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Proceeding Paper

Antimigratory Activity of Royal Jelly on HCT-116 Colorectal Cancer Cells †

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Abstract: Royal jelly (RJ) is a natural product, consumed as a functional food and in the form of a food supplement with multiple biological potentials. Apitherapy presents a complementary medical approach using bee products in the treatment of diseases, including cancer. Cancer metastasis implies the acquisition of migratory potential of cancer cells, and RJ already showed remarkable antimetastatic effects. We aimed to investigate the effects of RJ on the migration of colorectal cancer cells and key proteins involved in this process, E- and N-cadherin. Experiments were done 24 h after treatment with two selected concentrations. RJ suppressed the migratory potential of colorectal cancer cell line HCT-116, and enhanced the expression of anti-migratory protein, E-cadherin, while significantly inhibiting the promigratory marker, N-cadherin.

Keywords: N-cadherin; Transwell assay; apitherapy; migration; natural product

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1. Introduction

Royal jelly (RJ) has been employed throughout history as a natural product with multiple benefits for human well-being. In the present day, it is mainly consumed as a functional food, an active factor of daily supplements, and other formulations because of its various beneficial biological activities [1]. Apitherapy presents a complementary medical approach that involves the application of bee products in various diseases, including cancer [2]. RJ has been considered an important agent in apitherapeutic practices [2], especially when there are experimental studies proving its antimetastatic effects [3]. The pivotal process underlying metastasis is the acquisition of migratory potential, activating markers hallmarks of migration, on gene and protein levels. Colorectal cancer is of particular interest for research, as it is one of the deadliest types of cancer in the world and affects both males and females. Treatment of this disease is complicated especially when metastases are already present within the body. Metastasis is instigated by the detachment of malignant cells from the primary cancer site due to a reduction or loss of intercellular adhesion molecules, such as E-cadherin, and replaced by pro-migratory protein, N-cadherin. Consequently, the cells acquire high motility, enabling their dissemination into distant organs [4]. Therefore, scientists are focusing on alternative and complementary types of therapies, often using natural products with already-proven anticancer activity. This is the reason why the present report aims to investigate the effects of RJ (sampled from Serbia) on the migration of colorectal cancer cells (HCT-116) and explain the possible cellular mechanism involved in cancer cell motility.

2. Materials and Methods

Fresh royal jelly (*Apis mellifera* L.) was sampled from beehives located in Central Serbia (44°20′02″ N, 21°04′36″ E) and prepared in a Phosphate Buffer Solution (PBS) to obtain a stock solution (concentration 1 mg/mL), followed by further dilution in a complete medium (consisting of Dulbecco's modified Eagle medium—DMEM (Lonza, Basel, Switzerland), 10% Foetal bovine serum—FBS, and supplemented with 100 U/mL penicillin and 100 µg/mL streptomycin) to obtain two working solutions: 10 and 100 µg/mL. Colorectal cancer cell line HCT-116 was obtained from the American Tissue Culture Collection (ATCC, Manassas, VA, USA) and cultured according to standard procedure in a complete culture medium in a humified atmosphere at 37 °C and 5% CO_2. When it reached 90% confluency, the cells were seeded for assays. For purpose of investigating RJ effects on migratory potential, the Transwell test [5] was employed. Upper chambers of inserts (8 µm pore size; Greiner Bio-One, Frickenhausen, Germany) consisted of 1×10^5 cells treated with two RJ concentrations (10 and 100 µg/mL), while cells that were treated only with the complete media were used as the control. Lower chambers consisted only of 20% FBS that was used as an attractant. Results were obtained using an ELISA reader (RT-6100; Rayto Life and Analytical Sciences Co., Helmbrechts, Germany). Evaluation of protein expression was done using the immunofluorescent method [5], where cells were seeded on coverslips (4×10^4) and after 24 h treated with two RJ concentrations of 10 and 100 µg/mL. Effects were assessed 24 h after the treatment followed by the fixation of cells with 4% p-formaldehyde, permeabilization with 0.1% Triton-X, and blockage of non-specific binding sites with 1% Bovine serum albumine. Immunofluorescent micrographs were obtained using a TI-Eclipse microscope (Nikon Instruments Inc, Melville, NY, USA) and were analyzed by ImageJ software (1.53v, LOCI, Madison, WI, USA).

Statistical analysis implied the use of IBM SPSS statistical software, whereat the One-way ANOVA test was applied. Results are presented as mean ± standard error (SE). Between compared values, * $p > 0.005$ was considered a significant difference.

3. Results

3.1. Effects of RJ on Migratory Potential

Results obtained after applying the Transwell assay showed that RJ was a potent inhibitor of HCT-116 cell migration (Figure 1). Both applied concentrations were able to suppress the motility of these cells 24 h after treatment when compared to the control values. Lower RJ concentration (10 µg/mL) was more potent in this inhibition than higher (100 µg/mL).

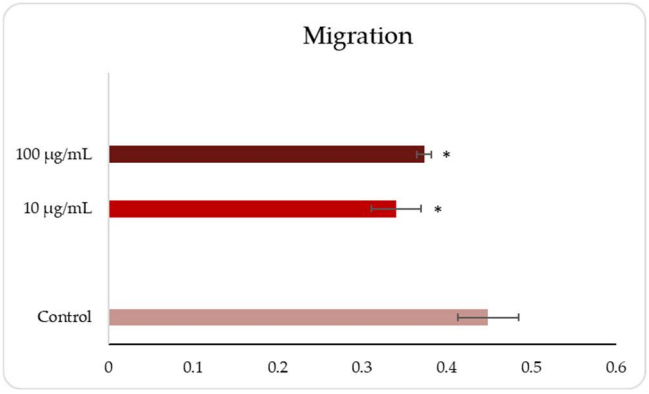
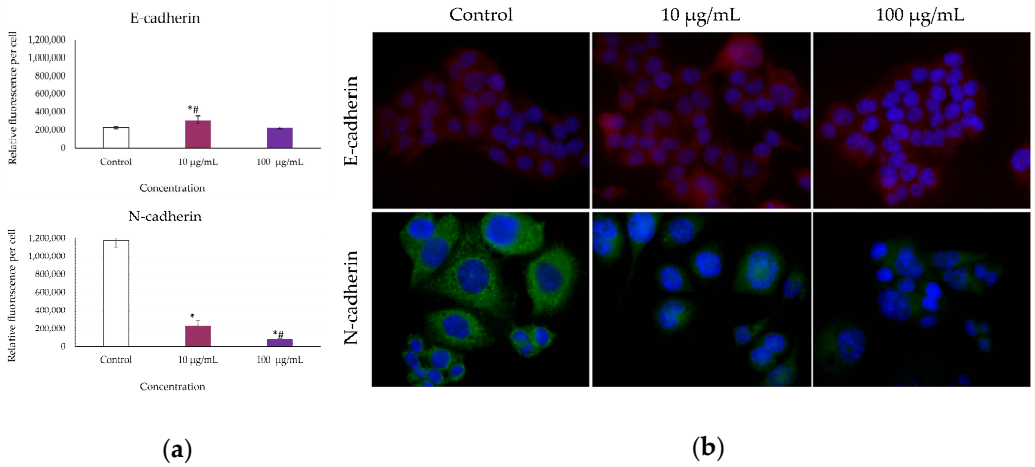


Figure 1. Effects of RJ on the migratory potential of HCT-116 cells. * statisticly significant difference between control and treatment values.

3.2. Effects of RJ on Protein Expression

According to our results obtained by using the immunofluorescent method, significantly enhanced expression of anti-migratory protein E-cadherin by lower RJ concentration

(10 µg/mL) can be noticed, while higher concentration slightly increased the level of this protein in the HCT-116 cell line when compared to the control (untreated cells). Meanwhile, notable suppression of promigratory marker, N-cadherin, was induced by both applied concentrations of this treatment, and a higher (100 µg/mL) concentration was more effective (Figure 2a,b).



(**a**) (**b**)

Figure 2. Protein expression of E-cadherin and N-cadherin in HCT-116 cells 24 h after treatment with RJ. Results expressed as relative fluorescence per cell (means ± SE; * $p > 0.005$ statistically significant difference compared to control, # $p > 0.005$ statistically significant difference between treatment concentrations (**a**). Representative micrographs showing fluorescently labeled target proteins; cell nuclei are stained blue; E-cadherin is stained red; N-cadherin is stained green (**b**).

4. Discussion

It is known that E-cadherin is a transmembrane protein with a role in the maintenance of intercellular connections when bound to β-catenin by its intracellular domain. This complex is present in epithelial cells that are tightly bound to each other. However, when the cell acquires migratory potential, this protein becomes lowered, and E-cadherin/β-catenin complexes are disrupted. Consequently, bonds between cells are loosened and cells are able to detach from the primary cancer site, invade through tissue, intravasate into blood vessels, and inhabit other distant organs. When this happens, E-cadherin becomes replaced with N-cadherin, a protein that disables intercellular bonds, allowing enhanced cell motility (known as "cadherin switch") [4].

According to our results, RJ treatment was able to elevate E-cadherin, presumably causing the restoration of cell-cell junctions while simultaneously lowering N-cadherin, which resulted in the inhibition of the migratory activity of the highly motile and invasive colorectal HCT-116 cell line. This is a valuable result of the present study and elucidates the anti-migratory activity of this natural product on colorectal cancer cells. Considering that RJ is a mixture of water, sugars, proteins, lipids, and minerals [4], and there are no experimental data investigating the effects of RJ on anti- and promigratory protein markers in cancer, we can conclude that this activity might be the result of one of RJ's active components, such as unsaturated fatty lipid acid 10HDA, that already exerted antimetastatic activity in vitro [6,7].

5. Conclusions

This is the first report regarding the effects of royal jelly on the migration of HCT-116 colorectal cancer cells and the presented findings reveal that the application of royal jelly showed significant anti-migratory effects against colorectal cancer cell line in vitro when compared to untreated cancer cells. As far as we know, our study is the first to elucidate the possible molecular mechanism of this important RJ property via inhibition of migratory marker N-cadherin. RJ might be proposed as a promising agent in the suppression of

colorectal cancer migration, however, the limitation of this study was an investigation in vitro, therefore additional investigations should be conducted on in vitro model systems to approve these findings.

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