

ORIGINAL ARTICLE

# The *in vivo* Effect of High-Intensity Endurance Training and *Trachyspermum ammi* (Ajwain) Seed Supplementation on Expression of Specific Zinc Transporter Genes

Araz Nazari<sup>1\*</sup>, Abbas Ghanbari-Niaki<sup>2</sup>, Khadijeh Nasiri<sup>2</sup>, Yazgaldi Nazari<sup>3</sup>

1. Department of Physical Education, University of Saravan, Saravan, Iran

2. Department of Exercise Physiology, Faculty of Sport Sciences, University of Mazandaran, Babolsar, Mazandaran, Iran

3. Department of Sports Sciences, Faculty of Humanities and Social Sciences, Golestan University, Gorgan, Iran

## ARTICLE INFO

### Keywords:

*Trachyspermum ammi*  
Ajwain  
Zinc transporter genes  
Gastric tissue  
Rat

### \*Corresponding author:

Araz Nazari, PhD;  
Department of Physical Education,  
Saravan University,  
Saravan, Iran.  
Tel: +98-9112799464  
Email: [Araznazari@gmail.com](mailto:Araznazari@gmail.com)  
Received: November 10, 2025  
Revised: February 5, 2026  
Accepted: February 11, 2026

## ABSTRACT

**Background:** Zinc as an essential trace element plays a vital role in cellular metabolism and various biological processes. Zinc homeostasis in cells is regulated by two families of transporter proteins, Slc30a (ZnT) and Slc39a (Zip). This study investigated the effects of high-intensity endurance training and supplementation with an aqueous extract of *Trachyspermum ammi* (Ajwain) seeds on the expression of key zinc transporter genes in the gastric tissue of male rats.

**Methods:** Twenty-four male Wistar rats were divided into four groups of Saline-Control (SC), Saline-Training (ST), Ajwain-Control (AC), and Ajwain-Training (AT). The training groups underwent a treadmill running protocol for eight weeks (5 days/week, 60 minutes/session). The Ajwain groups received a daily oral gavage of 200 mg/kg body weight of aqueous *T. ammi* seed extract. The gene expression levels of ZnT5, ZnT6, ZnT8, ZnT9, Zip7, Zip8 and Zip14 were measured using Real-Time PCR.

**Results:** The results indicated that training and Ajwain supplementation significantly affected the expression of ZnT6, ZnT8, ZnT9, Zip8 and Zip14 genes in the gastric tissue, whereas the expression of ZnT5 and Zip7 genes did not show significant changes.

**Conclusion:** Supplementation with *T. ammi* and high-intensity endurance training distinctly modulated the expression of zinc transporter genes and may play a role in the regulation of zinc homeostasis and gastric health. These findings provide new insights into the effects of nutrition and exercise on the gastrointestinal system.

Please cite this article as: Nazari A, Ghanbari-Niaki A, Nasiri K, Nazari Y. The *in vivo* Effect of High-Intensity Endurance Training and *Trachyspermum ammi* (Ajwain) Seed Supplementation on Expression of Specific Zinc Transporter Genes. Int J Nutr Sci. 2026;11(1):137-145. doi: 10.30476/ijns.2026.107953.1536.

## Introduction

Zinc, as the second most abundant trace element in the mammalian body, is a vital micronutrient that plays an essential role in enzymatic catalysis, the structural stability of proteins (particularly zinc-

finger transcription factors), and the regulation of cellular signaling pathways (1, 2). Cytosolic and organellar zinc homeostasis is chiefly maintained by two opposing membrane transport protein families of SLC30A (ZnT) and SLC39A (ZIP).

ZnT proteins (10 mammalian members) primarily reduce cytosolic zinc by efflux or sequestration into organelles (e.g., Endoplasmic Reticulum (ER), Golgi apparatus, secretory vesicles). Conversely, ZIP proteins (14 mammalian members) elevate cytosolic zinc via influx or release from organellar stores (3).

ZnT5 and ZnT6 are predominantly localized to the ER and Golgi apparatus, often functioning as heterodimers to supply zinc for the activation of enzymes resident in the secretory pathway (such as alkaline phosphatases) (4). ZnT8 is primarily found in the secretory granules of the pancreas (and other endocrine glands) and is vital for the storage and secretion of peptides like insulin (5). ZnT9 has also been identified as a zinc transporter in mitochondria, playing a role in maintaining the function of this organelle (6). From the ZIP family, the transporter Zip7 is situated in the ER and Golgi membranes; through the controlled release of zinc into the cytosol (regulated by phosphorylation via Casein Kinase 2 (CK2), it activates signaling pathways associated with cell proliferation and survival (7-9). Zip8 is found in the plasma membrane and acidic organelles (such as lysosomes) and, in addition to transporting zinc, is involved in immune and inflammatory responses, with its expression being upregulated by inflammatory stimuli (10). Zip14 is also located in the plasma membrane and transports not only zinc, but also non-heme iron and manganese. Its expression is strongly regulated by the pro-inflammatory cytokine IL-6, rendering it a key interface between systemic inflammation and metal metabolism (11, 12).

Intense and prolonged physical activity, such as endurance running, constitutes a major physiological stressor that can affect bodily homeostasis at various levels, including zinc metabolism (13, 14). There are reports of decreased serum zinc concentrations and increased zinc excretion following exercise, indicating an augmented requirement or redistribution of zinc in active individuals (15, 16). These alterations can have implications for athletic performance and general health, as zinc is involved in energy metabolism, antioxidant function (as a cofactor for superoxide dismutase), and the immune response (17, 18). Intense exercise particularly affects the gastrointestinal tract, potentially leading to "Exercise-Induced Gastrointestinal Syndrome" (EIGS). This syndrome involves functional disturbances and structural damage, characterized by diminished splanchnic perfusion, heightened mucosal permeability, localized inflammation, oxidative stress, and modified gastrointestinal motility (19). As the primary segment following the oesophagus, gastric tissue experiences mechanical

stress, exercise-induced relative hypoxia due to blood flow redistribution, and pH alterations. Such conditions can impair mucosal barrier function, increasing vulnerability (20-22).

Medicinal plants with antioxidant and anti-inflammatory properties have garnered attention as potential strategies for mitigating exercise-induced physiological stress and enhancing training adaptations (23-25). *Trachyspermum ammi* (Ajwain), an Apiaceae family member, is traditionally employed for digestive, respiratory, and inflammatory conditions (26). Pharmacological study has validated the antioxidant, anti-inflammatory, antispasmodic, gastroprotective, and antimicrobial attributes of Ajwain seed extract, largely due to bioactive constituents like thymol, carvacrol, and other phenolics and terpenoids. Moreover, Ajwain seeds reportedly contain essential minerals, including zinc (27).

A recent study in 2025 has highlighted the potent bioactive properties of *T. ammi*. For instance, its significant antibacterial and antioxidant potential has been reaffirmed, underscoring its role as a natural protective agent (28). Moreover, comprehensive reviews have documented its emerging pharmacological benefits, solidifying its traditional use in gastrointestinal health (29). The rich phytochemistry of Ajwain is believed to be responsible for its wide range of bioactivities, making it a compelling candidate for interventions aimed at mitigating physiological stress (30).

There is a need to examine the concurrent impact of an intensive endurance running regimen and aqueous Ajwain extract supplementation on the gastric tissue gene expression profile of pivotal zinc transporters (Znt5, Znt6, Znt8, Znt9, Zip7, Zip8, and Zip14). This lacuna is significant giving gastric tissue's susceptibility to intense exercise as a critical function of zinc transporters in cellular homeostasis, and the Ajwain's medicinal properties. So this study investigated the *in vivo* effect of high-intensity endurance training and *T. ammi* (Ajwain) seed supplementation on expression of specific zinc transporter genes in gastric health.

## Materials and Methods

Twenty-four male Wistar rats (189±39 g), sourced from the Pasteur Institute of Iran (Amol), were acclimatized for one week at the University of Mazandaran's animal facility. Animals were group-housed (five per cage) under controlled conditions (20±2°C, 50±5% humidity, 12h light/dark cycle) with ad libitum access to water and standard chow. Subsequently, rats were randomly allocated to four groups (n=6/group): 1) Saline-Control (SC): saline

gavage, no exercise; 2) Saline-Training (ST): saline gavage, treadmill exercise; 3) Ajwain-Control (AC): aqueous Ajwain seed extract (200 mg/kg) gavage, no exercise; 4) Ajwain-Training (AT): Ajwain extract gavage, treadmill exercise. Daily gavage occurred 30 minutes post-exercise for ST and AT groups, and at a comparable time for SC and AC groups. All procedures adhered to International Animal Care Guidelines and were approved by the University of Mazandaran's Biomedical Research Ethics Committee (IR.UMZ.REC.1403.115). All procedures were conducted in accordance with the ARRIVE guidelines for reporting animal research.

Ajwain seeds, cultivated in Isfahan, were procured from Sari's traditional market (Mazandaran, Iran) and authenticated via morphological comparison with herbarium specimens. The washed, shade-dried seeds were later powdered. The aqueous extract was prepared by adding 10 mL boiling distilled water per gram of powder, followed by three-day incubation at  $50\pm 1^\circ\text{C}$  and one hour of gentle boiling. After cooling, the mixture was filtered sequentially through muslin cloth (single, double, triple-layer) and Whatman No. 1 filter paper. The filtrate was oven-concentrated to one-third its initial volume and stored at  $4^\circ\text{C}$  in dark glass containers until use (31). AC and AT groups received 200 mg/kg BW/day of the extract (32).

An eight-week aerobic treadmill exercise program was conducted five days per week, with 60-minute sessions. It began with a one-week adaptation phase (10-15 min/day walking at 10 m/min,  $0^\circ$  incline). This was followed by a two-week progressive phase (weeks 2-3), starting at 20 min/day running at 15 m/min ( $0^\circ$  incline), gradually increasing to 60 min/day at 32 m/min ( $0^\circ$  incline) by week three's end. The final phase (weeks 4-8) maintained constant intensity: 60 min/day running at 32 m/min ( $0^\circ$  incline) (33). Thirty-six hours

post-final training/intervention, following a 12-hour overnight fast (water ad libitum), rats were deeply anaesthetized via intraperitoneal ketamine/xylazine injection. Gastric tissue was then promptly dissected, rinsed with cold physiological saline, placed in sterile microtubes, and snap-frozen in liquid nitrogen for subsequent analysis.

Approximately 50 mg of frozen gastric tissue was homogenized in liquid nitrogen. Total RNA was extracted using a Dena Zist Asia Co. kit as per manufacturer's protocol. Complementary DNA (cDNA) was synthesized from 1  $\mu\text{g}$  total RNA with an oligo-dT primer and a Yekta Tajhiz Azma kit, following instructions, then stored at  $-80^\circ\text{C}$ . Specific primers for target genes (Slc30a5, Slc30a6, Slc30a8, Slc30a9, Slc39a7, Slc39a8, Slc39a14) and the reference gene ( $\beta$ -actin) were designed using Primer Premier 5 and synthesized by Bioneer (South Korea) (Table 1). Quantitative Real-Time PCR (qPCR) was performed using SYBR Green master mix (Ampliqon) on a Rotor-Gene Corbett 6000. The thermal profile included initial denaturation ( $95^\circ\text{C}$ , 15 min), then 40 cycles of denaturation ( $95^\circ\text{C}$ , 20s), annealing ( $57^\circ\text{C}$ , 30s), and extension ( $72^\circ\text{C}$ , 30s). Melt curve analysis confirmed product specificity. Cycle threshold ( $C_t$ ) values ( $\geq 2$  technical replicates/sample) were determined. Relative mRNA expression was calculated via the  $2^{-\Delta\Delta C_t}$  method, normalized to  $\beta$ -actin. Statistical analyses were conducted using SPSS software (IBM Corp., version 27, Chicago, IL, USA). Data normality was evaluated via the Shapiro-Wilk test, with results presented as mean $\pm$ standard deviation. One-way ANOVA was employed to assess mean differences among the four groups. Significant overall differences prompted Tukey's HSD post-hoc test for pairwise comparisons. Statistical significance was set at  $p < 0.05$  for all analyses.

**Table 1:** Primer sequences used for Real-Time PCR analysis.

Gene	Forward and Reverse primer	Accession number	Product length (bp)
Slc30a5 (Znt5)	F-5'-TGACACAAACATGCTGACACC-3' R-5'-CATGACTGTGGGCGTGACT-3'	NM_001106404.1	89
Slc30a6 (Znt6)	F-5'-TGCTAAACTTCTCAGACCACCA-3' R-5'-TCACATTTTTCCCAGGCGTATT-3'	NM_001277279	141
Slc30a8 (Znt8)	F-5'-TTCTTTATGGTGGCAGAGGTG-3' R-5'-GCAGGAAACTAGTCAGGTCAATTA-3'	NM_001130538.1	100
Slc30a9 (Znt9)	F-5'-ACCAATGGAATCCCTGCTATG-3' R-5'-ATTGCCTGTTATGGAGGTAAGG-3'	NM_001109088.1	247
Slc39a7 (Zip7)	F-5'-CTCATTCGCACGATACTCCG-3' R-5'-TGTCTCACAACTTCTCCACCAC-3'	NM_001164744.1	133
Slc39a8 (Zip8)	F-5'-ATAAGAAGTCGTATTTCCCAAGA-3' R-5'-GTAAAATCCACCAAACACAGCAAC-3'	NM_001011952.1	165
Slc39a14 (Zip14)	F-5'-CAATTATGTCTCCAAGTCTGCTGT-3' R-5'-GTCCGTGATGGTCTCGTT-3'	NM_001107275.1	110
$\beta$ -actin	F-5'-GTGTGACGTTGACATCCGTAAAGAC-3' R-5'-TGCTAGGAGCCAGGGCAGTAAT-3'	NM_031144.3	119

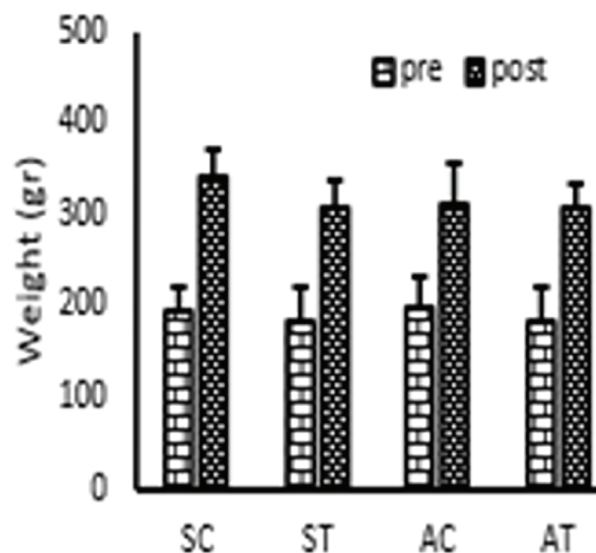
**Results**

Mineral analysis of the *T. ammi* seeds revealed significant zinc content of 53.4 µg/g. The analysis also identified high concentrations of other essential minerals, including calcium (14829.3 µg/g), potassium (5531.5 µg/g), magnesium (3906 µg/g), and phosphorus (2876.1 µg/g), as well as sodium (1621.3 µg/g), iron (72.2 µg/g), manganese (25.39 µg/g), copper (7.3 µg/g), and selenium (1.0 µg/g). The data demonstrated that the rat's weight significantly increased at the end of the experimental period compared to their initial weight. However, no significant differences were observed between the groups regarding their initial and final weights throughout the study (Figure 1).

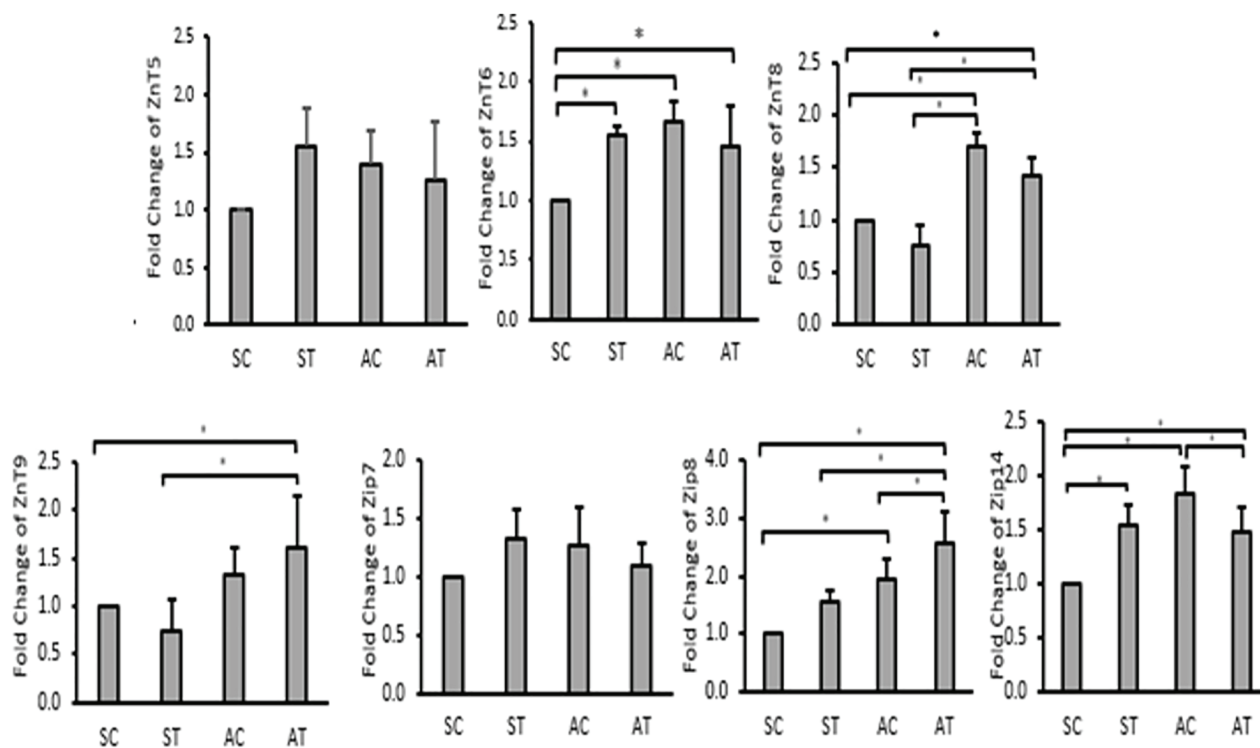
Investigation of the expression profile of selected genes from the ZnT and Zip families in gastric tissue revealed that all studied genes, including Znt5, Znt6, Znt8, Znt9, Zip7, Zip8, and Zip14, were expressed in this tissue. However, the expression of these genes in gastric tissue did not follow a uniform pattern. An interesting observation was the presence of relatively similar patterns of change in expression levels between the gene pairs Znt5 and Zip7, as well as between Znt6 and Zip14, in response to the applied treatments, which may suggest coordinated regulation or related roles for these gene pairs. The results of ANOVA analysis indicated that changes in the expression of Znt6, Znt8, Znt9, Zip8, and Zip14 genes were statistically

significant among the different groups ( $p < 0.05$ ), whereas the expression of Znt5 and Zip7 genes did not show significant differences ( $p > 0.05$ ) (Figure 2).

Tukey's HSD post-hoc test revealed significant differences ( $p < 0.05$ ) between specific groups. The expression of the Znt6 gene showed that all three intervention groups (ST, AC, and AT) were significantly higher than the control group (SC).



**Figure 1:** Mean±SD of body weight in the different groups in relation to initial and final weights. Saline-Control (SC), Saline-Training (ST), Ajwain-Control (AC), and Ajwain-Training (AT).



**Figure 2:** Relative mRNA expression of zinc transporter genes in gastric tissue after eight weeks of endurance training and Ajwain supplementation. Saline-Control (SC), Saline-Training (ST), Ajwain-Control (AC), and Ajwain-Training (AT). Data were presented as mean±standard deviation (Mean±SD) for each group (n=6). \* indicates a statistically significant difference between the specified groups at  $p < 0.05$ .

The expression of the *Znt8* gene indicated that supplementation with aqueous Ajwain seed extract in both groups (AC and AT) showed a significant increase compared to the saline groups (SC and ST). Furthermore, Tukey's post-hoc test for *Znt9* gene expression demonstrated that only the combined Ajwain supplementation with training group (AT) demonstrated a significant increase compared to both saline groups (SC and ST); although the AC group also illustrated an increase compared to both saline groups (SC and ST), and this increase was not statistically significant. Examination of *Zip8* gene expression revealed that the AC group showed a significant increase compared to the SC group.

Moreover, the AT group exhibited the highest expression level that showed a significant increase when compared to all three other treatment groups (SC, ST, and AC). Analyses of *Zip14* indicated that the gene expression in the SC group was lower than all other groups, meaning all three treatment groups (ST, AC, and AT) had significantly increased expression when compared to SC. Additionally, the AC group had the highest expression level among the treatments, denoting to a significant increase even compared to the AT group. In contrast, the observed changes in the expression of *Znt5* and *Zip7* genes did not reach statistical significance between any of the groups ( $p < 0.05$ ) (Figure 2).

## Discussion

*T. ammi* (Ajwain) is well-regarded for its rich phytochemical profile, which includes bioactive constituents like thymol, carvacrol, and various phenolics and terpenoids. These compounds are largely responsible for its validated antioxidant, anti-inflammatory, and gastroprotective properties (26, 27). Recent literature further supports these attributes, highlighting its potential to counteract oxidative and microbial challenges (28, 30). The significant mineral content of Ajwain seeds, particularly zinc, as identified in our analysis, suggests a dual mechanism of action by providing essential micronutrients; while its bioactive compounds modulate cellular pathways. This makes it a particularly interesting supplement in the context of exercise-induced stress, which is known to elevate oxidative and inflammatory responses (29).

The present study investigated the expression profile of selected zinc transporter genes (*ZnT* and *Zip* families) in the gastric tissue of male rats subjected to supplementation with aqueous Ajwain seed extract and high-intensity endurance running. The first key finding was the expression of all studied genes, namely *Znt5*, *Znt6*, *Znt8*, *Znt9*, *Zip7*, *Zip8*, and *Zip14*, in the gastric tissue. This observation

underscores the potential importance of these transporters in zinc homeostasis and the maintenance of physiological functions within this vital organ of the gastrointestinal tract. However, a more detailed analysis revealed that the expression pattern of these genes was not uniform in response to the applied treatments (exercise, Ajwain supplementation, and their combination), exhibiting considerable diversity. This heterogeneity, indicative of complex and possibly physiologically-conditioned regulation of these transporters in the stomach, aligns with findings from previous studies in other tissues such as the testis (27) and liver (34, 35), although the specific response pattern of each gene can be distinctly tissue-dependent.

Furthermore, it must be considered that physical activity per se, even at moderate intensity and for a relatively short duration (e.g., 1 hour), can lead to significant alterations in gastrointestinal function. It was demonstrated that endurance exercise can disrupt normal gastric electrical activity and delay oro-caecal transit time (OCTT). These exercise-induced functional changes, likely occurring via neuroendocrine pathways (increased sympathetic activity) and reduced splanchnic blood flow (splanchnic hypoperfusion), could underlie the observed changes in zinc transporter gene expression in the exercise group (ST) of the current study (15).

In this study, one-way ANOVA indicated that the expression of *Znt6*, *Znt8*, *Znt9*, *Zip8*, and *Zip14* genes was significantly altered by the treatments ( $p < 0.05$ ). The significant changes in *Znt6* gene expression in gastric tissue were noteworthy, as similar studies employing the same treatments on testis (27) and liver (34) tissues did not observe significant changes in *Znt6* expression. This difference may suggest a specific role or a distinct regulatory mechanism for *Znt6* in the gastric epithelium compared to other tissues. Tukey's post-hoc test revealed that *Znt6* expression was significantly higher in the ST, AC, and AT groups compared to the SC group. This increase across all three intervention groups suggests a potential role for *Znt6* in the gastric response to metabolic stresses induced by exercise or the bioactive compounds in Ajwain. Moreover, the observation of a relatively similar pattern of change between *Znt6* and *Zip14* (both significantly affected by treatments) in the present study might allude to their coordinated roles in zinc transport within specific cellular compartments or response to common signals in gastric tissue, although such co-regulation was not reported in this manner in liver (34) or testis (27) studies.

The expression of the *Znt8* gene also exhibited significant alterations, and Tukey's test revealed

multiple significant differences between groups (SC vs. AC, SC vs. AT, ST vs. AC, ST vs. AT, and AC vs. AT). The means displayed a complex pattern; while the exercise factor resulted in decreased *Znt8* gene expression in both the ST group when compared to the SC group and the AT group in comparison to the AC group. The expression changes in the saline groups (SC vs ST) in gastric tissue were similar to those in rat testis (27); however, in contrast to gastric tissue, *Znt8* gene expression in the testis showed a significant increase in the AT group compared to the AC group. Considering the role of *ZnT8* in pancreatic beta-cells and testicular Leydig cells, its specific role in the stomach and the reasons for these contrasting responses to exercise and supplementation warrant further investigation (36).

The *Znt9* gene also changed significantly, with significant differences observed between the SC vs. AT and ST vs. AT group pairs. The means indicated that the expression of this gene decreased in the ST group but reached its highest level in the AT group, which was significantly higher than both the SC and ST groups. This robust increase in the combined group (AT) is consistent with findings from Ajwain supplementation in the testis (27) (significant increase in *Znt9* in the AT group compared to AC and ST) and also with pumpkin seed extract supplementation in liver tissue, which increased *Znt9* gene expression (35). This underscores the responsiveness of this gene, essential for mitochondrial function, to metabolic and nutritional interventions (4).

The significant change in *Zip8* expression in the stomach, which showed significant differences between the SC vs. AC, SC vs. AT, ST vs. AT, and AC vs. AT group pairs, aligns with findings from Ajwain supplementation and high-intensity endurance activity in liver (34) and testis (27) tissues, where *Zip8* expression was significantly affected by treatments. The results indicate that *Zip8* gene expression gradually increased from the SC group, reaching the highest level in the AT group. Furthermore, the increasing pattern of *Zip8* in gastric and liver tissues was similar. This upward trend, particularly the strong increase in the Ajwain-receiving groups (AC and AT), highlights the potential role of *Zip8*, which functions in the plasma membrane and lysosomes in zinc homeostasis and lysosomal function, in response to exercise and especially the bioactive compounds present in Ajwain across different tissues (7).

Finally, *Zip14* gene expression was also significantly affected by the treatments, with significant differences observed between the SC vs. ST, SC vs. AC, SC vs. AT, and AC vs. AT group pairs. The means showed that expression was

significantly higher in the ST and AC groups than in the SC group. Interestingly, however, in the AT group, its expression was lower than in the ST and AC groups, although it remained higher than in the SC group. This result contrasts with findings from research involving Ajwain supplementation in testis tissue (27) and also chickpea and pumpkin seed extract supplementation in liver tissue (35), where no significant changes in *Zip14* were observed. This disparity again emphasizes tissue-dependent regulation and possibly supplement-type dependency (Ajwain versus pumpkin/chickpea). The complex response pattern in the combined group (AT) for *Zip14* in the stomach requires further investigation.

In contrast to the aforementioned genes, the expression of *Znt5* and *Zip7* genes did not show significant differences between groups in the present study ( $p < 0.05$ ). The lack of significant change in *Zip7* is fully consistent with research findings in testis (27) and liver tissue (34). This finding, that *Zip7* expression in the stomach was not significantly altered by the treatments, is noteworthy, especially considering the vital role of this transporter, located in the ER and Golgi apparatus (37), which is regulated by phosphorylation (via CK2) to release zinc into the cytosol and activate signalling pathways (5). The study in skeletal muscle cells showed that reduced *Zip7* expression significantly impaired glucose metabolism and the insulin signaling pathway (reduced expression of *Insr*, *Irs1*, *Irs2*, and Akt phosphorylation), underscoring its important metabolic role (6).

The lack of a significant response of *Zip7* in the stomach in our study could indicate differential regulation or a less prominent metabolic role in this tissue under the specific applied stresses, or that its basal expression in the stomach was more stable to these interventions. However, the absence of a significant change in *Znt5* in the stomach contrasts with the results of studies on both Ajwain supplementation with high-intensity endurance running in the testis (27) and liver (34), both of which reported significant changes. Yet, it aligns with research on chickpea extract supplementation and pumpkin seed extract supplementation in liver tissue, where no significant changes were observed (35).

*ZnT5* is located in the early secretory pathway and Golgi apparatus and, along with *ZnT7* or *ZnT6*, plays a role in activating alkaline phosphatase (38). This inconsistency indicates the need for further investigation into the role and regulation of *Znt5* specifically in the stomach. Although the overall changes in *Znt5* and *Zip7* were not significant, the observation of a relatively similar pattern of change in their mean expression levels across groups might

reflect their shared role or coordinated regulation in the early secretory compartments (ER/Golgi), as alluded to at the beginning of the results section.

The prominent differences observed in the response of zinc transporter genes in the present study among gastric, testis (27), and liver (34, 35) tissues with similar or related treatments clearly demonstrate the importance of tissue-specific regulation of zinc homeostasis. Factors such as the basal zinc status in each tissue, the different metabolic needs of the cells in each organ, and their dominant signaling pathways can contribute to these differential responses. As previously mentioned, endurance exercise alone can affect gastric function (e.g., reduced myoelectrical activity) and intestinal transit time (15). These functional disturbances resulting from the physiological stress of exercise could, independently or in interaction with the effects of Ajwain supplementation, lead to the observed changes in zinc transporter gene expression in the stomach. Furthermore, Ajwain extract is rich in active compounds and valuable minerals such as zinc and iron (27, 34).

This extract also possesses notable antioxidant and anti-inflammatory properties. The diverse physiological effects of high-intensity endurance exercise (such as induced oxidative stress, changes in inflammatory status, and cellular energy demands) can influence the expression of these genes in the stomach via various molecular pathways. The details of significant differences between specific groups, as identified by Tukey's test, provide more precise insights into which treatments or treatment combinations had the most pronounced effect on each specific gene in the stomach and can guide future studies to investigate more precise regulatory mechanisms.

This study had several limitations that should be acknowledged. First, our findings are based on a rodent model, and the direct translation of these results to human physiology requires caution. Further clinical studies are needed to confirm these effects in athletes. Second, we measured gene expression (mRNA levels), which does not always directly correlate with protein levels or functional transporter activity. Future research should include proteomic analyses to validate these findings. Finally, this study used a single dosage of Ajwain extract; while dose-response studies can provide deeper insights into its optimal therapeutic range for modulating zinc homeostasis in response to exercise.

## Conclusion

Supplementation with *T. ammi* and high-intensity endurance training distinctly modulated the

expression of zinc transporter genes and may play a role in the regulation of zinc homeostasis and gastric health. These findings provide new insights into the effects of nutrition and exercise on the gastrointestinal system.

## Acknowledgement

The present research is the result of the approved research project No. 10576/16, entitled "The effect of exercise and *Trachyspermum ammi* seed supplementation on the gene expression of some zinc transporters in the gastric tissue of rats," which was financially supported by the Vice-Chancellor for Research of Saravan University. The authors hereby express their gratitude.

## Funding

This study was financially supported by the Vice-Chancellor for Research of Saravan University (research project No. 10576/16). The funding body had no role in the study design; in the collection, analysis, or interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

## Authors' Contribution

All authors made substantial contributions to this work. AN and YN conceived and designed the study. AN and KN performed the experiments and collected the data. AGN and AN analyzed and interpreted the data. All authors contributed to drafting the article and revising it critically for important intellectual content. All authors gave final approval of the version to be submitted.

## Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## References

- 1 Kambe T, Hashimoto A, Fujimoto S. Current understanding of ZIP and ZnT zinc transporters in human health and diseases. *Cell Mol Life Sci.* 2014;71:3281-95. DOI: 10.1007/s00018-014-1617-0. PMID: 24710731.
- 2 Moradpour GR, Minaei MR, Haghghat N, et al. The Effect of Gastric Bypass and Sleeve Gastrectomy Surgery on the Level of Vitamin A, Iron, Copper, Ferritin, Hemoglobin, Vitamin D, Vitamin B12, Zinc and Cardiometabolic Factors in Patients with Morbid Obesity. *Int J Nutr Sci.* 2025;10:467-475. DOI: 10.30476/ijns.2025.104992.1386.

- 3 Kambe T, Tsuji T, Hashimoto A, et al. The physiological, biochemical, and molecular roles of zinc transporters in zinc homeostasis and metabolism. *Physiol Rev*. 2015;95:749-84. DOI: 10.1152/physrev.00035.2014. PMID: 26084690.
- 4 Deng H, Qiao X, Xie T, et al. SLC-30A9 is required for Zn<sup>2+</sup> homeostasis, Zn<sup>2+</sup> mobilization, and mitochondrial health. *Proc Natl Acad Sci U S A*. 2021;118:e2023909118. DOI: 10.1073/pnas.2023909118. PMID: 34433664.
- 5 Taylor KM, Hiscox S, Nicholson RI, et al. Protein kinase CK2 triggers cytosolic zinc signaling pathways by phosphorylation of zinc channel ZIP7. *Sci Signal*. 2012;5:ra11. DOI: 10.1126/scisignal.2002585. PMID: 22317921.
- 6 Myers SA, Nield A, Chew GS, et al. The zinc transporter, Slc39a7 (Zip7) is implicated in glycaemic control in skeletal muscle cells. *PLoS One*. 2013;8:e79316. DOI: 10.1371/journal.pone.0079316. PMID: 24265765.
- 7 Liu MJ, Bao S, Gálvez-Peralta M, et al. ZIP8 regulates host defense through zinc-mediated inhibition of NF-κB. *Cell Rep*. 2013;3:386-400. DOI: 10.1016/j.celrep.2013.01.009. PMID: 23403290.
- 8 Mehrabani D, Sholehvar F, Yaghmaei P, et al. The impact of acemannan, an extracted product from Aloe vera, on proliferation of dental pulp stem cells and healing of mandibular defects in rabbits. *Am J Stem Cells*. 2024;13:75-86. DOI: 10.62347/UAF3719. PMID: 38765804.
- 9 Jalli R, Mehrabani D, Zare S, et al. Cell Proliferation, Viability, Differentiation, and Apoptosis of Iron Oxide Labeled Stem Cells Transfected with Lipofectamine Assessed by MRI. *J Clin Med*. 2023;12:2395. DOI: 10.3390/jcm12062395. PMID: 36983399.
- 10 Fujishiro H, Yano Y, Takada Y, et al. Roles of ZIP8, ZIP14, and DMT1 in transport of cadmium and manganese in mouse kidney proximal tubule cells. *Metallomics*. 2012;4:700-8. DOI: 10.1039/c2mt20024d. PMID: 22534978.
- 11 Chu A, Petocz P, Samman S. Zinc status at baseline is not related to acute changes in serum zinc concentration following bouts of running or cycling. *J Trace Elem Med Biol*. 2018;50:105-10.
- 12 Zare S, Mehrabani D, Jalli R, et al. MRI-Tracking of Dental Pulp Stem Cells In Vitro and In Vivo Using Dextran-Coated Superparamagnetic Iron Oxide Nanoparticles. *J Clin Med*. 2019;8:1418. DOI: 10.3390/jcm8091418. PMID: 31505807.
- 13 Maynar M, Muñoz D, Alves J, et al. Influence of an acute exercise until exhaustion on serum and urinary concentrations of molybdenum, selenium, and zinc in athletes. *Biol Trace Elem Res*. 2018;186:361-9. DOI: 10.1007/s12011-018-1327-9. PMID: 29623649.
- 14 Sheikhan-Shahin H, Mehrabani D, Ashraf MJ, et al. The healing effect of bone marrow-derived stem cells and aquatic activity in Achilles tendon injury. *J Hellenic Vet Med Soc*. 2019;70:1373-1380.
- 15 Gaskell SK, Burgell R, Wiklendt L, et al. Impact of exercise duration on gastrointestinal function and symptoms. *J Appl Physiol*. 2023;134:160-71. DOI: 10.1152/jappphysiol.00393.2022. PMID: 36476157.
- 16 Sheikhan Shahin H, Koushkie Jaromi M, Kardeh E, et al. The effect of aerobic exercise on bone mineral density and bone mineral content in female athlete patients following kidney transplantation in Shiraz, southern Iran. *World Appl Sci J*. 2013;27:23-27.
- 17 Fallah A, Mohammad-Hasani A, Colagar AH. Zinc is an essential element for male fertility: a review of Zn roles in men's health, germination, sperm quality, and fertilization. *J Reprod Infertil*. 2018;19:69-81. PMID: 30009140
- 18 Dabidi Roshan V, Hekmat F, Memar Moghadam M, et al. The Effect Of Running Exercise And Calcium Supplementation In Tibial Bone Strength In Ovariectomized Mouse. *Olympic*. 2010;18:121-135.
- 19 Yavari A, Javadi M, Mirmiran P, et al. Exercise-induced oxidative stress and dietary antioxidants. *Asian J Sports Med*. 2015;6:e24898. DOI: 10.5812/asjms.24898. PMID: 25883776.
- 20 Boskabady MH, Alitaneh S, Alavinezhad A. Carum copticum L.: a herbal medicine with various pharmacological effects. *Biomed Res Int*. 2014;2014:569087. DOI: 10.1155/2014/569087. PMID: 25089273.
- 21 Masoumi SJ, Mehrabani D, Moradi F, et al. The prevalence of dyspepsia symptoms and its correlation with the quality of life among Qashqai Turkish migrating nomads in Fars Province, southern Iran. *Pak J Med Sci*. 2015;31:325-330. DOI: 10.12669/pjms.312.6956. PMID: 26101484.
- 22 Masoumi SJ, Khademolhosseini F, Mehrabani D, et al. Correlation Of Quality Of Life With Gastroesophageal Reflux Disease Amongst Qashqai Nomads In Iran. *Arch Iran Med*. 2012;15:47-50. PMID: 23199245.
- 23 Asif HM, Sultana S, Akhtar N. A panoramic view on phytochemical, nutritional, ethanobotanical uses and pharmacological values of Trachyspermum ammi Linn. *Asian Pac J Trop Biomed*. 2014;4:S545-S53.
- 24 Rahnema M, Mehrabani D, Japoni S, et al. The healing effect of licorice (glycyrrhiza glabra)

- on *Helicobacter pylori* infected peptic ulcers. *J Res Med Sci.* 2013;18:532-533. PMID: 24250708.
- 25 Panjehshahin MR, Owji AA, Mehrabani D, et al. Effect Of Curcumin On Cholesterol Gall-Stone Induction In Rats. *J Appl Anim Res.* 2003;23:75-80.
  - 26 Ranjbaran A, Kavooosi G, Mojallal-Tabatabaei Z, et al. The antioxidant activity of *Trachyspermum ammi* essential oil and thymol in murine macrophages. *Biocatal Agric Biotechnol.* 2019;20:101220.
  - 27 Nazari A, Ghanbari-Niaki A, Nasiri K. The Selected Zinc Transporters (ZnT and ZIP) Gene Expression, Zinc, Iron and Glycogen Concentrations in Healthy Rat Testis: Effect of Aqueous Ajwain (*Trachyspermum ammi*) Seeds Powder Extraction and High-intensity Treadmill Running. *J Chem Health Risks.* 2024;14:711-725. DOI: 10.60829/jchr.2024.3121949.
  - 28 Irshad A, Jawad R, Mushtaq Q, et al. Determination of antibacterial and antioxidant potential of organic crude extracts from *Malus domestica*, *Cinnamomum verum* and *Trachyspermum ammi*. *Sci Rep.* 2025;15:976. DOI: 10.1038/s41598-024-83506-0. PMID: 39762362.
  - 29 Agarwal U, Pannu A, Tonk RK. Emerging Traditional and Pharmacological Health Benefits of *Trachyspermum Ammi* (Ajwain): Future Aspects. *J Funct Foods.* 2025;4. DOI: 10.2174/0126668629357338250113095001.
  - 30 Elsalhy GE, Sharaf El-din MG, Abdelhameed RF, et al. *Trachyspermum ammi* (L.) Sprague: A Comprehensive Review of Its Phytochemistry and Bioactivity. *Rec Pharm Biomedical Sci.* 2025;9:83-109.
  - 31 Handa SS, Khanuja SP, Longo G, et al. Extraction technologies for medicinal and aromatic plants: Earth. *JMSET.* 2008;1:21-5.
  - 32 Javed I, Iqbal Z, Rahman Z, et al. Comparative antihyperlipidaemic efficacy of *Trachyspermum ammi* extracts in albino rabbits. *Pak Vet J.* 2006;26:23-9.
  - 33 Ghanbari-Niaki A, Ghanbari-Abarghooi S, Rahbarizadeh F, et al. Heart ABCA1 and PPAR- $\alpha$  genes expression responses in male rats: effects of high intensity treadmill running training and aqueous extraction of black crataegus-pentaegyna. *Res Cardiovasc Med.* 2013;2:153-9. DOI: 10.5812/cardiovascmed.13892. PMID: 25478513.
  - 34 Niaki AG, Nazari A, Nasiri K. Intense Endurance Running Training and Supplementation with the Aqueous Extract of Ajwain Seed: Effect on the Levels of Zinc and Some zinc Transporters in the Liver Tissue of Male Wistar Rats. *Anim Biol.* 2024;16:83-97.
  - 35 Khavidaki AD, Ghanbari-Niaki A, Nasiri K, et al. Zinc Transporters in the Livers of Healthy Male Wistar Rats: An Investigation of the Effects of Aerobic Exercise and Supplementation with Pumpkin Seed and White Pea. *Zahedan J Res Med Sci.* 2023;26:e137982
  - 36 Zhang X, Guan T, Yang B, et al. A novel role for zinc transporter 8 in the facilitation of zinc accumulation and regulation of testosterone synthesis in Leydig cells of human and mouse testicles. *Metab.* 2018;88:40-50. DOI: 10.1016/j.metabol.2018.09.002. PMID: 30236453.
  - 37 Taylor KM, Morgan HE, Johnson A, et al. Structure-function analysis of HKE4, a member of the new LIV-1 subfamily of zinc transporters. *Biochem J.* 2004;377:131-9. DOI: 10.1042/BJ20031183. PMID: 14525538.
  - 38 Fukunaka A, Kurokawa Y, Teranishi F, et al. Tissue nonspecific alkaline phosphatase is activated via a two-step mechanism by zinc transport complexes in the early secretory pathway. *J Biol Chem.* 2011;286:16363-73. DOI: 10.1074/jbc.M111.227173. PMID: 21402707.