INTRODUCTION

Royal jelly is a bee product that is traditionally used as a dietary supplement but also as a potential remedy. Although it is widely used, experimental data that supports its therapeutic potential is lacking. Also, many studies have examined individual fractions and isolated substances from royal jelly, but there are very few studies that examine the native form of royal jelly, the one used in the daily diet. Our aim was to examine the effects of royal jelly, in the form of lyophilized powder product commercially available on the market, on the viability and proliferation of different cell lines in vitro. Our results showed that examined royal jelly product did not influence the cell viability of examined cell lines in examined concentrations while acted anti-proliferative in concentration-dependent manner on HeLa, cancer cell line but not on MDCK, non-cancer cell line. We can conclude that royal jelly contains some compounds that could exert certain activity towards cancer cells indicating its potential to which further studies should be directed.

Key words: royal jelly; in vitro; HeLa cells; MDCK cells; proliferation; viability.

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In vitro analysis of the biological activity of royal jelly on different cell lines

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Abstract

Royal jelly is a bee product that is traditionally used as a dietary supplement but also as a potential remedy. Although it is widely used, experimental data that supports its therapeutic potential is lacking. Also, many studies have examined individual fractions and isolated substances from royal jelly, but there are very few studies that examine the native form of royal jelly, the one used in the daily diet. Our aim was to examine the effects of royal jelly, in the form of lyophilized powder product commercially available on the market, on the viability and proliferation of different cell lines in vitro. Our results showed that examined royal jelly product did not influence the cell viability of examined cell lines in examined concentrations while acted anti-proliferative in concentration-dependent manner on HeLa, cancer cell line but not on MDCK, non-cancer cell line. We can conclude that royal jelly contains some compounds that could exert certain activity towards cancer cells indicating its potential to which further studies should be directed.

Key words: royal jelly; in vitro; HeLa cells; MDCK cells; proliferation; viability.

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The most abundant vitamins in royal jelly are vitamin B5 (pantothenic acid), followed by B3 (niacin) and B6 (pyridoxine) while small amounts of vitamins A, C, E, B1 (thiamine), B2 (riboflavin), B8 (biotin), B9 (folic acid), and B12 were found in royal jelly [2,7].

Many biological activities are attributed to major royal jelly proteins (MRJPs) and free fatty acids [4]. Reported in vivo and potential mechanisms, both in vitro and in vivo studies have shown that royal jelly and its components have estrogenic activity [2,15]. It has been reported that royal jelly has beneficial effect on female reproductive health due to estrogen-like activity, has anti-aging and wound healing activity and may be used as protective agent in neurodegenerative and aging diseases [14]. Some activities such as anti-tumor, immunomodulatory, estrogenic and neurogenic are attributed to royal jelly fatty acids [16]. Animal studies showed that royal jelly may be used for prevention of osteoporosis and for the improvement of bone strength due to the effects of royal jelly on bone metabolism [2,17,18]. Some studies have shown that royal jelly stimulates the growth of glial cells [19] and neurogenesis of stem cells in the brain [20]. There is also preliminary evidence that royal jelly lowers blood cholesterol levels [21,22] and has anti-hypertensive effect [2]. Immunomodulatory activity of royal jelly is attributed to the fatty acids [23,24] that are the main lipid components of royal jelly. Studies have shown that 10H2DA, inhibits VEGF-induced angiogenesis in vitro [25] and has a protective effect on oxidative stress caused by cisplatin in rats [26] while 3,10-dihydroxy-decanoic acid (3,10-DDA) was proved to stimulate maturation and Th1 polarizing capability of human monocyte-derived dendritic cells in vitro, which could be beneficial for anti-tumour activity [27]. In addition to having a potentially beneficial effect, on both healthy people and people suffering from certain diseases, royal jelly may exert some side effects, because it has many allergens [28], so it can cause allergic reactions, asthma, and even anaphylaxis [29].

In our recent study, we made a review of the characteristics and biological activities of propolis, bee product rich in biologically active substances and widely used in traditional medicine [30], which is often combined with other bee products such as royal jelly. Chemical composition and subsequent biological activity of propolis are reported to vary to great extent based on geographical origin and plant sources from which bees collect the material to produce propolis [30]. Unlike propolis, composition of royal jelly is reported to be independent of these environmental factors [16], and its composition is more or less standard-
examined in this study. It was dissolved directly in cell culture medium (complete DMEM) and examined in the concentration range from 32 to 2,000 μg/mL.

**Viability and proliferation assay**

HeLa and MDCK cells were detached using trypsin-EDTA solution and appropriate cell density was adjusted using Trypan Blue Dye Exclusion method. HeLa cells were seeded at density 100,000 cells per well (in viability assay) and 20,000 cells per well (in proliferation assay) while MDCK cells were seeded at density 60,000 cells per well (in viability assay) and 15,000 cells per well (in proliferation assay), in 96 well plates (Greiner Bio-One, Germany). Twenty-four hours after cultivation of cells under the standard cell culture conditions, royal jelly was added to the cells in examined concentrations. Cells cultured in standard cell culture medium (complete DMEM) without royal jelly (untreated cells) under the same conditions, were used as control cells. Each concentration of royal jelly, as well as control medium, was tested in four to eight replicates, and experiment was repeated three times. Cells were incubated with royal jelly and complete medium for the next 24 hours (in viability assay) or 72 hours (in proliferation assay), under the standard cell culture conditions, respectively. The cells were observed and photographed before and after the incubation with royal jelly, on inverted light microscope (Observer Z1, Carl Zeiss, Germany).

**MTT test**

After incubation of cells ended in both assays, cell viability and proliferation were assessed by MTT test. The cells were first washed with phosphate buffer saline followed by addition of 100 μL of MTT solution (concentration 1 mg/mL). The cells were incubated with MTT for the next three hours at 37 °C followed by removal of MTT solution and dissolution of formed formazan crystals with 100 μL of 2-propanol per well. The amount of formed formazan crystals is in direct correlation with the percentage of viable cells. The absorbance of dissolved formazan was measured on Multiskan Ascent Plate Reader (ThermoLab Systems, Finland) at a wavelength of 540 nm. The mean absorbance values were calculated for each examined concentration, as well as for the control cells. The results are expressed as a percentage of cell viability that was calculated according to the formula: % of viable cells / cell proliferation = (absorbance of treated cells/absorbance of control cells)*100.

**Statistical analysis**

The results of MTT test were analyzed using one-way analysis of variance (ANOVA) and expressed as a percentage of cell viability/proliferation with relative standard deviation, calculated according to the control culture of cells for which cell viability and proliferation rate were considered to be 100%. As statistically significant differences we considered those for which p < 0.05.

**RESULTS**

The results of viability assay are presented in Figure 1 while the results of proliferation assay are presented in Figure 2.

Royal jelly did not affect significantly the cell viability of the used cell lines at examined concentrations and conditions.

![Figure 1](image1.png)

**Figure 1.** The effect of royal jelly on cell viability.

Royal jelly showed concentration-dependent effect on cell proliferation and acted anti-proliferative on HeLa cells at higher examined concentrations (1,000 and 2,000 μg/mL) but not on MDCK cells. This decrease in HeLa cells’ proliferation was significant (p < 0.01) when compared to % of MDCK cells’ proliferation at concentrations 1,000 and 2,000 μg/mL as well as at a concentration 32 μg/mL, although the difference in the response of cells of both cell lines was noticed at all examined concentrations.

![Figure 2](image2.png)

**Figure 2.** The effect of royal jelly on cell proliferation; (*) p < 0.01.
DISCUSSION

In recent years, many researches have focused on biological properties of bee products in general and their beneficial effects on human health. Royal jelly is used as a dietary supplement and is usually combined with honey which preserves the fresh royal jelly. Although it has great history of being used as functional food and wide traditional use, research on the potential benefits on human health has been intensifying in recent years.

There are very few studies on the effects of royal jelly in its original natural form, as a mixture of biologically active substances, on cell growth in vitro. Most of the literature data reported the action of individual fractions and isolated components of royal jelly on cells in culture. Our results are unique since they show how royal jelly in its complete composition affects the growth of different epithelial cells. This is especially important to know if you keep in mind that preparations of bee products, including royal jelly, are applied mainly to the epithelium. Also, we analyzed commercially available product, in the form of lyophilized powder, and we show that it is biologically active. There is an emerging requirement for searching the potential compounds, favorable from natural sources that would have potential to inhibit the growth of cancer cells that can be used as addition to the standard chemotherapy. That was the reason why we choose two cell lines, one cancer and the other non-cancer cell line, to examine possible selective effect on the cancer cells. Although the results are preliminary, and for any concrete conclusion we need to perform more studies on other cancer cell lines and non-cancer cells but also to examine the royal jelly in different in vitro cell culture systems as well as in vivo on experimental animals, obtained results may be indicative and towards the course of further research in that direction.

In one of the rare studies of the effects of royal jelly on cell proliferation, an inhibitory effect of royal jelly on bisphenol-A-induced breast cancer cell proliferation was found, without an anti-proliferative effect on non-induced cell proliferation [32]. Izuta et al. found that bee products, including royal jelly, act by suppressing the proliferation of HUVEC cells induced by VEGF, as well as the process of angiogenesis [33]. The above findings on the negative effect of royal jelly fractions on the development of blood vessels, including our findings on the anti-proliferative action of royal jelly on cancer cells, suggest that royal jelly should be further examined for potential use as addition to chemotherapy. Moubarak et al. showed that royal jelly alone is relatively non-toxic to normal cells, but decreased the viability of human breast cancer cell line MDA-MB-231 in higher concentrations (200 μg/mL) as estimated by Trypan blue exclusion assay, while this decrease in cancer cells' viability was enhanced when royal jelly was combined with thymoquinone [34].

When it comes to the testing of the activity of certain fractions obtained from royal jelly, data have been published on the cytotoxicity of some protein fractions on HeLa cells [35]. On the other hand, the 57-kDa protein fraction has been shown to act as a mitogen, causing increased DNA synthesis in rat hepatocytes, with increased albumin synthesis in these cells. The authors of this study saw the potential role of royal jelly in liver regeneration and hepatocyte cytoprotection in this finding [36].

Having in mind the results of our study and reported literature data, it can be concluded that the effect of royal jelly depends on the cell type on which it is examined, the dose/concentration, the way of application of royal jelly and system and method used for assessing the effects.

CONCLUSION

Based on obtained results we can conclude that royal jelly exerts concentration-dependent effect on cell cultures in vitro. In examined concentrations, royal jelly did not affect cell viability, however, acted anti-proliferative on HeLa cells in higher examined concentrations that may suggest its selective effect toward cancer cells. Since we used only one cancer and one non-cancer cell line, we could not make strong conclusions about specific activity. Further analysis on greater number of different cell lines but also on more complex systems such as 3D cell cultures and animal models in vivo, together with detailed chemical profiling is needed.

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In vitro analiza biološke aktivnosti matičnog mleča na različitim ćelijskim linijama

Kratak sadržaj
Matični mleč je pčelinji proizvod koji se tradicionalno koristi kao dodatak ishrani, ali i kao lek. Iako je u širokoj upotrebi, nedostaju eksperimentalni podaci koji podržavaju njegov terapeutski potencijal. Takođe, mnoge studije su se bavile ispitivanjem pojedinih frakcija i izolovanih supstanci iz matičnog mleča, ali retke su studije u kojima se ispituje nativna forma matičnog mleča, ona koja se koristi u svakodnevnoj ishrani. Naš cilj je bio da ispitamo efekat matičnog mleča, u obliku proizvoda koji sadrži liofilizovani matični mleč i koji je komercijalno dostupan na tržištu, na vijabilnost i proliferaciju ćelija dve različite ćelijske linije in vitro. Naši rezultati su pokazali da ispitivani proizvod matičnog mleča nije uticao na vijabilnost ćelija ispitivanih ćelijskih linija u ispitivanim koncentracijama, dok je delovao antiproliferativno na HeLa, kancerske ćelije, ali ne i na MDCK, ne-kancerske ćelije. Možemo zaključiti da matični mleč sadrži neka jedinjenja koja bi mogla da ispolje određenu aktivnost prema kancerskim ćelijama što ukazuje na potencijal matičnog mleča na koji bi trebalo usmeriti dalja istraživanja.

Ključne reči: matični mleč; in vitro; HeLa ćelije; MDCK ćelije; proliferacija; vijabilnost.

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