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The Effect of Swimming Training and Royal Jelly Supplementation on Tumor Necrosis Factor-Alpha Gene Expression in the Lung Tissue of Mice with Benzo[a]pyrene-Induced Lung Cancer

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Abstract

Background: Lung cancer is associated with the activation of inflammatory pathways and increased levels of the pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-α). The present study aimed to investigate the effects of swimming training and royal jelly supplementation on TNF-α gene expression in the lung tissue of an animal model of lung cancer.

Methods: In this experimental study, 42 male Balb/C mice, aged eight weeks, were divided into the following groups: healthy control, lung cancer (BZP), swimming exercise (ST), royal jelly 50 mg/kg (RJ50), royal jelly 100 mg/kg (RJ100), swimming + royal jelly 50 mg/kg (ST.RJ50), and swimming + royal jelly 100 mg/kg (ST.RJ100). Lung cancer was induced via intraperitoneal injection of 100 mg/kg benzo[a]pyrene. Swimming training was performed three days per week for 12 weeks, and royal jelly was administered intraperitoneally at doses of 50 and 100 mg/kg. Forty-eight hours after the last exercise session, animals were sacrificed, lung tissues were collected and frozen, and TNF-α gene expression was measured using real-time PCR.

Results: TNF-α levels were significantly elevated in the BZP, RJ50, RJ100, and ST groups compared to the healthy control group. TNF-α expression in the ST.RJ50 group was also significantly higher than the healthy control. In the intervention groups (ST, ST.RJ50, and ST.RJ100), TNF-α expression was significantly lower than in the BZP group. Moreover, TNF-α expression in the ST.RJ50 and ST.RJ100 groups was significantly lower than in the RJ50 and RJ100 groups.

Conclusion: Swimming training, especially when combined with royal jelly, can reduce TNF-α expression in the lung tissue of Mice with benzo[a]pyrene-induced lung cancer. This combination may serve as a complementary anti-inflammatory and preventive strategy to control the progression of lung cancer.

Keywords: Swimming training, Royal jelly, Lung cancer, Tumor necrosis factor-alpha

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Introduction

Lung cancer remains a major global health concern, emphasizing the urgent need for effective strategies to reduce lung cancer–related mortality (1). The molecular pathogenesis of lung cancer is highly complex and heterogeneous. Lung cancer may arise as a consequence of several genetic and epigenetic alterations (e.g., point mutations, amplifications, insertions, deletions, and translocations). This process is particularly associated with the activation of growth-promoting pathways and inhibition of tumor suppressor pathways (2). Inflammatory parameters serve as pivotal indicators in the prognosis and management of lung cancer (3).

Tumor necrosis factor-alpha (TNF-α) was first identified in the 1970s as a serum mediator of innate immunity capable of inducing hemorrhagic necrosis in tumors (4). Today, a wide range of biological activities has been attributed to this molecule, and its clinical application has primarily focused not on using it for cancer therapy but rather on inhibiting its effects for the treatment of autoimmune diseases (4, 5). In addition to its well-established role in immune responses, TNF-α regulates key cellular processes such as apoptosis and proliferation through the activation of various intracellular signaling pathways (such as MAPK, Akt, and NF-κB) via complex formation by ligand-activated TNFα receptors (6). TNF-α has been shown to exert dual effects on cancer cells, as it can activate both pro-survival and anti-survival pathways depending on various contextual factors such as cell type, concentration, and cell density. A precise understanding of TNF-α signaling phenomena is essential to elucidate its multifaceted role in malignancies and its potential as a therapeutic target or anticancer agent (5).

Despite remarkable advances in screening, diagnosis, surgery, radiotherapy, targeted therapy, and immunotherapy, the incidence of lung cancer has declined in some countries with a high human development index; however, the five-year survival rate remains below 20% in most regions (7). Prolonged exposure to air pollution has been associated with an increased risk of lung cancer, and this effect is modulated by lifestyle factors or genetic risk (8). Therefore, further research is required to explore therapeutic approaches, including lifestyle interventions, for the prevention and mitigation of lung cancer complications (9, 10). A potential strategy to combat the growing prevalence of various cancers, including lung cancer, is the adoption of preventive measures—particularly the maintenance of a healthy lifestyle, such as maintaining an optimal body weight, adhering to a healthy diet, engaging in regular physical activity, avoiding smoking and alcohol consumption, and using nutritional supplements to enhance immune function (10, 11).

Proper nutrition and exercise each have independent positive effects on patients with lung cancer (11–13). Ester et al. reported that exercise improves physical and psychological health, while nutrition enhances nutritional status—both contributing to improved quality of life in these patients. Moreover, combining these two interventions may produce synergistic effects, simultaneously improving physical performance, reducing disease symptom burden, and enhancing quality of life, although further research is needed to fully elucidate their combined effects (12).

Previous studies have demonstrated that regular exercise training can complement cancer treatment (14), and research indicates that physical activity may play a role in both the prevention and treatment of lung cancer, although the precise mechanisms remain incompletely understood (15, 16). Among the exercise modalities used for lung cancer management, swimming training has been shown to improve pulmonary capacity and function, reduce inflammation, and alleviate complications while enhancing the quality of life in patients with chronic respiratory diseases and in post–lung cancer rehabilitation (16–20). Reports suggest that aerobic exercise can significantly reduce TNF-α expression, making it a potentially effective strategy for lung cancer prevention or adjunct therapy (21).

Furthermore, royal jelly—one of the natural products derived from honeybees—contains a rich composition of antioxidants and anti-inflammatory compounds. Its antioxidant and anti-inflammatory effects have been reported in both animal and in vitro studies (22, 23). Specifically, royal jelly has been shown to modulate oxidative stress markers and reduce TNF-α levels, thereby inhibiting tumor growth in murine models of lung cancer (24). Recent studies have also reported that the combined use of royal jelly and swimming exercise exerts greater efficacy against lung cancer, including a more pronounced reduction in caspase-3 expression in the lung tissue of tumor-bearing mice (25).

Given the high prevalence and mortality rate of lung cancer, it represents one of the major global health challenges, exerting broad impacts on individuals, families, and healthcare systems (1, 2). The current inconsistencies in findings have created an impasse for strategies targeting the TNF-α pathway in combination with immunotherapy for lung cancer treatment, underscoring the need for further research on biomarkers that can guide rational therapeutic combinations (26). Considering the pivotal role of TNF-α in the pathogenesis of lung cancer (5) and the limited evidence regarding the combined effects of interventions on this pleiotropic variable, a more comprehensive understanding of the potential synergistic effects of swimming exercise and royal jelly supplementation on this cytokine may provide a foundation for future clinical investigations and the development of low-cost, low-side-effect adjunctive therapeutic strategies that contribute to better control of lung cancer progression.

Accordingly, this preclinical study was designed to compare the effects of swimming exercise and royal jelly supplementation on TNF-α gene expression in the lung tissue of mice exposed to benzo[a]pyrene.

Materials and Methods

In the present experimental study, which was conducted using a post-test design with a control group, forty-eight male Balb/C mice with an average age of 8 weeks and a body weight of 18–22 grams were purchased from the Laboratory Animal Breeding and Reproduction Institute of the Pasteur Institute of Tehran and transferred to the animal laboratory of the Pishtazan Higher Education Institute in Shiraz.
To adapt to the laboratory environment, the animals were kept in polycarbonate cages for one week. During this period, the samples were maintained under temperature conditions of 22 ± 3 °C, humidity of 40–60%, with proper ventilation and a 12/12-hour light/dark cycle, and had free access to food and water.
Of the 42 mice, 6 mice were assigned to the healthy control group (It should be noted that the healthy control group did not receive any intervention, including BZP, whereas the control group did receive BZP.), and the remaining 36 mice, after induction of lung cancer by injection of benzo[a]pyrene, were divided into the following groups: lung cancer, swimming training, royal jelly 50, royal jelly 100, swimming training + royal jelly 50, and swimming training + royal jelly 100 (It should be noted that the effects of royal jelly at doses of 50 mg/kg and 100 mg/kg have been reported in previous studies.)

For the induction of lung cancer, after 12 hours of fasting, the mice received an intraperitoneal injection of benzo[a]pyrene at a dose of 100 mg/kg (Sigma-Aldrich, Germany, economic code B1760). For this purpose, 24 mg of benzo[a]pyrene was first dissolved in 1.2 mL of corn oil, and after complete dissolution, 10 international units of the solution were injected into each mouse (25).
Animal care and handling were performed in accordance with the ethical guidelines for research in animals and based on the standard principles of laboratory animal care approved by the Islamic Azad University, Shiraz Branch, and the Helsinki Declaration.

Before starting the experimental period and in a preliminary study, 4 mice were subjected to the mentioned dose of benzo[a]pyrene and 4 healthy mice were used as controls. Fourteen days after the benzo[a]pyrene injection, the mice in both groups were dissected after anesthesia with ketamine and xylazine, and their lung tissue was evaluated pathologically to confirm the induction of lung cancer by benzo[a]pyrene.

Mice in the swimming training, swimming training + royal jelly 50, and swimming training + royal jelly 100 groups performed swimming training for 12 weeks, five sessions per week. Swimming exercise was carried out in a special animal pool with dimensions of 110 cm in width and 80 cm in depth, and water temperature maintained at 32 °C. This protocol included two stages: an adaptation stage and the main protocol. In the adaptation stage, the experimental mice were familiarized with the water environment and swimming for 10 days. Then, during the main protocol period, the mice trained for 12 weeks, three sessions per week. In this protocol, the mice swam for 15 minutes in the first week. Then, swimming duration was gradually increased from 15

minutes to 30 minutes up to the tenth week. In the last two weeks, the training duration increased to 40 minutes. To observe the principle of progressive overload, the weight load was zero (no load) during weeks 1 to 4, 2% of body weight during weeks 5 to 8, and 5% of body weight during weeks 9 to 12, attached to the tails of the mice. This program was adopted with minor modifications based on previous studies (27, 28).

To prepare the royal jelly extract, 10 grams of royal jelly was added to 1000 cc of deionized distilled water, and the mixture was incubated for 16 hours at 50 °C. Then, the solution was filtered, and it was stored at 4 °C and injected intraperitoneally at doses of 50 and 100 mg/kg daily (25, 29). To minimize the inflammatory effects caused by exercise, 48 hours after the last training session, the samples were anesthetized by intraperitoneal injection of ketamine (75 mg/kg) and xylazine (25 mg/kg) (Alfasan Company, Netherlands). After confirming complete anesthesia, the thoracic cavity of the animals was opened. Then, by separating the connective tissues, the lung tissue was completely removed. The lung tissue was immediately washed and transferred to −70 °C. For measuring the TNF-α gene expression, total RNA was extracted from lung tissue using the column RNA extraction kit (FavorPrep™ Tissue Total RNA Kit, catalog number FATRK 001, Taiwan). Gene expression was calculated and expressed using the ΔΔCt method.

For data analysis, one-way analysis of variance (ANOVA) and the LSD post hoc test were used. All computations were performed using SPSS software version 26, and a significance level of $P \leq 0.05$ was considered. In the present study, TNF-α gene expression levels were measured using the RT-PCR method.

Results

According to the results obtained from the one-way analysis of variance test, there was a significant difference in the expression of the TNF-α gene among the experimental groups ($F = 9.209$; $p < 0.001$).

In order to determine the location of the differences, the LSD post hoc test was used. The results of this test showed a significant increase in the level of TNF-α in the BZP, RJ50, RJ100, and ST groups compared to the healthy control group.

Also, the level of TNF-α in the ST.RJ50 group was significantly higher than that of the healthy control group.

In the intervention groups ST, ST.RJ50, and ST.RJ100, the TNF-α gene expression was significantly lower compared to the BZP group.

Furthermore, TNF-α gene expression in the ST.RJ50 and ST.RJ100 groups was significantly lower than that in the RJ50 and RJ100 groups (Figure 1).

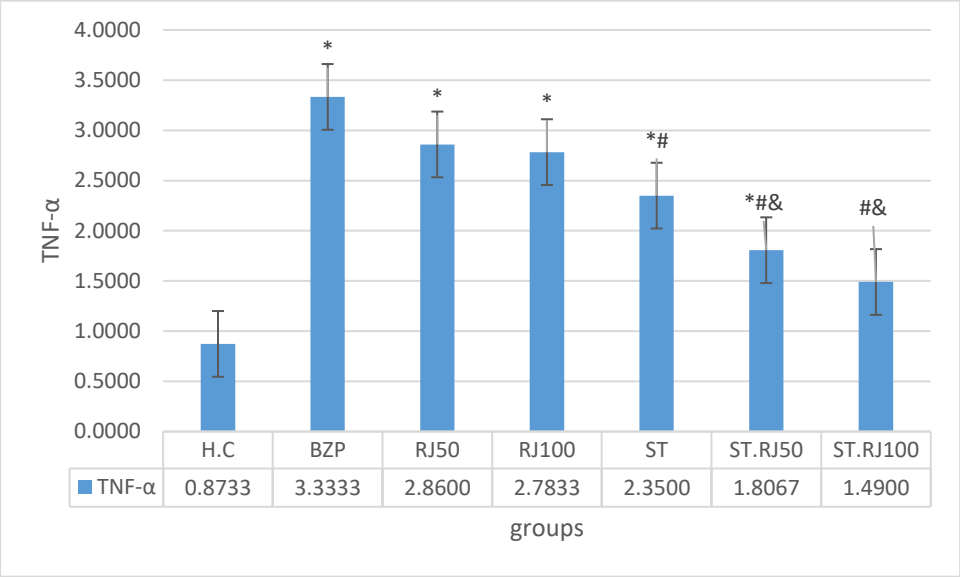

Figure 1. Changes in gene expression across the experimental groups.

*: Significant difference compared with the HC group;

#: Significant difference compared with the BZP group;

&: Significant difference compared with the RJ100 group.

Discussion

The results of the present study showed that exposure to benzo[a]pyrene caused a significant increase in TNF-α gene expression in lung tissue compared to the healthy control group. Exposure to benzo[a]pyrene induces structural and physiological alterations in the lungs, which ultimately lead to the development of chronic pulmonary diseases, including lung cancer (30). The main mechanism underlying this effect is the increased production of reactive oxygen species (ROS) and oxidative stress. Liu et al. also reported that benzo[a]pyrene elevates ROS production (31).

The findings of our study demonstrated that royal jelly supplementation at doses of 50 and 100 mg/kg, although leading to a reduction in TNF-α, did not produce a statistically significant difference compared with the benzo[a]pyrene group. However, in the swimming exercise and combined intervention groups (swimming exercise with royal jelly supplementation at both 50 and 100 mg/kg doses), a reduction in TNF-α levels was observed in the lung tissue of mice with lung cancer. These results indicate a positive role of swimming exercise in reducing TNF-α levels in lung tissue as an inflammatory factor in mice with lung cancer.

Regular exercise activates the body's endogenous antioxidant systems, leading to a reduction in oxidative stress within lung tissue. This effect occurs mainly through an increase in the activity of

major antioxidant enzymes (32). Consistent with the findings of the present study, Fashi et al. also reported that four weeks of aerobic exercise significantly reduced TNF-α expression in a mouse model of lung cancer induced by PM10 exposure (21), which aligns with our results.

One of the notable findings of the present study was the greater reduction in TNF-α gene expression observed in the combined intervention groups (swimming exercise and royal jelly supplementation). This reduction was so prominent that TNF-α gene expression in the combined groups, particularly in the swimming training group with the higher royal jelly dose (ST.RJ100), approached the level of the healthy control group. Furthermore, the reduction in TNF-α in the combined groups compared to the royal jelly groups (50 and 100 mg/kg) was statistically significant, indicating a synergistic effect of exercise and supplementation—especially exercise—on TNF-α gene expression in the lung tissue of mice with lung cancer.

Royal jelly can reduce oxidative stress and inhibit inflammatory cytokines such as TNF-α, thereby decreasing ROS production (24, 33). Aslan et al. also reported that royal jelly supplementation at a dose of 100 mg/kg reduced the expression of inflammatory proteins such as TNF-α (33). Moreover, it has been shown that royal jelly at doses ranging from 50 to 150 mg/kg can enhance antioxidant enzyme activity, reduce oxidative stress, and exert a protective role against lung tissue damage induced by arsenic trioxide (34). Although these studies are not entirely consistent with our findings regarding the independent effect of royal jelly supplementation, they may justify the synergistic effect of swimming exercise and royal jelly on TNF-α gene expression in lung cancer. Rashidi et al. also reported the synergistic effect of swimming exercise and royal jelly on caspase-3 in a mouse model of benzo[a]pyrene-induced lung cancer, which supports the results of the present study.

TNF-α can bind to two distinct receptors, TNFR1 and TNFR2. Its membrane-bound form (tmTNF-α) predominantly binds to TNFR2 with higher affinity. Following proteolytic cleavage of tmTNF-α by TNF-α converting enzyme (TACE), its soluble form (sTNF-α) is released, which has a higher affinity for TNFR1. This unique feature enables TNF-α to mediate a wide range of dual and sometimes opposing functions, including the promotion of tumor cell survival or the induction of apoptosis, stimulation or suppression of antitumor immune responses, and facilitation of angiogenesis and metastasis (26).

Various studies have demonstrated that increased expression and activity of TNF-α in lung tissue are directly associated with lung cancer progression, invasion, and metastasis, identifying this molecule as a potential therapeutic target (5). Studies have reported that selective blockade of sTNF-α by the compound INB03 can reduce the incidence and growth rate of carcinogen-induced tumors (35, 36). In addition, evidence indicates that sTNF-α and TNFR1 exert deleterious effects on effector CD8+ T cells infiltrating melanoma tissue and contribute to immune-related adverse events (irAEs); this supports the rationale for combined TNF-α blockade with immunotherapy in melanoma treatment (26).

In preclinical studies, both agonists and antagonists of TNFR2 have shown antitumor effects, suggesting that the feasibility of targeting and modulating TNFR2 as a potential strategy in lung cancer therapy remains valid (37–39). Overall, abundant evidence indicates that the TNF-α signaling pathway plays a critical role in lung tumor progression and response to immunotherapy (26). Based on this, it can be assumed that implementing swimming exercise interventions, especially in combination with royal jelly supplementation, may exert beneficial effects by reducing TNF-α levels and modulating its signaling pathway in lung cancer.

However, in the present study, changes related to TNF-α receptors and specific indices of its signaling pathway were not measured, which should be considered one of the main limitations of this research.

Conclusion

The findings of the present study demonstrated that lung cancer induction by benzo[a]pyrene led to an increase in TNF-α gene expression and pathological alterations in lung tissue. Swimming exercise significantly attenuated this increase, whereas royal jelly alone exerted a weaker effect. The combination of swimming exercise and royal jelly, particularly at the higher dose, exhibited a synergistic effect, reducing TNF-α expression to a level comparable with that of the healthy control group. Therefore, the concurrent application of these two interventions may represent a complementary strategy for mitigating inflammation and inhibiting the progression of lung cancer. Nevertheless, further studies at the genomic and proteomic levels are required to confirm these findings and elucidate the underlying molecular mechanisms.

Ethical Considerations

In the present study, all ethical principles regarding the handling of laboratory animals — including the availability of food and water and appropriate housing conditions — were strictly observed. This study was approved by the Ethics Committee of Islamic Azad University, Shiraz Branch under the code number IR.IAU.SHIRAZ.REC.1404.055, and all experimental procedures were conducted in accordance with the guidelines for the care and use of laboratory animals.

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Conflict of Interest

The authors declare that there is **no conflict of interest** regarding the publication of this article.

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