Medical Laboratory Journal
Online ISSN: 2538-4449
Golestan University of Medical Sciences

The effect of nettle extract consumption and endurance training on the expression of IFN-*γ* and Endostatin in the liver tissues of mice with melanoma

Vahid Zolghadri [1], Alireza Barari [1*], Asieh Abbasi Daloii [1], Hosein Abed Natanzi [2]

1. Department of Sport Physiology, Am.C., Islamic Azad University, Amol, Iran
2. Department of Sport Physiology, SR.C., Islamic Azad University, Tehran, Iran
* Correspondence: Alireza Barari. Department of Sport Physiology, Am.C., Islamic Azad University, Amol, Iran. Tel: +989111277793; Email: alireza54.barari@gmail.com

Abstract

Background: Melanoma is an aggressive malignancy that results from the transformation of pigment-producing melanocytes. This study aimed to investigate the effect of nettle extract consumption and aerobic exercise on the gene expression of *IFN-γ* and *Endostatin* in the liver tissues of mice with melanoma.

Methods: Twenty male BALB/c mice were randomly divided into four groups, including control, endurance, nettle, and endurance+nettle. The training program included running on a treadmill for 30 minutes at a speed of 16 meters per minute. The speed was increased by one meter per minute each week, reaching 22 meters per minute in the eighth week. Melanoma cells were injected subcutaneously into the left side of the mice. The experimental groups received 30 mg/kg/day of nettle ethanol extract orally for eight weeks. Real-time PCR was used to assess the expression of *IFN-γ* and *Endostatin*.

Results: *IFN-γ* expression levels in the experimental groups were not different from the control group, while *Endostatin* levels were significantly reduced ($p = 0.142$, $p < 0.001$, respectively). *IFN-γ* expression levels in the experimental groups were higher than in the control group, but did not reach a significant level. Also, *Endostatin* expression levels in training and combination groups were significantly lower than in the control group ($p = 0.022$, $p < 0.001$, respectively).

Conclusion: The results showed that endurance training combined with nettle extract may inhibit angiogenesis and capillary tissue formation in the tumor tissue of mice with melanoma by increasing IFN-γ and decreasing Endostatin.

Article Type: Research Article

Article History

Received: 11 April 2023
Received in revised form: 25 August 2023
Accepted: 12 September 2023
Available online:
DOI: 10.29252/mlj.19.X.X

Keywords

Interferon-gamma
Endostatins
Endurance training
Nettle extract
Melanoma cancer

Introduction

Melanoma is a skin cancer caused by the malignant transformation of melanocytes. The incidence of melanoma is rapidly increasing worldwide, posing public health problems. Primary extra cutaneous melanomas may occur in ocular, gastrointestinal, mucosal, genitourinary, and lymphatic tissues. The relationship between ultraviolet (UV) radiation and melanoma development is complex, and intermittent sun exposure strongly increases the risk. It is the fifth most common cancer in men and the sixth most common type of cancer in women (1). Early detection of skin cancer greatly reduces mortality and morbidity. Treatment and follow-up for melanoma patients may vary depending on tumor stage and the characteristics of the initial lesion (2). Interferons (IFNs) are cytokines with pleiotropic effects that play an important role in intercellular communication during innate and acquired immune responses and defense against viral and bacterial infections (3-5). In the tumor microenvironment (TME), IFN-γ continuously regulates antitumor immunity. IFN-γ acts as a cytotoxic function and cytokine, along with Granzyme B and Perforin, to initiate apoptosis in tumor cells (6). In the environment of inflamed tissue or tumor, secreted pro-inflammatory cytokines bind to their receptors on IFN-γ-producing cells (6-9).

In this context, Endostatin is considered a new biomarker for melanoma (10). Endostatin has been described as an inhibitor of tumor angiogenesis and is a proteolytic fragment of 183 amino acids produced from collagen XVIII precursor (11). Previous studies have described multiple roles of Endostatin in modulating endothelial cell behavior. For example, Endothelin induces endothelial cell apoptosis and acts as a regulator of tube formation and endothelial cell migration and growth. Therefore, Endothelin interferes with tumor proliferation by inhibiting the activity of tumor-stimulating growth factors (12). In addition, several studies have shown that Endostatin inhibits angiogenesis and metastasis by limiting blood supply to tumors. As a result, it deprives tumors of nutrients and is considered a potential anticancer agent in the treatment of malignant tumors (13). Physical activity affects the risk of various cancers. Available evidence strongly supports the role of regular physical activity in reducing the risk of colon, breast, and endometrial cancers to a lesser extent, lung, and pancreatic cancers. A similar protective effect has also been suggested for other cancers (14). However, the effect of physical activity on IFN-γ levels has varied in different studies.

Zamani et al. (2017) reported that moderate activity increases the production of IFN-γ and interleukin-12 (IL-12) in peripheral blood mononuclear cells (15). Vijayaraghava and Radhika (2014) investigated the effect of exercise intensity on IFN-γ production. The results showed that plasma IFN-γ decreases after a moderate acute exercise session. However, severe acute exercise causes a sharp decrease. Regular moderate exercise increases IFN-γ (16). Golzari et al. (2010) investigated the effect of combined exercises on the level of IFN-γ and IL-17 in blood plasma in women with multiple sclerosis. They found that combined exercise training decreased IFN-γ and IL-17 levels in plasma and peripheral blood mononuclear cells (17). Shakoor et al. (2018) investigated the effect of physical activity on IFN-γ, body fat, and BMI in kidney transplant patients. The results showed that physical activity had no significant effect on IFN-γ levels (18). Also, Gu JW et al. (2004) investigated the effect of physical activity on Endostatin levels in healthy people. They found that circulating Endostatin can be significantly increased by exercise in proportion to maximal oxygen consumption in physiological conditions in healthy volunteers (19).

The use of medicinal plants for the prevention and treatment of some diseases has been the focus of traditional medicine specialists since ancient times (20,21). The nettle plant is rich in essential amino acids, several minerals such as calcium, iron, magnesium, and phosphorus, and

also contains vitamins such as C, B, and K. In addition, it contains Flavonoids that show antioxidant activity in addition to the anti-inflammatory activity of its polysaccharides (22,23). For example, a study by Mohammadi et al. showed that the dichloromethane extract of nettle leaves inhibited the growth and proliferation of human prostate cancer cells (PC3) after 48 hours of treatment (24). Although herbal medicines still lack strong evidence for their role in cancer treatment and patient recovery, their use has become popular among cancer patients worldwide. The use of herbal medicines is supported by a large body of evidence from clinical studies reporting the benefits of several herbal medicines in reducing chemotherapy-induced toxicities (25,26). Considering the effectiveness of safe and low-cost interventions, regular exercise and the use of herbal supplements can make the physiological effects of these interventions more specific as a cost-effective method. As a result, this study aimed to investigate the effect of endurance training and nettle extract consumption on the gene expression of *IFN-γ* and *Endostatin* in the liver tissue of mice with melanoma.

Methods

The subjects of this research project were adult male BALB/c mice, 6-8 weeks old, with an average initial weight of 350-300 grams. After purchase, these animals were transferred to the laboratory animal breeding and maintenance center. This research was approved by the Ethics Committee of the Islamic Azad University, Marvdasht branch, with ethics number IR.IAU.M.REC.1399.008. After entering the research environment and getting familiar with the new environment and how to work on the treadmill for two weeks, the animals were randomly divided into four groups: control, endurance, nettle, and endurance+nettle. All stages of keeping and killing mice were done according to the Animal Ethics Committee of the Neuroscience Research Center of Shahid Beheshti University. Since laboratory mice are very sensitive to respiratory diseases; therefore, proper ventilation was placed in the storage area to prevent the accumulation of ammonia from animal urine. Endurance training was done four days after the start of supplementation for six weeks, five sessions a week on a treadmill. Mice in the training group exercised on a treadmill for 10 to 15 minutes for one week at a speed of 10 m/min for five days. From the second week, the overload phase was applied for three weeks until the end of the fourth week. The overload phase was such that on each training day, 3 minutes were added to the activity time and 1 meter per minute to the treadmill speed. By the end of the fourth week, the speed of the treadmill reached 28 meters per minute for 60 minutes of activity. From the fourth to the sixth week, the stabilization phase continued for three weeks at a speed of 28 m/min and for one hour.

B16F10 cells were purchased from the Pasteur Institute of Iran. These cells were chosen due to the same cell type as the studied mouse species. The cells were cultured in M199 medium and when the cell density reached 80%, they were prepared for injection into mice. The number of living cells before injection was counted by trypan blue staining. On the study day, 106 melanoma cells were subcutaneously injected on the left side of the mice (27). To prepare nettle extract, some of the stems and leaves of the nettle plant were collected after being cut into small pieces and washed, then dried in the open air and made into powder with a machine.

In the present research, to prepare nettle extract, some of the stems and leaves of the nettle plant were collected after being cut into small pieces and washed, then dried in the open air and made into powder with a machine. Then, 60 grams of nettle plant powder was placed in a 2.5-liter beaker and 2 liters of distilled water were added to it and the beaker was placed on a special heater (Model MR3001 K, Heidolphl, Germany). After boiling, the intended decoction was filtered with filter paper. Extraction inside the distillation apparatus was placed in a rotary vacuum (Laboratory 4003 made by Heidolf, Germany) with a temperature of 45 °C and a vacuum pressure of 65 mbar and a speed of 20 rpm. To prepare the solution, the aqueous extract of the nettle plant was dissolved in distilled water to dissolve completely and obtain a thin and smooth solution, we placed it inside the falcon and on the vortex so that the obtained solution could easily pass through the insulin syringe. To prepare the desired extract, the above steps were repeated several times. The experimental groups received nettle extract for six weeks in the amount of 30 mg per kilogram of body weight per day.

To prepare the solution, we dissolved the aqueous extract of the nettle plant in distilled water and put it inside the Falcon tube and on the vortex so that it dissolves completely and a thin and smooth solution is obtained. In order to make the obtained solution easily pass through the insulin syringe, the above steps were repeated several times to prepare the desired extract. The experimental groups received nettle extract for six weeks in the amount of 30 mg per kilogram of body weight per day. Sampling was done 48 hours after the last session of endurance activity. Mice were anesthetized by intraperitoneal injection of a combination of ketamine (70 mg/kg) and xylosin (5.3 g/kg) and were taken out of the chamber and transferred to the operating table for tissue sampling. For liver tissue sampling, subjects were placed on their backs on a fixed laboratory table and liver tissue was drawn directly after cutting the abdomen. The desired tissues were removed and frozen in the nitrogen tank at -80 °C.

Real-time polymerase chain reaction (Real-time PCR) is commonly used to measure gene expression. The first step in a real-time PCR reaction is the conversion of RNA to complementary DNA (cDNA); this process is known as reverse transcription. The next step uses fluorescent reporters and a PCR reaction to amplify and detect specific genes. Quantitation of RNA by kitRNX-Plus (SinaClone; RN7713C), and it was extracted according to the manufacturer's instructions. Then RNA extracted by the nanodrop spectrophotometer (ND-1000; Thermo Sci., Newington, NH) was investigated. A part of the extracted RNA sample was electrophoresed on a 1% agarose gel, and after staining with Ethidium Bromide, the RNA quality was checked. To check gene expression first from RNA, cDNA was made, which is complementary to RNA and can be used for this field's real-time PCR. The cDNA Reverse Transcription Kit and Cell and Tissue RNA Isolation Kit were used with the brand of Kiagene Fanavar.

The real-time PCR technique was used by Rotor Gene 6,000 (Corbett Research, Australia) for 40 cycles to check gene expression. Primers of three genes with one control or reference gene, GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) were designed and ordered for synthesis from Synaclon Company (Table 1). For PCR, 2x master mix buffer, forward and reverse primer combination, cDNA, and injection water were used. The resulting mixture was prepared in the amount of 10 µl in a special vial of the Corbett machine, and then it was placed in the router of the machine. The level of mRNAs of each gene was calculated relative to the level of mRNAs of the GAPDH gene. Primer sequences for each gene are summarized in Table 1.

Statistical analysis

Quantitative description of data was done using central dispersion indices such as mean and standard deviation (SD), and the Shapiro-Wilk test was used to determine the normality of data distribution and Levine's test was used to assess the homogeneity of variances. Also, the one-way analysis of variance method was used to check the significant changes between different groups. If a statistically significant difference was observed, Tukey's post hoc test was used in the ANOVA to determine the location of the difference between groups. The significance level for all calculations was considered $p < 0.05$. All statistical operations were performed using SPSS software version 20.

Table 1. Primer sequences for each gene

Gene	Primer sequence (5'->3')	
	Forward	Reverse
INF-γ	GAGTGTGGAGACCATCAAGGAAG	CTCACACCTCTGGTAGTTCCTTC
Endostatin	GGGGAATTCCACAGCCACCGCGACTTCCAG CGACTTCCAG	CCCCTTAAGGTGTCGGTGGCGCCTCAAGGTCGCTGAAGGTC
GAPDH	AAGTTCAACGGCACAGTCAAGG	TTCAAGTTGCCGTGTCAGTTCC

Results

The data obtained from the research variables for five groups are presented descriptively in Table 1. The mean and SD of *IFN-γ* and *Endostatin* gene expression levels in the different groups showed that the lowest level of *IFN-γ* was observed in the control group and the highest levels were observed in the combined group. Also, the lowest level of *Endostatin* was observed in the combined group and the highest levels were observed in the control group. Data analysis using one-way analysis of variance showed that the expression of the *IFN-γ* gene in the experimental groups was not different from the control group. While Endostatin level had a significant decrease (p=0.142, p <0.001, respectively). The expression levels of the *IFN-γ* gene in the experimental groups increased compared to the control group, but did not reach a significant level. Also, the expression levels of the *Endostatin* gene in the training and combination groups were significantly decreased compared to the control group (p=0.022, p <0.001, respectively) (Table 2 and Figure 1, Table 3 and Figure 2).

Table 2. *IFN-γ* gene expression in different groups (Arbitrary units)

Variable		Sum of squares	Degree of freedom	Mean square	F-test	p
IFN-γ	Between groups	9.876	3	3.292	2.089	0.142
	Within group	25.214	16	1.576		
	Total	35.09	19	4.868		

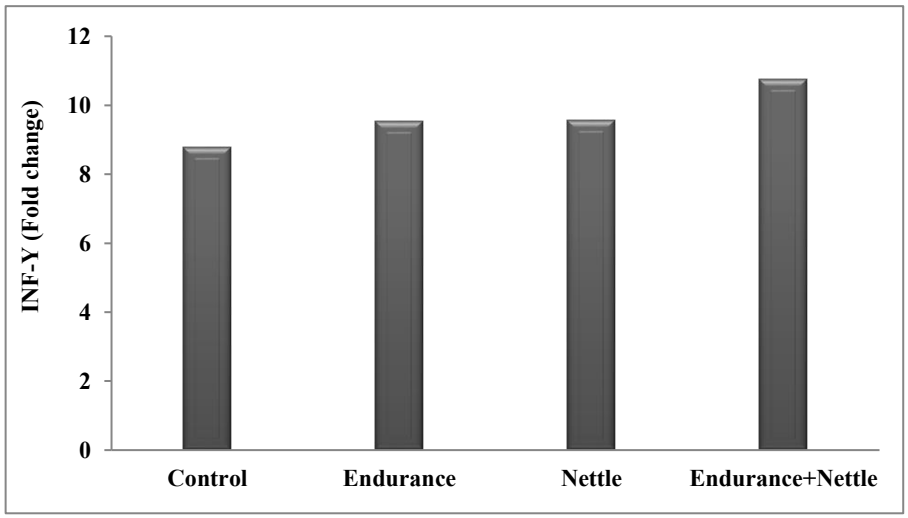

Figure 1. *IFN-γ* gene expression changes in different groups

Table 3. Endostatin gene expression in different groups (Arbitrary units)

Variable		Sum of squares	Degree of freedom	Mean square	F-test	p
Endostatin	Between groups	40.808	3	13.903	11.109	0.000
	Within group	19.592	16	1.224		
	Total	60.4	19	15.127		

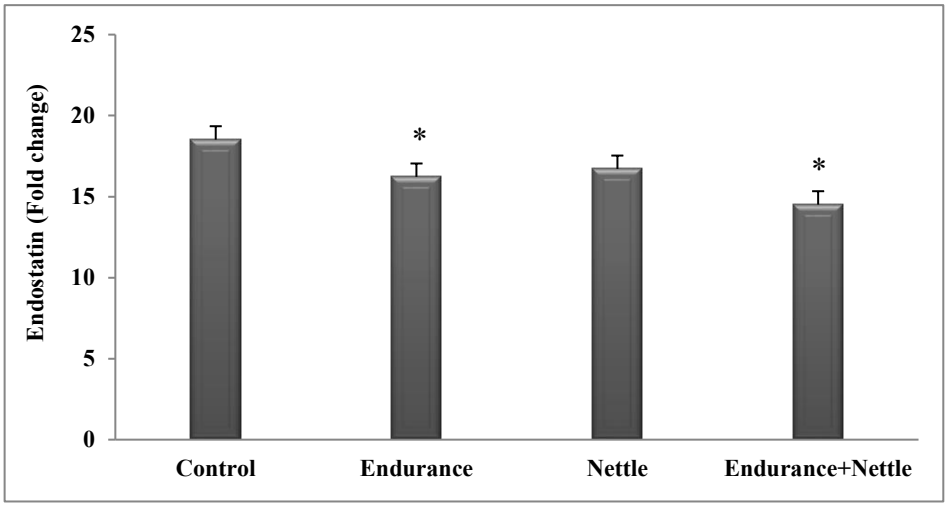

Figure 2. *Endostatin* gene expression changes in different groups

* Difference with the control group (p=0.000).

Discussion

The results of the present research showed that nettle extract and endurance exercises have no significant effect on the level of ***IFN-γ*** gene expression in different groups. Although a non-significant statistical result was observed in the experimental groups compared to the control group. IFN-γ is a pro-inflammatory cytokine produced by cytotoxic T cells, $CD4^+$ T cells, and NK cells following immune system activation and inflammatory stimuli, and acts against viral and bacterial infections (28). The results of the present study do not agree with the findings of several studies. Golzari et al. showed that eight weeks of combined strength-endurance training in women with atherosclerosis led to a significant decrease in IFN-γ levels. Most likely to related to other physiological parameters (17). Wang et al. showed that 12 weeks of exercise training did not change IFN-γ levels and did not reduce inflammatory markers. Wang et al.'s findings support the findings of this research (29). However, Golzari and Wang's findings showed that eight weeks of concurrent training had beneficial anti-inflammatory effects by reducing the production of IFN-γ and IL-17. Physical activities increase the capacity of oxidative enzymes in muscles due to increased mitochondrial density. In addition, increasing the activity of the electron transport chain enzymes increases the activity of the enzymes involved in fat oxidation, especially the enzymes of the beta oxidation cycle and protein lipase (17,29). It seems that the difference of different reports about the response of IFN-γ levels to sports activities originate from different factors that were different in different studies and thus brought contradictory results. Among these factors, we can mention the use of different protocols with different intensities in exercise in human or animal sample. It seems that the effect of immunosuppressive factors did not change IFN-γ in animal subjects with melanoma. It seems that the lack of change in IFN-γ levels is due to the intensity, duration and type of sports activities, and consumption of herbal medicines (30,31). Also, the results of the research showed that endurance training and nettle extract have a decrease in the level of *Endostatin* gene expression in the experimental groups compared to the control group, which reached a significant level in the training and combination groups. Previous studies describe multiple roles of Endostatin in modulation of endothelial cells' behavior. For example, Endothelin induces endothelial cell apoptosis and regulates endothelial cell migration and growth (32). In addition, several studies have shown that Endostatin inhibits tumor angiogenesis and tumor metastasis by limiting blood supply to tumors. It can deprive tumors of nutrients and is considered a potential anticancer agent in the treatment of malignant tumors. However, higher liver tissue Endostatin levels were found in various malignancies such as breast cancer, non-small-cell lung cancer, renal cell carcinoma, and soft tissue sarcoma. In addition, Endostatin tissue levels are significantly increased in patients with gastric cancer (33). In the present study, Endostatin levels were higher in the control group (Melanoma) than in other groups. Therefore, it is possible that an increase in tissue Endostatin levels can be used as a biomarker for early detection and prediction of various types of cancer (10). Several studies showed a close relationship between increased Endostatin tissue levels and tumor stage in gastric cancer (34). However, other studies have shown conflicting results (10). Endostatin has various antitumor functions through the regulation of various receptors, including inhibition of angiogenesis and suppression of migration and invasion of tumor cells (35). Consequently, elevated liver Endostatin levels in advanced cancer may be attributed to production by cancerous tissues during tumor progression (36). On the other hand, a recent study provided strong evidence that Endostatin was a potent mediator of systemic inflammation, and this could better explain the association with advanced tumor stages. The results show that Endostatin tissue levels may play a role in cancer progression. In accordance with our findings, Fujita et al. also found that serum levels of Endostatin increased in gastric cancer patients. It also shows that Endostatin serum levels can be an important prognostic biomarker in predicting the survival of patients with gastric cancer (36). Consistent with our findings, Feldman et al. showed an increase in plasma Endostatin levels in colorectal patients with liver metastasis (37). Although Endostatin is a potent antiangiogenic agent in tumor lymph node involvement, circulating levels of Endostatin may not be sufficient to tip the angiogenic balance toward anti-angiogenesis. Previous research has shown that the effect of Endostatin on endothelial cells depends on the duration of its exposure. In addition, Endostatin level is important for optimal inhibitory effect. Celik et al. showed that higher and lower doses of Endostatin have a lower inhibitory effect on lymph node involvement (38). Another reason for conflicting findings between studies in different cancers may be differences in angiogenic pathways in distinct tumor types (39). Plants contain several active chemical compounds at the same time and unlike chemical drugs, they can have synergistic effects and thus affect different aspects of disease pathology simultaneously. In other words, plant extracts rich in biologically active compounds can reduce the growth of cancer cells and induce apoptosis in them at the same time. These plants lead to tumor eradication by preventing angiogenesis and metastasis. Interactions of active compounds in plant extracts with tumors can give the immune system the opportunity to recognize and respond to the tumor cell. Plants contain several bioactive molecules capable of inducing cell protection and response to stresses such as antioxidant enzymes and apoptosis (40). In the present study, the levels of IFN-γ and Endostatin in the extract group did not change significantly compared to the control group. The lack of a significant difference in the extract group may be due to the use of animal samples as well as the dosage and duration of extract consumption. Nettle plant extract may exert biological anticancer activities through various mechanisms, such as antioxidant and anti-mutagenic properties and induction or inhibition of key processes in cell metabolism (40). The most related explanation for the significant anticancer effect of nettle plant is the known content of flavonoids. Among the bioactive molecules of nettle plant, flavonoids are polyphenolic compounds that are able to induce anticancer effects through various mechanisms, such as antioxidant activity, apoptosis induction, and inhibition of cell growth. In fact, several flavonoid-rich plants have disease-preventive and therapeutic properties, and consumption of flavonoid-rich vegetables and fruits in particular is associated with reduced cancer risk. Stinging nettle may be used as a bioactive nutrient in cancer therapy to prevent or reduce cancer without providing side effects of current anticancer treatments. However, further studies are needed to identify the pure bioactive molecules in this plant to better understand its multiple anticancer actions and explore these potentials in fighting human cancers.

Conclusion

The results of the study showed that endurance training combined with the consumption of nettle extract may inhibit angiogenesis in tumor tissue of melanoma-infected mice by reducing endostatin. Also, nettle extract may exert its anticancer activities through antioxidant properties and anti-apoptotic effects.

Acknowledgement

We would like to thank and appreciate the efforts of Abed Natanzi, who helped us in the implementation of this research group work, and the efforts of the animal laboratory department of Khatam Al Anbia Hospital.

Funding sources

This article is part of the title of the doctoral thesis of Ayatollah Amoli Branch and its budget was fully paid personally by Vahid Zolghadri.

Ethical statement

This research was approved by the Ethics Committee of the Islamic Azad University, Marvdasht branch, with ethics number IR.IAU.M.REC.1399.008.

Conflicts of interest

There is no conflict of interest.

Author contributions

V. Z. drafted the manuscript, and his supervisors and advisors provided guidance and advice in writing all parts of the thesis and article.

Data availability statement

The data used in the research is available in the text of the student's thesis and in the central library of Ayatollah Amoli Branch of Islamic Azad University.

References

1. Qadir MI. Skin cancer: Etiology and management. Pak J Pharm Sci. 2016;29(3):999-1003. [View at Publisher] [PMID] [Google Scholar]
2. Ahmed B, Qadir, MI, Ghafoor S. Malignant Melanoma: Skin Cancer-Diagnosis, Prevention, and Treatment. Crit Rev Eukaryot Gene Expr. 2020;30(4):291-7. [View at Publisher] [DOI] [PMID] [Google Scholar]
3. Pestka S, Krause CD, Walter MR. Interferons, interferon-like cytokines, and their receptors. Immunol Rev. 2004;202:8-32. [View at Publisher] [DOI] [PMID] [Google Scholar]
4. Lilkova E, Petkov P, Ilieva N, Krachmarova E, Nacheva G, Litov L. Molecular modeling of the effects of glycosylation on the structure and dynamics of human interferon-gamma. J Mol Model. 2019;25(5):127. [View at Publisher] [DOI] [PMID] [Google Scholar]
5. Mendoza JL, Escalante NK, Jude KM, Bellon JS, Su L, Horton TM, et al. Structure of the IFNγ receptor complex guides design of biased agonists. Nature. 2019;567(7746):56-60. [View at Publisher] [DOI] [PMID] [Google Scholar]
6. Ivashkiv LB. IFNγ: signalling, epigenetics and roles in immunity, metabolism, disease and cancer immunotherapy. Nat Rev Immunol. 2018;18(9):545-58. [View at Publisher] [DOI] [PMID] [Google Scholar]
7. Tau GZ, Cowan SN, Weisburg J, Braunstein NS, Rothman PB. Regulation of IFN-γ signaling is essential for the cytotoxic activity of CD8+ T cells. J Immunol. 2001;167(10):5574-82. [View at Publisher] [DOI] [PMID] [Google Scholar]
8. Thieu VT, Yu Q, Chang H, Yeh N, Nguyen ET, Sehra S, et al. Stat4 is required for T-bet to promote IL-12-dependent Th1 fate determination. Immunity. 2008;29(5):679-90. [View at Publisher] [DOI] [PMID] [Google Scholar]
9. Burke JD, Young HA. IFN- γ : a cytokine at the right time, is in the right place. Semin Immunol. 2019;43:101280. [View at Publisher] [DOI] [PMID] [Google Scholar]
10. Masiak W, Szponar A, Chodorowska G, Dąbrowski A, Pedowski T, Wallner G. Evaluation of Endostatin and EGF serum levels in patients with gastric cancer. Pol Przegl Chir. 2011;83(1):42-7. [View at Publisher] [DOI] [PMID] [Google Scholar]
11. Zhuo W, Chen Y, Song X, Luo Y. Endostatin specifically targets both tumor blood vessels and lymphatic vessels. Front Med. 2011;5(4):336-40. [View at Publisher] [DOI] [PMID] [Google Scholar]
12. Wan YY, Tian GY, Guo HS, Kang YM, Yao ZH, Li XL,et al. Endostatin, an angiogenesis inhibitor, ameliorates bleomycin-induced pulmonary fibrosis in rats. Respir Res. 2013;14(1):56. [View at Publisher] [DOI] [PMID] [Google Scholar]
13. Bai YJ, Huang L-Z, Zhou A-Y, Zhao M, Yu W-Z, Li X-X. Antiangiogenesis effects of Endostatin in retinal neovascularization. J Ocul Pharmacol Ther. 2013;29(7):619-26. [View at Publisher] [DOI] [PMID] [Google Scholar]
14. McTiernan A. Mechanisms linking physical activity with cancer. Nat Rev Cancer. 2008;8(3):205-11. [View at Publisher] [DOI] [PMID] [Google Scholar]
15. Zamani A, Salehi I, & Alahgholi-Hajibehzad M. Moderate Exercise Enhances the Production of Interferon-γ and Interleukin-12 in Peripheral Blood Mononuclear Cells. Immune Netw. 2017;17(3):186-91. [View at Publisher] [DOI] [PMID] [Google Scholar]
16. Vijayaraghava A, K R. Alteration of Interferon Gamma (IFN-γ) in Human Plasma with Graded Physical Activity. J Clin Diagn Res. 2014;8(6), BC05-7. [View at Publisher] [DOI] [PMID] [Google Scholar]
17. Golzari Z, Shabkhiz F, Soudi S, Kordi MR, Hashemi SM. Combined exercise training reduces IFN-γ and IL-17 levels in the plasma and the supernatant of peripheral blood mononuclear cells in women with multiple sclerosis. Int Immunopharmacol. 2010;10(11):1415-9. [View at Publisher] [DOI] [PMID] [Google Scholar]
18. Shakoor E, Salesi M, Koushkie Jahromi M, Sadegh H, Karimi MH, Yusof A. The Effect of Exercise on Interferon Gamma, Body Fat and BMI of Kidney Transplant Patients. Rev Bras Med Esporte. 2018;24(5):333-7. [View at Publisher] [DOI] [Google Scholar]
19. Gu JW, Gadonski G, Wang J, Makey I, Adair TH. Exercise increases Endostatin in circulation of healthy volunteers. BMC Physiol.2004:4:2. [View at Publisher] [DOI] [PMID] [Google Scholar]
20. Khosroshahi AY, Habibi Khaniani B, Naghdibadi H. Review on taxol as the most important anticancer natural drug. J. Med. Plants 2006, 5(18): 1-10 [View at Publisher] [Google Scholar]
21. Bourgeois C, Leclerc ÉA, Corbin C, Doussot J, Serrano V, Vanier J.-R, et al. Nettle (Urtica dioica L.) as a source of antioxidant and anti-aging phytochemicals for cosmetic applications. C R Chim. 2016;19(9):1090-100. [View at Publisher] [DOI] [Google Scholar]
22. Fattahi S, Ardekani AM, Zabihi E, Abedian Z, Mostafazadeh A, Pourbagher R, et al. Antioxidant and apoptotic effects of an aqueous extract of Urtica dioica on the MCF-7 human breast cancer cell line. Asian Pac J Cancer Prev. 2013;14(9):5317-23. [View at Publisher] [DOI] [PMID] [Google Scholar]
23. Levy A, Sivanesan D, Murugan R, Jornadal J, Quinonez Y, Jaffe M. Urtica dioica Induces cytotoxicity in human prostate carcinoma LNCaP Cells: Involvement of oxidative stress, mitochondrial depolarization and apoptosis. Trop J Pharm Res. 2014;13(5):711-7. [View at Publisher] [DOI] [Google Scholar]
24. Mohammadi A, Mansoori B, Aghapour M, Shirjang S, Nami S, Baradaran B. The Urtica dioica extract enhances sensitivity of paclitaxel drug to MDA-MB-468 breast cancer cells. Biomed Pharmacother. 2016; 83:835-42. [View at Publisher] [DOI] [PMID] [Google Scholar]
25. Samuels N, Morag O, Maimon Y. Use of herbal medicine for cancer treatment-related toxicities]. Harefuah. 2015;154(1):43-6, 67. [View at Publisher] [PMID] [Google Scholar]
26. Yin SY, Wei WC, Jian FY, Yang NS. Therapeutic applications of herbal medicines for cancer patients. Evid Based Complement Alternat Med. 2013; 2013:302426. [View at Publisher] [DOI] [PMID] [Google Scholar]
27. Amjadi F, Javanmard SH, Zarkesh-Esfahani H, Khazaei M, Narimani M. Leptin promotes melanoma tumor growth in mice related to increasing circulating endothelial progenitor cells numbers and plasma NO production. J Exp Clin Cancer Res. 2011;30(1):21. [View at Publisher] [DOI] [PMID] [Google Scholar]
28. Thomson AW, Lotze MT. The Cytokine Handbook, Two-Volume Set. Hudson, Texas: Gulf Professional Publishing; 2003. [View at Publisher] [Google Scholar]
29. Wang MY, An LG. Effects of 12 weeks'tai chi chuan practice on the immune function of female college students who lack physical exercise. Biol Sport. 2011;28(1):45-9. [View at Publisher] [DOI] [Google Scholar]
30. Irving BA, Lanza IR, Henderson GC, Rao RR, Spiegelman BM, Nair KS. Combined training enhances skeletal muscle mitochondrial oxidative capacity independent of age. J Clin Endocrinol Metab. 2015;100(4):1654-63. [View at Publisher] [DOI] [PMID] [Google Scholar]
31. Gopal A, Mondal S, Gandhi A, Arora S, Bhattacharjee J. Effect of integrated yoga practices on immune responses in examination stress-A preliminary study. Int J Yoga. 2011;4(1):26-32. [View at Publisher] [DOI] [PMID] [Google Scholar]
32. Jiang W-G, Lu X-A, Shang B-Y, Fu Y, Zhang SH, Zhou D, et al. Genetically engineered Endostatin-lidamycin fusion proteins effectively inhibit tumor growth and metastasis. BMC Cancer. 2013:13:479. [View at Publisher] [DOI] [PMID] [Google Scholar]
33. Li L-X, Zhang Y-L, Zhou L, Ke M-L, Chen J-M, Fu X, et al. Antitumor efficacy of a recombinant adenovirus encoding Endostatin combined with an E1B55KD-deficient adenovirus in gastric cancer cells. J Transl Med. 2013:11:257 [View at Publisher] [DOI] [PMID] [Google Scholar]
34. Woo IS, Kim K-A, Jeon H-M, Hong SH, Young Rho S, Jin Koh S,et al. Pretreatment serum Endostatin as a prognostic indicator in metastatic gastric carcinoma. Int J Cancer. 2006;119(12):2901-6 [View at Publisher] [DOI] [PMID] [Google Scholar]

35. Xu R, Ma N, Wang F, Ma L, Chen R, Chen R,et al., "Results of a randomized and controlled clinical trial evaluating the efficacy and safety of combination therapy with Endostar and S-1 combined with oxaliplatin in advanced gastric cancer. Onco Targets Ther. 2013:6:925-9. [View at Publisher] [DOI] [PMID] [Google Scholar]
36. Fujita T, Gotohda N, Kato Y, Kinoshita T, Takahashi S, Konishi M, et al. Clinicopathological features of stomach cancer with invasive micropapillary component. Gastric Cancer. 2012;15(2):179-87. [View at Publisher] [DOI] [PMID] [Google Scholar]
37. Feldman AL, Alexander HR Jr, Bartlett DL, Kranda KC, Miller MS, Costouros NG, et al. A prospective analysis of plasma Endostatin levels in colorectal cancer patients with liver metastases. Ann Surg Oncol. 2001;8(9):741-5. [View at Publisher] [DOI] [PMID] [Google Scholar]
38. Celik I, Sürücü O, Dietz C, Heymach JV, Force J, Höschele I, et al. Therapeutic efficacy of Endostatin exhibits a biphasic dose-response curve. Cancer Res. 2005;65(23):11044-50. [View at Publisher] [DOI] [PMID] [Google Scholar]
39. Tjin Tham Sjin RM, Naspinski J, Birsner AE, Li C, Chan R, Lo K-M, et al. Endostatin therapy reveals a U-shaped curve for antitumor activity. Cancer Gene Ther. 2006;13(6):619-27. [View at Publisher] [DOI] [PMID] [Google Scholar]
40. Esposito S, Bianco A, Russo R, Di Maro A, Isernia C, Pedone PV. Therapeutic Perspectives of Molecules from Urtica dioica Extracts for Cancer Treatment. Molecules. 2019;24(15):2753. [View at Publisher] [DOI] [PMID] [Google Scholar]

Cite this article as:
Zolghadri V, Barari A, Abbasi Daloii A, Abed Natanzi H. The effect of nettle extract consumption and endurance training on the expression of IFN-γ and Endostatin in the liver tissues of mice with melanoma. *Med Lab J.* 2025;19(X):X. http://dx.doi.org/10.29252/mlj.19.X.X