological Activities of Royal Jelly - Review

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Abstract

Royal jelly is a secretion product of the cephalic glands of nurse bees that has been used for centuries for its extraordinary properties and health effects. This bibliographic study aims to review many of the scientific findings and research that prove many of the remarkable various actions, effects and some uses of royal jelly. There are taken into consideration numerous biological properties and effects of royal jelly: antioxidant, neurotrophic, hypoglycemic, hypocholesterolemic and hepatoprotective, hypotensive and blood pressure regulatory, antitumor, antibiotic, anti-inflammatory, immunomodulatory and anti-allergic, general tonic and antiaging. Royal jelly is one of the most studied bee products, but there still remains much to reveal about its biochemistry and biological activity in future research for our health and life benefit.

Keywords: biological activity, royal jelly (RJ), scientific evidence.

1. Introduction

Royal jelly (RJ) is a secretion product of the cephalic glands of nurse bees and serves as the most important part of honeybee larvae diet, playing a major role in caste differentiation [1]. For the first 2-3 days RJ is the only food given to all young larvae in their maturation process, while for the queen it is the specific food for her whole life period. This is the reason for the longer life of queen bee over other bee. RJ, one of the most effectual and beneficial remedy for human beings, is widely used both in folk and in official medicine and it is a controversial dietary supplement. Due to its complex composition (water, proteins, lipids, carbohydrates, amino acids, mineral salts, vitamins, enzymes, hormones, oligo-elements, natural antibiotics), RJ has a

multitude of pharmacological activities: antioxidant, neurotrophic, hypoglycemic, hypocholesterolemic and hepatoprotective, hypotensive and blood pressure regulatory, antitumor, antibiotic, anti-inflammatory, immunomodulatory and anti-allergic, general tonic, antiaging etc [2]. RJ was not quite as well studied as other hive products like honey, bee pollen or propolis and the history of reports on use and biochemistry of RJ has insufficient scientific data for proving all of its effects. It is gratifying that in the last two decades the interest in RJ research has been increasing and new evidences have appeared regarding its action mechanisms that support some of its traditional uses. The present article aims to present a brief review of new and older research studies on RJ biological activities.

2. Royal Jelly biological activities and effects

RJ biological activities have been studied on *in vitro* experimental models, on laboratory

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animals (mouse, rat, rabbit, hamster), farm animals (ewe, chicken) and in clinical trials. The scientific evidence found in bio-therapeutical activities and effects of RJ are presented below in a sequence that allows a medical fluency.

2.1. Antioxidative activity

Recently enzymatic hydrolysates, water and alkaline extracts of RJ have been tested for antioxidant properties [3, 4]. It has been shown that the RJ collected 24 hours after the larval transfer has the strongest anti-oxidative action [5]. The RJ antioxidative activity has also been proven in various in vitro experimental models on plants and yeast [6], on rats [7] and protection against oxidative stress has been confirmed in experiments on laboratory animals [7, 8, 9]. Also, lipid peroxidation was inhibited in vitro and in experiments on rats.

RJ has been found to protect tissue DNA against the oxidative damage. Studying the effect of the RJ diet on mice, it has been showed that after feeding RJ to mice for 16 weeks, the levels of 8-hydroxy-2-deoxyguanosine (an oxidative stress marker) were significantly reduced in kidney DNA and serum and the average life span of C3H/HeJ mice life expectancy was increased through the mechanism of reduced oxidative damage [10].

There is no clinical data on antioxidative activity.

2.2. Neurotrophic action

RJ has been traditionally known to improve memory, prevent senility, increase energy, reduce anxiety and calm hyperactive subjects [11]. In this area there are studies on RJ and some of its compounds that have effects on neural cells.

RJ contains large quantities of acetylcholine, a neurotransmitter in both the peripheral and central nervous systems and the only neuromodulator used in the motor division of the somatic nervous system.

Recently it has been reported that RJ increases the differentiation of all types of brain cells from neural stem cells, while 10-hydroxy-trans-2-decanoic acid (HDEA), an unsaturated fatty acid characteristic of RJ, increased the generation of neurons and decreased that of astrocytes from neural stem cells [12]. Also, RJ has played neurotrophic and neuroprotective roles on the hippocampus of the adult mouse brain [13]. Oral

administration of RJ has been proven to be responsible for the increase of the number of granular cells in the dentate gyrus of the hippocampus and for the simultaneous improvement of the affected cognitive processes [13, 14].

AMP N1-oxid from RJ stopped the proliferation of PC 12 cells and stimulates the expression of neurofilaments-M, a protein specific to mature neurons, which proves AMP N1-oxid's stimulatory action of PC 12 cells neuronal differentiation induction [15].

Trans-10-hydroxy-2-decanoic acid stimulated the in vitro differentiation of nervous stem cells into neurones [12].

2.3. Insulin-like action

Especially the Chinese and Japanese medicine uses RJ to combat diabetes and to keep the blood sugar levels normal.

RJ can reduce blood sugar level via insulin-like peptides and other compounds (like chromium, sulphur, vitamins B3 and H). RJ is also capable to sustain the optimal blood level of sugars by taking part in the oxidation of glucose to obtain energy, through the insulinic effect [16] of insulin-like peptides found in it. Furthermore, the insulin found in RJ very closely resembles the insulin found in mammals [17].

In insulin-resistant diabetes patients, RJ produced an important decrease of sanguine sugar levels to 33%, 3 hours after administration through injection [18].

Some tests were carried out to confirm that RJ reduced effects of diabetes on animals. RJ has reduced alloxan induced diabetes which affects rats. The simultaneous administration of RJ and a fructose solution for 8 weeks to insulin-resistant rats significantly reduced plasmatic concentration of insulin and triglycerides and reduced systolic arterial pressure, without affecting the blood levels of glucose or total cholesterol.

These results suggest that RJ can be a functional dietary treatment for the prevention of insulin resistance associated to developing hypertension in diabetes patients [19, 20].

Hypo-cholesterolemic and hepato-protective action RJ is efficacy use in lowering and controlling triglycerides and cholesterol in humans [21, 22] and there are many reports that have showed these effects of RJ.

Administration of 50-100 mg RJ/day lowered serum levels of total cholesterol by 14% and of total lipids by 10% in patients with atherosclerosis [23]. In elderly people, eating 10 g of RJ daily for 14 days raised the levels of serum high density lipids (HDL) and improved the levels of low density lipids (LDL), without affecting serum triglycerides [24]. Another study indicates that ingestion of 6 g RJ/day for 4 weeks led to the lowering of serum total cholesterol and LDL, but had no effecting on the HDL or on the triglycerides [25].

The action mechanism of RJ in decreasing cholesterol levels is by attaching the phytosterols like biosterol in the intestinal tract [26-28].

A study on mice suggest that the cholesterol levels decrease effect is seemingly due to the adjustment of squalene epoxidase enzyme (SQLE) and of the low density lipoprotein receptors (LDLR) which are involved in cholesterol incorporation in the liver [29].

RJ stimulates cellular growth, especially the hepatocytes [30]. 57 kDa protein seems to be responsible for this effect.

There are other studies concerning the protective action of RJ against different toxics in rats. RJ prevented nicotine-1 induced cholesterol levels rise [31] and has lowered serum cholesterol in rats fed with a cholesterol enriched diet [32]. RJ also had a dose-dependent protective effect against fumosins (mycotoxins) in rats, inducing significant histological and histochemical improvements in the liver and in the kidneys and of the serum levels of renal and hepatic parameters [7].

In experimental animals and/or humans RJ sustained rebuilding glycogen and helped ammonia and lactic (responsible for fatigue) detoxification [33], increased the oxygen flow to the liver [23] and promoted liver health and hepatocytes growth [16, 9].

2.4. Hypotensive and blood regulatory actions

RJ acts on the cardio-vascular system and on the blood as a blood pressure regulator, it stimulates and invigorates the organism. Alternative medicine recommend RJ in anaemia (a 2-3 weeks treatment significantly improves the number and quality of the red blood cells [34]), in hypo- and hyper-tension and atherosclerosis [22].

Researchers in Japan have investigated the effect of certain peptides obtained through enzymatic

hydrolysis of RJ on experimental animals with high blood pressure. Peptides have acted in a inhibitory manner on the angiotensin 1 conversion enzyme (ACE) and the sanguine pressure has been decreased after the repetitive oral administration of RJ doses to spontaneous hypertensive mice [35,36]. The anti-hypertensive effect went as high as 38% [37].

Other studies suggest that the trans-2-octenoic acid and the hydroxydecanoic acid from RJ may be responsible for the anti-hypertensive action, with different RJ fractions exercising bigger or smaller effects on the duration of the action. RJ has also been associated with protective and therapeutic actions in cases of adrenalin-induced arrhythmia; still, there haven't been any observations of an effect on the heart rate [38].

2.5. Antitumoral action

Literature regarding the influence of RJ on neoplastic growth is not consensual and not enough to clinch this subject. Antitumoral action has been studied on experimental models. However, there have been no human studies to show comparable results, so claims cannot be substantiated for these results.

Recently, the mechanism of antitumoral action was attributed to the 10-hydroxy-2 decenoic acid (10HDA) contained in the RJ, a substance that demonstrates inhibitory action on the VEGF (vascular endothelial growth factor) induced angiogenesis, thus cancelling both cell proliferation and cell migration, which leads to tumor vascularization inhibition [39].

It has also been observed that apalbumin-1 and apalbumin-2, two major proteins in RJ, stimulate mouse macrophages to release TNF- α (tumor necrosis factor) [40].

Another antitumoral evidence is that RJ has an immunostimulating effect by preventing the myelosuppression induced by tumor evolution and by splenic hematopoiesis suppression in mice carriers of Ehrlich ascetic tumor (EAT) and prologues the survival period, depending on the length of the treatment and of the RJ doses [41].

Antitumoral effects of RJ in mice have been investigated also, by oral administration of this substance and by the use of transplantable tumor stems of advanced leukemia L1210 and P388 strains, sarcoma 180 ascities, Ehrlich and solid tumor strains. In the experiments with

leukemia, the RJ has not shown an antitumoral effect, but in the case of the other tumor strains, post transplant life expectancy has significantly risen, varying according to the RJ administration protocol in use), correlated with the decrease of E2 prostaglandin levels following the RJ treatment [42].

Mixing RJ with tumoral cells before the inoculation completely stopped the development of AKR leukemia and of three types of ascitic tumors in mice, fractionation studies establishing that this antitumoral activity is due to the main fatty acid of RJ, the 10-hydroxydecanoic acid [43].

Following an experiment on CBA mice using spontaneous mammary carcinoma and metilcolantren-induced fibrosarcoma transplantable tumors, the RJ has not influenced the formation of metastases when it was administered intraperitoneally or subcutaneously, but simultaneous intravenous administration of RJ with the tumor cells has significantly inhibited the occurrence of metastases [44].

There has been demonstrated even an anti-estrogenic activity of RJ by the inhibition of the effect of bisphenol A – an estrogen which stimulates the MCF-7 mammary cancer cells proliferation [45, 46]. But, on the other hand, treatment of the MCF-7 cells with the lipid estrogenic RJ component has increased the proliferation of these cells [47].

The RJP30 proteic fraction is another part of RJ that has been found to play an antitumoral role. It was cytotoxic for HeLa cells of cervical-uterine carcinoma, decreasing 2.5 times the initial cell density after 7 days of treatment [48].

RJ has shown a slight protective action against irradiation X-rays in rats [49].

Antibiotic effect

After venom and propolis, RJ shows a strong antibacterial activity. RJ cannot exhibit antibiotic and anti-inflammatory effects by ingestion, due to the destruction of the active substances involved through digestion or neutralisation through pH changes [50, 51]. No clear evidence from controlled experiments exists to support claims of internal usefulness of RJ.

It has been proved a strong antibacterial *in vitro* action of the royalisin protein from RJ against Gram-positive bacteria (*Lactobacillus helveticus*, *Clostridium*, *Corynebacterium*, *Leucnoscoc*,

Stafilococcus, *Streptococcus*), but not against the Gram-negative ones [52].

The 10-hidroxi- α 2-decenoic acid had also antibiotic activity against several bacteria and fungi (among which *Micrococcus pyogenes*, *Escherichia coli*, *Neurospora sitophila*) [53].

Jeleines -I, -II, -III presented exclusive antimicrobial activity towards yeast, Gram positive and negative bacteria, while jeleine-IV did not have such properties [54].

Hydrosoluble components of RJ, like proteins and peptides, present a high capacity of inhibiting Gram positive bacteria and fungi [55]. It is assumed that all these antibacterial components take part in the defensive immune system of bees.

In vitro cumulative or synergic effects of both RJ and starch against *Streptococcus aureus* and *E. coli* [56], and RJ and honey against *Staphylococcus aureus* [57] have been demonstrated.

A human study led on 60 diabetes patients with limb-threatening diabetic foot infection suggests that cutaneous ulcers and deep tissular infections can be successfully treated with an RJ and panthenol ointment [58].

Anti-inflammatory action and wound healing effect

Antioxidative, antibacterial, anti-inflammatory and wound healing effects make RJ an ideal component of cosmetics and skin care products.

On the anti-inflammatory activity of RJ scientific reports are a few and quite recent.

In vitro evidence revealed that supernatants of RJ suspensions added to mice peritoneal macrophage culture treated with lipopolisacharides and IFN- γ , efficiently inhibit the production of pro-inflammatory cytokines (TNF- α , IL-6, IL-1), in a dose dependent manner, without cytotoxic effects on the macrophages, which suggests that RJ contains factors (MRJP3 among them) which suppress the secretion of pro-inflammatory cytokines [59].

RJ has an anti-inflammatory action and increases wound healing capacity by decreasing exudation and collagen formation in granulation tissue formation. RJ shorted the healing period of desquamated skin lesions [60]. Inhibiting capillary permeability is another mechanism which explains the anti-inflammatory and wound healing effects.

In an *in vitro* study, RJ has promoted the collagen production of skin fibroblasts in the

presence of ascorbic acid-2-O- α -glucoside (AA-2G), the increase of the collagen production being induced by 10-hydroxy-2-decenoic acid (10H2DA) and 10-hydroxydecanoic acid (10HDA) in a dose-dependent manner. 10H2DA induced the production of TGF- β 1, a transforming growth factor important to collagen production [61]. In another study, ointments with different RJ concentrations have significantly improved the recuperation of 5-fluorouracil induced damage in a dose dependent manner, which suggest that topical application of RJ has a healing effect on severe oral mucositis induced by chemotherapy [62].

2.6. Immunomodulatory and antiallergic activity

Various *in vitro* and *in vivo* experiments have explored and proved the support effects of RJ and its components on the immune system.

RJ contains amino and gamma globulin, unsaturated fatty acids, hormones, enzymes, proteins, vitamin E and A which helps the immune system fight infections.

It has been recently showed that fatty acids isolated from RJ (10HDA and 3-10-dihydroxydecanoic acid) modulate the immune response in rat dendritic cell and T-cell cocultures, in different ways depending on concentration (they stimulate the proliferation of T cells, but, in high concentrations, they inhibit it, decrease IL-2 production and increase IL-10) [63]. Similar effects of different components isolated from RJ were been found on rat T-cell and suggest that water extract possesses the most potent immunomodulatory activity *in vitro* [64].

The DIII protein of RJ has stimulated the growth of 5 cellular lines of human lymphocytes in serum deprived mediums [65] and of the U-937 human myeloid cell line [66].

MRJP 1, the most abundant protein of RJ, stimulated the proliferation of human monocytes (U 937 cell line) [67]. The 70 kDa glycoprotein modulated the *in vivo* and *in vitro* immune response, being able to suppress IL-4, IL-2 and gamma-IFN production, along with the simultaneous inhibition of T lymphocytes proliferation. In another experimental model on mice, the intraperitoneal administration of MRJP 3 has suppressed (IgE și IgG1) ovalbumin induced spontaneous hypersensitivity [68].

In chicks, RJ administration for 28 days has led to an increase of the number of circulating leucocytes and lymphocytes, with the decrease in serum globulins levels [69].

In the case of animal immunization with sheep erythrocytes (SRBC), in chickens there has been noticed a significant increase in antibodies production among those birds who were administered RJ [70] and in rats and mice the serum levels of total proteins and of immunoglobulins have significantly dropped and there has been an increase in plaque-forming splenocytes, in the weight of the inguinal lymph node and in the number of peripheral lymphocytes [71]. In tumor bearing mice RJ administration increased the survival period of and proved myeloprotection through positive effects on stem cells of bone marrow and on the hematopoiesis in induced splenic tumors [41].

RJ suppressed the local reaction of immediate hypersensitivity in DNP-KLH immunized mice by restoring macrophage function and improving cellular response. Oral administration of RJ (1g/kg) significantly decreased the serum levels of specific Ig E, inhibited histamine discharge and lower the macrophage production of PG E₂, improves the Th1/Th2 ration in favor of Th1 [72]. In systemic lupus erythematosus in mice, RJ has inhibited auto-immunity, determining a significant drop in the serum levels of IL-10, of anti-DNA antibodies (mono and double catenary) and of anti-erythrocyte antibodies, and a reduction of the number of splenic autoreactive B lymphocytes. Administrated orally, before the onset of the illness, it has significantly delayed it, it has decreased the proteinuria and prolonged the life span, while administrated after the onset of the illness, RJ has produced a significant amelioration of the renal symptoms, leading to the prolongation of life [73].

The immunomodulatory role played by RJ in Grave's disease (autoimmune hypothyroidism) has been studied *in vitro* using lymph cells from both healthy volunteers and from Grave's disease patients. RJ has produced the proliferation of healthy lymphocytes and the increased secretion of various cytokines (gamma-IFN), while decreasing the production of others. In lymphocytes from patients treated with RJ, the ratio between Th1/Th2 (IFN γ /IL-4) cytokines has changed in favor of Th1 and the levels of the antibodies against the thyroid stimulating hormone

receptor (Ac-TSHR) have registered a significant drop. These results suggest that the effect of RJ is similar to that of the usual medication used to lower Ac-TSHR [74].

RJ stops the evolution of cutaneous lesions from the category of atopic dermatitis, lowering the hypertrophy, hyperkeratosis and epidermis and dermis inflammatory cells infiltration levels, possibly through a blend of TNP-specific low adjustment of the IFN-gamma specific production and of High adjustment of iNOS expression [75]. In addition, it is known that RJ stimulates the collagen production of the cutaneous fibroblasts in the presence of ascorbic-1-O-alfa-glicozid acid. Also, it has been reported that the effect of purified RJ is similar to that of 10HDA, the former being able to stimulate fibroblasts to produce the growth factor - beta 1 transformation, this being an important factor in collagen production [76].

2.7. Effects on the reproductive system and fertility

RJ allows the complete development of the larvae in their brood cells and maintains its ovulatory capacity over its entire life-span [2]. This unusual property of RJ has spurred a possible connection to fertility and we know that RJ has always been used as a stimulator of fertility.

RJ is effective in perimenopausal symptoms, osteoporosis, improving hormonal equilibrium and fertility in men and women by increasing ovules and sperm quality [77] and helps in cases of low libido and impotence, especially in the elderly [34]. These pharmacological effects of royal jelly are similar to those caused by the hormone estrogen, but RJ been found to exhibit a low estrogenic activity. [78].

A series of studies have assessed RJ on mice for improving menopause symptoms in the 1970's. Even so, recent clinical studies in humans are insufficient.

A study on 99 couples with asthenospermia caused infertility has concluded that simple and efficient way of treating this condition is the intravaginal administration of RJ and honey [79].

RJ is an important source of para-aminobenzoic acid which increases fertility in women who regularly consume this product for at least 6 months. Together with the pantothenic acid (vitamin B5), this acid induces protein usage for

healthy hair growth and for its regimentation and that of the skin's.

Several fatty acids from RJ has found to mimic human estrogens [47], but RJ has been found to have a low estrogenic activity mediated through the interaction with estrogen receptors which leads to modified gene expression and cell proliferation [78].

The potential estrogenic activity of RJ has been investigated using different approaches. There have been recent studies on laboratory animals and ewe. It has been found that RJ contains stimulating factors for the female mice reproductive system's development [80]. Feeding rats with a RJ supplement has led to a slight hypertrophy of the uterine luminal epithelium [47].

Oral administration of RJ can counter "summer infertility" (significant improvement of a series of spermography parameters) and improve physiological status in male rabbits [81].

Different studies in ewe have proved similar comparative results on the intravaginal administration of RJ and of progesterone, improving the estral response and gestation rates [82]. RJ had similar effects likewise as the equine chorionic gonadotropin, by inducing the estrus and improving gestation and nativity rates [83, 84]. However, the oral administration of RJ has not been effective in improving the estrus in sheep during the transition between the inactive and the active reproductive seasons [85].

In experimental models of tissular cultures and on ovariectomized mice, a positive effect of RJ against osteoporosis has been registered. The increase in bone calcium levels and the recuperation of bone mass were interpreted as the result of an improved intestinal absorption of calcium, to the disadvantage of the opposite effect of the parathyroid hormone [86].

2.8. Fortifying and tonic action

Through its complex composition, RJ is a nutritional vitaminising and natural anabolism stimulant. In human, it has been noted that RJ multidirectionally or selectively restores the homeostasis, having a cumulative effect of increasing energy and mental activity (after a minimum 3 months treatment) [87]. RJ was likely to increase muscular effort capacity, vital capacity, respiratory function and energy levels [16], it improved the appetite, the strength and the

body mass in malnutrition, underweight, depression and anorexia cases [88]. Also, it increased the feeling of being energetic in patients with chronic fatigue syndrome [16]. RJ had very good results in convalescence and postsurgical recuperation periods [89] and in anemia (a 2-3 weeks treatment significantly improved the number and quality of erythrocytes [34]). Other studies suggest that it induces euphoria, comfort feelings, an increase in strength and appetite of heart disease patients [21].

In humans, the pantothenic acid is converted to coenzyme A, which helps the body to metabolize lipids and to improve its stress response capacity, by supporting the suprarenal glands. The pantothenic acid (B5) content of RJ is higher than in any other known source [90].

There is one experiment on mice, in which RJ has prevented physical fatigue, decreasing the lactate and serum azote accumulations [33].

2.9. Anti-aging effect

It has been believed from ancient times that RJ can prolong life through a similar effect as the one that it provides a lot longer life of queens than the worker bees.

Clinical studies regarding the role of RJ in geriatrics are few, although there is much talk about the anti-aging potential of this product. Some physicians acknowledged RJ as a great ally of geriatrics (2-3 weeks treatments repeated every 3 months are sufficient to “activate” the organism of elder people) [34].

In a C3H/HeJ mice model study, a 16 weeks diet with RJ had risen the average life expectancy, protecting the DNA and lowering the oxidative stress [10].

2.10. Allergic side effects

Allergic reactions are the most frequent side effects of RJ. Their incidence, however, is not well known [91].

Following the oral administration of RJ, allergic reactions may vary from minor disorders (light gastro-intestinal problems, atopies) to severe reactions, including asthma, anaphylactic shock, intestinal bleeding and even death [92-96]. Even if there haven't been reported any fatalities in patients with bronchial asthma who have ingested RJ during bouts, this is not recommended [91]. It has been reported that the risk of developing an

allergy to RJ is greater in individuals who already have other allergies [97] or strong allergies to other bee products [98, 97, 96]. Individuals allergic to pollen, honey, venom should not orally ingest RJ. In patients with bronchial asthma, RJ should not be taken during an attack [99]. Topical applications of RJ (as ointments or in its pure state) can cause skin rashes and eczemas [100].

Conclusions

RJ is a natural bee product with a great potential for use in medicine. RJ has numerous precious therapeutic properties used from ancient times until today. Some of its biological and therapeutic activities have been confirmed, but scientists have only begun to uncover the many health benefits of this amazing superfood and there are just a few solid evidences for those claims. On the other hand, the chemistry and bioactive compounds RJ are not sufficiently known. Further experimentation (*in vitro*, in animal research and on clinical trials) and validation would be needed to prove any useful benefit and action mechanism of native RJ and of isolated compounds as well.

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