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Does Royal jelly affect tumor cells?

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ABSTRACT

Introduction: Royal jelly is a substance that appears to be effective on immune system and it appears to be effective on both prevention and growth of cancer cells. In this study, we aimed to carry out a research to investigate the effect of royal jelly on the growth of WEHI-164 fibrosarcoma cell in syngenic Balb/c mice.

Methods: In an experimental study, 28 male Balb/c mice were designated into four equal groups. The mice were subcutaneously injected with $5x10^5$ WEHI-164 tumor cells on the day zero in the chest area of the animal. Animals in groups 1 to 4 were orally given 100, 200, 300 mg/kg of royal jelly or vehicle, respectively. In every individual mouse, the tumour size was measured every 2 days from day 5 (days 5, 7, 9, 11, 13, 15 and 17). Data were statistically analyzed using Kruskal-Wallis and Mann Whitney-U tests.

Result: Our results showed that the mean size of tumor in case group was significantly smaller than the control group in days 11, 13, 15 and 17 ($P<0.05$). No metastasis was seen in test and control groups.

Conclusion: With emphasize on antitumor effect of royal jelly, it seems that royal jelly has important role in control and regression of fibrosarcoma cells. Since royal jelly showed a delayed effect in control of fibrosarcoma, we suggest that royal jelly be used at least 10 days before tumor inoculation

Implication for health policy/practice/research/medical education:
Royal jelly demonstrated an important activity in control and regression of fibrosarcoma cells in preclinical study. Therefore, clinical studies are recommended to evaluate its beneficial effects in prevention and treatment of different cancer types.

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Introduction

Fibrosarcoma is a tumor of mesenchymal cell origin that is composed of malignant fibroblasts in a collagen background. This tumor often involves muscle fascia deeply. The tumor may be infinitely large when it is diagnosed. The tumor usually appears in people younger than 20 years (1).

One of the known treatments for fibrosarcoma is radiotherapy, which prevents the rapid growth of cancer cells and causes the death of cancer cells (2,3). All the methods of treatment, including surgery, amputation, radiotherapy and chemotherapy have side effects such as hair loss, nausea, vomiting, skin rash and an increased risk of infection in result of weakening immune system, while biological methods are non-invasive and effective (4). Enhancing of the immune system (immunotherapy) is one of the surveyed and effective treatments of the cancer. This method is focused on strengthening the immune system and stimulating factors of this system. Strengthening the immune system prevents or reduces cancer cells growth. Meanwhile, one of the materials which seems to be effective in enhancing the immune system is royal jelly (5,6). Royal jelly is one of the Bee products which is a milky white substance with a sharp smell and fruity taste and is nutritionally abundant. This gel contains all water-soluble B vitamins such as thiamine (B1), riboflavin (B2), and pyridoxine (B6) as well as niacin, biotin, folic acid, inositol, and minerals (including sodium, potassium, chromium, magnesium, nickel) and 20 essential amino acids, sugars, sterols, phosphorus compounds, acetylcholine, gamma globulin, nucleic acids and nutrients needed for health (7,8). Royal jelly has a strong antibiotic activity against bacteria and fungi (9-12). Royal jelly effects on atherosclerosis, arthritis, diabetic foot ulcers, tissue repair, collagen, warts and estrogen-like effects have also been demonstrated (13-18).

Regarding the anti-tumor effect of royal jelly, a group of Japanese researchers gave royal jelly to one of two groups of laboratory mice, prior to tumor cell implantation and

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observed that the gel had no effect on leukemia cells but it had effects on sarcoma cells and increased the lifespan of mice by about 20%. In this study, the size of tumors decreased about 50% (19). Other studies also have pointed to royal jelly's anti-tumor effects (21,22). In a study by Tamura et al, they found no antitumor effect for royal jelly, but it increased the lifespan of mice by as much as 19.3% and restricted tumor growth greatly (22). In a study conducted by Bincoletto et al, it was shown that royal jelly modulates the immune system and is effective anti-tumor response with a long treatment regimen (23).

Fibrosarcoma is a soft tissue tumor with no pain which growths deeply and therefore its diagnosis happens later. On the other hand, at the diagnosis time it is such a big tumor that can involve muscle fascia and its boundaries are not clear, but early detection and treatment can increase the survival rate of patients. Since current treatments are not effective in the treatment of fibrosarcoma singly and the need for new treatments or a combination therapy are crucial, according to the antitumor and anti metastatic effects of royal jelly in many studies, It seems that this material is also effective against fibrosarcoma. Thus, this study aimed to determine the effects of royal jelly on WEHI-164 fibrosarcoma, in male mice Balb/c.

Material and Methods

In this study, 4 groups of 7 male Balb/c mice from similar age which obtained by the Pasteur Institute of Iran were selected. The mice were injected $5x10^5$ 164-WEHI tumor cells under the skin in the chest region. Each group except the control group received royal jelly orally at doses of 100, 200 and 300 mg/kg at the beginning and through the study, after tumor cells inoculation (20). Bee royal jelly was obtained from the Agricultural Research Center of Chaharmahal and Bakhtiari. In order to measure the growth of malignant cells from the second day after the injection, each mouse was examined for the tumor mass by touching the injection site. When the tumor was touched by finger skin, its diameter was measured by caliper in two directions immediately. The study of the rats regarding the existence of the tumor and its diameter continued till two weeks after the injection. At this period, the tumor diameter was measured seven times and at the days 5, 7, 9, 11, 13, 15 and 17 after the injection of tumor cells. In order to calculate the average tumor area, two perpendicular diameters were measured, added together and the sum was divided by the number 4. The result of the division was squared and was multiplied by 3.14, eventually (24). In order to compare the mean of the tumor areas in treated and control groups, Mann-Whitney and Kruskal-Wallis tests were used.

Results

The results of the growth of WEHI-164 tumor cells in Balb/c mice showed that no tumor growth was observed in the first, second and third days after tumor inoculation. The tumor was palpable on day fourth the fifth. On the day seven tumor masses were observed in all animals.

The results of measuring the size of tumor after the fifth day and for 7 consecutive times and every other day showed that the mean tumor area of control group (the group which did not receive the royal jelly) on days 11, 13, 15 and 17 were significantly higher than those of other three groups (the groups receiving 100, 200 and 300 mg/kg royal jelly) ($P<0.05$; Figure 1).

In order to show the metastasis of tumor in the lungs and livers of the animals, the organ cells were culture in tissue culture medium. No tumor cells growth were detected. In this study, all animals survived until the end of the study, and even after that.

Discussion

In this study, the effects of royal jelly on the growth and metastasis of malignant fibrosarcoma cells in Balb/c mice were studied. The results showed that the average size of tumors in mice receiving royal jelly was smaller than the control group. No Similar study is conducted to survey the effects of royal jelly on WEHI-164 fibrosarcoma in Balb/c mice, but the effect of royal jelly on other types of cancer in animal models was investigated. In a study conducted by a group of Japanese researchers the effects of royal jelly on cancer was investigated. In this study one of two groups of laboratory rats received royal jelly prior to tumor cell inoculation. The study shows that royal jelly has no effect on leukemia cells but has therapeutic effects on sarcoma cells (19). The results obtained in this study also show that the size of tumors in the control group is larger than their size in treated groups. It seems that during the study soft tissue cells have had no metastatic properties, because the metastasis in soft tissue fibrosarcoma is serotinal (21) or it occurs in longer periods of time. Therefore, In this study the anti-metastatic effect of royal jelly has been observed.

In a study conducted by Tamvra and et al, royal jelly did not show any antitumor effects, but the lifetime has increased 3.19 percent and the use of prophylaxis-therapy inhibits tumor growth, 1.49-1.56% (22). These findings also confirm the findings of the present study.

Other study conducted by Bincoletto et al showed that royal jelly modulates body immune response and long-term treatment regimen resulted in anti-tumor effects. In this study, the prescribed royal jelly for 33 days at doses of 500, 1000 and 1500 mg/kg increased the survival respectively 38, 85 and 71%

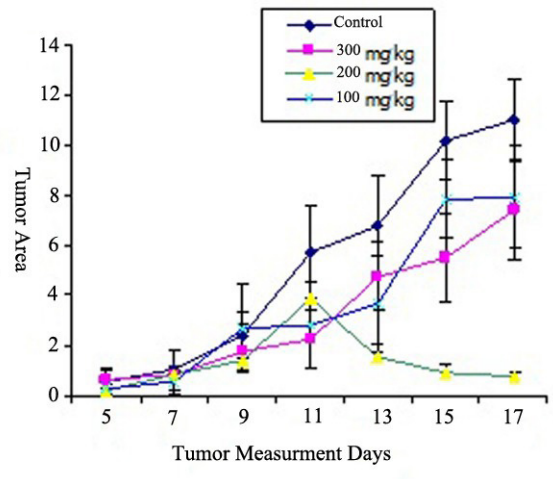

Figure 1. The comparison of different concentrations of royal jelly on the growth of WEHI-164 fibrosarcoma tumor in Balb/c mice.
100, 200 and 300 mg/kg of royal jelly reduced the tumor size significantly at days 11, 13, 15 and 17 compare to control group ($P<0.05$).

in the same dose for 23 and 28 days. The same dose for 23 and 28 days increased survival rates by 19 and 23%. The increased survival rate may be due to the Prostaglandin E2 after the treatment. The results of the study changed royal jelly effects from a biological response to an anti-tumor agent (23). In the study, all the rats survived until the end of the study, and even after that. The tumor used in this study is possibly according to the clashes of soft tissue and not observing metastasis have been ineffective on the survival of the rats.

Considering that the early use of royal jelly had no effect on tumor growth, it seems that the effect of royal jelly on the immune system and its anti-tumor activity takes time and because of the small size of tumors in the early days, it's not possible to comment about the effect of this material on tumor growth, but during the time and due to the effects of royal jelly on immune system, tumor growth decreases in compare to control group. Therefore it is suggested to begin treatment regimen of royal jelly sometimes before tumor injection in the future studies in order to make it ready to progress and prevent tumor growth.

Also it is recommended to study the anti fibrosarcoma effects of royal jelly in the future researches in other laboratory animals, with different injection method.

Conclusion

The results of the study show that 100, 200 and 300 kg/mg doses of royal jelly are effective on WEHI164 fibrosarcoma in Balb/c mice. This effect introduces the possible therapeutic and dietary use of royal jelly.

Authors' contributions

RK, NSh, MN, MSh carried out experiments and participated in design of the study. MSh revised and approved the manuscript. RK, NSh, MN, helped in draft of the manuscript.

Conflict of interests

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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References

1. Wong SL. Diagnosis and management of desmoid tumors and fibrosarcoma. J Surg Oncol 2008; 97(6): 554-8.
2. Loh ML, Ahn P, Perez-Atayde AR, Gebhardt MC, Shamberger RC, Grier HE. Treatment of infantile fibrosarcoma with chemotherapy and surgery: results from the Dana-Farber Cancer Institute and Children's Hospital, Boston. J Pediatr Hematol Oncol 2002;24(9): 722-6.
3. Mirra JM, Marcove RC. Fibrosarcoma: review of five cases. J Bone Joint Surg 1999; 56-287.
4. Mattil-Fritz S, Scharner D, Piuko K, Thönes N, Gissmann L, Müller H, et al. Immunotherapy of equine sarcoid: dose-escalation trial for the use of chimeric papillomavirus-like particles. J Gen Virol 2008; 89(Pt 1): 138-47.
5. Sver L, Orsolic N, Tadic Z, Njari B, Valpotic I, Basic I. A royal jelly as a new potential immunomodulator in rats and mice. Comp Immunol Microbiol Infect Dis 1996; 19(1): 31-8.
6. Vucevic D, Melliou E, Vasilijic S, Gasic S, Ivanovski P, Chinou I, et al. Fatty acids isolated from royal jelly modulate dendritic cell-mediated immune response in vitro. Int Immunopharmacol 2007;7(9): 1211-20.
7. Ishii R, Horie M, Murayama M, Maitani T. Analysis of tetracyclines in honey and royal jelly by LC/MS/MS. Shokuhin Eiseigaku Zasshi 2006;47(6): 277-83.
8. Kobayashi N, Unten S, Kakuta H, Komatsu N, Fujimaki M, Satoh K, et al. Diverse biological activities of healthy foods. In Vivo 2001;15(1): 17-23.
9. Fontana R, Mendes MA, de Souza BM, Konno K, César LM, Malaspina O, et al. Jelleines: a family of antimicrobial peptides from the royal jelly of honeybees (Apis mellifera). Peptides 2004;25(6): 919-28.
10. Melliou E, Chinou I. Chemistry and bioactivity of royal jelly from Greece. J Agric Food Chem 2005;53(23): 8987-92.
11. Kamakura M, Mitani N, Fukuda T, Fukushima M. Antifatigue effect of fresh royal jelly in mice. J Nutr Sci Vitaminol (Tokyo) 2001;47(6): 394-401.
12. Fujiwara S, Imaj J, Fujiwara M, Yaeshima T, Kawashima T, Kobayashi K. A potent antibacterial protein in royal jelly. Purification and determination of the primary structure of royalisin. J Biol Chem 1990;265(19): 11333-7.
13. Abdelatif M, Yakoot M, Etmaan M. Safety and efficacy of a new honey ointment on diabetic foot ulcers: a prospective pilot study. J Wound Care 2008;17(3): 108-10.
14. Hidaka S, Okamoto Y, Uchiyama S, Nakatsuma A, Hashimoto K, Ohnishi ST, et al. Royal jelly prevents osteoporosis in rats: beneficial effects in ovariectomy model and in bone tissue culture model. Evid Based Complement Alternat Med 2006;3(3): 339-48.
15. Koya-Miyata S, Okamoto I, Ushio S, Iwaki K, Ikeda M, Kurimoto M. Identification of a collagen production-promoting factor from an extract of royal jelly and its possible mechanism. Biosci Biotechnol Biochem 2004; 68(4): 767-73.
16. Mishima S, Suzuki KM, Isohama Y, Kuratsu N, Araki Y, Inoue M, et al. Royal jelly has estrogenic effects in vitro and in vivo. J Ethnopharmacol 2005; 101(1-3): 215-20.
17. Maly E, Pacenovska M. Successful treatment of warts by royal jelly. Cesk Dermatol 1966; 41(1): 36-9.
18. Madar J, Maly E, Neubauer E, Moscovic F. Effect of bee royal jelly (gelee royale) on the cholesterol level, total lipids in the serum and on the fibrinolytic activity of plasma of elderly arteriosclerotic patients. Z Alternsforsch 1965; 18(2): 103-8.
19. Taniguchi Y, Kohno K, Inoue S, Koya-Miyata S, Okamoto I, Arai N, et al. Oral administration of royal jelly inhibits the development of atopic dermatitis-like skin lesions in NC/Nga mice. Int Immunopharmacol 2003; 3(9): 1313-24.
20. Shirzad H, Shahinfard N, Naficy MR, Karami M. Comparison of royal jelly effects with Gentamicin and Ceftriaxone on the growth of *Escherichia coli, Bacillus*

cereus, Pseudomonas aeruginosa, Staphylococcus aureus, in a laboratory environment. 5th Europian congress on Tropical Medicine and International Health. Amsterdam ;2007.
21. Mark RJ, Sercarz JA, Tran L, Selch M, Calcaterra TC. Fibrosarcoma of the head and neck. The UCLA experience. Arch Otolaryngol Head Neck Surg 1991; 117(4):396-401.
22. Tamura T, Fujii A, Kuboyama N. Antitumor effects of royal jelly (RJ). Nippon Yakurigaku Zasshi 2000; 89(2): 73-80.
23. Bincoletto C, Eberlin S, Figueiredo CA, Luengo MB. Effect produced by royal jelly (RJ) on haematopoiesis, relation with host resistance against Ehrlich ascites tumour challenge. Nutr Cancer 2005;5(4): 679-88.
24. Shirzad H, Burton RC, Smart YC, Rafieian-kopaei M, Shirzad M. Natural cytotoxicity of NC-2(+) cells against the growth and metastasis of WEHI-164 fibrosarcoma. Scand J Immunol 2011;73(2): 85-90.