Research Article

Modulatory effects of aerobic exercise and Urtica dioica hydroalcoholic extract on tumor growth and Interleukin-10 levels in a Murine melanoma model

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Abstract

To investigate the individual and combined effects of six weeks of aerobic exercise and hydroalcoholic extract of Urtica dioica (nettle) on tumor volume, body weight, and interleukin-10 (IL-10) levels in both serum and tumor tissue in male C57BL/6 mice bearing B16F10 melanoma tumors. Thirty-two male C57BL/6 mice (6–8 weeks old, 12–14 g) were randomly assigned to four equal groups (n=8): Control, Aerobic Exercise (AE), Nettle Extract (NE), and Combined (AE+NE). Melanoma was induced via surgical implantation of B16F10 tumor fragments. The AE protocol consisted of treadmill running for 6 weeks (5 days/week, progressing from 20 to 30 minutes/session at 6–16 m/min). The NE group received intraperitoneal injections of hydroalcoholic nettle extract (200 mg/kg body weight). The combined group received both interventions. The combined AE+NE group showing a significant reduction in tumor volume compared to the control group (p=0.0186). One-way ANOVA revealed significant differences in serum IL-10 across groups (F=9.811, p=0.0001), with significant increases observed in the AE (p=0.0003) and AE+NE (p = 0.0005) groups compared to controls. For tumor IL-10, a significant difference was also found across groups (F=3.047, p= 0.0451), with the combined AE+NE group showing a significant decrease (p =0.0435) compared to the control group. No significant correlation was found between serum and tumor IL-10 levels in any group (p>0.05). The combined intervention significantly suppressed tumor growth compared to controls and was associated with increased serum IL-10 levels but decreased IL-10 levels within the tumor microenvironment, suggesting a complex, compartment-specific immunomodulatory effect.

Key Words: Exercise, Melanoma, IL-10, Nettle extract

Introduction

Melanoma remains one of the most biologically aggressive malignancies, characterized by rapid proliferation, high metastatic potential, and a remarkable capacity to evade immune surveillance. Despite advances in targeted therapy and immune checkpoint inhibition, therapeutic resistance and tumor relapse continue to limit long-term survival, highlighting the need for adjunctive strategies that modulate the tumor microenvironment (TME) (Qasim et al., 2025; Sikorski et al., 2025). The TME is a complex network of tumor cells, stromal components, and immune mediators, where cytokines play a central regulatory role in tumor progression or suppression (Abdul-Rahman et al., 2024). Among these, interleukin-10 (IL-10) has emerged as a pleiotropic cytokine with context-dependent effects, capable of both dampening anti-tumor immunity and promoting cytotoxic responses under specific conditions (Rallis et al., 2022). Its dualistic behavior makes IL-10 a critical target for understanding how systemic and local immune responses can be differentially regulated in melanoma (Emmerich et al., 2012).

Lifestyle-related interventions, particularly aerobic exercise, have gained attention as modulators of cancer biology through systemic immunological and metabolic adaptations (Greco et al., 2025). Exercise induces transient increases in circulating catecholamines and myokines, which mobilize immune effector cells such as natural killer (NK) cells and cytotoxic T lymphocytes into circulation and tumor sites (Koivula; Lynn, 2019). Additionally, regular aerobic activity reduces chronic low-grade inflammation and alters cytokine profiles, including upregulation of anti-inflammatory mediators such as IL-10 (Gonzalo-Encabo et al., 2021). These systemic adaptations may translate into improved immune surveillance and reduced tumor growth, as demonstrated in preclinical melanoma models where exercise reduced tumor burden via enhanced immune cell infiltration and vascular normalization (Hojman, 2017; Pedersen et al., 2016).

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In parallel, increasing interest has been directed toward plant-derived bioactive compounds as complementary anti-cancer agents. Urtica dioica (stinging nettle) contains a diverse array of phytochemicals, including flavonoids, lignans, phenolic acids, and sterols, which exhibit antioxidant, anti-inflammatory, and anti-proliferative properties (Grauso et al., 2020). Experimental studies suggest that nettle extracts can interfere with key oncogenic pathways such as NF-κB signaling, oxidative stress responses, and apoptosis regulation (Wójciak et al., 2024). Importantly, these compounds may also influence cytokine production and immune cell function, thereby reshaping the tumor microenvironment (FranciškoviĆ et al., 2017). However, the interaction between exercise-induced physiological adaptations and phytochemical-mediated signaling remains insufficiently explored. Therefore, investigating their combined effects on tumor progression and IL-10 dynamics may provide new insights into integrative approaches for melanoma control.

Materials and Methods

Animals and ethical statement

This experimental and fundamental study was designed to investigate the effects of aerobic exercise and nettle (Urtica dioica) extract on male laboratory mice bearing melanoma tumors. A total of 32 male C57BL/6 mice, aged 6 to 8 weeks and weighing between 12 and 14 grams, were obtained from the Pasteur Institute of Iran and transferred to the animal facility at Baqiyatallah University of Medical Sciences, Tehran. Following a one-week acclimatization period under controlled laboratory conditions (temperature: 22±1.4°C, relative humidity: 55%, and a 12:12-hour light-dark cycle), the animals were randomly assigned to four equal groups (n=8 per group): Control, Aerobic Exercise, Nettle Extract, and Combined Aerobic Exercise + Nettle Extract. Throughout the study, all animals had ad libitum access to standard rodent chow and water. All procedures were approved by the Institutional Animal Care and Use Committee of Islamic Azad University, Tehran, Iran (Ethical code: IR.IAU.M.REC .1399.008). The study adhered to the NIH Guide for the Care and Use of Laboratory Animals (8th edition, 2011). Every effort was made to minimize animal suffering and to reduce the number of animals used.

Cell line preparation and culture

In this study, the murine melanoma cell line B16F10 was obtained from the Pasteur Institute of Iran. Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and antibiotics (penicillin and streptomycin). The resulting cell pellet was resuspended in complete culture medium and transferred to sterile culture flasks. Flasks were incubated at 37°C in a humidified atmosphere containing 5% CO_2. Cell attach-ment and morphology were monitored regularly under an inverted microscope, and the medium was refreshed routinely. Once the cell confluency reached approximately 70%, cells were washed with sterile phosphate-buffered saline (PBS), detached using trypsin-EDTA solution, neutralized with serum-containing medium, centrifuged, and subcultured into new flasks to expand the population for experimental use.

Viability assessment of cell suspension

To determine the viability of B16F10 cells prior to injection, the trypan blue exclusion assay was employed. A 1:5 mixture of cell suspension and 0.4% trypan blue in normal saline was prepared and incubated for 1–2 minutes. Viable cells (unstained) and non-viable cells (blue-stained) were counted using a Neubauer hemocytometer under a light microscope. The viability of the cell suspension was calculated to be >95%. The number of viable cells per milliliter of culture medium was calculated by multiplying the average cell count in the 16-square grid by the dilution factor, based on the known volume of the counting chamber. This method ensured accurate quantification of cell density and viability prior to tumor induction.

Tumor stock preparation via subcutaneous injection

To establish a tumor-bearing animal model, a suspension containing 700,000 viable B16F10 cells per milliliter of sterile PBS was prepared and injected subcutaneously into the flank region of five male C57BL/6 mice (syngeneic to the experimental animals). Prior to injection, the target area was sterilized with alcohol swabs, and the cell suspension was administered using insulin syringes. Post-injection, mice were housed individually under controlled conditions (25°C, standard diet and water ad libitum) for three weeks. Daily monitoring was conducted to assess general health and nutrition, and weekly physical examinations were performed to evaluate tumor development and confirm successful engraftment.

Tumor induction via surgical implantation

In this study, tumor induction was performed using a surgical implantation method to establish a stable melanoma model in laboratory mice. This approach was selected because surgical implantation of tumor fragments produces a more consistent and homogeneous tumor take rate compared to direct cell injection, better recapitulates the heterogeneous architecture of solid melanomas, and minimizes the risk of cell leakage or non-uniform engraftment. A total of 32 male C57BL/6 mice (6–8 weeks old) were obtained from the Pasteur Institute in Karaj. Prior to surgery, the flank region of each mouse was prepared by shaving with an electric clipper followed by depilation using a chemical hair removal cream. To minimize skin irritation, animals were allowed to rest for 24 hours under laboratory conditions before undergoing surgery. Tumor tissue was harvested from five donor

mice (male C57BL/6) previously injected with B16F10 melanoma cells. These mice were euthanized via cervical dislocation, and subcutaneous tumors were sterilized with ethanol, excised using sterile forceps and scissors, and dissected into 2–3 mm fragments free of fat and vasculature. The tumor fragments were stored in sterile Petri dishes containing physiological saline until implantation. For implantation, mice were anesthetized with an intraperitoneal injection of a ketamine-xylazine mixture (ratio 2:1), with each mouse receiving 100 µL of the solution. Once fully anesthetized, a small incision was made in the prepared flank area, and a subcutaneous tunnel was created using sterile instruments. A tumor fragment was inserted at the distal end of the tunnel, and the incision was closed using surgical adhesive and skin staples. Postoperatively, mice were housed individually at 25°C and monitored daily. The surgical site was disinfected with povidone-iodine to prevent infection. This method enabled the establishment of a reproducible and localized tumor model suitable for evaluating therapeutic interventions across four experimental groups.

Experimental interventions: Aerobic exercise and nettle extract administration

In this study, the aerobic exercise protocol consisted of six weeks of treadmill running, structured into three progressive two-week phases. During the initial phase (weeks 1–2), mice underwent familiarization training at a gradually increasing speed of 6 to 8 meters per minute, for 20 minutes per session, five days per week. In the second phase (weeks 3–4), continuous aerobic training was implemented at speeds ranging from 10 to 12 meters per minute, with each session lasting 25 minutes. In the final phase (weeks 5–6), the intensity was further increased to 14 –16 meters per minute, and the duration extended to 30 minutes per session. The exercise protocol (6–16 m/min) was selected based on prior studies defining moderate-intensity aerobic exercise for C57BL/6 mice (Hojman et al., 2017); however, we did not perform direct physiological validation (e.g., blood lactate or $\%VO_2max$), which is a limitation. To control for the psychological stress associated with treadmill exposure, mice in the control group were placed on a stationary treadmill for an equivalent duration. However, we acknowledge that this does not fully control for noise and vibration, which is a limitation of the study.

In the extract-treated groups, tumor-bearing mice received intraperitoneal (IP) injections of hydroalcoholic nettle (Urtica dioica) extract at a dose of 200 mg/kg body weight. Although oral gavage would be more clinically relevant, the IP route was chosen to ensure consistent bioavailability and to avoid first-pass metabolism that could alter the phytochemical composition. This is acknowledged as a limitation. The extract was prepared from air-dried leaves of nettle plants collected from in Amol, Iran. The dried plant material was ground into powder and subjected to hy--droalcoholic extraction using 70% methanol via percolation. The resulting extract was concentrated using rotary evaporation and stored under refrigerated conditions until administration.

Body weight measurement

Body weight was measured at end of the six-week intervention period using a calibrated digital scale with appropriate sensitivity for laboratory animals. Weighing was conducted at consistent time points under controlled environmental conditions (stable temperature, minimal stress, and absence of external stimuli). Each mouse was individually removed from its cage, allowed to calm briefly, and then placed on the scale. Body weights were recorded in grams and documented separately for each experimental group (Control, Aerobic Exercise, Nettle Extract, and Combined Intervention). All procedures were performed in accordance with ethical standards to minimize stress and prevent harm to the animals.

Tumor volume measurement

Tumor volume was assessed at end of study. Two perpendicular dimensions of each tumor were measured using a digital caliper: the longest axis was recorded as the tumor length (L), and the axis perpendicular to it as the width (W). Tumor volume (V) was calculated using the standard ellipsoid formula:

$$V = \frac{\pi}{6} \times W \times L^2$$

This method enabled consistent, non-invasive, and quantitative monitoring of tumor growth dynamics across the experimental period.

Measurement of serum and tissue IL-10 concentration

The concentration of interleukin-10 (IL-10) in both serum and tumor tissue samples was quantified using a mouse specific enzyme linked immunosorbent assay (ELISA) kit (R&D Systems, catalog number M1000B, sensitivity 2.0 pg/mL, intra assay CV 4.5%, inter assay CV 7.2%) following the manufacturer's protocol. For serum analysis, whole blood was collected and centrifuged at 4 °C to separate the serum, which was carefully aliquoted and stored at −80 °C until analysis. For tumor tissue assessment, each tumor was homogenized in phosphate buffered saline (PBS) containing 0.05% Tween 20 and a protease inhibitor cocktail (Roche, Basel, Switzerland). The homogenate was centrifuged at 12,000 × g for 15 min at 4 °C, and the supernatant was collected. Total protein concentration was determined using a BCA assay (Thermo Fisher Scientific), and IL-10 levels were normalized to total protein (pg/mg protein). A defined volume of each sample was added to ELISA microplate wells pre coated with IL-10 specific capture antibodies. All samples were run in duplicate. The assay procedure included in-cubation, washing

steps, addition of enzyme conjugated secondary antibodies, and substrate development. The resulting colorimetric reaction, directly proportional to IL-10 concentration, was measured at a wavelength of 450 nm using a microplate reader (BioTek ELx800, USA). Final concentrations were calculated by referencing a standard curve generated from known IL-10 concentrations using the kit's dedicated analysis software.

Statistical analysis

All statistical analyses were performed using GraphPad Prism 9. Data are presented as mean ± standard deviation (SD) or standard error of the mean (SEM). Comparisons among multiple groups were carried out using one-way analysis of variance (ANOVA), followed by appropriate post-hoc tests (Tukey's test) when significant differences were found. To evaluate the relationship between two continuous variables, Pearson's correlation coefficient was calculated. A p-value of less than 0.05 was considered statistically significant for all tests.

Results

The levels of the evaluated variables including body weight, tumor volume, and serum and tissue concentrations of IL-10 are presented as mean ± standard deviation in Table 1. The results of the one-way ANOVA test indicated a significant difference in tumor volume among the study groups. Post-hoc Tukey test revealed that the combined aerobic exercise and nettle extract group exhibited a significant reduction in tumor volume compared to the control group (1480.35±718.27 mm³ vs. 3179.81±882.44 mm³, p=0.0186), representing a 53.4% decrease. Although the aerobic exercise alone group (2244.71 ± 438.68 mm³,) and nettle extract alone group (2194.64 ± 282.24 mm³) showed reductions compared to controls, these differences did not reach statistical significance (p>0.05). No significant difference was observed between the AE and NE groups (p>0.05) (Table 1).

The changes in serum IL-10 levels across different research groups are shown in Figure 1. The results of the one-way ANOVA test indicated a significant difference in this factor among the study groups (F=9.811, p=0.0001). The post-hoc Tukey test results showed that the aerobic training alone group (p=0.0003) and the combined aerobic training and nettle extract group (p=0.0005) exhibited a significant increase in serum IL-10 levels in animals with melanoma model. Meanwhile, changes in this factor between the other groups were not significant (p>0.05).

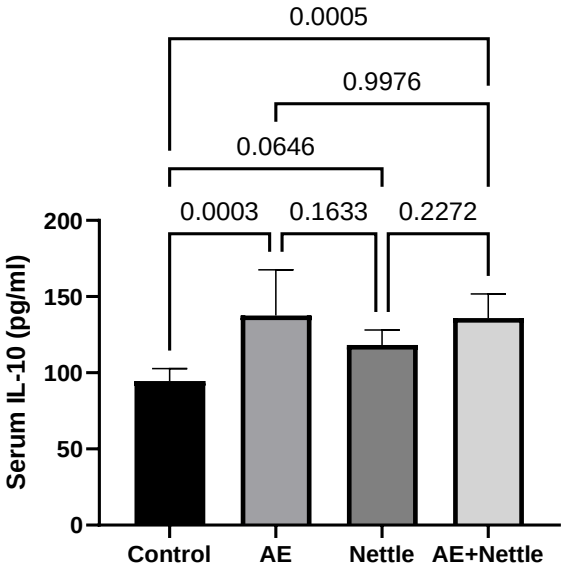

Figure1. Serum levels of IL-10 at different groups of study. Data are show as means±SD. * show the significant difference P<0.05, *** p<0.0001. Abbreviation: AE: Aerobic Exercise.

Changes in tumor IL-10 levels in different research groups are shown in Figure 2. The results of a one-way ANOVA indicated that there was a significant difference in this factor among the different research groups (F=3.047, p=0.0451). The results of Tukey's post hoc test showed that only the combination of aerobic exercise and nettle extract (p=0.0435) caused a significant decrease in tumor IL-10 levels in animals with melanoma model. However, changes in this factor were not significant among the other groups (p>0.05).

Correlation

In the present study, the Pearson correlation statistical method was also used to investigate the relationship between serum IL-10 levels and tumor tissue IL-10 levels among different study groups (Figure 3). The results of this statistical test indicated that there is no correlation between these variables in serum and tissue (p>0.05). The Pearson's r and p-values for each group were as follows: Control group: r=0.435, p=0.280; Aerobic Exe-

Table 1. Descriptive statistics of the studied variables across experimental groups (Mean ± Standard Deviation)

Groups	Weight(gr)	Tumor. Volume(mm3)	IL.10.serumi(pg/ml)	IL.10.Tumor(pg/ml)
Control	15.8±1.10	3179.81±882.44	94.63±14.94	133.31±2.97
AE	15.2±0.84	2244.71±438.68	137.65±45.28	117.03±4.37
Nettle	15.75±1.14	2194.64±282.24	118.24±3.5	117.74±2.86
AE+Nettle	14.6±1.14	1480.35±718.27*	135.96±6.43	113.20±4.92*

Abbreviation: AE: Aerobic Exercise. Significant post hoc comparisons (Tukey's test): For tumor volume: AE+NE vs. Control p = 0.0186 (*). For serum IL-10: AE vs. Control p = 0.0003 (*), AE+NE vs. Control p = 0.0005 (*). For tumor IL-10: AE+NE vs. Control p = 0.0435 (*).

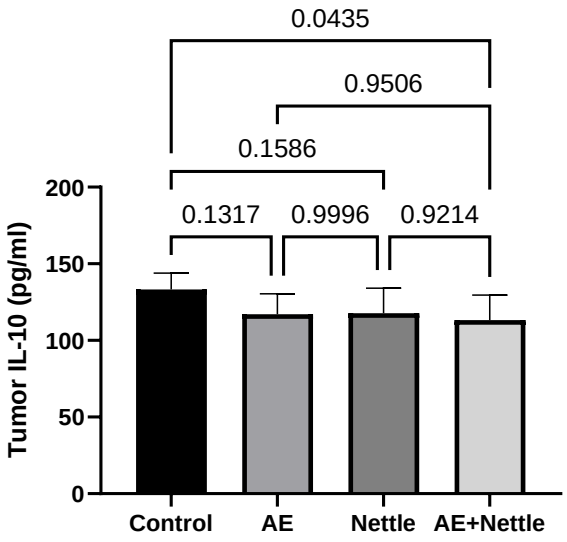

Figure 2. Tumor levels of IL-10 at different groups of study. Data are show as means ± SD. * show the significant difference P<0.05, *** p<0.0001. Abbreviation: AE: Aerobic Exercise.

-rcise group: r=-0.324, p=0.4332; Nettle Extract group: r=-0.125, p=0.7674; Combined group: r=-0.575, p=0.1353.

Discussion

The most striking finding of the present study is the significant 53.4% reduction in tumor volume observed in the combined aerobic exercise and nettle extract group compared to controls, with the combined intervention demonstrating superior anti-tumor efficacy relative to either intervention alone. This synergistic effect is clinically meaningful and suggests that the physiological adaptations induced by exercise and the bioactive phytochemicals present in Urtica dioica may converge on complementary pathways that suppress melanoma growth more effectively than either modality alone. The observed tumor volume reduction in the combined group (1480.35±718.27 mm³) compared to controls (3179.81±882.44 mm³) represents a substantial anti-tumor effect. Although both the aerobic exercise alone group (2244.71±438.68 mm³, 29.4% reduction) and nettle extract alone group (2194.64±282.24 mm³, 31.0% reduction) showed moderate reductions that did not reach statistical significance, the lack of significance may be attributable to the relatively small sample size and the high variability within the control group. The fact that the combined group achieved both statistical significance and a greater magnitude of effect strongly supports an additive or synergistic interaction between these two interventions.

Several mechanisms may explain this enhanced tumor suppression. First, aerobic exercise has been shown to improve tumor perfusion and reduce hypoxia, limiting hypoxia-inducible factor-1α (HIF-1α) signaling which drives angiogenesis and metabolic adaptation in tumors (Jia et al., 2021). Second, the combined group demonstrated reduced intratumoral IL-10 levels, which may relieve immunosuppression within the tumor microenvironment, allowing more effective immune-mediated tumor clearance. Third, the concurrent increase in serum IL-10 (observed in both AE and AE+NE groups) suggests enhanced systemic resolution of inflammation, which may prevent chronic immune exhaustion and preserve anti-tumor immunity. The divergence between systemic and intratumoral IL-10 levels provides a plausible mechanistic explanation for the superior tumor suppression observed in this group.

It is noteworthy that the combined intervention achieved significant tumor reduction despite the fact that neither exercise alone nor nettle extract alone reached statistical significance. This pattern strongly suggests that these interventions operate through distinct but complementary mechanisms. One possibility is that exercise enhances immune cell mobilization and trafficking (Pedersen et al., 2016), while nettle extract sensitizes tumor cells to immune-mediated killing or directly inhibits proliferation via apoptotic pathways (Hodroj et al., 2020). The reduction in intratumoral IL-10 observed only in the combined group may reflect a shift from M2-like to M1-like macrophage polarization, which would enhance anti-tumor immunity and contribute to reduced tumor burden. However, because we did not measure macrophage polarization markers (iNOS, Arg1, CD86, CD206) or quantify immune cell infiltration, these mechanistic interpretations require direct experimental validation in future studies.

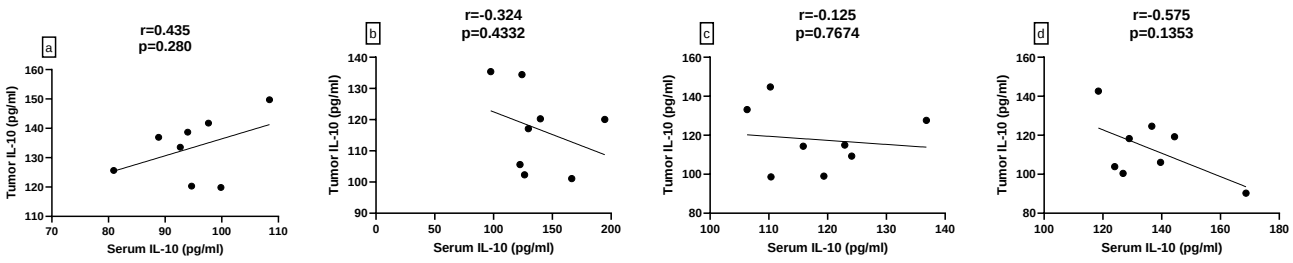

Figure 3. Pearson correlation between IL-10 at serum and tumor tissue at different groups of study (a: Control group, b: Aerobic exercise group, c: Nettle extract group, d: Aerobic exercise+ Nettle extract group). Data are show as means ± SD.

The present findings demonstrate that the combination of aerobic exercise and Urtica dioica extract exerts a stronger inhibitory effect on melanoma tumor growth than either intervention alone, suggesting a synergistic interaction at the cellular and molecular levels. One plausible mechanism involves the modulation of tumor metabolism and vascularization. Aerobic exercise improves tumor perfusion and reduces hypoxia, which in turn can limit hypoxia-inducible factor-1α (HIF-1α) signaling (Jia et al., 2021). Concurrently, bioactive compounds in nettle extract may suppress angiogenic pathways such as VEGF signaling and inhibit endothelial cell proliferation. Together, these effects likely contribute to a less favorable microenvironment for tumor expansion (Jia et al., 2021; Savage et al., 2023). However, the present study did not measure perfusion, hypoxia, HIF-1α, or VEGF; therefore, these proposed mechanisms remain speculative.

A notable observation in this study is the divergence between systemic and intratumoral IL-10 levels. Aerobic exercise, alone or combined with nettle extract, increased circulating IL-10, which is consistent with its role in resolving systemic inflammation and preventing excessive immune activation (Savage et al., 2023). At the cellular level, IL-10 can inhibit NF-κB signaling in macrophages and dendritic cells, reducing the production of pro-inflammatory cytokines such as TNF-α and IL-6 (Wang et al., 1995). This systemic anti-inflammatory effect may improve immune efficiency by preventing chronic immune exhaustion. However, within the tumor microenvironment, reduced IL-10 levels in the combined group suggest a shift toward a more immunostimulatory milieu, potentially enhancing antigen presentation and cytotoxic T-cell activity (Itakura et al., 2011). Nevertheless, because we did not measure macrophage polarization markers (iNOS, Arg1, CD86, CD206) or quantify immune cell populations (NK cells, CD8+ T cells), these interpretations are preliminary and require direct experimental validation.

This compartment-specific behavior of IL-10 may also reflect changes in immune cell polarization (Chuang et al., 2016). Tumor-associated macrophages (TAMs) exist along a spectrum from pro-inflammatory (M1-like) to anti-inflammatory (M2-like) phenotypes (Hernández-Peralta et al., 2024). High intratumoral IL-10 is typically associated with M2 polarization, which supports tumor growth, angiogenesis, and immune suppression (Sun et al., 2024). The reduction of IL-10 within tumor tissue observed in the combined intervention group may indicate a shift toward M1 polarization, characterized by increased production of reactive oxygen species, enhanced antigen presentation, and tumoricidal activity. Exercise has been shown to influence macrophage polarization through AMP-activated protein kinase (AMPK) activ-ation, while phytochemicals in nettle may further reinforce this effect via inhibition of STAT3 signaling (Manoharan & Perumal, 2024; Sag et al., 2008). Another important mechanism involves the mobilization and redistribution of immune effector cells. Exercise-induced increases in epinephrine and IL-6 promote the trafficking of NK cells and CD8+ T cells into tumor sites, where they exert direct cytotoxic effects (Miao et al., 2024; Pedersen et al., 2016). These cells release perforin and granzymes, inducing apoptosis in tumor cells. Simultaneously, nettle-derived compounds may sensitize tumor cells to apoptosis by modulating mitochondrial pathways, including upregulation of Bax and downregulation of Bcl-2 proteins (Hodroj et al., 2020). This dual mechanism may explain the pronounced reduction in tumor volume observed in the combined group.

Oxidative stress regulation also provides a mechanistic link between the interventions. While moderate exercise enhances endogenous antioxidant defenses through activation of pathways such as Nrf2, excessive oxidative stress within tumors can promote DNA damage and tumor progression (Dizdaroglu, 2015). Nettle extract, rich in phenolic antioxidants, may help restore redox balance by scavenging reactive oxygen species and inhibiting lipid peroxidation. At the cellular level, this balance is crucial, as controlled oxidative stress can trigger apoptosis, whereas excessive ROS may promote mutagenesis and tumor adaptation. The interplay between exercise-induced hormesis and phytochemical antioxidant activity likely contributes to improved cellular homeostasis (Wójciak et al., 2024).

Finally, the absence of correlation between serum and tumor IL-10 levels highlights the complexity of cytokine regulation in cancer. Cytokine signaling is highly localized, and systemic measurements may not accurately reflect intratumoral dynamics. This finding aligns with emerging evidence that immune responses in cancer are spatially regulated, with distinct cytokine gradients influencing cell behavior within different compartments. Therefore, therapeutic strategies should consider both systemic and local immune modulation. The combined use of exercise and plant-derived compounds appears to achieve this dual regulation, enhancing systemic anti-inflammatory effects while simultaneously promoting a local tumor environment with reduced IL-10 levels.

The mechanisms underlying the observed additive effect of exercise and nettle extract on tumor growth and compartment-specific IL-10 modulation remain to be elucidated. Based on published literature, several hypotheses can be proposed for future investigation. First, aerobic exercise may improve tumor perfusion and reduce hypoxia, potentially limiting HIF-1α signaling (Jia et al., 2021), and nettle extract might suppress angiogenic pathways such as VEGF signaling – but these possibilities require direct measurement in future studies. Second

, the reduction in intratumoral IL-10 could reflect a shift from M2-like to M1-like macrophage polarization; future work should include phenotyping of tumor-associated macrophages using markers such as iNOS, Arg1, CD86, and CD206. Third, exercise-induced increases in epinephrine and IL 6 might promote NK cell and CD8+ T cell trafficking into tumors (Pedersen et al., 2016; Miao et al., 2024), and nettle compounds could sensitize tumor cells to apoptosis via Bax/Bcl 2 modulation (Hodroj et al., 2020) – both need direct confirmation using flow cytometry and apoptosis assays. Fourth, oxidative stress regulation via Nrf2 and antioxidant pathways may contribute to the observed effects. The present study was not designed to test these molecular mechanisms, and we strongly encourage future research to address these hypotheses using appropriate pharmacological inhibitors, gene knockdowns, or knockout models.

The present study has several limitations. First, the hydroalcoholic extract of Urtica dioica used in this study was not fully characterized phytochemically. Due to laboratory constraints, we did not perform HPLC or LC-MS analysis to determine the fingerprint of major bioactive compounds (e.g., chlorogenic acid, rutin, caffeic acid derivatives), nor did we measure the extraction yield percentage or verify residual solvent (methanol) levels. Additionally, storage conditions are only described as "refrigerated" without specifying the exact temperature or duration of stability. The lack of batch-to-batch quality control and a voucher specimen number for plant authentication further limits reproducibility. Therefore, the lack of detailed chemical characterization of the nettle extract is a significant limitation of this study. Future studies should include comprehensive phytochemical profiling to enable replication and comparison of findings. Second, although we observed significant effects, the sample size was relatively small, and only male mice were used, which limits generalizability to female subjects. Third, no a priori sample size calculation was performed; however, post hoc power analysis for the primary outcome (tumor volume) revealed a Cohen's d effect size of 2.55 and a power of 0.94 (α=0.05), indicating that the sample size of n=8 per group was sufficient to detect the observed effect. Nevertheless, the relatively small sample size remains a limitation, and future studies should include larger and more diverse samples (including females). Fourth, weekly tumor volume measurements were not recorded; only final tumor volume at the endpoint was measured. Consequently, we cannot provide a figure showing tumor growth over time, and the lack of longitudinal data limits our ability to assess growth trajectories or early intervention effects. Future studies should include serial tumor measurements to monitor dynamic changes. Fifth, the forced treadmill running protocol may have induced stress that could influence immune responses, and although a stationary treadmill was used for controls, we did not control for noise or vib--ration. Sixth, the intraperitoneal route of extract administration, while ensuring consistent bioavailability, does not reflect the oral consumption typical of human use, and future studies should compare IP with oral gavage. Despite these limitations, the observed synergistic effects justify further investigation with more rigorous extract characterization.

Conclusion

The present study demonstrates that the combination of aerobic exercise and Urtica dioica extract produces a synergistic anti-tumor effect in a murine melanoma model, characterized by significant suppression of tumor growth and distinct modulation of IL-10 in systemic versus tumor compartments. Critically, the combined intervention achieved superior tumor reduction compared to either modality alone, with only the combination reaching statistical significance, underscoring the additive or synergistic potential of integrating exercise with phytochemical therapy. These findings suggest that the integration of physiological and phytochemical interventions can differentially regulate immune responses, enhancing systemic anti-inflammatory status while promoting a more immunologically active tumor microenvironment. At the cellular level, mechanisms likely involve improved immune cell trafficking, modulation of macrophage polarization, inhibition of pro-tumor signaling pathways, and restoration of redox balance. The significant tumor volume suppression observed in this study provides direct evidence that combining aerobic exercise with nettle extract may be an effective complementary strategy for melanoma management, warranting further investigation into the molecular mechanisms underlying this synergistic effect. Collectively, this integrative approach represents a promising complementary strategy for melanoma management and warrants further investigation in translational and clinical settings.

What is already known on this subject?

Melanoma progression is strongly influenced by the interaction between the tumor microenvironment and systemic immune regulation, and both aerobic exercise and plant-derived bioactive compounds have been shown to modulate inflammatory and anti-inflammatory cytokines, including IL-10, in ways that may affect tumor growth. Tissue crosstalk is now recognized as a key biological mechanism in disease, meaning signals exchanged between tumor tissue, immune cells, and distant organs can shape cancer behavior rather than acting in isolation.

What this study adds?

This study adds evidence that aerobic exercise combined with Urtica dioica extract can suppress melanoma growth producing a 53.4% reduction in tumor volume while producing a compartment

-specific IL-10 response, with higher serum IL-10 but lower tumor IL-10, suggesting that systemic and local immune signaling are regulated differently. This is the first demonstration that the combination of exercise and nettle extract achieves statistically significant tumor suppression where neither intervention alone reaches significance, supporting a synergistic interaction. It also supports the idea that beneficial crosstalk between muscle activity, phytochemical exposure, and the tumor microenvironment may contribute to anti-tumor effects through coordinated immunomodulation rather than a single isolated pathway.

Organ Cross-Talk Tips:

- The opposite pattern of IL-10 in serum and tumor tissue suggests compartment-specific communication, showing that systemic anti-inflammatory responses do not always match local tumor signaling
- The combined effect of aerobic exercise and nettle extract supports a multi-organ regulatory network in which exercise-induced myokines and plant-derived bioactive compounds may jointly reshape the tumor microenvironment.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest in the present research.

Ethical approval

All procedures were approved by the Institutional Animal Care and Use Committee of Islamic Azad University, Tehran, Iran (Ethical code: IR.IAU.M.REC.1399.008).

Informed consent Animal study.

Author contributions

Conceptualization: S.S., Methodology: S.Sh., Software: S.S., Validation: H.A.,; Formal analysis: S.S.,; Investigation: S.Sh.,; Resources: S.S.,; Data curation: H.A.,; Writing - original draft: S.S.,; Writing–review & editing S.Sh.,; Visualization: S.S.,; Supervision: H.A.; Project administration: S.Sh.,.; Funding acquisition: H.A.

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