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# Expression of β-Catenin Marker in Colorectal Cancer Cells after Treatment with Royal Jelly †

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**Abstract:** Deregulation of Wnt/*β*-catenin signal pathway is common in colorectal cancer, while β-catenin, its crucial component, is target for development of many anticancer therapies. Here, we showed that royal jelly, as well known beneficial natural product, is able to affect β-catenin at gene and protein level in HCT-116 colorectal cancer cell line. Our results indicate effectiveness of royal jelly in targeting crucial marker responsible for cancer development and progression. Therefore, royal jelly presents promising agent for development of supplementary anticancer therapy.

**Keywords:** HCT-116 cell line; qRT-PCR; immunofluorescence; nuclear export

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## 1. Introduction

Wnt/β-catenin signaling pathway is crucial in colorectal carcinoma, and its central component β-catenin is the focus of many targeted anticancer therapies [1]. In healthy cells, Wnt signaling pathway is not active, and β-catenin is mainly located in the peripheral part of the cytoplasm. In the cell membrane area, β-catenin is bound to E-cadherin, thus forming a complex with a role in connecting neighboring cells, which reflects its importance in the epithelium tissue. If found free in the cytoplasm, it becomes immediately degraded by the APC complex [2]. However, in cancer cells, the Wnt pathway is deregulated and if the APC or β-catenin are mutated or absent, β-catenin is not degraded. It becomes accumulated in cell cytoplasm and transported into nucleus where acts as transcription factor regulating cell proliferation, migration and invasion, thus formation of metastases [1].

Literature data report existance of many natural products as source of biologically active molecules originating from plants and animals with already proved effects on Wnt signaling. Inhibitory properties of these products have been described and are desired when it comes to Wnt signal pathway. These features designate significant therapeutic potential and make them candidates for succesful targeted therapies against cancer [3].

One of recognized natural products reported to be useful as anticancer agent is royal jelly. This special food derived from bees and intended for their consumption showed inhibitory effects on cancer cell proliferation, tumor growth and cell invasion [4].

We examined the effect of this natural product, royal jelly (RJ) sampled in Serbia, on β-catenin expression and concentration, since previous studies have shown that RJ has certain suppressive properties on cancer, as well as on its progression in terms of metastasis.

In this study, we examined the effects of RJ on β-catenin gene and protein expression in colorectal cancer cell line.

## 2. Methods

*β-catenin* gene expression was evaluated 24 h after treatment with two RJ concentrations (10 and 100 μg/mL) using the qRT-PCR method, as previously described in detail [5]. Results are presented as the fold change in mRNA expression in a target sample normalized to a reference gene (*β-actin*) and relative to the control sample. Values of relative gene expression were calculated according to $2^{-\Delta\Delta Ct}$ method.

Meanwhile, protein level and localization of β-catenin fractions in the nucleus and cytoplasm were determined by immunofluorescent assay [5] 24 h after treatment of cells with two RJ concentrations (10 and 100 μg/mL). Results of protein expression are presented as relative fluorescence intensity (%).

## 3. Results

### *3.1. Gene Expression*

The results showed that this natural treatment caused a significant increase in β-catenin gene expression in both applied concentrations (Figure 1). A dose-dependent effect of this treatment compared to the level of the control housekeeping gene *β-actin* can be observed in Figure 1.

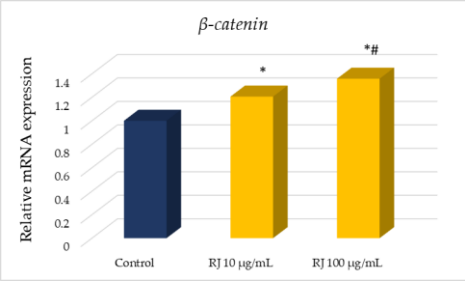


**Figure 1.** Gene expression of *β-catenin* in untreated (control) and HCT-116 cells treated with RJ after 24 h.

### *3.2. Protein Expression and Localization*

Our results show that RJ treatment had an impact on total β-catenin concentration when compared to control values. Namely, both applied RJ concentrations (10 and 100 μg/mL) were able to decrease β-catenin, as it can be observed in Table 1.

**Table 1.** Level of total β-catenin in HCT-116 cells 24 h after treatment with RJ. Results are presented as relative fluorescence intensity per cell.

| Treatment | Value |
|---|---|
| Control | 235,214.6 |
| RJ 10 μg/mL | 186,172.9 |
| RJ 100 μg/mL | 199,708.6 |

We noticed a significant decrease of nuclear β-catenin in HCT-116 cells after treatment with RJ (Figure 2). Meanwhile, dose-dependent increase in cytoplasmic fraction of this protein was evident after 24 h of treatment with RJ when compared to control values (Figure 2).

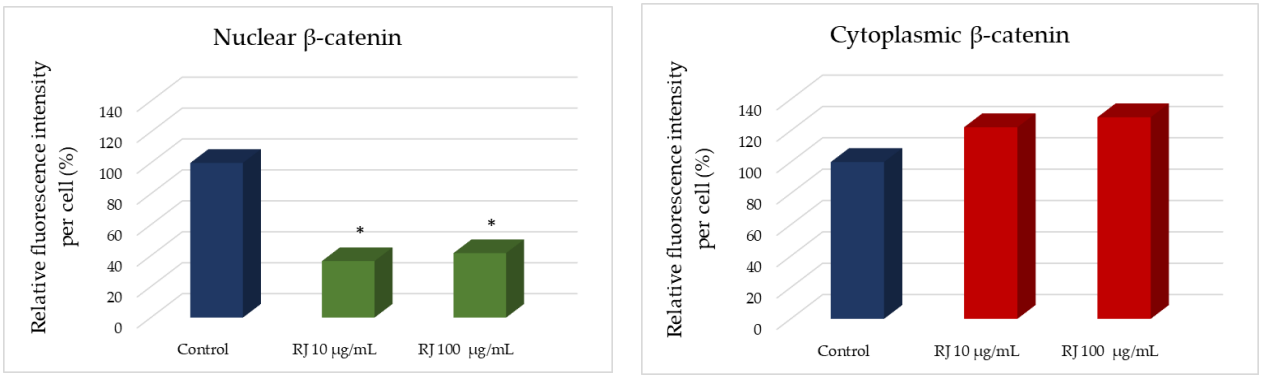


**Figure 2.** Nuclear and cytoplasmic β-catenin protein expression in control (untreated) and HCT-116 cells treated with RJ after 24 h.

Immunofluorescent technique showed that localization of nuclear β-catenin was not limited only to nucleus of control (untreated) HCT-116 cells but was also found in cell cytoplasm. When these cells were treated with lower RJ concentration, the localization of this protein was found in both cell compartments again, however its concentration was lower than in control cells. Furthermore, higher RJ concentration induced mostly cytoplasmic localization of this protein (Figure 3a).

Meanwhile, we observed the localization of cytoplasmic β-catenin in control and HCT-116 cells treated with RJ and it can be concluded that this protein was mostly concentrated on cell membrane area (Figure 3b).

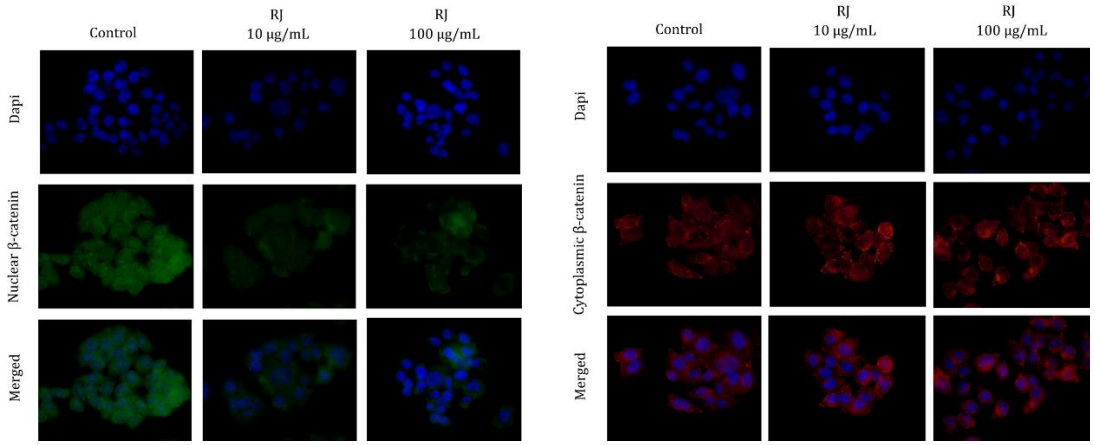


**Figure 3.** Nuclear (**a**) and cytoplasmic (**b**) β-catenin protein expression and localization in control (untreated) and HCT-116 cells treated with RJ after 24 h.

## 4. Discussion

The Wnt/*β*-catenin signal pathway is conserved signaling and its dysregulation has crucial role in cancer development and progression. Hence, agents that are able to target its components are focus of the worldwide researchers increasing attention as innovative therapeutic drugs in combating cancer [6].

Considering that royal jelly, as natural product with proved beneficial effects on human wellness, have already showed significant anticancer activity [4], we aimed to test its potential to modulate Wnt/*β*-catenin pathway in selected colorectal cancer cell line.

Firstly, HCT-116 cell line possess one mutated *β-catenin* gene allele, while *APC* gene is wild type [1,2]. Therefore, this cell line represents good model system for examining the impact of royal jelly on central component of Wnt signal pathway, β-catenin.

Our results indicate that the treatment caused the obvious decrease in *β-catenin* gene expression indicating royal jelly's efficacy in targeting nuclear processes, which is prominent result that should not be neglected. Previous study conducted by Wang et al. [7] proved that bee products are able to downregulate *β-catenin* mRNA level in cancer cells, which concords with our results.

Considering total β-catenin protein level in these cells, we observed decrease of this marker in HCT-116 cells induced by royal jelly. Furthermore, lower concentration of β-catenin was found in the cell nucleus and slightly increased level of this protein in cytoplasmic area. This could indicate that treatment started APC-independent signaling pathways responsible for export of β-catenin from the nucleus, or passive export could occurred as result of treatment effect. This could be consequence of reduced interaction of β-catenin with transcription factors and interaction with DNA in nucleus. It is known that when present in cell nucleus, this protein regulates expression of many markers with significant role in cell proliferation, invasion and migration. Therefore, it is very important that level of β-catenin is on minimum when found in cell nuclei, in order to inhibit cancer development and progression [2].

Also, its relocation from nucleus to the cytoplasm most probably indicates its migration to the membrane in order to bind to E-cadherin and restore intercellular connections. Besides, it is also possible that after its export from nucleus, royal jelly activated APC protein complexes responsible for degradation of one portion of β-catenin protein resulted from wild type gene allele that is substrate capable for degradation by APC, therefore its lower concentration in cell cytoplasm.

In conclusion, royal jelly proved to be powerful agent in affecting β-catenin gene expression and export of this protein from nucleus to the cytoplasm where it can be degraded. Similar results in HCT-116 cells treated with natural products have been published earlier by Šeklić et al. [1] confirming potential that can be found in agents originating from nature regarding targeted activity in cancer cells.

## 5. Conclusions

Obviously, this natural product was effective in targeting important component included in significant signal pathway already deregulated in colorectal cancer cells, however, enormous examinations yet remain to be completed. We anticipate that these findings will be focus of increasing attention in both scientific and clinical field of research.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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