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REVIEW ARTICLE

Royal jelly and its hormonal effects in breast cancer: a literature review

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Abstract

Background Breast cancer is the most common cancer in women which can be cured in most individuals with early-stage non-metastatic disease. Imbalance in estrogen signaling pathways and propagating levels of estrogens has important roles in breast cancer development. Targeting the estrogen receptor signaling pathway is linked to breast cancer treatment. Royal jelly is one of the bee products containing 10-hydroxy-2-decenoic acid, a structure similar to mammalian estrogen, allowing it to attach to estrogen receptors. It is considered as a general tonic and immunomodulator which may be helpful in reducing the side effects of cancer treatments. Currently, there are controversial data regarding the pros and cons of royal jelly in cancer. Here we provide an overview of the effects of royal jelly on sex hormones and its possible role in breast cancer.

Methods Electronic databases including PubMed, Scopus, and Web of Science were searched with the search terms royal jelly, cancer, and sexual hormones. All preclinical and clinical studies regarding the hormonal effects of royal jelly were included.

Results According to the collected preclinical data, consumption of royal jelly at daily doses below 200 mg/kg can be useful to decrease the risk of breast cancer since it reduces the serum level of estrogen; whereas increases progesterone, which subsequently decreases the expression of ERs on the ER-positive cells.

Conclusion Future clinical studies are essential to confirm the safe dose of royal jelly as an adjuvant therapy in breast cancer.

Keywords Bee · Honey · Neoplasm · Estrogen · Progesterone

Introduction

Breast cancer is the most prevalent malignant tumor in women with an increasing trend in the incidence, especially in developed countries. According to epidemiological studies, 2.089 million of breast cancer female patients were diagnosed in 2018. The crude rate of the disease varies from a low rate in Bhutan (5 out of 105) and Gambia (6.5 out of 105), to a moderate rate in Japan (58/105), and higher rates in European countries (an average of 84 out of 105) and United states (85/105). France (99/105), Belgium (113/105), and Australia (94/105) seem to have the highest crude rate of breast cancer all over the world [1]. It is worthy to mention that although the morbidity has a higher rate in industrialized countries, the mortality rate of breast cancer is higher in developing countries, mostly African countries [1].

The current comprehension of breast cancer etiopathogenesis is that invasive cancers occur through a series of molecular variation at the cell level. These changes cause uncontrolled growth in breast epithelial cells and immortal specifications [2]. Breast cancers spread in the breast tissue,

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including mammary glands and the lobes, and may be diagnosed during a screening checkup, either before symptoms have developed, or after a woman discovered a lump [3]. Currently, mammography is considered to be the choice for breast cancer screening which provides up to 95% of sensitivity and specificity. Magnetic resonance mammography, ultrasound examination, and tumor biopsy are also considered for a better diagnosis in complicated cases [1]. In spite of attempts to categorize breast cancer according to its molecular and histological properties, it is almost considered as a dilemma in treatment. Various standard protocols are introduced for treatment of breast cancer such as surgical methods, chemotherapy, radiotherapy as well as endocrine therapy [4]. Although breast conserving therapy is preferred in early stages of cancer, major surgeries maybe inevitable in severe cases. Depending on the stage of the disease, physicians may decide to perform a tumor excision, a total mastectomy, or removal of the lymph nodes along with breast. Also, surgeries are usually followed by pharmacotherapy and/ or radiotherapy to fully suppress the cancerous cells [1]. A number of drugs and carriers have been developed for the management of breast cancer; however, neither of them is fully safe as these drugs are also toxic for normal cells.

Nowadays, public and medical attentions have focused on nutraceuticals with anticancer properties for primary/ secondary prevention of cancers. Moreover, their effects on alleviating cancer complications have received much attention. One of these nutraceuticals is Royal jelly (RJ). RJ, a secretion of hypopharyngeal gland of nurse bees, is an exclusive food for the queen honey bee (*Apis mellifera*) and larva. The composition of RJ depends on geography and climate and typically composed of about 12% to 15% antibacterial proteins, 60% to 70% water, 10% to 16% sugar, 3% to 6% fats, and 2% to 3% vitamins, salts, and amino acids [5]. Additionally, RJ contains many bioactive substances such as 10-hydroxy-2-decenoic acid (10-HDA) that has immunomodulatory properties [6]. RJ possesses several medicinal properties and can be used for hay fever, asthma, liver diseases, type 2 diabetes, kidney diseases, pancreatitis, stomach ulcers, bone fractures, skin diseases, gynecological disorders, diabetic foot ulcers, insomnia, hypercholesterolemia, as well as different types of cancer and its complications [7, 8]. It has been reported that RJ may influence the proliferation of breast cancer cell lines [9]. Some studies illustrated that this substance is able to inhibit the growth and proliferation of some breast cancer cell lines; whereas others indicate that RJ displays the estrogenic effects and may enhance the proliferation of MCF-7 mammary cancer cells [10–12]. In this study, we first review the currently-available studies on the effects of royal jelly on sex hormones in different cancerous and non-cancerous tissues in preclinical and clinical settings and then, discuss the possible role of this compound in the management of breast cancer.

Hormonal background of breast cancer

Several hormone-related factors, such as age at menarche, menopause, and parity can be determinative in breast cancer diagnosis. It has also been hypothesized that higher circulating levels of endogenous sex hormones, especially estrogens, may increase breast cancer risk [13].

Post-menopausal women diagnosed with breast cancer have shown more elevated estradiol and estrone levels than those of the healthy post-menopausal women [14]. A two-fold increase in breast cancer risk is observed in women with high estradiol levels. Indeed, it is proved that circulating estrogens and androgens are positively correlated with breast cancer risk in pre-menopausal women [15, 16]. Moreover, sex hormones can influence the behavior of breast cancer cells. For instance, sex steroids stimulate the proliferation of breast cancer cells. It is also proved that endogenous levels of sex steroids are associated with breast cancer risk and sustained tumor growth in post-menopausal women [17].

Hormones such as estrogen and progesterone influence some types of breast cancers cells. Some of these cells genetically overexpress specific protein receptors. Estrogen and progesterone easily bind to these receptors and triggers specific growth signals in cells [18]. According to the types of sex-hormone receptors, the breast cancer cells are divided into four groups; estrogen receptor (ER)-positive, which possess estrogen receptors, progesterone receptor (PR)-positive, which have progesterone receptors, hormone receptor-positive, that have either or both of the receptors mentioned, and hormone receptor-negative, which have neither the estrogen nor the progesterone receptors [19]. Since estrogen stimulates the proliferation of hormone-receptor-positive breast cancer cells, decreasing the level of this hormone can assist the inhibition of cancer's growth or relapse [20]. In hormone receptor-positive breast cancers, antagonists of these hormones are employed to inhibit hormone attachment to its receptors, an approach called endocrine/ hormone therapy. The patients with higher levels of tumor receptors responded better to hormonal therapy than those with only small amounts of ER [21]. Tamoxifen is recognized as a selective blocker of ERs on breast cancer cells and consequently prevents cell growth and proliferation. Today, this drug is used to treat women with hormone receptor-positive invasive breast cancer [22, 23]. Toremifene (Fareston) is another selective ER modulator that functions in a similar way, but is only approved to treat metastatic breast cancer in post-menopausal women [24, 25]. Another well-known drug is Fulvestrant, that blocks and deteriorates ERs. Fulvestrant does not act selective, but acts as an anti-estrogen throughout the body. This drug is currently approved only for post-menopausal women and

sometimes "off-label" in pre-menopausal women, often mixed with a LH-releasing hormone (LHRH) agonist to turn off the ovaries [26].

Aromatase inhibitors (AIs) are another class of medicines that hinder the production of estrogen. As aforementioned, before menopause, the highest amount of estrogen is produced by ovaries. In women diagnosed with dysfunction of ovaries, a little amount of estrogen is still produced in the fat tissue by aromatase enzymes. AIs act via inhibition of aromatase from producing estrogen [27]. LHRH analogs, such as goserelin (Zoladex) and leuprolide (Lupron), are considered as other choices for the suppression of ovaries, along with an AI. Indeed, they hamper the biological signals to the ovaries to produce estrogen that results in temporary menopause [28, 29]. These drugs can be administrated in pre-menopausal women with or without other hormone therapies [30].

Search strategy

In order to collect related data on the hormonal effects of RJ, a systematic search from inception until March 2023 was performed in electronic databases including PubMed, Scopus, and Web of Science. The search formula was as follow:

("royal jelly" OR "10-HDA" OR "10-hydroxy-2-decenoic acid") AND ("cancer" OR "breast cancer" OR "sex hormone" OR "progesterone" OR "estrogen" OR "testosterone" OR "follicle stimulating hormone" OR "luteinizing hormone").

Primary search results were screened by two independent investigators according to the title/abstract and then, full-texts. In case of disagreement, a third researcher decided on whether or not to include the paper in the review. References of the final papers were also reviewed to find more relevant studies. Inclusion criteria were any original in vitro/ in vivo/ clinical study in which the effect of RJ or its active compounds was assessed on one of the sex hormones, FSH, LH, or breast cancerous cells. Exclusion criteria were any study on RJ irrelevant to sex hormones or breast cancer, papers with full-texts in languages other than English, and review articles. Data extracted from the finally-included articles are summarized in Table 1. Due to the heterogeneity in the nature of the included studies, quantitative analysis of the reported data was not possible. Figure 1 shows the flow diagram of article selection process. To critically assess the quality and validity of the included studies, Animal Research: Reporting of in vivo Experiments (ARRIVE) essential 10 checklist was employed for animal studies (Table 2).

Hormonal effects of royal jelly

Preclinical evidence

Until now, many investigations have explored the effects of RJ on sex hormones in females. The therapeutic effect of RJ on polycystic ovary syndrome (PCOS)-related hormonal and biochemical changes was investigated in rats with PCOS. PCOS increased estradiol and nitric oxide (NO) levels; while decreased progesterone and ferric-reducing antioxidant potential (FRAP) levels. Administration of RJ to rats with PCOS caused a significant increase in progesterone and FRAP levels; whereas reduced estradiol and NO levels. In addition, there was a significant rise in the number of mature follicles and corpus luteum, as well as a reduction in weight of the ovaries and uterus [31]. Serum levels of ovarian hormones after feeding female Wistar rats with RJ was examined. It was observed that RJ increased the serum level of estradiol and progesterone, with the highest effect at 200 mg/kg dosage [31]. This effect seems to be mediated through stimulating effect on folliculogenesis and ovulation. It is demonstrated that progesterone decreases ERs on the ER-positive cell line, such as MCF-7 cell line, diminishing their response to estrogen [60]. Another study in rats with PCOS also showed that simultaneous administration of RJ can increase follicle stimulating hormone (FSH); while decreases luteinizing hormone (LH), estradiol, and exogenous testosterone in comparison to the animals receiving only exogenous testosterone, showing the antiandrogenic properties of RJ. It is noteworthy to mention that amongst the three tested dose levels (100, 200, and 400 mg/kg), the medium dose (200 mg/kg) showed the highest potency [32].

Husein et al. investigated the impact of RJ co-administered with progesterone on some reproduction parameters in Awassi ewes. Prior to the RJ administration, the animals were treated with intravaginal progesterone sponges for 12 days [49]. From the first day of progesterone sponge insertion, ewes were orally and intramuscularly treated with RJ at 12 equal daily doses of 250 mg per day. The results demonstrated that RJ, in both administration routes, strongly promotes follicular growth and, consequently, secretion of estradiol. In another investigation in the same settings, RJ was compared with equine chorionic gonadotropin (eCG). It was observed that RJ has a higher potency to decrease progesterone; while has a similar effect to eCG with respect to pregnancy and lambing rate [51].

There are also several reports about the effects of RJ on the sex hormones of males [39, 41]. In males, testosterone is secreted by adult Leydig cells under LH and FSH stimulation and plays a pivotal role in spermatogenesis [61]. Moreover, testosterone levels may be raised because of the administration of exogenous testosterone containing

Table 1 A summary of the studies on the effects of royal jelly on sex hormones and estrogen receptors

Aim of the study	Preparation	Design	RJ Dosage	Duration	Hormonal effects	Refs.
Investigation of the therapeutic effect of RJ on hormonal and biochemical changes in a rat model of PCOS	Aqueous solution of RJ	Female rats with PCOS	100, 200 mg/kg	21 d	Serum progesterone levels and FRAP ↑, serum estradiol level and NO levels ↓ mature follicles ↑ corpus luteum ↑ ovarian and uterus weight ↓	[31]
Investigation of the therapeutic effect of RJ on hormonal and biochemical changes in a rat model of PCOS	Aqueous solution of RJ	Female rats with PCOS receiving exogenous testosterone	100, 200, 400 mg/kg/day	4 w	FSH ↑ LH, testosterone, and estradiol ↓ Reduction of oxidative damage Improvement of follicular development	[32]
Investigation of the effect of RJ on HMG and the dose–response relationship of RJ in the treatment of HMG	Aqueous solution of RJ	Female rats with estrogen and progesterone-induced HMG	100, 200, 400, and 800 mg/kg/day	30 d	RJ at 100 and 800 mg/kg/day: improving the expansion of acinar and breast tissue ducts doses of RJ at 100 and 800 mg/kg/day: inhibit serum estrogen and prolactin secretion + serum progesterone secretion and the expression of ERβ in the breast tissue ↑ RJ at 800 mg/kg/day: hypothalamic GnRH ↓ pituitary GnRH-R ↑ RJ at 100 mg/kg/day: GnRH-R mRNA expression ↑	[33]
Evaluation of the effects of RJ on a set of reproductive parameters in immature female rats	Aqueous solution of RJ	Immature female Wistar rats orally treated with RJ	100, 200, 400 mg/kg/day	14 d	Serum estradiol level ↑ serum progesterone levels ↑ NO ↓ FRAP levels ↑	[34]
Assessing the immunomodulatory response of camellia RJ on a 4T1 murine breast cancer model	Aqueous solution of RJ	4T1 breast cancer-bearing female mouse model orally pre-treated or post-treated with RJ	0.5, 1.5 g/kg/day	42 d	No hormonal effect RJ (0.5 g/kg): LPO ↓ IgG ↑ TNF-α ↑ IL-6 ↓ IL-10 ↓ cytokines production ↓ Th2 response ↓	[35]

Table 1 (continued)

Aim of the study	Preparation	Design	RJ Dosage	Duration	Hormonal effects	Refs.
Evaluation of the effect of RJ on 4T1 murine breast cancer model	Fresh RJ	4T1 breast cancer-bearing female mouse model orally pre-treated or post-treated with RJ	0.5, 1.0, 1.5 g/kg/day	28 d	No hormonal effect thymus and spleen weight ↓ IL-2 (RJ 0.5 and 1.5 g/kg) ↑ IFN –α ↑ SOD ↑ TAOC↑ IL-4 (RJ 0.5 and 1.5 g/kg) ↓ IL-10 (RJ 1.0 g/kg) ↓ activities of GR ↑	[8]
Investigation of protective effect of RJ against $AlCl_3$ toxicity on pituitary, thyroid and sex hormones in addition to histological sections of testis	Aqueous solution of RJ	$AlCl_3$-induced toxicity in rat	400 mg/kg/day	8 w	FSH ↑ LH ↑ Testosterone ↑	[36]
Investigating pre-treatment and co-treatment with RJ on Cd-induced damage to male sexual organs	RJ	Cd-induced toxicity in male rat	100 mg/kg/day	56 d	Testes weight ↑ FSH ↑ LH ↑ Testosterone ↑ 3β hydroxysteroid dehydrogenase and 17β hydroxysteroid dehydrogenase ↑ TNF-α, LPO, NO ↓ SOD ↑	[37]
Investigation of the effect of RJ on reproductive parameters of testicular tissue in diabetic male rats	Aqueous solution of RJ	Streptozotocin-induced diabetes in male rats	100 mg/kg	6 w	Serum testosterone levels ↑ testicular weight ↑ sperm count↑ MDA ↓→LPO ↓ sperm deformity ↓ DNA integrity ↓ chromatin abnormality ↓	[38]
Evaluation of the protective effect of RJ on sperm parameters, testosterone level, and MDA production	Fresh RJ	Administration of RJ in OXM-induced oxidative stress in adult mice	100 mg/kg daily	30 d	Testosterone level ↑ immature sperms ↓ sperm DNA damage ↓ LOP ↓ Fertility ↑	[39]
Investigation of the effects of RJ on oxidative stress in testis	Fresh RJ	OXM-induced oxidative injury in mice testis	100 mg/kg/day	28 d	CAT activity ↑ LPO level ↓ Serum testosterone level ↑	[40]
Evaluation of the protective effect of RJ on sperm parameters and MDA production	Fresh RJ	Animal study on bleomycin-treated male rats	100 mg/kg daily	48 d	Testosterone ↑ Sperm count ↑ LPO ↓	[12]

Table 1 (continued)

Aim of the study	Preparation	Design	RJ Dosage	Duration	Hormonal effects	Refs.
Examination of the influence of RJ on the reproductive function of puberty male rats	Aqueous solution of RJ	Animal study in puberty male rats	200, 400, and 800 mg/kg	4 w	Serum FSH levels ↓ LH levels ↑ in the high-dose of RJ estrogen content ↑ in the high-dose of RJ Testosterone level ↑ in low-dose of RJ Organ coefficient for the testis ↓in high-dose of RJ Organ coefficient for the prostate ↑ (All doses) Organ coefficient for the seminal vesicles ↑ in moderate-dose group Numbers of spermatogenic epithelium ↑ with RJ dose increasing Abnormal sperm percentages ↑ in moderate and high RJ	[41]
Evaluation of the effect of addition of RJ in presence of heparin on buffalo (*Bubalus bubalis*) sperm motility, acrosome reaction and in vitro fertilization of buffalo oocytes	Aqueous solution of RJ	In vitro study on frozen semen which suspended in a fertilization medium containing 100 µg/mL heparin and 0.4% RJ	0.4% solution of RJ in distilled water	1, 2, 3 h	Motility, acrosomal reaction of buffalo spermatozoa ↑ in vitro fertilizing capacity ↑	[42]
Evaluation of the ability of RJ feeding to counteract "summer infertility"	Aqueous solution of RJ	Animal study on heat stress-induced male rabbits	200, 400, or 800 mg/kg/week	6 w	Testosterone serum level ↑ Ejaculated volume ↑ Seminal plasma fructose ↑ sperm motility ↑ sperm total output ↑ abnormal sperm ↓	[43]
Evaluating the effect of RJ on heat-induced infertility in male rabbit	Chinese RJ	Heat stress-induced infertility in male rabbit	50, 100, 150 mg/kg, twice weekly	20 w	Testosterone serum level ↑ sperm motility ↑ abnormal sperm ↓ Fertility after mating ↑	[44]
Evaluation of the efficacy RJ on hydrogen peroxide-induced damage in male reproductive system	Aqueous solution of RJ	Animal study on oxidative stressed-induced reproductive toxicity in adult male rats	1 g/kg	30 d	Testosterone level ↑ Sperm count ↑ glutathione level ↑ MDA ↓ → LPO ↓	[45]

Table 1 (continued)

Aim of the study	Preparation	Design	RJ Dosage	Duration	Hormonal effects	Refs.
Assessing the estrogenic activity of RJ and its fatty acids in vitro and in vivo	RJ, 10-hydroxy-trans-2-decenoic acid, 10-hydroxydecanoic acid, trans-2-decenoic acid, 24-methylenecholesterol from RJ	MCF-7 cells Animal study on 17α-ethinylestradiol-induced mild hypertrophy of the luminal epithelium in ovariectomized female rats	10-hydroxy-trans-2-decenoic acid, 10-hydroxydecanoic acid: 0.05–5 μM 24-methylenecholesterol: 0.025–2.5 μM trans-2-decenoic acid: 1–100 μM 2 doses of 17α-ethinylestradiol (0.1 and 3 mg/kg/day) or 1 g/kg/day RJ	24 h 3 d	Estrogenic effect: RJ fatty acids show ERβ-binding activity through activation of ERs (ERβ higher than ERα) proliferation of MCF-7 cells ↑ No change in uterus weight Luminal epithelium height ↑	[46]
Assessing the estrogenic activity of RJ fatty acids in vitro	10-HDA, 3,10-dihydroxydecanoic, and sebacic acid	MCF-7 and HeLa cells	10^{-10}–10^{-5} M	24 h	Absence of estradiol: Activation of ERβ but not ERα Presence of estradiol: Activation of both ERα and ERβ	[47]
Research on the effect of RJ on proliferation of human breast cancer cells	Heat-treated RJ solution in PBS	In vitro study on BPA-induced growth promotion in MCF-7 cells	0.1 g in 10 mL PBS	72 h	Proliferation of MCF-7 cells in the medium without BPA ↓ BPA-induced growth-promotion effect ↓	[10]
Evaluation of the effect of RJ on male infertility	RJ once daily at night in one teaspoonful	Clinical study on the semen of infertile men	25, 50 and 100 mg	90 d	Serum LH ↑ Testosterone ↑ Active sperm motility ↑ Sexual desire ↑	[48]
Comparing the effects of RJ and eCG treatments on reproductive performance	RJ solution injection	Animal study on Awassi ewes which was given an intramuscular treatment of 15 mg prostaglandinF2α and then received an intravaginal progesterone-releasing device	4.8 g RJ given in 12 equal doses of 400 mg per day	12 d	Pregnancy and lambing rates of RJ group ≈ pregnancy and lambing rates of eCG group (equal effects) follicular growth ↑ Estrus expression ↑ lambing rates ↑ time intervals to estrus ↓	[49]
Assessing the effectiveness of RJ and GnRH on the estrus synchronization and pregnancy rate	RJ paste	Awassi ewes pretreated with fluorogestone acetate impregnated sponge	3 g RJ paste in 12 equal doses of 250 mg	19 d	*compared to control group:* Progesterone profile ↓ on day 5,7 and 9 Plasma progesterone on day 17 and 19 ↑ estrus and first service conception rate in sheep via RJ + exogenous progesterone↑ Interval to detected estrus ↓	[50]
Determination of the role of RJ paste on reproductive responses	RJ capsule & RJ solution for injection	Animal study on Awassi ewes which were treated with progesterone sponges simultaneous with RJ administration	3 g of RJ given in 12 equal doses of 250 mg (250 mg RJ each capsule)	12 d	Follicular growth ↑ estradiol secretion ↑ Stimulation of reproductive responses via RJ ↑ Rate of lambed ↑	[51]

Table 1 (continued)

Aim of the study	Preparation	Design	RJ Dosage	Duration	Hormonal effects	Refs.
Evaluation of RJ activity in cisplatin-induced ovarian damage	RJ soft capsules	Cisplatin-induced ovarian damage in female rat	100 mg/kg/day	5 w	FSH and LH ↓ Progesterone ↑ Estradiol 2 ↑ Anti-mullerian hormone ↑ Improvement of ovarian and uterine tissue damage, GSH, SOD, and TAOC ↑ LPO ↓	[52]
In vivo and in vitro evaluation of RJ estrogenic activity	Freeze-dried RJ powder	Human choriocarcinoma JEG-3 cells expressing ERα and ERβ Estrogen-responsive transgenic reporter female mouse	0.3–30 µg/mL 4% in diet	24 h 7 d	No in vitro agonistic activity was observed against ERα or ERβ No estrogenic activity	[53]
Evaluating antiproliferative effects of 10-HDA in breast cancer cells	10-HDA	MCF-7 cell line	0.0125, 0.125, 1.25, 12.5, and 125 µg/mL	24 h	LD_{50}: 190 µg/mL c-MYC/BAX, cyclin D1 and CDK4 ↓ p53/BAX ↑ G0/G1 cell cycle arrest	[54]
Evaluating antiproliferative effects of 10-HDA in breast cancer cells in combination with doxorubicin	10-HDA	MCF-7 cell line	0.0125, 0.125, 1.25, 12.5, and 125 µg/mL of 10-HDA + 0.54 µg/mL of doxorubicin	24 h	Synergistic antiproliferative activity with doxorubicin	[55]
Assessing estrogenic activity of RJ in vitro and in vivo	Fresh RJ	MCF-7 cells expressing ERα and ERβ Ovariectomized female rat	0.1–1 mg/mL 1 g/kg	24 h Single dose	Affinity to ERα and ERβ similarly, but fivefold lower than diethylstilbestrol ER activity ↑ endogenous estrogen-responsive genes expression ↑ estrogen-responsive VEGF gene expression in uterus, but not in brain	[11]
Assessing the effect of RJ on nicotine-induced reproductive damage	Fresh RJ in normal saline	Nicotine-induced testicular damage in male mice	100, 150, 200 mg/kg/day	10 d	Testosterone ↑ FSH and LH ↑ NO and LPO ↓ sperm DNA fragmentation ↓ TAOC ↑	[56]
A clinical evaluation of the effect of RJ on testosterone level of healthy sedentary male subject	RJ	Randomized, placebo-controlled clinical study in 20 healthy sedentary young men aged 21–23	1 g/day	15 days	Testosterone vs. placebo and baseline levels ↑	[57]

Table 1 (continued)

Aim of the study	Preparation	Design	RJ Dosage	Duration	Hormonal effects	Refs.
Assessing the effect of RJ of hypothalamus-pituitary-ovarian axis	Fresh RJ	Ovariectomized female rats	750 mg/kg/day	8 w	Estrogen ↑ No significant change in FSH and LH Serotonin ↑ No significant change in dopamine	[58]
Assessing long-term impact of RJ in healthy volunteers of both genders	RJ drink	Randomized, double-blind, placebo-controlled clinical trial in healthy individuals of both genders	3000 mg/day	6 m	Log testosterone/ dehydroepiandrosterone sulfate ↑ in male subjects No significant change in log estradiol/testosterone	[59]

HMG Hyperplasia of the mammary gland; *RJ* Royal jelly; *BW* Body weight; *GnRH* Gonadotropin-releasing hormone; *GnRH-R* GnRH receptors; *MDA* Malondialdehyde; *LPO* Lipid peroxidation; *PCOS* Polycystic ovary syndrome, estrogen receptor; *ER*, *TNF* Tumor necrosis factor; *IL* Interleukin; *eCG* Equine chorionic gonadotropin; *PBS* Phosphate-buffered saline; *10-HDA* 10-hydroxy-2-dcenoic acid; *PMS* Premenstrual syndrome; *LH* Luteinizing hormone; *FSH* Follicle stimulating hormone; *IFN* Interferon; *TAOC* Total antioxidant capacity; *FRAP* Ferric reducing antioxidant potential; *AST* Aspartate transaminase; *ALT* Alanine transaminase; *SOD* Superoxide dismutase; *CAT* Catalase; *GR* Glutathione reductase; *GPx* Glutathione peroxidase; $AlCl_3$ Aluminum chloride; *NO* Nitric oxide; *BPA* Bisphenol A; *OXM* Oxymetholone

sources such as RJ. In fact, RJ consists of testosterone in the amount of 0.012 g/kg of fresh weight. In addition, it is confirmed that RJ abundantly contains zinc element, which participates in testosterone production [62]. Zinc is a co-factor of various fundamental enzymes and plays an important role in particular metabolic processes. Moreover, large concentrations of zinc are found in sexual organs such as testes, seminal plasma and prostate. In these organs, the presence of zinc affects the functions of some testicular enzymes. Hence, zinc deficiency may adversely influence the metabolism of sexual hormones [63]. Al-Eisa et al. investigated the effects of RJ on male rats, which were given a dose of Aluminum chloride ($AlCl_3$) [36]. $AlCl_3$ causes oxidative damage, prevents the activity of antioxidant enzymes and decreases the serum levels of sexual hormones. In this study, after 8 weeks of daily administration of 400 mg/kg RJ, the levels of FSH, LH and testosterone were measured. The serum levels of FSH and LH were significantly increased after RJ administration, which consequently increased the level of testosterone, as well. In an experiment by Yang et al., male rats were orally given 200, 400 and 800 mg/kg RJ for 4 weeks [41]. It was observed that, RJ at the dose of 800 mg/kg significantly decreased the levels of FSH and LH compared with controls. LH and FSH levels in two other doses did not significantly differ from control. In addition, the serum level of testosterone was remarkably reduced at the doses of 400 and 800 mg/kg RJ compared with control. However, in this study, it was suggested that long-term consumption of RJ at higher dose, might induce pituitary abnormalities and diminish the FSH, LH, and testosterone level in males.

Heat stress during summer adversely influences the fertility of rabbits, resulting in a significant reduction in production. In an experiment conducted by Elnagar et al. using heat stress-induced infertility, male rabbits were orally treated with 200, 400, or 800 mg/kg of RJ once a week for 8 weeks [43]. The findings indicated that, compared with the control group, RJ administration increased testosterone levels at all doses. However, compared with lower and higher doses, the middle dose of RJ showed a better effect. In another study in heat-induced infertility in male rabbits, three doses of RJ, i.e., 50, 100, and 150 mg/kg were administered two times per week for 20 weeks which showed a dose-dependent improvement of testosterone level [44].

In the same way, Kohguchi et al. fed golden male hamsters with an RJ-enriched diet containing 50 and 500 µg of RJ in each gram of the diet and monitored the variation of testosterone levels in animals [64]. It was observed that, after 44 weeks of treatment, the higher dose of RJ exhibited a higher increasing effect on the level of free testosterone compared with the lower dose.

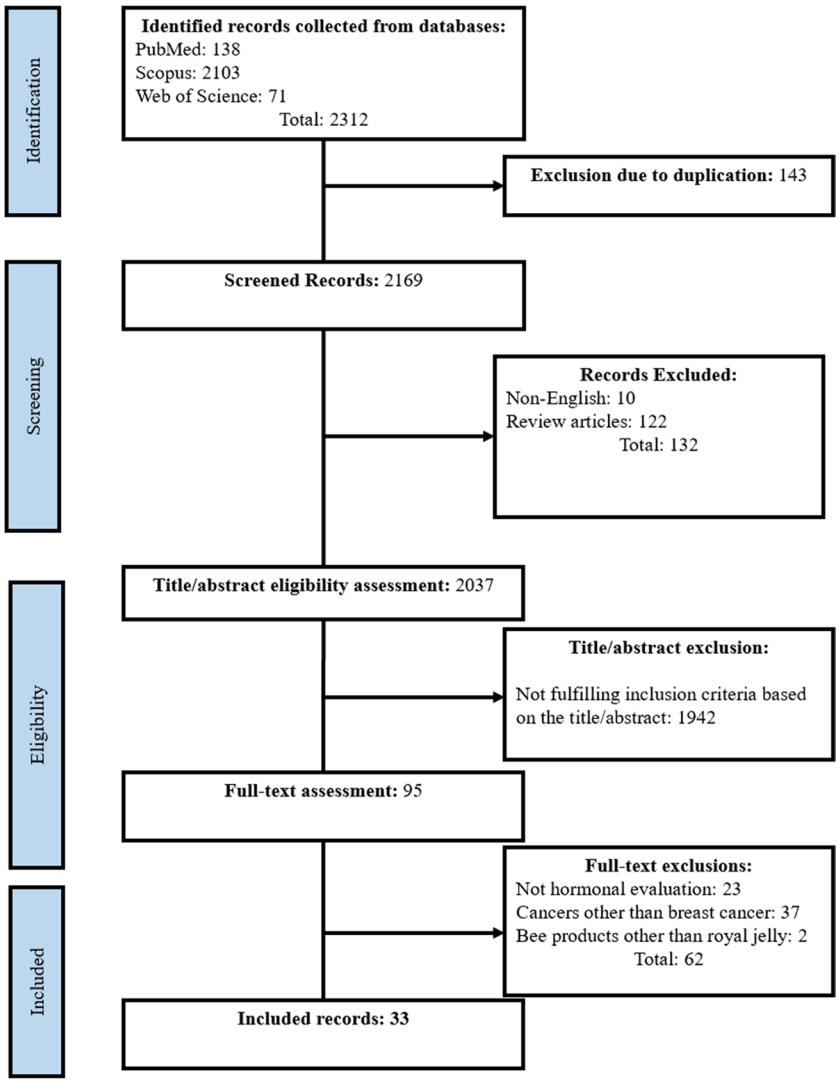

Fig. 1 Flow diagram of study selection process

Occasionally, some drugs or chemical therapeutic agents cause adverse effects on sex hormone secretion. In such conditions, the administration of supplements such as RJ may be promising to ameliorate these unfavorable effects [65]. For instance, Amirshahi et al. evaluated the effect of RJ on bleomycin-induced reproductive toxicity in male Wistar rats [12]. Bleomycin caused a dramatic decrease in testosterone level; though oral treatment with 100 mg/kg/day of RJ for 48 days could increase testosterone levels in the animals.

Diabetes is an endocrine disease associated with dysfunction of the male reproductive system via vacuolization in Sertoli cells and the induction of apoptosis in spermatogonia cells and spermatocytes in seminiferous tubules [66]. In addition to oxidative stress induced by diabetes, these events notably reduce the level of testosterone in males. In a study by Ghanbari et al., diabetic male Wistar rats were treated with oral RJ at a dose of 100 mg/kg for six weeks [38]. It was found out that RJ decreases peroxidation in testicular tissue and increases the serum level of testosterone in diabetic animals.

Hydrogen peroxide strongly induces lipid peroxidation in testes and thus, increases the level of malondialdehyde (MDA). Lipid peroxidation in testes establishes retardation in Leydig cells responsible for the secretion of steroids such as testosterone [67]. Accordingly, increased lipid peroxidation in testicular tissue is related to a reduction in testosterone levels. Hassan et al. conducted a study on the effect of RJ on hydrogen peroxide-induced toxicity in adult male rats [45]. Administration of RJ at a dose of 1 g/kg for 30 days significantly decreased lipid peroxidation of testes and increased testosterone level. Likewise, in the animal model of reproductive toxicity induced by oxymetholone, a synthetic testosterone derivative, 100 mg/kg of RJ administration during 28 days could significantly decrease lipid peroxidation and elevate the level of testosterone [40].

Table 2 Quality assessment of animal studies on the hormonal effects of royal jelly according to ARRIVE guideline Essential 10

Reference	Study design (2 p.)	Sample size (2 p.)	Inclusion and exclusion criteria (3 p.)	Randomization (2 p.)	Blinding (1 p.)	Outcome measures (2 p.)	Statistical methods (2 p.)	Experimental animals (2 p.)	Experimental procedures (4 p.)	Results (2 p.)	Score (Out of 22)
[11]	2	1	2	1	0	2	0	2	3	0	13
[12]	2	1	2	1	0	2	2	2	3	2	17
[31]	2	1	2	1	0	2	2	2	4	2	18
[32]	2	1	3	1	0	2	2	2	4	1	18
[49]	1	1	3	1	0	2	2	2	3	2	17
[51]	1	1	3	1	0	2	2	2	3	2	17
[41]	2	1	2	1	0	2	1	2	3	2	16
[39]	2	1	2	0	0	2	1	2	3	2	15
[36]	1	1	0	0	0	2	0	1	3	2	10
[43]	2	1	2	1	0	2	2	2	3	2	17
[44]	2	1	2	1	0	2	2	2	4	2	18
[38]	2	1	3	1	0	2	2	2	4	2	19
[45]	2	1	3	1	0	2	1	2	3	2	17
[40]	2	1	2	0	0	2	2	2	3	2	16
[33]	2	1	3	1	0	2	2	2	4	2	19
[46]	2	1	2	0	0	1	1	2	4	2	15
[53]	2	1	3	0	0	2	2	2	3	2	17
[8]	2	1	2	0	0	2	2	2	3	2	16
[35]	2	1	2	0	0	2	2	2	3	2	16
[34]	2	1	3	1	0	2	2	2	3	2	18
[42]	2	1	3	1	0	2	2	2	3	2	18
[50]	1	1	3	1	0	2	2	2	3	2	17
[52]	2	1	2	0	0	2	2	2	4	2	17
[56]	2	1	2	1	0	2	2	2	4	2	18
[58]	2	1	2	1	0	2	2	2	4	2	18

Clinical evidence

In addition to animal studies, some clinical studies were also conducted to evaluate the influence of RJ on the serum level of sexual hormones. Al-Sanafi assessed the effect of RJ consumption on infertile men [48]. In this study, eighty-three infertile men were treated with 25, 50 and 100 mg of RJ for 90 days. It was observed that in the group treated with daily 25 mg RJ, the levels of LH and testosterone were significantly increased by 20.3% and 22.01%, respectively, in comparison to baseline values. Interestingly, no significant effect was observed in patients treated with other doses. The authors have suggested that these observations are due to the central effect of RJ. In other words, RJ contains acetylcholine, which stimulates LH secretion at the hypothalamus level. In another clinical study, 20 sedentary and healthy young men aged 21–23 were randomly treated with either 1 g RJ or placebo daily for 15 days. A significant elevation of testosterone level was observed in the RJ-treated group both in comparison to their baseline testosterone level, as well as the placebo group [57]. In a clinical study in healthy male and female subjects, RJ with a daily dose of 3 g was administered for a period of six months. It was observed that in male subjects, log testosterone/ dehydroepiandrosterone sulfate was significantly higher; while no significant difference was observed in log estradiol/testosterone in any of the genders [59].

To sum up, RJ exhibits an increasing effect on LH serum levels and testosterone in males. Accordingly, in female, RJ consumption, at moderate doses, results in raising the level of estrogens, progesterone, LH, and FSH; while decreasing the level of testosterone. It is also demonstrated that the extent of this enhancing effect in both sexes depends on the administered dose of RJ. It was demonstrated that the administration of high doses of RJ might cause adverse effects on sexual hormones. Hence, RJ administration at moderate doses has a stimulatory effect on the secretion of sex hormones; while it causes a reduction of sex hormones via inhibition of LH and FSH secretion from the pituitary gland.

Interaction of royal jelly with ERs: direct evidence

RJ affects sex hormone levels, possibly due to the specific fatty acids mimicking the behaviors of the estrogen hormone. An animal study in immature female ICR mice demonstrated that 45-day supplementation with major RJ proteins at a dose of 125, 250, and 500 mg/kg can accelerate estrus, with 250 mg/kg dose showing the highest activity. Considering the gene expression of estrogen receptor (ERs) in ovarian tissues of the animals, a significant elevation in the gene expression of ERβ was observed in animals treated with major RJ proteins; while no significant change was observed in ERα gene expression [68].

In a survey by Liu et al., the effect of RJ on hyperplasia of the mammary gland (HMG) and the dose–response relationship of RJ in the treatment of HMG were investigated. As a result, RJ improves the expansion of acinar and breast tissue ducts, particularly at 100 and 800 mg/kg/day. Furthermore, these two doses also inhibit serum estrogen and prolactin secretion and increase serum progesterone secretion and the expression of ERβ in the breast tissue. In addition, 800 mg/kg/day decrease and increase the mRNA expression of hypothalamic gonadotropin-releasing hormone (GnRH) and pituitary GnRH receptors (GnRH-R), respectively. The lowest dosage (100 mg/kg/day) increases GnRH-R mRNA expression as well [33]. Some cells, such as MCF-7 cell lines, express a higher number of ERs; therefore, can highly represent the effect of ER ligands. In another study by Mishima et al., the potential estrogenic effect of RJ was evaluated through in vitro and in vivo assays [11]. In the in vitro assay, two series of MCF-7 cells were treated with diethylstilbestrol and RJ for 24 h and their ER binding affinity were compared with estrogen. The results of Ligand Binding assay using Ligand Screening System Estrogen Receptor α and β, illustrated that RJ could prevent the binding of estrogen to ER, but its affinity to ERs were approximately five times lower than those for diethylstilbestrol. In addition, the results of reverse transcription polymerase chain reaction (RT-PCR) suggested that RJ administration at the dose range of 0.1 to 1 mg/mL triggered ER activation and then caused increased transcription of a reporter gene via estrogen-responsive components. According to the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, it was also observed that the MCF-7 proliferation was increased after RJ administration at concentration ranges between 0.5 to 1 mg/mL. For in vivo evaluation, ovariectomized rats were subcutaneously administrated with a single dose of RJ. Estrogen-responsive vascular endothelial growth factor (VEGF) gene expression in uterus and brain, the main targets for estrogen, were measured. The results showed that RJ restored VEGF expression in the uterus, but not in the brain. RJ showed estrogenic effects via interaction with ER, such as ERα and ERβ, which causes stimulation in estrogen-sensitive gene expression and cell proliferation and function. In a similar study, Suzuki and coworkers isolated 10-HDA, 10-hydroxydecanoic acid, *trans*-2-decenoic acid, and 24-methylenecholesterol from RJ and treated MCF-7 cells with these compounds. According to the results of a ligand-binding assay for ERβ, these compounds represented estrogenic activity, and all of these compounds prevented binding of 17β-estradiol to ERβ; however, they did not affect the binding of 17β-estradiol to ERα. It was concluded that RJ competes with estrogenic compounds to bind to ERs [46]. In another study, 10-HDA,

3,10-dihydroxydecanoic, and sebacic acid were added to MCF-7 and HeLa cells in absence or presence of estradiol. In the absence of estradiol, RJ-derived fatty acids could interact with ERβ but not ERα; whereas they interacted with both ER receptors in the presence of estradiol. RJ fatty acids also inhibited estradiol-induced estrogen response element-mediated transactivation. At the molecular level, interaction of the suitable ligand with ER modifies the structure of receptor for an agonistic activity. RJ fatty acids could act as suitable ERβ agonists in absence or presence of estradiol; while they only showed estrogenic activity in presence of estradiol in ERα [47]. Nakaya et al. treated MCF-7 cell line with Bisphenol A, followed by RJ administration at a dose of 0.1 mg [10]. Bisphenol A is an environmental estrogen that stimulates the proliferation of MCF-7 cells via binding to ERs. RJ showed competitive inhibition of Bisphenol A binding to ERs and thus prevented growth-promoting impact of this compound in MCF-7 cells [10]. On the other hand, a recent study by Ishida et al., using genomic signaling assessments in in vitro and in vivo models showed a controversial result. In the in vitro evaluations, human choriocarcinoma JEG3 cells expressing ERα and ERβ receptors were exposed to a concentration range of 0.3–30 µg/mL of RJ. No agonistic activity of RJ was observed against these receptors. In the animal study, estrogen-responsive reporter mice were treated with dietary RJ (4%) for a period of one week; while it showed no significant ER-dependent genomic action. Both in vitro and in vivo studies used estradiol as positive control to assure the acceptable sensitivity of the models for detecting ER-dependent activities. The negative results obtained in this study may be due to the different concentration/dose of RJ in comparison to those studies with opposite results [53]. In another study in MCF-7 breast cancer cells, 10-HDA, the active ingredient of RJ, showed a LD_{50} value of 190 µg/mL [54]. Also, the 125 µg/mL concentration of 10-HDA in combination with 0.54 µg/mL of doxorubicin showed a synergistic antineoplastic activity in these cells which was 1.6-fold higher than doxorubicin alone [55]. Although these two studies assessed mechanisms other than hormonal receptors in the antiproliferative properties of 10-HDA, the involvement of 10-HDA interaction with sex hormone receptors cannot be ruled out since MCF-7 cells express both ER and progesterone receptors. On the other hand, it is demonstrated that not all types of breast cancer cell lines are responsive to the estrogenic effects of RJ. In a study performed by Zhang et al., 4T1-bearing mice were administered with daily doses of RJ (0.5, 1.0, and 1.5 g/kg) for 28 days [8]. RJ could inhibit the growth of 4T1 cells and reduce tumor size in animals; though, since 4T1 is a triple-negative breast cancer cell line, these activities cannot be attributed to the interaction of RJ with hormonal receptors. The inhibitory effect of RJ on the 4T1 murine breast cancer model is due to the immunomodulatory effects of RJ [35] since RJ at a dose of 0.5 g/kg augmented the level of tumor necrosis factor (TNF)-α, immunoglobulin (Ig)-G and decreased the level of interleukin (IL)-6. It can be concluded that in absence of hormone receptors on breast cancer cells, RJ can still show beneficial effect via anti-inflammatory and immunomodulatory properties.

It is concluded that RJ represents estrogenic effects on specific cell types. The extent of this effect dramatically depends on the presence of ERs on the surface of cells. Cells, such as the MCF-7 cell line that genetically express ERs, are susceptible to RJ estrogen-like compounds. Hence, some functions, such as cell proliferation in ER containing cells, can be directly stimulated by RJ consumption.

Discussion

Breast cancer is one of the most commonly diagnosed cancers in women and might be malignant if it invades surrounding tissues and metastasizes to distant areas. To treat breast cancer, a number of drugs have been developed; however, neither of them is entirely safe as these drugs are cytotoxic and could be toxic for normal cells. Nowadays, besides the conventional therapies, many people are inclined to use complementary and alternative medicine to quickly relieve side effects, decrease pain, and improve their quality of life. RJ has represented specific characteristics for breast cancer treatment with no effects on metastases. Since sex hormones are highly involved in the progression of breast cancer, effect of RJ on these hormones is of a great importance. Figure 2 shows the hormonal targets of RJ in reproductive system. RJ competed with estrogen for binding to ERs and stimulated the endogenous estrogen-responsive genes pS2. It is also demonstrated that the administration of RJ has some undesirable effects on breast cancer, such as stimulation of MCF-7 cell proliferation and triggering ER activity. On the other hand, it is reported that higher circulating levels of endogenous sex hormones, especially estrogens, may increase breast cancer risk. Besides, a two-fold increase in breast cancer risk is observed in women with high estradiol levels. Indeed, some types of breast cancers cells overexpress specific protein receptors. Estrogen and progesterone easily bind to these receptors and trigger specific growth signals in cells.

The effects of RJ on sex hormones as well as breast cancer cells directly depend on the dose of administration. RJ consumption at daily doses below 200 mg/kg can be useful to decrease the risk of breast cancer. At these dose levels, the serum level of estrogen becomes reduced; whereas the serum level of progesterone is increased, which subsequently decreases the expression of ERs on the ER-positive cell line, such as MCF-7 cell line and diminishes their response to estrogen. It is also suggested

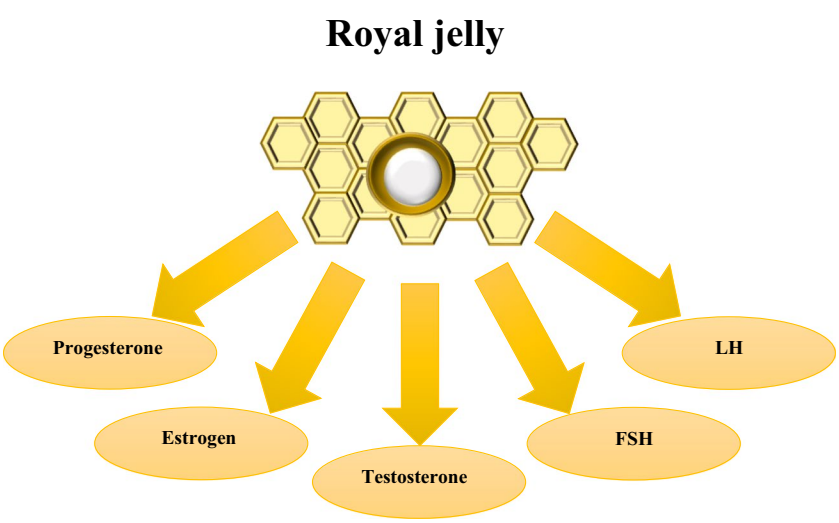

Fig. 2 Hormonal targets of royal jelly in reproductive system

that the interaction of RJ with ERβ is higher than ERα. It should be noted that current available literature mostly includes in vitro and in vivo studies which provide a lower level of evidence compared with clinical studies. In the current literature review, only three human studies met the inclusion criteria; while two studies have performed the evaluations in male subjects. No valid clinical trial on the effects of RJ on hormonal parameters of female subjects is available; thus, there is a big gap in the clinical evidence regarding the effects of RJ on sex hormones. One of the reasons for the few numbers of human studies on RJ may be the high price of this medicinal material. RJ is one of the most expensive bee products and due to the high doses needed in clinical trials, these studies may be costly. Nevertheless, the best way to assess the effects of RJ on sex hormones seem to be well-designed clinical trials in healthy female subjects to measure the changes in serum levels of these hormones in response to short-term and long-term intake of RJ. Results of such studies- along with preclinical evidence discussed in this paper- can pave the way for further human studies in patients with diseases related to dysregulation of sex hormones, as well as those with breast cancer.

Taken together, current evidence regarding the effects of RJ on sex hormones and ERs is controversial and mostly limited to preclinical studies; therefore, future clinical trials are needed to provide a higher level of evidence regarding the proper human dose of RJ as a safe and effective intervention in integrative oncology.

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