





The Combined Effects of Cisplatin and Royal Jelly on Lung Cancer: Growth, Proliferation, Gene Expression, and Apoptosis

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Received: 9 March, 2025; Revised: 20 May, 2025; Accepted: 24 May, 2025

Abstract

Background: Royal jelly (RJ) exhibits diverse biological properties and may enhance the efficacy of chemotherapeutic drugs through synergistic effects, particularly in inhibiting cancer growth and mitigating drug-related side effects

Objectives: This study investigated the combined effects of cisplatin and RJ on the growth, proliferation, gene expression, and apoptosis of A549 lung cancer cells.

Methods: The A549 cells (ATCC CCL-185) were treated with cisplatin, RJ, or their combination at various concentrations for 24, 48, and 72 hours. IC₅₀ and EC₅₀ values were determined, and cell viability was assessed using the MTT assay after 48 hours. Expression of chromobox protein 3 homolog (CBX3) and mitochondrial DNA (MT-ND1) genes was quantified by real-time PCR, and apoptosis/necrosis was evaluated via flow cytometry.

Results: After 48 hours, cisplatin significantly reduced gene expression, while RJ increased it. The 1:1 combination treatment increased gene expression compared to cisplatin alone but decreased it relative to RJ alone. Apoptosis rates were 1.70% for cisplatin, 1.66% for RJ, and 6.61% for the combination, with apoptotic cell percentages of 54.2%, 11.9%, and 53.5%, respectively.

Conclusions: The combination of cisplatin and RJ significantly enhances apoptosis by approximately sixfold, suggesting a synergistic effect that may improve lung cancer treatment outcomes.

Keywords: CBX3 Gene, MT-ND1 Gene, MTT Assay, Real-time PCR, Flow Cytometry

1. Background

In 2023, the FDA approved cisplatin for the 45th time, underscoring its role as a cornerstone in cancer therapy. Widely used to treat early-stage ovarian cancer, non-small cell lung cancer (NSCLC), head and neck cancer, advanced bladder cancer, and testicular cancer, cisplatin achieves a 100% cure rate when cancer is detected early. Although its precise mechanism remains incompletely understood, cisplatin induces cancer cell death through four key processes: Cellular uptake, activation by aquation, DNA binding, and processing of DNA lesions. Notably, cisplatin causes significant mitochondrial DNA (MT-ND1) damage, triggering mitochondria-mediated apoptosis (1). Cisplatin binds to DNA, causing damage, and induces mitochondria-mediated apoptosis. The MT-

ND1 is greatly impacted by cisplatin (2). Cisplatin-sensitive cells contain higher mitochondrial content and elevated levels of mitochondrial reactive oxygen species (mtROS) compared to cisplatin-resistant cells. Increasing mtROS by inhibiting or destroying the ROS-protective uncoupling protein UCP2 enhances cisplatin-induced apoptosis (3).

To mitigate cisplatin's side effects, recent studies have proposed royal jelly (RJ) as an effective antioxidant capable of inhibiting free radicals. The RJ, produced and secreted by the hypopharynx and mandibular glands of worker honey bees (*Apis mellifera*), contains essential compounds such as free amino acids, proteins, sugars, fatty acids, minerals, and vitamins. These confer multiple biological activities, including vasodilation, blood pressure reduction, cholesterol lowering,

antimicrobial, anti-allergenic, anti-inflammatory, and antioxidant properties (4).

Chromobox protein 3 homolog/heterochromatin protein γ (CBX3/HP1 γ), a member of the evolutionarily conserved heterochromatin protein family, is a DNA-binding factor that regulates gene transcription. Overexpression of this gene promotes cancer cell proliferation and plays a central role in tumorigenesis. The CBX3 expression is a poor prognosis marker in human cancers, including glioblastoma multiforme (GBM), NSCLC, ovarian cancer, breast cancer, osteosarcoma, hepatocellular carcinoma, gastric cancer, pancreatic adenocarcinoma, and prostate cancer (5).

NADH dehydrogenase subunit 1, encoded by MT-ND1, is the largest subunit of respiratory complex I (6). Located in the inner mitochondrial membrane, it facilitates electron transfer and proton pumping. Variations in this gene are associated with diseases such as Alzheimer's disease, colorectal cancer, Leber optic atrophy, Leigh syndrome, mitochondrial encephalopathy, lactic acidosis, stroke-like episodes, diabetes, dystonia, and hypertrophic cardiomyopathy (7).

2. Objectives

This study aimed at investigating the effects of cisplatin, RJ, and their combination on the growth, proliferation, and apoptosis of A549 lung cancer cells, focusing on CBX3 and MT-ND1 gene expression.

3. Methods

3.1. Preparation of Cisplatin and Royal Jelly

Cisplatin, obtained in a 50 ml vial from Pars Daro Derman Trading Company, and RJ, supplied in a 10 g package from Moein Tajhiz Pajoh Company, were used. Treatment solutions were prepared at concentrations of 5, 10, 20, 30, 40, 50, 75, 100, 250, and 500 $\mu\text{g}/\text{mL}$ for both cisplatin and RJ. Cisplatin was dissolved in deionized distilled water to ensure biocompatibility with the A549 cell line and to avoid solvent-related cytotoxicity or interference with gene expression, which can occur with DMSO or DMF at certain concentrations. Solutions were freshly prepared to maintain stability and efficacy.

3.2. Cell Cultivation and Treatment

The adherent human lung carcinoma cell line A549 (ATCC CRM-CCL-185) was propagated in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and maintained at 37°C with 95%

relative humidity and 5% CO₂. For the MTT assay, 10⁴ cells were seeded into each well of a 96-well plate and allowed to adhere for 24 hours. Cells were treated with cisplatin, RJ, or a 1:1 combination at concentrations of 5, 10, 20, 30, 40, 50, 75, 100, 250, and 500 $\mu\text{g}/\text{mL}$ for 24, 48, or 72 hours. After treatment, the medium was replaced with 100 μL of fresh DMEM containing 10 μL of 5 mg/mL MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] solution (Sigma-Aldrich, USA). Plates were incubated for 4 hours at 37°C, allowing viable cells to reduce MTT to formazan crystals. The medium was removed, and 100 μL of DMSO was added to dissolve the formazan. Absorbance was measured at 570 nm using a microplate reader (BioTek, USA). Cell viability was calculated as a percentage relative to untreated controls, with three replicates per condition.

3.3. Cell Treatment and Gene Expression Examination

A549 cells (1×10^6 cells/well) were seeded in 6-well plates for 24 hours, followed by treatment with IC₅₀ and EC₅₀ concentrations for 48 hours. Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. To eliminate contamination of genomic DNA, RNA samples were treated with DNase I (Fermentas, Burlington, Canada). RNA quantity and integrity were assessed using a NanoDrop spectrophotometer (Thermo Scientific, USA) and agarose gel electrophoresis, ensuring an A260/A280 ratio of 1.8 - 2.0. cDNA was synthesized from 1000 ng of RNA using the RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, Burlington, Canada). Expression levels of B2M (housekeeping gene), CBX3, and MT-ND1 were quantified using SYBR Green-based qPCR in a 25 μL reaction containing 12.5 μL of master mix (Bioneer, Korea), 1 μL of cDNA, 1 μL of forward and reverse primers (10 μM , Table 1), and DEPC-treated water. Reactions were performed in triplicate on a Rotor-Gene system with the following cycling conditions: 95°C for 10 minutes, followed by 40 cycles of 95°C for 20 seconds, 57°C for 40 seconds, and 72°C for 40 seconds. No-template and minus-reverse-transcriptase controls were included to confirm the absence of DNA contamination.

Primers for CBX3 and B2M (Table 1) were designed using Primer3 software and validated for specificity using NCBI BLAST against the human RefSeq mRNA database. Primers specific to MT-ND1 (Table 1) were obtained from previously published studies (8, 9).

3.4. Detection of Apoptosis Using Annexin V-FITC Test

Table 1. Primer Sequence Used in the Study

Symbol	5' Sequence 3'
CBX3	
Forward primer	GAGATGCTGCTGACAAACCA
Reverse primer	TATTTGCCTCTTTGCCAGC
MT-ND1	
Forward primer	CCACTCTAGCCTAGCCGTTTAC
Reverse primer	GGTCATGATGGCAGGAGTAAT
β-2 microglobulin	
Forward primer	CCACTGAAAAAGATGAGTATGCCT
Reverse primer	CCAATCCAATGCGGCATCTCA

Abbreviations: CBX3, chromobox protein 3 homolog, MT-ND1, mitochondrial DNA.

A549 cells (1×10^5 cells/well) were seeded in 6-well plates and cultured with IC₅₀ and EC₅₀ concentrations for 48 hours. Untreated controls were included. After the treatment, cells were washed twice with PBS. Apoptosis and necrosis were assessed using an Annexin V-FITC kit (Roch, Penzberg, Germany). The instructions were followed carefully. Flow cytometry from Biocompare, San Francisco, and USA was used for analysis and data were processed using Flow Jo software.

3.5. Statistical Analysis

Data were analyzed using GraphPad Prism 9 software and one-way ANOVA. Gene expression levels in treated and untreated cells were compared with Tukey's HSD post-hoc test used for pairwise comparisons. Results were reported as mean \pm standard deviation (SD) from three replicates. A P-value < 0.05 was considered statistically significant.

4. Results

4.1. Cell Viability

The cytotoxic and anti-proliferative effects of cisplatin and RJ are shown in [Figure 1](#). Cisplatin's effects on cell growth were studied after 24, 48, and 72 hours, as were those of RJ. Cisplatin's concentration and treatment duration influenced cell growth inhibition. After 24 hours with a concentration of 10 μ g/mL, and 48 and 72 hours with a minimum of 5 μ g/mL, cell viability significantly decreased compared to the control group.

IC₅₀ values for cisplatin were 72.89, 10.06, and 1.68 μ g/mL at 24, 48, and 72 hours, respectively. The RJ increased cell proliferation at 50, 40, and 30 μ g/mL, with significant growth compared to controls. EC₅₀ values for RJ were 557.23, 489.03, and 173.92 μ g/mL at 24, 48, and 72

hours, respectively. The 1:1 combination treatment significantly reduced cell viability, with IC₅₀ values of 66.75 μ g/mL at 24 hours, 30.83 μ g/mL at 48 hours, and 6.85 μ g/mL at 72 hours.

4.2. Expression of Chromobox Protein 3 Homolog and Mitochondrial DNA Genes After 48 Hours

Cells were treated with 10 μ g/mL cisplatin (IC₅₀), 489 μ g/mL RJ (EC₅₀), or a 1:1 combination for 48 hours. RNA was extracted, cDNA synthesized, and gene expression analyzed via qPCR. Cisplatin significantly reduced CBX3 and MT-ND1 expression ($P < 0.05$), while RJ increased expression compared to controls ($P < 0.05$), consistent with MTT assay results. The combination treatment increased gene expression compared to cisplatin alone but decreased it compared to RJ ($P < 0.05$). Results are shown in [Figure 2](#).

4.3. Apoptosis Induction

Apoptosis in A549 cells was assessed after 48 hours of treatment with cisplatin, RJ, or their 1:1 combination at IC₅₀ and EC₅₀ concentrations. Flow cytometry showed 79.7% healthy cells in the control group, compared to 18.3% for cisplatin, 73.4% for RJ, and 25.5% for the combination. Statistically significant differences were observed for cisplatin ($P = 0.0008$), RJ ($P = 0.0247$), and the combination ($P = 0.0016$) compared to controls. Gene expression patterns and flow cytometry results were consistent, confirming the reliability of the treatments' effects ([Figure 3](#)).

5. Discussion

Cancer, a leading cause of death worldwide, is commonly treated with radiotherapy, surgery, and chemotherapy, all of which can cause side effects. A

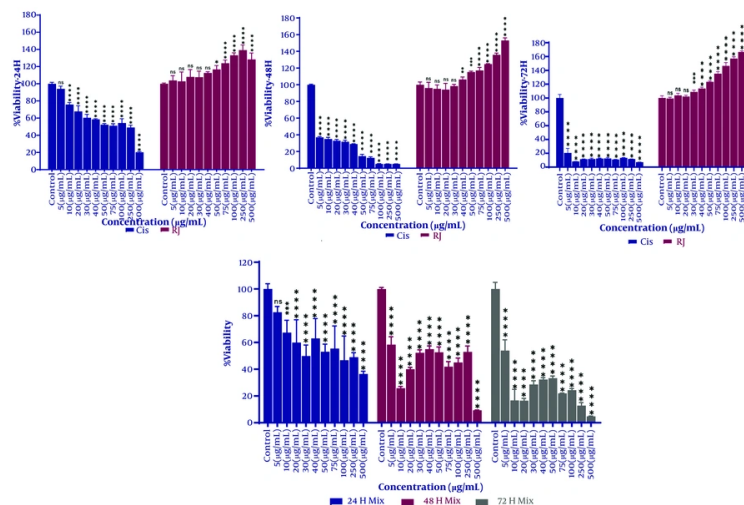


Figure 1. Cell viability of A549 cells after treatment with cisplatin, royal jelly (RJ), or a 1:1 combination at 24, 48, and 72 hours. The X-axis represents treatment concentrations (µg/mL), and the Y-axis shows cell viability (% of control). Statistical significance compared to the control group is indicated by * P < 0.05, ** P < 0.01, or *** P < 0.001.

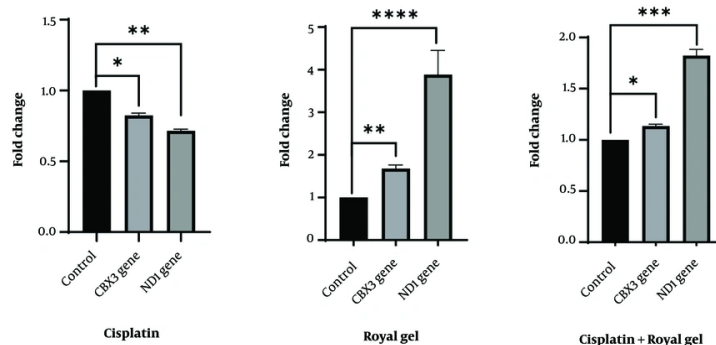


Figure 2. Expression of chromobox protein 3 homolog (CBX3) and mitochondrial DNA (MT-ND1) genes in A549 cells treated with cisplatin, royal jelly (RJ), or a 1:1 combination for 48 hours. The X-axis represents treatment conditions, and the Y-axis shows relative gene expression (fold change). Cisplatin decreased CBX3 (P = 0.034) and MT-ND1 (P = 0.006) expression, while RJ increased CBX3 (P = 0.009) and MT-ND1 (P < 0.0001). The combination increased expression compared to cisplatin (CBX3: P = 0.046; MT-ND1: P = 0.0007) but decreased it compared to RJ. Significance is indicated by * P < 0.05, ** P < 0.01, or *** P < 0.001, **** P < 0.0001.

healthy lifestyle, particularly a proper diet, is crucial for cancer prevention. (10). Combining plant-derived compounds with chemotherapy can enhance efficacy while reducing toxicity to healthy tissues. The RJ, with its potent antioxidant properties and ability to inhibit free radicals, has garnered significant attention (2). Despite ongoing research into natural products combined with anti-cancer drugs, definitive results remain elusive.

The RJ contains numerous bioactive compounds and exhibits various biological and pharmacological properties. Due to its low toxicity compared to other bee products (10), this study compared its effects with cisplatin on A549 cell viability, CBX3 and MT-ND1 gene expression, and apoptosis/necrosis induction

Cisplatin alone significantly reduced cell growth, while RJ increased proliferation at specific concentrations (50 µg/mL at 24 hours, 40 µg/mL at 48 hours, and 30 µg/mL at 72 hours) with statistically

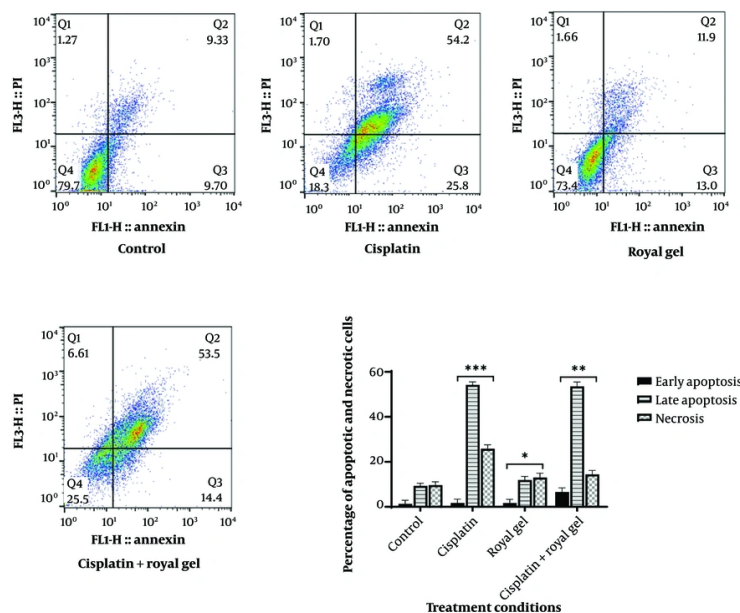


Figure 3. Flow cytometry analysis of A549 cells treated with cisplatin, royal jelly (RJ), or a 1:1 combination for 48 hours. The X-axis represents treatment conditions (control, cisplatin, RJ, combination), and the Y-axis shows the percentage of cells in each state (healthy, apoptotic, necrotic). The apoptosis rate was 54.2% for cisplatin, 11.9% for RJ, and 53.5% for the combination, with necrosis rates of 25.8%, 13%, and 14.4%, respectively; * P < 0.05, ** P < 0.01, or *** P < 0.001.

significant differences compared to controls. In a study by Salazar-Olivo and Paz-Gonzalez, extracted crude protein from RJ increased the population doubling rate of Tn-5B1-4 insect cells by 6.5 times per milligram of protein, compared to 2.55 times for fetal bovine serum, suggesting that RJ’s bioactive compounds promote cell growth, differentiation, and survival in various cell types (11).

The compound 10-hydroxy-2-decenoic acid, structurally similar to mammalian estrogen, may contribute to RJ’s proliferative effects and reduce side effects of cancer treatment (12). This fatty acid exhibits anti-cancer, anti-inflammatory, immune-modulating, and antimicrobial properties (13). Although some studies have suggested estrogenic activity (14-16), Ishida et al. found that RJ does not activate ER α or ER β , indicating that its estrogen-like effects are not mediated through these receptors (17).

The 1:1 combination of cisplatin and RJ significantly reduced cell growth, mirroring cisplatin’s effects, indicating RJ’s cytotoxic potential. Similar results were observed in bladder cancer cells, where RJ reduced viability, migration, and matrix metalloproteinase 9 (MMP-9) expression and protein levels in HTB 5637 cells (18).

Overexpression of CBX3 is linked to cancer progression (19, 20). Niu et al. found correlations between CBX3 expression and survival prognosis, DNA methylation, protein phosphorylation, and immune cell infiltration, suggesting CBX3 as a biomarker and therapeutic target (19). MT-ND1, encoding the largest subunit of mitochondrial complex I, is critical for oxidative phosphorylation. Its mutations make it a significant cancer biomarker (21).

Flow cytometry showed apoptosis rates of 1.17% for cisplatin, 1.66% for RJ, and 6.61% for the combination, with 53.5% of combination-treated cells in late apoptosis and 14.4% in necrosis. The combination reduced viable cells to 25.5%, compared to 18.3% for the cisplatin treatment alone.

In line with the present study, Icariside II (IS) combined with cisplatin inhibits cell proliferation and induces apoptosis in NSCLC without significant toxicity. The IS enhances cisplatin-induced apoptosis partly by promoting endoplasmic reticulum (ER) stress signaling (22).

The RJ proteins, including RJG-1 (a glucosylceramidase) and RJG-2 (major RJ protein 1), play key roles in cell death; RJG-1 disrupts cell membrane glucosylceramide, causing necrosis (23). Additionally, RJ

induces programmed cell death in acute lymphoblastic leukemia (ALL) and nervous system cancers (24). In neuroblastoma and glioblastoma cells, increased apoptosis is linked to changes in macromolecular composition, protein structure, and phosphorylation (25).

This study was limited to RJ's effects on cancer cell viability, gene expression, and apoptosis. Further research is needed on the antioxidant and anti-inflammatory roles of its combination with cisplatin and the molecular mechanisms behind the increased apoptosis and related immune responses.

5.1. Conclusions

Cisplatin's side effects, including excessive free radical production, can damage healthy tissues. The RJ, a potent free radical scavenger, may mitigate these effects. This study demonstrates that combining cisplatin with RJ reduces A549 cell viability comparably to cisplatin alone while inducing a sixfold increase in apoptosis. These findings suggest that RJ could enhance cisplatin's efficacy in lung cancer treatment. Further clinical studies are warranted to explore the therapeutic potential of this combination and its impact on immune responses.

Acknowledgements

We express gratitude to the organizations and individuals whose invaluable support facilitated the completion of this research project.

Footnotes

Authors' Contribution: Study concept and design: Z. T. F.; Acquisition of data: Z. T. F. and F. A.; Analysis and interpretation of data: Z. T. F., M. H., and F. A.; Drafting of the manuscript: Z. T. F.; Critical revision of the manuscript for important intellectual content: Z. T. F.; Statistical analysis: Z. T. F.; Administrative, technical, and material support: F. A., M. H., and Z. T. F.; Study supervision: Z. T. F.

Conflict of Interests Statement: The authors declare no conflict of interest.

Data Availability: The dataset presented in the study is available on request from the corresponding author during submission or after publication.

Ethical Approval: The study received ethics code IR.IAU.PS.REC.1401.138 from the Research Ethics

Committees of Pharmacy and Pharmaceutical Branches Faculty, Islamic Azad Tehran Medical Sciences University.

Funding/Support: All financial and material support for the research was provided by F. A. (Master of Science).

References

- Gandin V, Hoeschele JD, Margiotta N. Special Issue "Cisplatin in Cancer Therapy: Molecular Mechanisms of Action 3.0". *Int J Mol Sci.* 2023;**24**(9). [PubMed ID: 37175624]. [PubMed Central ID: PMC10178266]. <https://doi.org/10.3390/ijms24097917>.
- Podratz JL, Knight AM, Ta LE, Staff NP, Gass JM, Genelin K, et al. Cisplatin induced mitochondrial DNA damage in dorsal root ganglion neurons. *Neurobiol Dis.* 2011;**41**(3):661-8. [PubMed ID: 21145397]. [PubMed Central ID: PMC3031677]. <https://doi.org/10.1016/j.nbd.2010.11.017>.
- Kleih M, Bopple K, Dong M, Gaissler A, Heine S, Olayioye MA, et al. Direct impact of cisplatin on mitochondria induces ROS production that dictates cell fate of ovarian cancer cells. *Cell Death Dis.* 2019;**10**(11):851. [PubMed ID: 31699970]. [PubMed Central ID: PMC6838053]. <https://doi.org/10.1038/s41419-019-2081-4>.
- Karadeniz A, Simsek N, Karakus E, Yildirim S, Kara A, Can I, et al. Royal jelly modulates oxidative stress and apoptosis in liver and kidneys of rats treated with cisplatin. *Oxid Med Cell Longev.* 2011;**2011**:981793. [PubMed ID: 21904651]. [PubMed Central ID: PMC3166579]. <https://doi.org/10.1155/2011/981793>.
- Bosso G, Cipressa F, Tullo L, Cenci G. Co-amplification of CBX3 with EGFR or RAC1 in human cancers corroborated by a conserved genetic interaction among the genes. *Cell Death Discov.* 2023;**9**(1):317. [PubMed ID: 37633946]. [PubMed Central ID: PMC10460438]. <https://doi.org/10.1038/s41420-023-01598-5>.
- Xu YC, Su J, Zhou JJ, Yuan Q, Han JS. Roles of MT-ND1 in Cancer. *Curr Med Sci.* 2023;**43**(5):869-78. [PubMed ID: 37642864]. <https://doi.org/10.1007/s11596-023-2771-0>.
- Lou X, Zhou Y, Liu Z, Xie Y, Zhang L, Zhao S, et al. De novo frameshift variant in MT-ND1 causes a mitochondrial complex I deficiency associated with MELAS syndrome. *Gene.* 2023;**860**:147229. [PubMed ID: 36717040]. <https://doi.org/10.1016/j.gene.2023.147229>.
- Bragoszewski P, Kupryjanczyk J, Bartnik E, Rachinger A, Ostrowski J. Limited clinical relevance of mitochondrial DNA mutation and gene expression analyses in ovarian cancer. *BMC Cancer.* 2008;**8**:292. [PubMed ID: 18842121]. [PubMed Central ID: PMC2571110]. <https://doi.org/10.1186/1471-2407-8-292>.
- Wallace L, Mehrabi S, Bacanamwo M, Yao X, Aikhionbare FO. Expression of mitochondrial genes MT-ND1, MT-ND6, MT-CYB, MT-COI, MT-ATP6, and 12S/MT-RNR1 in colorectal adenopolyps. *Tumour Biol.* 2016;**37**(9):12465-75. [PubMed ID: 27333991]. [PubMed Central ID: PMC5661973]. <https://doi.org/10.1007/s13277-016-5101-3>.
- Salama S, Shou Q, Abd El-Wahed AA, Elias N, Xiao J, Swillam A, et al. Royal Jelly: Beneficial Properties and Synergistic Effects with Chemotherapeutic Drugs with Particular Emphasis in Anticancer Strategies. *Nutrients.* 2022;**14**(19). [PubMed ID: 36235818]. [PubMed Central ID: PMC9573021]. <https://doi.org/10.3390/nu14194166>.
- Salazar-Olivo LA, Paz-Gonzalez V. Screening of biological activities present in honeybee (*Apis mellifera*) royal jelly. *Toxicol In Vitro.* 2005;**19**(5):645-51. [PubMed ID: 15893447]. <https://doi.org/10.1016/j.tiv.2005.03.001>.
- Aavani F, Rahimi R, Goleij P, Rezaeizadeh H, Bahramsoltani R. Royal jelly and its hormonal effects in breast cancer: a literature review. *Daru.* 2024;**32**(2):745-60. [PubMed ID: 38717683]. [PubMed Central ID: PMC11555035]. <https://doi.org/10.1007/s40199-024-00513-2>.

13. Albalawi AE, Althobaiti NA, Alrdahe SS, Alhasani RH, Alaryani FS, BinMowyna MN. Anti-Tumor Effects of Queen Bee Acid (10-Hydroxy-2-Decenoic Acid) Alone and in Combination with Cyclophosphamide and Its Cellular Mechanisms against Ehrlich Solid Tumor in Mice. *Molecules*. 2021;**26**(22). [PubMed ID: 34834112]. [PubMed Central ID: PMC8617861]. <https://doi.org/10.3390/molecules26227021>.
14. Hanai R, Matsushita H, Minami A, Abe Y, Tachibana R, Watanabe K, et al. Effects of 10-Hydroxy-2-decenoic Acid and 10-Hydroxydecanoic Acid in Royal Jelly on Bone Metabolism in Ovariectomized Rats: A Pilot Study. *J Clin Med*. 2023;**12**(16). [PubMed ID: 37629354]. [PubMed Central ID: PMC10456009]. <https://doi.org/10.3390/jcm12165309>.
15. Suzuki KM, Isohama Y, Maruyama H, Yamada Y, Narita Y, Ohta S, et al. Estrogenic activities of Fatty acids and a sterol isolated from royal jelly. *Evid Based Complement Alternat Med*. 2008;**5**(3):295-302. [PubMed ID: 18830443]. [PubMed Central ID: PMC2529378]. <https://doi.org/10.1093/ecam/nem036>.
16. Balan A, Moga MA, Dima L, Toma S, Elena Neculau A, Anastasiu CV. Royal Jelly-A Traditional and Natural Remedy for Postmenopausal Symptoms and Aging-Related Pathologies. *Molecules*. 2020;**25**(14). [PubMed ID: 32698461]. [PubMed Central ID: PMC7397171]. <https://doi.org/10.3390/molecules25143291>.
17. Ishida K, Matsumaru D, Shimizu S, Hiromori Y, Nagase H, Nakanishi T. Evaluation of the Estrogenic Action Potential of Royal Jelly by Genomic Signaling Pathway in Vitro and in Vivo. *Biol Pharm Bull*. 2022;**45**(10):1510-7. [PubMed ID: 35922913]. <https://doi.org/10.1248/bpb.b22-00383>.
18. Fazili N, Soheili ZS, Malekzadeh-Shafaroudi S, Samiei S, D.Alipoor S, Moshtaghi N. [Royal Jelly Decreases MMP-9 Expression and Induces Apoptosis in Human Bladder Cancer 5637 Cells]. *J Cell Molecular Res*. 2021;**13**(1):36-43. FA. <https://doi.org/10.22067/jcmr.2021.68523.1000>.
19. Niu H, Chen P, Fan L, Sun B. Comprehensive pan-cancer analysis on CBX3 as a prognostic and immunological biomarker. *BMC Med Genomics*. 2022;**15**(1):29. [PubMed ID: 35172803]. [PubMed Central ID: PMC8851738]. <https://doi.org/10.1186/s12920-022-01179-y>.
20. Peng W, Shi S, Zhong J, Liang H, Hou J, Hu X, et al. CBX3 accelerates the malignant progression of glioblastoma multiforme by stabilizing EGFR expression. *Oncogene*. 2022;**41**(22):3051-63. [PubMed ID: 35459780]. <https://doi.org/10.1038/s41388-022-02296-9>.
21. Xu Y, Zhou J, Yuan Q, Su J, Li Q, Lu X, et al. Quantitative detection of circulating MT-ND1 as a potential biomarker for colorectal cancer. *Bosnian Journal of Basic Medical Sciences*. 2021. <https://doi.org/10.17305/bjbms.2021.5576>.
22. Tang Z, Du W, Xu F, Sun X, Chen W, Cui J, et al. Icariside II enhances cisplatin-induced apoptosis by promoting endoplasmic reticulum stress signalling in non-small cell lung cancer cells. *Int J Biol Sci*. 2022;**18**(5):2060-74. [PubMed ID: 35342361]. [PubMed Central ID: PMC8935239]. <https://doi.org/10.7150/ijbs.66630>.
23. Yuan Y, Wang W, Fan R, Jiang J, Feng S, Yin H, et al. Ethanol-soluble proteins from the royal jelly of Xinjiang black bees. *Protein Sci*. 2021;**30**(2):291-6. [PubMed ID: 33131155]. [PubMed Central ID: PMC7784752]. <https://doi.org/10.1002/pro.3985>.
24. Yazdanparast S, Bashash D, Bahrami AN, Ghorbani M, Izadirad M, Bakhtiyaridovvombaygi M, et al. Royal jelly induces ROS-mediated apoptosis in acute lymphoblastic leukemia (ALL)-derived Nalm-6 cells: Shedding light on novel therapeutic approaches for ALL. *Iranian journal of basic medical sciences*. 2024;**27**(7):801.
25. Simsek Ozek N. Exploring the in vitro potential of royal jelly against glioblastoma and neuroblastoma: impact on cell proliferation, apoptosis, cell cycle, and the biomolecular content. *Analyst*. 2024;**149**(6):1872-84. [PubMed ID: 38349213]. <https://doi.org/10.1039/d3an01840g>.