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Evaluation of the Biological Activities of Royal Jelly on Prostate and Breast Cancer Cells

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Abstract: Due to their high toxicity to healthy body cells, chemotherapy drugs used to treat cancer pose a serious threat to the organism. Recent studies have encouraged the utilization of bee products to prevent and treat the cancer. The specific food for the queen honeybee larva (*Apis mellifera*), royal jelly (RJ) is a yellow, milky, cremy product of the bee which is stowed from the mandibular and hypopharyngeal glands of the worker honeybee. According to the reports, RJ may have cytotoxic effects in animal models. However, RJ's cytotoxic and antioxidant properties on PC-3 prostate cancer and MCF-7 breast cancer cell lines have yet to be thoroughly investigated at the cellular level. Therefore, the antioxidant and anti-proliferative activities of RJ in PC-3 prostate cancer cells and MCF-7 breast cancer cells were evaluated to investigate a chemopreventive strategy to increase cancer therapy efficacy. WST-1 assay was used to measure cell proliferation, and levels of reactive oxygen species (ROS) and lipid peroxidation (LPO) were analyzed to look at antioxidant activities. According to the data of this research, RJ act as prooxidants in PC-3 and MCF-7 cancer cell lines by lessening cellular viability and raising ROS and LPO formation.

Arı Sütünün Prostat ve Meme Kanseri Hücreleri Üzerindeki Biyolojik Aktivitelerinin Değerlendirilmesi

Anahtar Kelimeler
Arı sütü,
WST-1 testi,
Prostat kanseri,
Meme kanseri,
Lipid peroksidasyonu

Öz: Kanser tedavisinde kullanılan kemoterapi ilaçları, sağlıklı vücut hücrelerine yüksek toksisiteleri nedeniyle organizma için ciddi bir tehdit oluşturmaktadır. Son zamanlarda yapılan çalışmalar, kanserin önlenmesi ve tedavisi için arı ürünlerinin kullanımını teşvik etmiştir. Kraliçe bal arısı larvası (*Apis mellifera*), arı sütü için özel gıda, işçi bal arısının çene ve hipofarenks bezlerinden salgılanan sarımsı, sütlü, kremsi bir arı ürünüdür. Literatür verilerine göre, RJ'nin hayvan modellerinde sitotoksik etkilerinin olduğu söylenebilir. Bununla birlikte, arı sütünün PC-3 prostat kanseri ve MCF-7 meme kanseri hücre dizileri üzerindeki sitotoksik ve antioksidan özellikleri henüz hücresel düzeyde tam olarak araştırılmamıştır. Bu nedenle, bu çalışmada arı sütünün PC-3 prostat kanseri hücrelerinde ve MCF-7 meme kanseri hücrelerinde antioksidan ve anti-proliferatif aktiviteleri araaşrırılmıştır. Çalışmada, hücre proliferasyonunu analiz etmek için WST-1 testi kullanıldı ve antioksidan aktivitelerini analiz etmek için reaktif oksijen türlerinin (ROS) ve lipid peroksidasyonunun (LPO) seviyeleri analiz edildi. Bu araştırmanın verilerine göre arı sütü hücre canlılığını azaltarak ve ROS ve LPO oluşumunu artırarak PC-3 ve MCF-7 kanser hücre hatlarında prooksidan görevi görmektedir.

1. INTRODUCTION

Royal Jelly (RJ) contains primarily proteins (between 27 and 41 percent). More than 80% of these are soluble proteins. These proteins are thought to be the key macromolecules behind the unique physiological functions of RJ during the growth of the queen honeybee. In earlier investigations, RJ proteins were found to stimulate cell proliferation [1-3]. Sebacic acid (SEA), 10-hydroxydecanoic acid (10-HDAA), and trans-

10-hydroxy-2-decenoic acid (10-H2DA) are three commonly occurring fatty acids in RJ.

The biophysiological properties of RJ comprise anti-cancer, anti-microbial, immune-modulatory, anti-oxidative and anti-hypertensive impacts. They also work in conjunction with proteins to contribute to these biological activities [4-8]. RJ also contains phenolic acids, carotenoids, flavonoids, organic acids [9], vitamins, and minerals [10-11]. The antioxidant action of RJ is mostly attributed to the phenolic and flavonoids found in it, including chrysin, acacetin, apigenin, quercetin, hesperidin and kaempferol [10-12]. Due to their strong anti-oxidative capacity and their ability to alter a number of signaling pathways, containing the inhibition of cell viability, stimulation of apoptotic pathways, and cell cycle arrest, flavonoids also have anticancer properties [13-16,11].

Recent studies have encouraged the utilization of the important products of bee (ie. honey, propolis) for preventing and treating the cancer [17-18]. RJ's antioxidant and anticancer properties on PC-3 prostate cancer and MCF-7 breast cancer cell lines, however, have not yet been thoroughly investigated at the molecular level. For that reason, the goal of this research was to examine RJ's anti-oxidant and anti-cancer effects on PC-3 prostate cancer and MCF-7 breast cancer cell lines as well as the mechanisms behind these effects.

2. MATERIAL AND METHOD

2.1. Materials

PC-3 and MCF-7 cancer cell lines were kindly provided by Tokat Gaziosmanpasa University. Local beekeepers who are members of the Bingöl Beekeepers Association provided Royal Jelly. Local beekeepers produced RJ in the spring of 2019 in Bingöl and kept it at -20 °C. Malondialdehyde bis and 2′,7′-Dichlorofluorescein diacetate (DCFH-DA) were purchased from Merck.

2.2. Cell Culture

In full endothelial growth media with 10% fetal bovine serum and 1% penicillin-streptomycin (10000 units/ml, 10 mg/ml streptomycin), HUVEC, human PC-3 prostate and MCF-7 breast cancer cells were cultured. A humidified incubator with 5 percent CO_2 was used to culture the cells, and it was monitored every two to three days. Using the EZPCR mycoplasma test kit, cells were examined for mycoplasma contamination (Biological Industries).

2.3. Cell Proliferation Assay

The cytotoxicity of Turkish RJ was tested on PC-3 and MCF-7 cells using a Water Soluble Tetrazolium-1 (WST-1) cell proliferation assay kit from Clontech Laboratories in the United States. The supplier's recommended protocol was followed during the experiments. In a 96-well plate, 10.000 PC-3 and MCF-7 cells were first grown. The cells were then exposed to RJ at concentrations ranging from 0.02 to 0.64 mg/ml. 5 µl of WST-1 was then injected into each well. Using a SpectraMax Plus 384 Microplate Reader, each well's absorbance was measured at 450 nm (the reference wavelength is 630 nm) following a 4-hour incubation [19] (Molecular Devices, USA).

2.4. ROS Assay

Using a 2′-7′ dichlorodihydrofluorescein diacetate (DCFH-DA) test kit bought from Abcam, MA, USA, cellular ROS production was assessed [19]. The cells were handled as it is explained in cell culture section. 1000000 HUVEC, PC-3 and MCF-7 cells were collected after treatment and cell growth, and 1 h at 37 °C in the presence of 2 M DCFH-DA was used to measure the fluorescence.

2.5. Measurement of Malondialdehyde Levels

A small modification to the previously published methodology was used to measure the malondialdehyde (MDA) level in RJ-exposed HT-29 cells [20]. In cell culture, the cells were grown as instructed. Centrifugation of the cells was carried out for 5 min. at 30,000 rpm. The LPO assay mixture was then added to the cell suspensions, which were then and there incubated for 0.5 hours at 95 °C with 1 ml of 0.8 percent w/v thiobarbituric acid and 70 percent w/v trichloroacetic acid. The solution was then chilled on an ice bath for about five minutes, and the cell suspensions were then centrifugated at $15x10^3$ rpm for ten minutes. At 532 nm, the supernatant's absorbance was measured.

2.6. Statistical Analysis

At least three times each of the experiments were repeated. With the aid of GraphPad Prism 5, statistical analysis and comparable data groups were evaluated using the one-way ANOVA Newman-Keuls post-hoc Test; $p < 0.05$ was regarded as significant.

3. RESULTS

3.1. Cell Proliferation Assay

On the human prostate cancer cell line PC-3, the growth inhibition of the PC-3 cells by various doses of RJ (0.02 mg/ml, 0.04 mg/ml, 0.16 mg/ml and 0.64 mg/ml) was investigated. RJ substantially and concentration-dependently decreased the viability of prostate cancer cells (Figure 1a). The most effective concentration of RJ on PC-3 cells were determined to be 0.02 mg/ml. We also looked into how RJ affected the viability of MCF-7 breast cancer cell line at the same concentrations of RJ, and the findings revealed that there was no significant impact on the cell proliferation at the tested concentrations except at concentration of 0.02 mg/ml of RJ (Figure 1b).

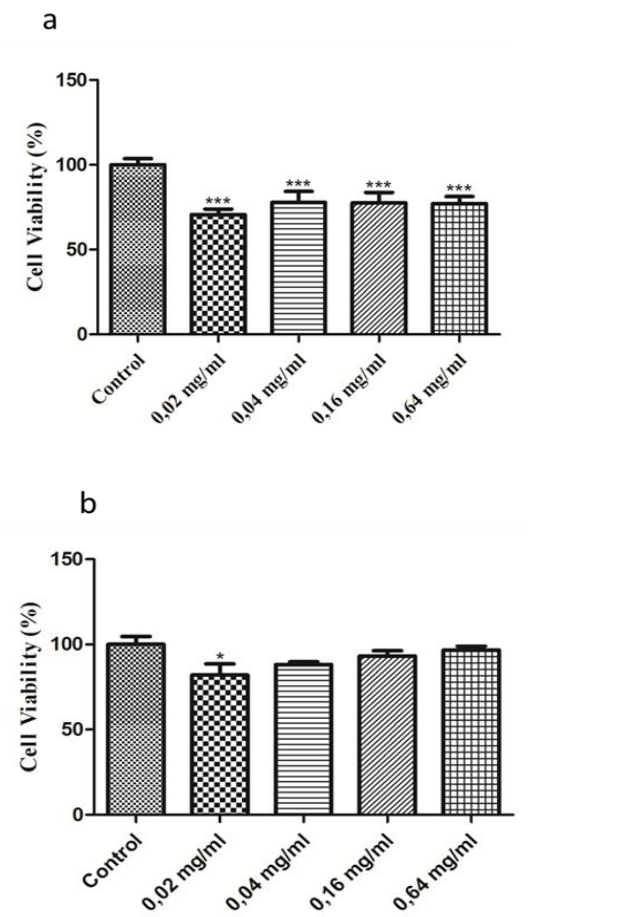

Figure 1. Effects of various Turkish RJ concentrations on the growth of PC-3 (a) and MCF-7 (b) cells. By using the WST-1 assay, the cell proliferation was evaluated. Experimental data are shown as mean SEM (three replicate treatments). ***p < 0.001, *p < 0.05 indicates statistically significant differences between Cont and other groups

3.2. ROS Assay

In PC-3 cells, the effects of RJ administration on ROS production were assessed. The findings showed that RJ dramatically enhanced ROS levels in PC-3 cells at a concentration of 0.02 mg/ml (Figure 2). The effects of RJ treatment on MCF-7 cell line were also examined within scope of this study. The findings revealed that 0.02 mg/ml of RJ significantly increased ROS generation in MCF-7 and HUVEC cells in comparison to the control (Figure 2).

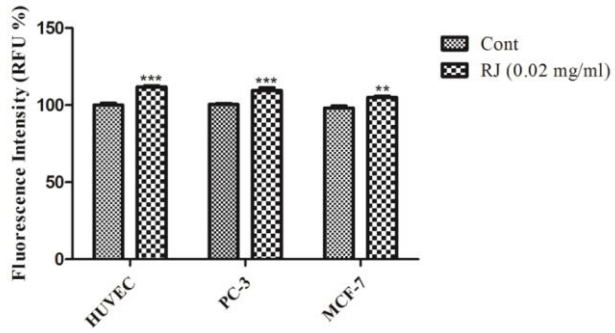

Figure 2. The impacts of RJ exposure on ROS production on PC-3 and MCF-7 cells. Experimental data are shown as mean SEM (three replicate treatments). ***p < 0.001, **p < 0.01 indicates statistically significant differences between Cont and other groups

3.3. Lipid Peroxidation Assay

Malondialdehyde (MDA) levels in controls, HUVEC, PC-3 and MCF-7 cells were measured using a lipid peroxidation technique to evaluate oxidative stress. The amount of thiobarbituric acid reactive substances (TBARS) produced by malondialdehyde was measured as part of the LPO assay (MDA). MDA concentration was evaluated in 0.02 mg/ml RJ treated HUVEC, PC-3 and MCF-7 cells to see whether RJ induced MDA formation in these cells. The results of our study indicated that 0.02 mg/ml of RJ significantly increased MDA generation in HUVEC, PC-3 and MCF-7 cells in comparison to the control (Figure 3).

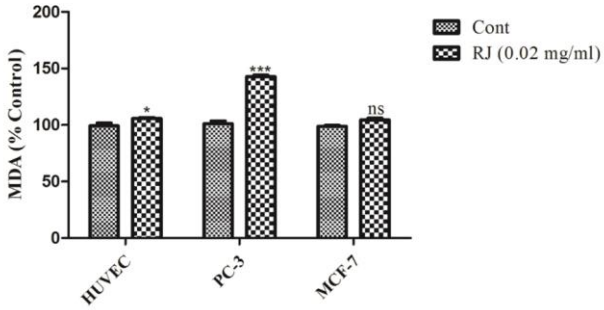

Figure 3. The impacts of RJ exposure on MDA production on PC-3 and MCF-7 cells. Experimental data are shown as mean SEM (three replicate treatments). Data were expressed as mean ± SEM, n = 4. ***p < 0.001, *p < 0.05, ns: nonsignificant indicates statistically significant differences between Cont and other groups

4. DISCUSSION AND CONCLUSION

Traditional medicine has long employed bee products extensively. Intriguing bioactivities, such as antimicrobial [21-22], anti-inflammatory [23], and anti-cancer [24] properties, have been demonstrated for the components, water or ethanolic extrats, and izolated biologicaly active ingredients from these products. Along with treating some tumors, bee products have also been widely used to treat a number of immune-related disorders [25]. Numerous malignant cell lines, including those from the prostate, breast, lung, and liver malignancies, have shown evidence of in vitro cellular apoptosis being induced by bee products. Thus, these biologically active natural compounds may prove useful as a component of cutting-edge, underfunded therapy for certain malignancies [16].

According to reports, RJ may have anticancer effects in mice [26,27]. Studies showed that RJ had anticancer properties because it caused tumor cells to develop apoptotic and antiproliferative pathways [12]. Biochemical methods are taken into consideration as a treatment option for cancer, whether it is colon, breast, prostate or another type. Nevertheless, anti-oxidant-rich products of bee species were utilized as bio-therapeutics to lessen cancer patients' risk of recurrence and side effects [28]. To our knowledge, there was no information related to the cytotoxic activity of RJ on PC-3 and MCF-7 cells, according to a thorough literature search. The purpose of this study was to examine the impact of

various RJ concentrations on PC-3 and MCF-7 cell viability, ROS production, and MDA levels.

The investigation into the anti-proliferative and cytotoxic activities of RJ against colon cancer cells revealed that, it might inhibit the physiologic development of the cancerous cells in a concentration-dependent manner with a discernment to cancerous and sarcoma cells. Leukaemia cells were not affected, but sarcoma cells were shown to respond therapeutically to Turkish RJ [29]. According to the extent of the exposure and the RJ concentrations, RJ has been shown to have an immune-stimulating impact via prevention the myelosuppression brought on by tumor evolution [30].

We show that RJ caused an increase in ROS and LPO levels, which is necessary to understand if the cellular processes of RJ treatment on cell survival can be connected to certain induction patterns of ROS and LPO. SOD, CAT, and glutathione are components of the anti-oxidant defence system in the human body, which maintains the balance between the neutralisation and creation of ROS [31]. Nevertheless, oxidative injury, which starts the evolution of cancer, is caused by an inequity between the generation of ROS and the anti-oxidant defence mechanisms [32]. According to [33], ROS have also been linked to the apoptotic pathway, which is essential for eliminating damaged cells during the course of cancer treatment.

Another study's findings showed that Bingöl RJ at a dose of 0.3 mg/ml dramatically increased the amount of ROS in SH-SY5Y cells, whereas RJ at a concentration of 0.5 mg/ml had essentially no influence on ROS levels. While 0.5 mg/ml RJ had no discernible effect on MDA levels in SH-SY5Y cells, the addition of 0.3 mg/ml RJ significantly enhanced the levels of malondialdehyde in those cells. The findings demonstrated that RJ treatment at 0.3 mg/ml dramatically decreased SOD and CAT activity while having little to no effect at 0.5 mg/ml, suggesting that RJ could protect cell membranes from radical-mediated cell damage [34, 35].

The biophysiological properties of RJ comprise anti-cancer, anti-microbial, immune-modulatory, anti-oxidative effects. The purpose of this study was to examine the impact of various RJ concentrations on PC-3 and MCF-7 cell viability, ROS production, and MDA levels. In this study, RJ treatment of these cancer cells reduced cell growth as demonstrated by WST-1 assay. ROS and LPO levels revealed to increase after the RJ treatment at a concentration of 0.02 mg/ml in both cancer cell lines suggesting RJ acts as a prooxidant in our study.

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