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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 natural product, consumed as a functional food and in form of food supplement with multiple biological potentials. Apitherapy presents a complementary medical approach using bee products in treatment of diseases, including cancer. Cancer metastasis implies acquisition of migratory potential of cancer cells, and RJ already showed remarkable antimetastatic effects. We aimed to investigate the effects of RJ on 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 HCT-116 migratory potential, and enhanced expression of antimigratory protein E-cadherin, while significantly inhibited 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 natural product with multiple benefits for human wellbeing. In present days, it is mainly consumed as a functional food, active factor of daily supplements and other formulations because of its various beneficial biological activities [1]. Apitherapy presents a complementary medical approach which imply application of bee products in various diseases, including cancer [2]. RJ has been considered as important agent in apitherapeutic practice [2], especially when there are experimental studies proving its antimetastatic effects [3]. Pivotal process underlying metastasis is acquisition of migratory potential, activating markers hallmarks of migration, on gene and protein level. Colorectal cancer is of particular interest for research, being one of the deadliest types of cancer in the world and affecting both male and female. Treatment of this disease is complicated especially when metastases are already present in the body. Metastasis is instigated by 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 the ability of 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 proved anticancer activity. This is the reason why the present report aimed to investigate the effects of RJ (sampled from Serbia) on

migration of colorectal cancer cells (HCT-116) and explain the possible cellular mechanism involved in cancer cell motility.

2. Materials and Methods

Royal jelly was sampled from beehives located in Central Serbia and prepared in Phosphate Buffer Solution (PBS) to obtain stock solution, followed by further dilution in complete medium to obtain working solutions. Colorectal cancer cell line HCT-116 was cultured according to standard procedure and when reached 90% confluency cells were seeded for assays. For purpose of investigation of RJ effects on migratory potential, Transwell test [5] was employed, while for evaluation of protein expression, immunofluorescent method [5] was used. Cells were treated with two RJ concentrations 10 and 100 μg/mL, and effects were tracked 24 h of treatment. Immunofluorescent micrographs were obtained using Nikon TI-Eclipse microscope, and were analyzed by ImageJ software.

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

3. Results

3.1. Effects of RJ on Migratory Potential

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

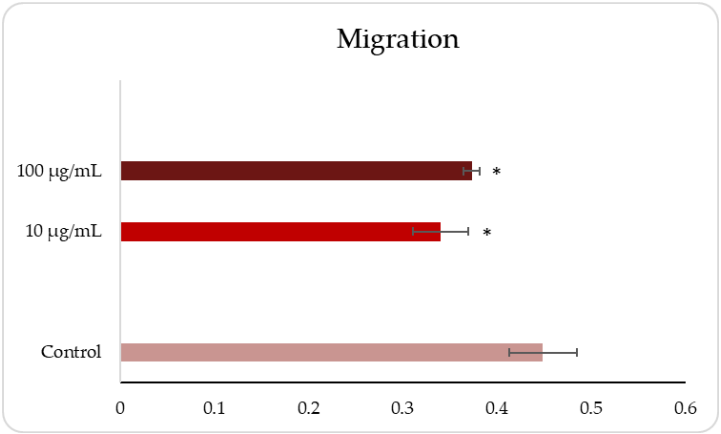

Figure 1. Effects of RJ on migratory potential of HCT-116 cells.

3.2. Effects of RJ on Protein Expression

According to our results obtained by using immunofluorescent method, significantly enhanced expression of antimigratory protein E-cadherin by lower RJ concentration (10 μg/mL) can be noticed, while higher concentration slightly increased the level of this protein in HCT-116 cell line, when compared to control (untreated cells). Meanwhile, notable suppression of promigratory marker N-cadherin was induced by both applied concentrations of this treatment, and higher (100 μg/mL) concentration was more effective (Figure 2a,b).

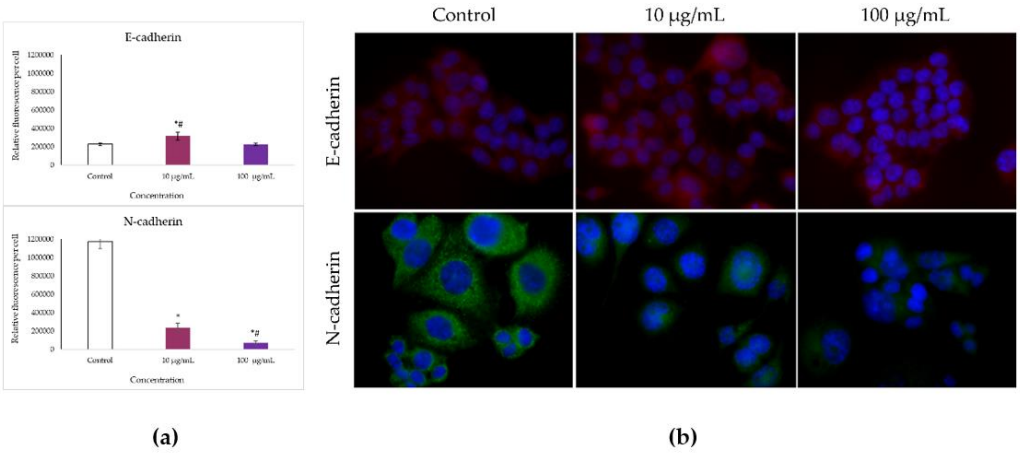

Figure 2. Protein expression of E-cadherin and N-cadherin in HCT-116 cells 24 h after treatment with RJ. Results expressed as relative fluorescence per cell (means ± SE; * $p > 0.005$ statistically significant difference compared to control, # $p > 0.005$ statistically significant difference between 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 transmembrane protein with role in 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 cell acquires migratory potential, this protein becomes lowered, and E-cadherin/β-catenin complexes are disrupted. Consequently, bonds between cells are loosen and cells are able to detach from primary cancer site, invade through tissue, intravasate into blood vessels and inhabit other distant organs. When this happens, E-cadherin become replaced with N-cadherin, protein that disables intercellular bond, allowing enhanced cell motility (known as "cadherin switch") [4].

According to our results, RJ treatment was able to elevate E-cadherin, probably causing restoration of cell-cell junctions, while simultaneously lower N-cadherin, which resulted in inhibition of migratory activity of highly motile and invasive colorectal HCT-116 cell line. This is valuable result of present study and elucidates antimigratory activity of this natural product on colorectal cancer cells. Considering that RJ is mixture of water, sugars, proteins, lipids and minerals [6], and there are no experimental data investigating 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 vivo [7,8].

5. Conclusions

The findings of the current study revealed that application of royal jelly showed significant antimigratory effects against colorectal cancer cell line in vitro and might be proposed as a promising agent against colorectal cancer migration. However, additional investigations should be conducted to approve these findings, especially on in vivo model systems.

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