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Inhibition of Migration of SW-480 Cells Induced by Royal Jelly Due to Reduction of β-Catenin †

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Abstract: Royal jelly (RJ), a natural bee product known for its abundance of bioactive compounds, is often referred to as a "superfood" and has been utilized in alternative medicine for centuries. Numerous studies have highlighted its therapeutic properties, including anticancer activity. A major challenge in standard cancer therapy is the migration of cancer cells, which leads to metastasis and the formation of secondary tumors with often fatal outcomes. Cancer cell migration is facilitated by the epithelial-to-mesenchymal transition (EMT) and the aberrant activation of the Wnt/β-catenin signaling pathway. A key component of this pathway, the transcription factor β-catenin, regulates the expression of various cellular components that play critical roles in cell motility. This study investigated the antimigratory potential of RJ on the colorectal cancer cell line SW-480 and its effects on β-catenin protein expression. RJ significantly suppressed the motility of SW-480 cells and markedly reduced β-catenin protein levels 24 h after treatment. These findings underscore the potential of RJ as a functional food to regulate colorectal cancer cell motility through modulation of β-catenin, thereby reducing disease aggressiveness.

Keywords: wound healing; colorectal cancer; immunofluorescence; migration; Wnt/β-catenin signal pathway; bee product

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1. Introduction

One of the most significant risk factors for the occurrence and development of colorectal cancer (CRC) is nutrition [1]. Dietary modification, such as the use of functional food supplements, can improve the effectiveness of cancer therapies [2]. Royal jelly (RJ) is a natural bee product, highly appreciated in human nutrition because of its abundance in bioactive substances, and it is also named as a superfood. It has been used in alternative medicine for centuries, and many studies have reported its therapeutic properties, including anticancer activity [3]. It has been found that RJ significantly reduces the metastatic potential of cancer by preventing the formation of metastasis in distant organs [4].

CRC is one of the most frequent types of cancer in both sexes, known as the most common cause of death in the world, with a high mortality rate [5]. With its increasing incidence, patients' survival is often poor because of the high metastatic activity, which presents the greatest problem in standard cancer therapy. Metastasis starts with the acquisition of the migratory potential of cancer cells through the epithelial-to-mesenchymal transition (EMT). This program is regulated by transcription factors that affect the suppression of epithelial markers and the stimulation of mesenchymal markers on gene levels [6,7]. One

of them, perhaps the most important in CRC, is the aberrantly activated Wnt/β-catenin signal pathway and its central component, β-catenin. This transcription factor controls the expression of many other cellular components, with a significant role in the motility of cells [8]. Molecular discoveries in CRC designated Wnt/β-catenin signaling as a key driver of CRC development, while abnormal Wnt pathway components have been identified as potential targets for the treatment of CRC [5,9].

In the case of healthy cells, when there is no role in connecting E-cadherin on the cell membrane with the actin cytoskeleton in the cytoplasm of the cell and when Wnt is not active, β-catenin in the cytoplasm is constantly phosphorylated by the GSK3β/CK I/Axin/APC complex, so it is degraded. However, if the Wnt pathway is activated, the degradation of β-catenin is blocked, it accumulates in the cytoplasm, and it translocates to the nucleus, where it transmits a signal in the form of the regulation of gene expression. The accumulation of β-catenin in the cell nucleus can also occur due to mutations in components of the degradation system (e.g., APC) [10]. In interaction with other regulatory factors, β-catenin defines the fate of the cancer cell by physically and functionally interacting with the SNAIL transcription factor in the nucleus, forming a SNAIL/β-catenin complex. This leads to the direct activation of the Wnt signaling pathway, as well as the initiation and progression of EMT in cancer [11].

All the above-mentioned factors led us to conduct this study with aim of assessing the antimigratory activity of RJ on CRC cell line SW-480 and identifying the role of β-catenin in this cancer cells' behavior.

2. Materials and Methods

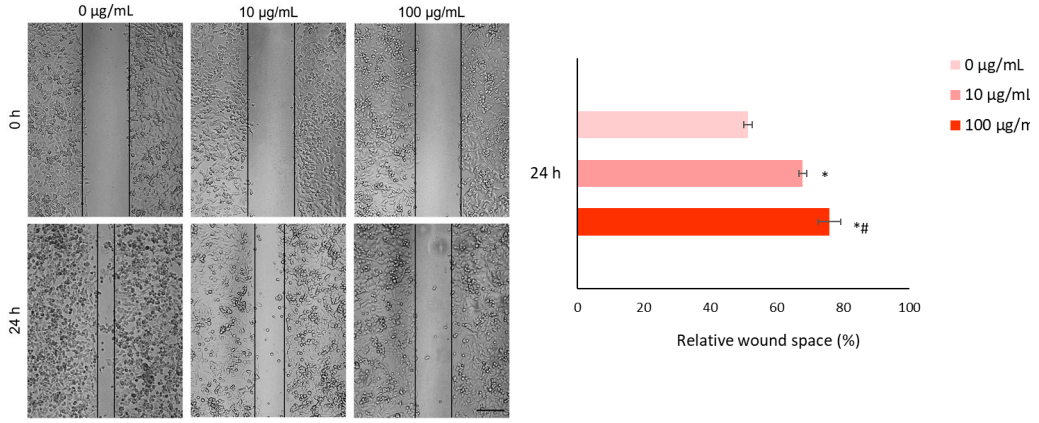
In this study, the SW-480 cells, originating from stadium II colorectal carcinoma (CRC), were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA), cultured according to the standard culturing procedure (complete culturing medium Dulbecco's Modified Eagle Medium—DMEM—supplemented with 10% fetal bovine serum—FBS—and penicillin/streptomycin) in a humified atmosphere at 37 °C and 5% of CO_2. When 90% confluence was reached, cells were treated with RJ (product by *Apis mellifera* L. species) sampled from the central region of Serbia, diluted in phosphate-buffered solution (PBS) and DMEM, resulting in obtaining two selected concentrations—10 and 100 µg/mL. These two selected concentrations showed no effect on cell viability in a previous study [12]. After 24 h, the motility of cells was examined by using a wound healing (scratch) assay, as described earlier [13], while the protein expression of β-catenin was acquired with the use of an immunofluorescent method according to a procedure already reported [9]. Inverted fluorescent microscope Eclipse Ti (Nikon Instruments Inc., Tokyo, Japan) was used to obtain micrographs (at 600× magnification), and the quantification of the relative fluorescence intensity of targeted proteins was performed using an ImageJ software package (ver. 1.52a) [9].

All experiments were performed in triplicate within two independent experiments, and data were analyzed using a one-way Anova test in an IBM SPSS statistical software package (ver. 20) (NY, USA). The obtained results are presented as mean ± standard error, where * $p < 0.05$ was considered as a statistically significant difference between treatments and the control, while # $p < 0.05$ designated a statistically significant difference between treatment concentrations.

3. Results

As can be observed on the presented micrographs (Figure 1a,b), results indicate a significant suppression of SW-480 cell motility by RJ in both applied concentrations 24 h after treatment when effects were compared to control values. However, among tested

concentrations, the higher one—100 µg/mL—had a more prominent anti-migratory effect on these cells.

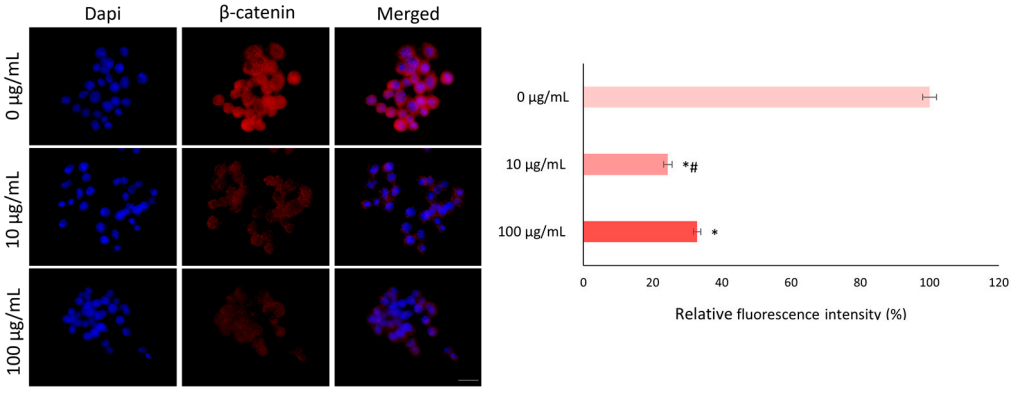


(a) **(b)**

Figure 1. Representative micrographs showing the migratory potential of control and SW-480 cells treated with RJ (**a**). Results are also presented as mean values ± standard error from two independent experiments performed in triplicate (**b**); * $p < 0.05$ is considered as statistically significant differences between treatments and control values and # $p < 0.05$ is considered as statistically significant differences between treatment concentrations. Scale bar: 30 µm.

Considering that the migratory capacity of cancer cells greatly depends on the concentration of regulatory factor β-catenin, this study aimed to determine whether RJ treatment affects this protein in SW-480 cells.

Immunofluorescent staining revealed the expression intensity of β-catenin in control (untreated) cells was at a high level, and the localization of this protein was in both cell nuclei and the cytoplasm, indicating its abundance in cells (Figure 2a). Meanwhile, RJ significantly suppressed the β-catenin expression rate below control values and restricted its localization only to the cell cytoplasm. Figure 2a shows that the presence of this protein was not observed in nuclei compartments. The fluorescence intensity of β-catenin expression was lower when a lower RJ concentration 10 µg/mL was applied (Figure 2b).



(a) **(b)**

Figure 2. Representative micrographs showing β-catenin protein expression and localization (**a**) and relative fluorescence intensity (**b**) in untreated and SW-480 cells treated with RJ. Results are presented as mean ± standard error from 2 independent experiments performed in triplicate, where * $p < 0.05$ is considered as statistically significant differences between treatments and control values and # $p < 0.05$ is considered as statistically significant differences between treatment concentrations. Scale bar: 50 µm.

4. Discussion

Royal jelly has been used in folk medicine for centuries, as a dietary supplement, and it has proved to be effective in treating cancer, considering it has already shown anticancer properties in suppressing cancer aggressiveness and metastatic rate [1]. Hence, this study showed the antimigratory potential of an RJ sample from Serbia on the CRC cell line SW-480, and the suppression of this cell behavior is a highly desirable approach for designing anticancer therapeutics.

The present study reports on the suppression of motility of tested colorectal cancer cells induced by treatment with RJ, obviously due to the lowered β-catenin protein level. Previous investigations confirm valuable anticancer effects of RJ attributed mainly to its prominent bioactive constituent 10H2DA. This unsaturated fatty acid has already shown the ability to bind to estrogen β receptors, which results in the regulation of the expression of β-catenin on gene levels [14], considering that this type of estrogen receptor is widely present in CRC [15].

However, it is also known that other bee products, such as melittin, the main component of bee venom, can lower the level of β-catenin and simultaneously affect the increase in E-cadherin in pancreatic cancer cells [16].

The SW-480 cell line, used in the present study, is positive for a mutation in the *APC* gene, so the degradation of β-catenin is greatly reduced in these cells, which results in the accumulation of this protein in the cell cytoplasm [1]. Overexpression of this protein in CRC is under the regulation of many signaling pathways, including Wnt/β-catenin. β-catenin is able to translocate from the cell cytoplasm into the nucleus, where it assumes the role of a transcription factor regulating the expression of pro-migratory/invasive markers such as vimentin and SNAIL. The synergistic action of β-catenin and SNAIL in the nucleus additionally enables cells to survive during the dissemination and invasion of cancer [8].

Our study shows that RJ caused the retainment of β-catenin in the cell cytoplasm, disabling its translocation to the nucleus, which is the most probable cause of resulting antimigratory effects of this natural product.

The obtained results are in concordance with a previous study conducted by Šeklić et al. [9], where several natural products, such as edible and medicinal mushroom species, were able to reduce the expression of β-catenin in the SW-480 cell line and, subsequently, the migratory capacity of these CRC cells.

5. Conclusions

Our results underscore the significant role of RJ in modulating the motility of CRC by targeting β-catenin, a key marker in CRC progression. This regulatory effect reduces the aggressiveness of CRC, highlighting the therapeutic potential of RJ. Notably, this study is the first to demonstrate the antimigratory effects of RJ on the SW-480 cell line, emphasizing its potential as a natural adjunct in anticancer strategies. These promising results warrant further investigation to fully elucidate the molecular mechanisms underlying RJ's effects and to explore its applicability in clinical settings.

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