Physical Activity and Hormones (J Physic Act Horm)
Vol. 6 Spring 2025

Swimming training and royal jelly effects on caspase-3 expression in lung cancer mice

Farjam Rashedi[1]
Department of Physical Education and Sports Medicine, Sh.C., Islamic Azad University, Shiraz, Iran

Elaheh Heidarnia[2]
Department of Physical Education and Sports Medicine, Sh.C., Islamic Azad University, Shiraz, Iran

Seyed Ali Hosseini[3]
Department of Physical Education and Sports Medicine, Marv.C., Islamic Azad University, Marvdasht, Iran

ABSTRACT

Introduction: Caspase-3 (CASP3) is a key regulator of apoptosis and plays a critical role in tumor growth and metastasis in lung cancer. This study aimed to investigate the effects of swimming training (ST) and royal jelly (RJ) consumption on CASP3 gene expression in lung tissue using a murine lung cancer model.

Material & Methods: In this experimental study, 24 male Balb/c mice (aged 8 months; weight: 18–22 g) were divided into healthy control, sham, and cancer-induced groups. Lung cancer was induced via benzo[a]pyrene (B[a]P) injection (100 mg/kg). The intervention groups received either ST (12 weeks, 3 sessions/week), RJ (50 or 100 mg/kg), or a combination of both. After the intervention period, lung tissue was dissected, and CASP3 gene expression was measured using real-time PCR. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test.

Results: B[a]P-induced cancer led to a significant decrease in CASP3 expression compared to the healthy control and sham groups ($P < 0.05$). However, the combination of ST and RJ significantly increased CASP3 expression compared to the B[a]P group and even the groups receiving individual interventions ($P < 0.05$).

Conclusion: The findings suggest that ST and RJ, particularly when combined, exert anticancer effects by restoring apoptosis suppression caused by B[a]P.

Keywords: Lung cancer, Apoptosis, Caspase-3, Exercise training, Royal jelly.

***Correspondence:** Heidarnia.elaheh@iau.ac.ir
Received: Feb 2025; **Revised:** Mar 2025; **Accepted:** Apr 2025.

DOI: ----

1. Introduction

Lung cancer is a type of cancer characterized by the uncontrolled growth of lung cells, leading to tumor formation. The disease can invade nearby tissues or metastasize to other parts of the body. It ranks among the most common and deadly cancers worldwide (1, 2). Although lung cancer is the second most frequently diagnosed cancer in both men and women, it remains the leading cause of cancer-related mortality. In 2020, it caused more deaths than breast, colorectal, and prostate cancers combined (1). Additionally, lung cancer is a major contributor to reduced life expectancy in affected patients (3). Despite declining mortality rates over the decades, it persists as the primary cause of cancer deaths (1).

Advances in oncology, including the development of cytotoxic agents, immunotherapy, and targeted therapies, have significantly improved cancer management (4). Benzo[a]pyrene (B[a]P), a polycyclic aromatic hydrocarbon, is a potent carcinogen present in cigarette smoke, tobacco, fossil fuel emissions, bitumen, asphalt, and industrial environments. It has been strongly linked to lung cancer and other malignancies (5, 6). Beyond promoting cancer stem cell proliferation, B[a]P exhibits immunosuppressive effects in lung cancer (2) and is commonly used in animal studies to induce the disease (6). A major challenge in cancer treatment is the development of drug resistance (4), compounded by the side effects and tumor resistance associated with chemotherapy (7). Thus, identifying effective therapeutic strategies remains a critical research priority.

Cancer pathogenesis often involves dysregulation of cell death mechanisms, including autophagy, necrosis, and apoptosis—the most tightly regulated form of programmed cell death. Apoptosis is marked by irreversible morphological and biochemical changes, such as phosphatidylserine externalization and DNA fragmentation, mediated by intrinsic (mitochondrial-dependent) and extrinsic (death receptor-dependent) pathways (8). As the predominant form of programmed cell death in vertebrates, apoptosis plays a pivotal role in suppressing tumor metastasis and is considered a key regulatory mechanism in cancer progression (9). Caspases, a family of cysteine protease enzymes, execute apoptosis in their activated form. Among them, caspase-3 (CASP3), an executioner caspase, is central to apoptotic processes and has emerged as a prime therapeutic target in cancer treatment (10). Modulating apoptosis through chemotherapy (8) or lifestyle interventions (11) represents a promising approach to cancer control.

Exercise has been proposed as a valuable adjunct to cancer therapy (12). The American Society of Clinical Oncology now recommends preoperative exercise for lung cancer patients (13). The mechanisms underlying exercise's anti-tumor effects are multifaceted, involving direct impacts on tumor growth/metastasis and systemic benefits (e.g., enhanced immune/metabolic function, reduced inflammation) as well as localized improvements (e.g., tumor vascularity and blood flow) (12, 14). Notably, exercise can regulate apoptosis in cancerous tissues, with evidence suggesting that regular physical activity induces apoptosis and suppresses tumor growth through diverse pathways (11, 15).

Dietary factors, such as royal jelly (RJ), may also play a role in cancer prevention and treatment. Produced by the hypopharyngeal and mandibular glands of nurse honeybees, RJ exhibits antioxidant, anticancer, anti-inflammatory, and antibacterial properties (16, 17). For instance, studies report that RJ enhances apoptosis in human hepatoma cell lines (18).

Despite the proposed benefits of exercise and antioxidant supplementation (e.g., RJ) in cancer management, their effects on lung cancer remain understudied. Moreover, CASP3, a critical regulator of apoptosis, is dysregulated in cancer, altering apoptotic rates in both tumor and healthy tissues. Investigating how therapeutic interventions influence CASP3 is therefore essential.

Given these gaps, the present study aimed to evaluate the effects of swimming training (ST) and RJ consumption on CASP3 levels in lung tissue using a murine cancer model.

2. Methodology

2.1. Materials and methods

This experimental study employed a post-test design.

2.2. Participants

This study involving 24 male Balb/c mice (average age: 8 months; weight: 18–22 g), including 3 healthy controls and 21 lung cancer model mice.

2.3. Measurements

Cancer Induction: Benzo[a]pyrene (B[a]P) (Sigma-Aldrich, Germany) was dissolved in olive oil and administered intraperitoneally (100 mg/kg) to fasting mice.

To confirm successful cancer induction, a pilot study was conducted: 8 mice (4 B[a]P-injected and 4 healthy controls) were euthanized after 4 weeks, and lung tissues were analyzed for tumorigenesis via pathological examination.

Following validation, 40 additional mice received B[a]P (6). The lung cancer models were then allocated into seven groups: B[a]P (cancer control), B[a]P + RJ (50 mg/kg), B[a]P + RJ (100 mg/kg), B[a]P + ST, B[a]P + ST + RJ (50 mg/kg), B[a]P + ST + RJ (100 mg/kg), B[a]P + RJ solvent (Sham).

2.4. Intervention

2.4.1 Exercise Protocol (Swimming Training, ST)

Conducted in a specialized animal pool (110 cm wide; water temperature: 32°C). Familiarization phase (10 days): Mice were acclimated to the aquatic environment. Main protocol (12 weeks, 3 sessions/week): Weeks 1–10: Gradual increase from 15 to 30 min/session. Weeks 11–12: Duration extended to 40 min/session. Mice carried a 2% body weight load attached to their tails during ST (19, 20).

2.4.2 Royal Jelly (RJ) Administration

Preparation: 10 g RJ was mixed with 1000 mL deionized distilled water, incubated (50°C, 16 h), filtered, and stored at 4°C. Dosage: Administered intraperitoneally at 50 or 100 mg/kg/day (21).

2.4.3 Tissue Collection and Analysis

Euthanasia: 48 hours post-last training session, mice were anesthetized (ketamine at a dose of 25 mg/kg and xylazine at a dose of 65 mg/kg, which is manufactured by Alfasan, Netherlands), and lung tissues were excised and stored at −80°C. RNA Extraction: Total RNA was isolated using the FavorPrep™ Tissue Total RNA Kit (FATRK 001, Taiwan). Gene Expression: CASP3 mRNA levels were quantified via real-time PCR and calculated using the ΔΔCt method.

2.5. Statistical Methods

Data were analyzed by one-way ANOVA followed by LSD post hoc tests (SPSS v26). Also, the significance level of all tests was considered to be 0.05 or less ($P \leq 0.05$).

3. Results

The result of comparison of caspase 3 levels between research groups in Table 1.

Table 1. *The result of comparison of caspase 3 levels between research groups*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.258	7	0.608	6.733	0.001
Within Groups	1.445	16	0.090		
Total	5.703	23			

According to the results of one-way analysis of variance (Table 1), a significant difference was observed between the levels of CASP3 between the research groups. In order to find the location of the difference, the LSD post hoc test was used (Figure 1). According to the results of the LSD post hoc test, the level of CASP3 in the B[a]P group was significantly lower than that in the healthy control and sham groups ($P = 0.013$; $P = 0.018$, respectively). The level of CASP3 in the B[a]P + ST + RJ (50 mg/kg) group was significantly higher than that in the B[a]P and B[a]P + ST groups ($P = 0.002$; $P = 0.027$, respectively). The level of CASP3 in the B[a]P + ST + RJ (100 mg/kg) group was significantly higher than the healthy control ($P=0.007$), sham ($P=0.005$), B[a]P ($P<0.001$), B[a]P + RJ (50 mg/kg) ($P<0.001$), B[a]P + RJ (100 mg/kg) ($P=0.002$), B[a]P + ST ($P<0.001$) and B[a]P + ST + RJ (50 mg/kg) groups.

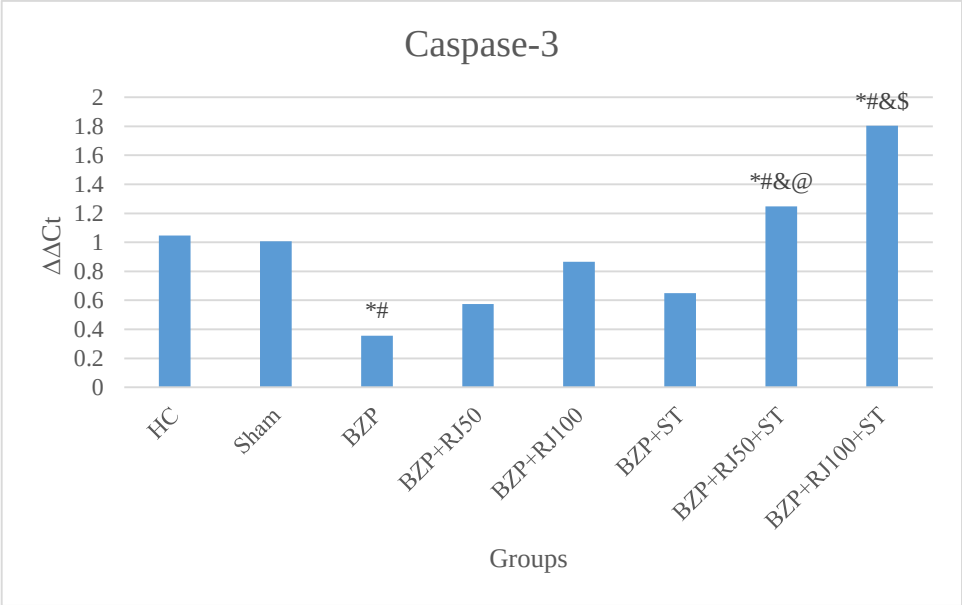

Figure 1. *Comparison of caspase-3 gene expression between research groups*

4. Discussion

The aim of the present study was to investigate the interactive effect of ST and consumption of RJ at different doses on CASP3 gene expression in the lungs of cancer model mice. The results showed that cancer induction with B[a]P caused a decrease in CASP3 gene expression in lung tissue. Cancer is characterized by the uncontrolled growth of normal cells due to dysregulated mechanisms in rapidly dividing cells (8). The reduction in CASP3 gene expression is one of the mechanisms by which B[a]P suppresses apoptosis and promotes tumor formation in lung tissue. In this regard, Chen et al. reported that B[a]P contributes to tumor progression in lung cancer by inhibiting apoptosis (22), which aligns with the findings of the present study. Thus, it can be inferred that cancer induction led to decreased CASP3 gene expression, likely due to diminished apoptotic activity, as this enzyme plays a central role in the execution pathway of programmed cell death. CASP3 is a key mediator of apoptosis, inducing cell death through the cleavage of critical cellular proteins (e.g., PARP, lamins, and other substrates) (23).

Regarding the effect of interventions on CASP3 gene expression, the results indicated that ST induced a non-significant increase in CASP3 expression compared to the B[a]P group, with no significant difference relative to the control and sham groups. The anticancer effects of exercise training may stem from multiple independent mechanisms that hinder cancer progression (24). The protective role of exercise in carcinogenesis is likely multifactorial, involving enhanced immune function, reduced chronic inflammation (both of which are known to suppress carcinogenesis), activation of DNA repair enzymes, and mitigation of oxidative stress via exercise-induced antioxidants. Hijin et al. also reported that tumors in exercising mice exhibited increased apoptosis, accompanied by elevated levels of active CASP3 (11), which is consistent with our findings. Exercise influences tumor cell apoptosis through complex mechanisms, including endocrine modulation, signaling pathway activation, tumor microenvironment improvement, and regulation of apoptosis-related genes (25).

In examining the effect of RJ consumption on CASP3, the results revealed non-significant changes compared to the control and sham groups. Alnomasi et al. reported that RJ at the IC50 concentration significantly increased CASP3 expression in apoptotic and necrotic hepatoma cells (18), supporting our observations. Osmaz et al. also found that RJ at a dose of 50 mg/ml inhibited cancer cell proliferation and induced apoptosis in ovarian cancer cells (26). Compelling evidence suggests that RJ enhances antioxidant capacity, thereby counteracting cancer-induced oxidative stress (17). In line with this, Aslan et al. demonstrated in a study on fluoride-damaged mouse lung tissue that RJ upregulates GSH and CAT activity, along with the expression of Bax, BDNF, CASP3, caspase-6, and caspase-9 proteins (27).

A notable finding in this study was the significant increase in CASP3 expression in the combined ST and RJ group compared to the B[a]P group, with levels even surpassing those in the exercise-only and RJ-only groups. These results suggest that the combination of exercise and RJ synergistically enhances apoptosis in cancerous lung tissue. Previous studies have reported that the interaction between exercise and RJ amplifies antioxidant defense and reduces lipid peroxidation in healthy active individuals (28). Given that cancer is an inflammatory disease (29) and chronic inflammation within the tumor microenvironment drives cancer initiation, progression, metastasis, and therapy resistance (30), reducing oxidative stress and inflammation is a key therapeutic target in

lung cancer. Although ST and RJ alone partially mitigated the B[a]P /induced suppression of CASP3, the combined intervention effectively reversed this effect.

5. Conclusion

The findings of this study demonstrate that ST and RJ interventions increase CASP3 gene expression, thereby promoting apoptosis in cancerous lung tissue. The combination of ST and RJ, particularly at higher doses, counteracts the effects of cancer by upregulating CASP3, underscoring their potential as anticancer therapies.

6. Acknowledgment

The researchers hereby thank and appreciate all those who contributed to this research.

Conflict of interests: The authors declare that there is no conflict of interest regarding the publication of this manuscript.

References

1. Kratzer TB, Bandi P, Freedman ND, Smith RA, Travis WD, Jemal A, Siegel RL. Lung cancer statistics, 2023. Cancer. 2024;130(8):1330-48. [https://doi.org/10.1002/cncr.35127]
2. Salem ML, El-Ashmawy NE, Abd El-Fattah EE, Khedr EG. Immunosuppressive role of Benzo [a] pyrene in induction of lung cancer in mice. Chemico-biological interactions. 2021;333:109330. [https://doi.org/10.1016/j.cbi.2020.109330]
3. Wolf AM, Oeffinger KC, Shih TYC, Walter LC, Church TR, Fontham ET, et al. Screening for lung cancer: 2023 guideline update from the American Cancer Society. CA: A Cancer Journal for Clinicians. 2024;74(1):50-81. [https://doi.org/10.3322/caac.21875]
4. Wei J-R, Lu M-Y, Wei T-H, Fleishman JS, Yu H, Chen X-L, et al. Overcoming cancer therapy resistance: From drug innovation to therapeutics. Drug Resistance Updates. 2025:101229. [https://doi.org/10.1016/j.drup.2024.101229]
5. Bukowska B, Mokra K, Michałowicz J. Benzo [a] pyrene—Environmental occurrence, human exposure, and mechanisms of toxicity. International journal of molecular sciences. 2022;23(11):6348. [https://doi.org/10.3390/ijms23116348]
6. Abekova A, Islamov R, editors. ChemoProtective effects of new iodine coordinated compound in benzo [a] pyrene-induced lung cancer in BALB/c mice. BIO Web of Conferences; 2024: EDP Sciences. [https://doi.org/10.1051/bioconf/202427401014]
7. Li Y, Wang Y, Yu X, Yu T, Zheng X, Chu Q. Radix tetrastigma inhibits the non-small cell lung cancer via Bax/Bcl-2/Caspase-9/Caspase-3 pathway. Nutrition and Cancer. 2022;74(1):320-32. [https://doi.org/10.1080/01635581.2021.1918410]
8. Chaudhry G-e-S, Akim AM, Sung YY, Muhammad TST. Cancer and apoptosis. Apoptosis and cancer: methods and protocols: Springer; 2022. p. 191-210.
9. Tan Z, Xue H, Sun Y, Zhang C, Song Y, Qi Y. The role of tumor inflammatory microenvironment in lung cancer. Frontiers in pharmacology. 2021;12:688625. [https://doi.org/10.3389/fphar.2021.688625]
10. Yadav P, Yadav R, Jain S, Vaidya A. Caspase-3: A primary target for natural and synthetic compounds for cancer therapy. Chemical biology & drug design. 2021;98(1):144-65. [https://doi.org/10.1111/cbdd.13813]
11. Higgins KA, Park D, Lee GY, Curran WJ, Deng X. Exercise-induced lung cancer regression: Mechanistic findings from a mouse model. Cancer. 2014;120(21):3302-10. [https://doi.org/10.1002/cncr.28889]
12. Yang L, Morielli AR, Heer E, Kirkham AA, Cheung WY, Usmani N, et al. Effects of exercise on cancer treatment efficacy: a systematic review of preclinical and clinical studies. Cancer research. 2021;81(19):4889-95. [https://doi.org/10.1158/0008-5472.CAN-20-3226]
13. Mancini A, Orlandella FM, Vitucci D, Luciano N, Alfieri A, Orrù S, et al. Exercise's impact on lung cancer molecular mechanisms: a current overview. Frontiers in Oncology. 2024;14:1479454. [https://doi.org/10.3389/fonc.2024.1479454]
14. Morielli AR, Courneya KS. Effects of exercise on cancer treatment completion and efficacy. Exercise oncology: Prescribing physical activity before and after a cancer diagnosis. 2020:209-27.
15. Banoon SR, Jasim SA, Ghasemian A. Effect of 12-week Aerobic Exercise on the Tumor Size and Expression of HIF-1α, BCL-2, Mir-15a, and VEGF Genes in BALB/C Female Mice with Breast Cancer. Journal of Chemical Health Risks. 2023;13(2).
16. 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):4166. [https://doi.org/10.3390/nu14194166]
17. Shakib Khoob M, Hosseini SM, Kazemi S. In vitro and in vivo antioxidant and anticancer potentials of royal jelly for dimethylhydrazine-induced colorectal cancer in Wistar rats. Oxidative Medicine and Cellular Longevity. 2022;2022(1):9506026. [https://doi.org/10.1155/2022/9506026]
18. Alnomasy SF, Al Shehri ZS. Anti-cancer and cell toxicity effects of royal jelly and its cellular mechanisms against human hepatoma cells. Pharmacognosy Magazine. 2022;18(79). [https://doi.org/10.4103/pm.pm_354_21]
19. Fei Z, Li D, Li K, Zhou M, Li Y, Li Y, Sun Z. Detraining after tumor-bearing accelerates tumor growth while continuous training decreases tumor growth in mice. Journal of Traditional Chinese Medical Sciences. 2020;7(1):75-81. [https://doi.org/10.1016/j.jtcms.2019.12.003]
20. Mirdar S, Arab A, Hedayati M, Hajizade A. The effect of pregnant rat swimming on hypoxia-inducible factor-1α levels of neonatal lung. Tehran University Medical Journal. 2012;69(12).
21. Shirzad M, Kordyazdi R, Shahinfard N, Nikokar M. Does Royal jelly affect tumor cells? Journal of HerbMed Pharmacology. 2013;2(2):45-8.
22. Chen W, Zhang H, Liu Y. Anti-inflammatory and apoptotic signaling effect of fucoxanthin on benzo (A) pyrene-induced lung cancer in mice. Journal of Environmental Pathology, Toxicology and Oncology. 2019;38(3). [https://doi.org/10.1615/JEnvironPatholToxicolOncol.2019030962]

23. Asadi M, Taghizadeh S, Kaviani E, Vakili O, Taheri-Anganeh M, Tahamtan M, Savardashtaki A. Caspase-3: structure, function, and biotechnological aspects. Biotechnology and Applied Biochemistry. 2022;69(4):1633-45. [https://doi.org/10.1002/bab.2305]
24. Granger CL, McDonald CF, Berney S, Chao C, Denehy L. Exercise intervention to improve exercise capacity and health related quality of life for patients with non-small cell lung cancer: a systematic review. Lung cancer. 2011;72(2):139-53. [https://doi.org/10.1016/j.lungcan.2011.08.002]
25. Luo Z, Wan R, Liu S, Feng X, Peng Z, Wang Q, et al. Mechanisms of exercise in the treatment of lung cancer–a mini-review. Frontiers in Immunology. 2023;14:1244764. [https://doi.org/10.3389/fimmu.2023.1244764]
26. Asmaz ED, Güler S, Zık B. Effects of royal jelly on ovary cancer cells proliferation and apoptosis. Medical Oncology. 2025;42(4):89. [https://doi.org/10.1007/s12032-025-02398-x]
27. Aslan A, Gok O, Beyaz S, Can MI, Parlak G, Gundogdu R, et al. Royal jelly regulates the caspase, Bax and COX-2, TNF-α protein pathways in the fluoride exposed lung damage in rats. Tissue and Cell. 2022;76:101754. [https://doi.org/10.1016/j.tice.2022.101754]
28. Kargaran ardakani S, Motamed, Pezhman, sadegh A-S. The effect of oral royal jelly supplementation and exercise training on antioxidant defense-related parameters in healthy active girls. Jundishapur Scientific Medical Journal. 2025;24(2):1-7.
29. Lee H-M, Lee H-J, Chang J-E. Inflammatory cytokine: an attractive target for cancer treatment. Biomedicines. 2022;10(9):2116 [https://doi.org/10.3390/biomedicines10092116]
30. Kartikasari AE, Huertas CS, Mitchell A, Plebanski M. Tumor-induced inflammatory cytokines and the emerging diagnostic devices for cancer detection and prognosis. Frontiers in oncology. 2021;11:692142. [https://doi.org/10.3389/fonc.2021.692142]