



Unsaturated Fatty Acid 10H2DA Content in Serbian Royal Jelly and Its Effects on Motility of Colorectal Carcinoma Cell Lines [†]

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Abstract: Royal jelly (RJ) is natural bee product with multiple medicinal properties that have been mainly attributed to its active compound fatty acid 10H2DA. Herein, we determined its content in RJ sampled in Serbia, and analyzed its anti-migratory activity on colorectal carcinoma cells. This is the first report of the 10H2DA concentration in Serbian RJ, which is 0.92%. This acid was potent in inhibiting the motility of very aggressive HCT-116 cell line in dose-dependent manner. Further studies are needed to be conducted, especially more detailed research regarding application of this natural product compound as complementary drug in anti-cancer treatment.

Keywords: 10H2DA; colorectal cancer; HPLC; royal jelly; migration



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1. Introduction

Royal jelly (RJ) is one of the most popular beehive products, besides propolis, honey, and bee venom. This natural product is also labeled as a superfood due to its complex composition: proteins, carbohydrates, lipids, vitamins, amino acids, minerals, hormones, and enzymes [1,2]. It has been traditionally used as dietary supplement and possess several confirmed pharmaceutical properties, among which are anti-inflammatory, anti-microbial, anti-oxidant, and anti-tumor properties [1,3]. Meanwhile, significant RJ properties are mainly attributed to unsaturated fatty acid 10H2DA, present only in RJ, and not found elsewhere in nature [1,3]. The presence of 10H2DA has been used as marker of quality of RJ [4]. This organic compound is one of the most active RJ constituents with proved antibacterial, anti-inflammatory, blood lipid lowering, immunity enhancing effects [5]. Moreover, its valuable anti-cancer properties have been investigated for many decades, and it is known that 10H2DA is able to suppress development of cancer metastasis in vivo [1], yet its anti-migratory effects on colorectal cancer cells have not been reported so far.

Colorectal cancer (CRC) is serious disease affecting both men and women and is among most frequent types of cancer globally [6]. At present, standard anti-cancer treatments include chemotherapy, radiation, and surgery, however it is not completely effective strategy. Thus, scientists are searching for complementary products that will ameliorate current approaches in fighting cancer [3].

When CRC cases are diagnosed, it is usually at advanced stage with metastatic process already ongoing throughout the body, which presents particular problem for treatment [7]. Therefore, understanding the migratory potential of CRC cells, its molecular mechanisms, and ways to prevent it is becoming important focus of research in the field of cancer [8].

The purpose of the present study was to evaluate content of 10H2DA in the RJ sample originated from Serbia, since data on this subject are scarce regarding Serbia. We also determined its anti-migratory potential on two different colorectal carcinoma cell lines by analyzing single cell migratory potential.

2. Methods

Content of 10H2DA in the RJ sampled from central part of Serbia was determined using high-performance liquid chromatography (HPLC) method. RJ sample was homogenized, dissolved in mixture of methanol and water: acetic acid, and filtered. 10H2DA was identified at wavelength 225 nm, whereat the UV/VIS spectra were set in range 200–400 nm.

10H2DA was commercially obtained from TCI Chemicals, Japan. After dissolving in DMSO and DMEM to obtain working concentrations, the potential of this unique RJ compound in suppressing motility of colorectal carcinoma cells (HCT-116, SW-480) was assessed by Transwell assay [9]. Cells were treated with two selected sublethal 10H2DA concentrations (10 and 100 μ M) and effects were tracked after 24 h of treatment.

3. Results and Discussion

By applying HPLC method, we observed that our tested RJ sample contains 0.92% w/w of 10H2DA (Figure 1). This result is in accordance with the literature data reporting that RJ mostly contains 0.33–6.5% of 10H2DA [10]. Flanjak et al. [10] reported in previous study concentrations of 10H2DA in RJs that were sampled from countries of the similar geographic positions as Serbia. Namely, Greek RJ contains between 0.8% and 6.5% of 10H2DA; Romanian RJ in range 1.35% to 2.03% of 10H2DA; Turkish 0.33–2.54% [10]. We can conclude that our Serbian RJ sample is of optimal quality concerning 10H2DA content, when compared to content of this fatty acid present in RJ samples mentioned above.

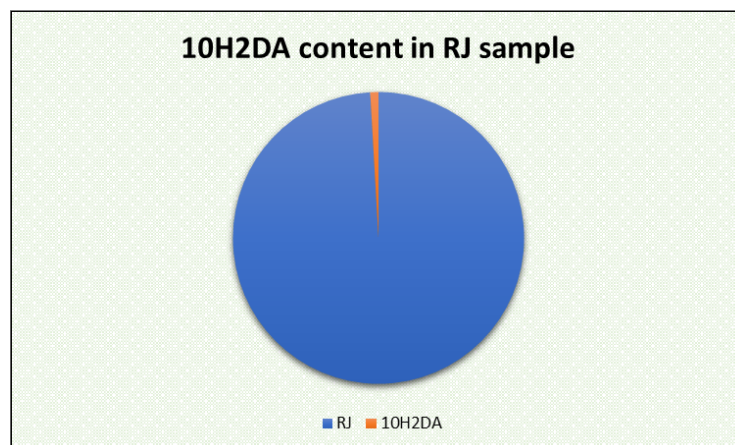


Figure 1. Content of 10H2DA in RJ sample originated from Serbia.

It is known that 10H2DA content in RJ mostly vary due to geographical, climatic, and floral differences worldwide [4]. Thus, it is possible that this value is a result of various environmental conditions that affected production of royal jelly fatty acid by worker bees.

When it comes to its biological activity, we evaluated potential of 10H2DA on migration of cancer cells. First of all, basal migratory potential of two tested colorectal cancer cell lines was obviously different (Figure 2). Namely, HCT-116 cells possess greater motility than SW-480 cell line. Nevertheless, both 10H2DA applied concentrations were able to significantly reduce motility of HCT-116 cells. On the other hand, the migratory potential of the SW-480 cells remained almost unchanged when treated with 10H2DA (Figure 2). Obviously, tested substance was able to inhibit motility of more aggressive HCT-116 cells than less mobile SW-480 cell line, which is a significant result of our study. As observed, HCT-116 cells proved to be more sensitive to the natural bioactive substances including RJ and 10H2DA, and their stronger sensitivity to the treatments, in comparison to SW-480 cell

line, was already confirmed in previous studies [9,11]. Antimigratory activity of 10H2DA was probably achieved via increase of anti-migratory and decrease of pro-migratory markers. In study conducted by Lin et al. [5], ability of 10H2DA to induce such response in human lung cancer cells was reported, and this effect was due to increase of E-cadherin and decrease of N-cadherin, Vimentin, and Snail.

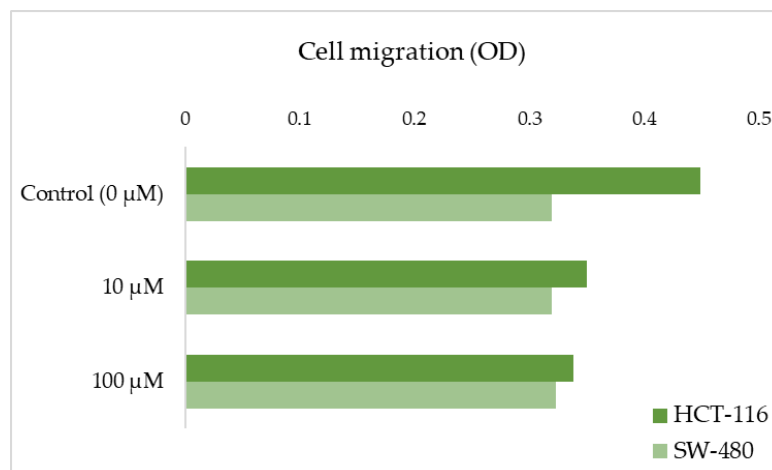


Figure 2. Effects of 10H2DA on HCT-116 and SW-480 cells motility, 24 h after treatment.

4. Conclusions

By search of literature data, we concluded that present study is the first report of 10H2DA content in RJ sample from Serbia, as far as we know. This very potent fatty acid 10H2DA possess significant anti-migratory effect on aggressive colorectal carcinoma cell line HCT-116, thus presents promising agent for future studies of bioactive components of RJ and application in anti-cancer treatment as complementary drug. However, exact molecular mechanism that underlies this important activity of 10H2DA remains to be elucidated furtherly.

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References

1. Oršolić, N.; Terzić, S.; Šver, L.; Bašić, I. Honey-bee products in prevention and/or therapy of murine transplantable tumours. *J. Sci. Food Agric.* **2005**, *85*, 363–370. [[CrossRef](#)]
2. Fratini, F.; Cilia, G.; Mancini, S.; Felicioli, A. Royal jelly: An ancient remedy with remarkable antibacterial properties. *Microbiol. Res.* **2016**, *192*, 130–141. [[CrossRef](#)] [[PubMed](#)]

3. Miyata, Y.; Sakai, H. Anticancer and protective effects of royal jelly for therapy-induced toxicities in malignancies. *Int. J. Mol. Sci.* **2018**, *19*, 3270. [[CrossRef](#)] [[PubMed](#)]
4. Kolayli, S.; Sahin, H.; Can, Z.; Yildiz, O.; Malkoc, M.; Asadov, A. A member of complementary medicinal food: Anatolian royal jellies, their chemical compositions, and antioxidant properties. *J. Evid. Based Complementary Altern Med.* **2016**, *21*, NP43–NP48. [[CrossRef](#)] [[PubMed](#)]
5. Lin, X.M.; Liu, S.B.; Luo, Y.H.; Xu, W.T.; Zhang, Y.; Zhang, T.; Xue, H.; Zuo, W.B.; Li, Y.N.; Lu, B.X.; et al. 10-HDA induces ROS-mediated apoptosis in A549 human lung cancer cells by regulating the MAPK, STAT3, NF- κ B, and TGF- β 1 signaling pathways. *BioMed Res. Int.* **2020**, 3042636. [[CrossRef](#)] [[PubMed](#)]
6. Kuipers, E.J.; Grady, W.M.; Lieberman, D.; Seufferlein, T.; Sung, J.J.; Boelens, P.G.; van de Velde, C.J.; Watanabe, T. Colorectal cancer. *Nat. Rev. Dis. Primers* **2015**, *1*, 15065. [[CrossRef](#)] [[PubMed](#)]
7. Mármol, I.; Sánchez-de-Diego, C.; Dieste, A.P.; Cerrada, E.; Yoldi, M.J.R. Colorectal carcinoma: A general overview and future perspectives in colorectal cancer. *Int. J. Mol. Sci.* **2017**, *18*, 197. [[CrossRef](#)] [[PubMed](#)]
8. Pijuan, J.; Barceló, C.; Moreno, D.F.; Maiques, O.; Sisó, P.; Martí, R.M.; Panosa, A.M.A. In vitro cell migration, invasion, and adhesion assays: From cell imaging to data analysis front. *Cell Dev. Biol.* **2019**, *7*, 107. [[CrossRef](#)] [[PubMed](#)]
9. Šeklić, D.S.; Stanković, M.S.; Milutinović, M.G.; Topuzović, M.D.; Štajn, A.Š.; Marković, S.D. Cytotoxic, antimigratory, pro- and antioxidative activities of extracts from medicinal mushrooms on colon cancer cell lines. *Arch. Biol. Sci.* **2016**, *68*, 93–105. [[CrossRef](#)]
10. Flanjak, I.; Jakovljević, M.; Kenjerić, D.; Cvijetić Stokanović, M.; Primorac, L.J.; Bilić Rajs, B. Determination of (2E)-hydroxydec-2-enoic acid in Croatian royal jelly by high-performance liquid chromatography. *Croat J. Food Sci. Technol.* **2017**, *9*, 152–157. [[CrossRef](#)]
11. Šeklić, D.S.; Obradović, A.D.; Stanković, M.S.; Živanović, M.N.; Mitrović, T.L.; Stamenković, S.M.; Marković, S.D. Proapoptotic and antimigratory effects of *Pseudevernia furfuracea* and *Platismatia glauca* on colon cancer cell lines. *Food Technol. Biotechnol.* **2018**, *56*, 421–430. [[CrossRef](#)] [[PubMed](#)]