

# A novel non-invasive combination of nutraceuticals and magnet therapy for Hashimoto's thyroiditis: preclinical evidence

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## Abstract

**Background:** Hashimoto's thyroiditis is a highly prevalent autoimmune disorder characterized by persistent chronic inflammation and elevated circulating anti-thyroid antibodies. Conventional hormone replacement therapies often fail to fully alleviate systemic clinical symptoms, such as chronic fatigue and underlying metabolic imbalances. This study sought to evaluate a novel, multifaceted therapeutic strategy combining a targeted natural supplement (comprising Vitamin C, Vitamin D3, selenium, zinc, and Nigella sativa extract) with localized magnet therapy in an experimental animal model.

**Methods:** Experimental autoimmune thyroiditis (EAT) was induced in mice. The animals were randomly assigned to four experimental cohorts: healthy controls, untreated EAT model, supplement-only treatment, and a combined supplement plus localized magnet therapy group. The nutritional supplement was administered daily via oral gavage for 4–6 weeks. Concomitantly, localized static magnet therapy (200–800 Gauss) was applied over the cervical region for 20–30 minutes daily. Serum concentrations of thyrotropin (TSH), thyroid hormones (T<sub>3</sub>, T<sub>4</sub>), anti-thyroperoxidase (anti-TPO), anti-thyroglobulin (anti-TG), and C-reactive protein (CRP) were quantified, complemented by histopathological analyses of thyroid tissue.

**Results:** Monotherapy with the natural supplement significantly attenuated auto-antibody titers, improved circulating thyroid hormone profiles, and suppressed systemic CRP levels. Notably, the integration of localized magnet therapy exerted a profound synergistic effect, yielding robust anti-inflammatory outcomes and significantly preserving thyroid follicular architecture. These findings indicate that the combined physical-nutraceutical intervention offers distinct therapeutic advantages over nutritional supplementation alone.

## Introduction

Hashimoto's thyroiditis stands as the primary etiology of hypothyroidism in iodine-sufficient regions. As a chronic autoimmune disorder, its pathogenesis is characterized by the generation of autoantibodies tar-

geting thyroid tissue, culminating in persistent lymphocytic infiltration, progressive fibrosis, and eventual glandular insufficiency [1]. Although standard clinical management relies almost exclusively on levothyroxine replacement therapy, a growing body of evidence indicates that dietary modifications, targeted

micronutrient supplementation, and complementary interventions can favorably alter the autoimmune trajectory and enhance patient quality of life [2].

Deficiencies in essential vitamins and minerals most notably Vitamin D3, Vitamin C, selenium, and zinc are highly prevalent among patients diagnosed with Hashimoto's thyroiditis. These micronutrient deficits have been directly linked to exacerbated inflammatory cascades, elevated antithyroid antibody titers, and unchecked oxidative stress [3]. Mechanistically, Vitamin D3 acts as a potent immunomodulator that regulates T-lymphocyte activity, decreases autoimmune reactivity, and supports baseline thyroid function [4]. Concurrently, Vitamin C functions as a major antioxidant, scavenging free radicals and mitigating tissue damage under chronic inflammatory conditions [5].

The essential trace elements selenium and zinc are similarly indispensable cofactors for thyroid hormone synthesis, peripheral deiodination, and the preservation of glutathione peroxidase activity within thyrocytes, thereby attenuating autoimmune tissue destruction [6,7]. In tandem with these micronutrients, *Nigella sativa* (black seed) extract has gained substantial attention due to its anti-inflammatory, antioxidant, and immunomodulatory properties. Thymoquinone, the chief bioactive constituent of *Nigella sativa*, plays a pivotal role in modulating inflammatory signaling pathways and maintaining cellular homeostasis [8]. Clinical evidence suggests that the administration of *Nigella sativa* extract can significantly lower thyrotropin (TSH) concentrations and optimize metabolic markers in patients with Hashimoto's disease [9].

The progressive destruction of thyroid architecture by autoantibodies eventually induces overt hypothyroidism. This state of hormone deficiency manifests clinically through a constellation of systemic symptoms, including chronic fatigue, cold intolerance, weight gain, constipation, mood disturbances,

and adverse alterations in skin and hair texture. Crucially, the disease trajectory typically begins subclinically and can remain asymptomatic for years, frequently escaping detection until substantial follicular destruction has already occurred [10,11].

The clinical progression of Hashimoto's thyroiditis spans five distinct stages: genetic predisposition, early immune activation, subclinical hypothyroidism, overt hypothyroidism, and potentially, progression toward broader systemic autoimmunity [3,12,13]. While the exact etiology remains multifactorial, it is driven by a complex interplay of genetic vulnerability and environmental triggers, such as radiation exposure, excessive iodine intake, viral infections, chronic stress, and systemic hormonal shifts [1,14].

In recent years, complementary physical modalities, including magnet therapy, have been investigated for their ability to enhance localized microcirculation, suppress inflammatory markers, and accelerate tissue repair processes. Although direct empirical data regarding magnetic stimulation of the thyroid gland remain sparse, research in other chronic inflammatory and musculoskeletal models indicates that static and pulsed magnetic fields can positively modulate cellular membrane permeability, ion channel dynamics, and cellular regeneration [15]. Consequently, utilizing a specialized magnetic device positioned over the cervical region represents an innovative, non-invasive approach to alleviate localized inflammation and restore physiological equilibrium.

The integration of a multi-ingredient natural supplement combining Vitamin D3, Vitamin C, selenium, zinc, and *Nigella sativa* extract—with localized magnet therapy represents a novel, multifaceted strategy for the management of Hashimoto's thyroiditis. By targeting multiple pathological arms simultaneously (modulating immune responses,

reducing oxidative stress, and protecting follicular morphology), this combinatorial approach aims to establish a safe, auxiliary framework alongside standard medical management to improve long-term patient outcomes.

## Materials and Methods

### Study Design and Ethical Approval

This experimental study was approved by the institutional animal ethics of Kimia Andishe Gene Pooyan Institute. All procedures complied with the ARRIVE guidelines and national regulations for laboratory animal care.

### Preparation of the Natural Supplement

#### Nigella sativa Extract

Black seeds (*Nigella sativa*) were cleaned, dried at room temperature, and ground to a fine powder (40–60 mesh size). Hydroalcoholic extraction was performed using 70% ethanol (1:10 w/v) for 48 hours at room temperature in darkness, with gentle stirring twice daily. The mixture was filtered (Whatman No. 1 filter paper), concentrated under reduced pressure at <40°C using a rotary evaporator, and freeze-dried. The dry extract was stored in airtight, dark containers at 4°C. Thymoquinone content was quantified by HPLC (C18 column, UV detection) for standardization.

#### Micronutrient Powders

Vitamin C, vitamin D3, selenium, and zinc powders were individually dried in a low-temperature oven, sieved (60 mesh), and stored in light-protected containers.

#### Supplement Formulation

A geometric dilution method was used. Due to its low dose, vitamin D3 was first diluted with microcrystalline cellulose. Then, vitamin C, selenium, zinc, and *Nigella sativa* extract were added sequentially with thorough mixing. The final blend was stored as powder in dark, airtight containers. For oral gavage, a fresh suspension was prepared daily by

dispersing the powder in 0.5% carboxymethylcellulose (CMC) solution using a magnetic stirrer.

### Quality Control

The supplement was tested for mixing uniformity, pH, total microbial count, physical stability (days 7 and 14), and thymoquinone concentration.

### Animal Model and Experimental Design

**Animals:** Male C57BL/6 mice (20–25 g) were obtained from an accredited laboratory animal center. They were housed under controlled conditions (22±2°C, 50–60% humidity, 12 h light/dark cycle) with free access to standard chow and water. A one-week acclimatization period preceded the experiment.

### Induction of Experimental Autoimmune Thyroiditis (EAT)

Hashimoto's model was induced by subcutaneous injection of porcine thyroglobulin emulsified with Complete Freund's Adjuvant (CFA) into the dorsal region. After two weeks, a booster injection with the same antigen in Incomplete Freund's Adjuvant (IFA) was given. Disease confirmation (elevated anti-thyroglobulin antibodies and altered thyroid hormones) was performed at week 4.

### Group Allocation

Mice were randomly assigned to four groups (n=4 per group): Group 1: Healthy control (no induction, no treatment), Group 2: EAT untreated, Group 3: EAT + natural supplement (oral gavage daily), Group 4: EAT + natural supplement + magnet therapy

### Supplement Administration

The supplement suspension was administered daily by oral gavage for 6–8 weeks. Doses were adjusted individually based on body weight (mg/kg).

### Magnet Therapy Protocol

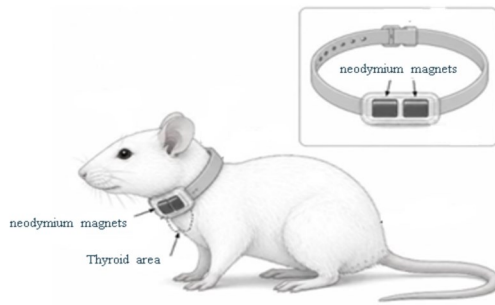


Figure 1: This system consists of an adjustable soft collar, a protective casing, and small neodymium magnets placed at the anterior aspect of the neck, directly over the thyroid gland. The device is designed to deliver a localized low-intensity static magnetic field to the thyroid region.

A custom-designed magnetic collar (soft silicone band) held two small neodymium magnets (200–500 Gauss static field) positioned over the anterior neck, directly above the thyroid gland. Magnets were encased in plastic to avoid skin contact. The collar was worn for 4–6 hours daily throughout the intervention period. Control animals (Groups 1–3) were not subjected to any magnetic field (Fig 1).

#### Sample Collection and Biochemical Analyses

##### Blood Collection

Blood samples were collected from the tail vein or retro-orbital sinus at baseline and at the end of the treatment period. Serum was separated by centrifugation and stored at  $-70^{\circ}\text{C}$ .

##### Hormonal and Immunological Assays

Commercially available ELISA kits (validated for mice) were used to measure: Thyroid hormones: TSH, total T3, total T4, free T3, free T4, Autoantibodies: anti-TPO, anti-thyroglobulin (anti-TG)

Inflammatory markers: TNF- $\alpha$ , CRP

##### Antimicrobial Assays (Quality Control)

To assess microbiological safety, the broth microdilution method was used to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the supplement

against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Candida albicans* (ATCC 10231). MIC was defined as the lowest concentration with no visible growth after 24 h at  $37^{\circ}\text{C}$ . MBC was determined by subculturing non-growing wells onto agar.

#### Histopathological Examination

At study end, mice were humanely euthanized, and thyroid glands were removed. Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned (5  $\mu\text{m}$ ), and stained with hematoxylin and eosin (H&E). A semi-quantitative scoring system (0–4) was used to evaluate lymphocytic infiltration, follicular destruction, colloid depletion, and overall structural changes.

#### Gene Expression Analysis

Thyroid tissue homogenates were used for RNA extraction and quantitative real-time PCR (RT-qPCR). mRNA expression levels of TNF- $\alpha$ , IL-6, IL-10, and FoxP3 were measured and normalized to GAPDH. Fold change was calculated relative to the healthy control group (set to 1.0).

#### Statistical Analysis

Data were expressed as mean  $\pm$  SD or SEM. Comparisons among groups were performed using one-way ANOVA followed by Tukey's post-hoc test (or appropriate non-parametric alternative). A p-value  $< 0.05$  was considered statistically significant. Analyses were conducted using SPSS or GraphPad Prism.

### Results

#### Baseline Characteristics

At study entry (week 0), no significant differences were observed among the four experimental groups in terms of body weight or thyroid hormone levels (TSH, T3, T4). All animals were healthy and acclimatized. Baseline data are summarized in Table 1.

Table 1. Baseline characteristics of animals (mean  $\pm$  SD)

Note: Numerical baseline values for body weight, T4, and T3 were not recorded; placeholders shown.

| Variable         | Healthy control (n=4) | Untreated EAT (n=4) | EAT + supplement (n=4) | EAT + supplement + magnet (n=4) | p-value |
|------------------|-----------------------|---------------------|------------------------|---------------------------------|---------|
| Body weight (g)  | —                     | —                   | —                      | —                               | >0.05   |
| TSH (mIU/L)      | 1.18 $\pm$ 0.10       | 1.25 $\pm$ 0.12     | 1.23 $\pm$ 0.11        | 1.22 $\pm$ 0.09                 | 0.72    |
| T4 ( $\mu$ g/dL) | —                     | —                   | —                      | —                               | >0.05   |
| T3 (ng/dL)       | —                     | —                   | —                      | —                               | >0.05   |

Table 2. Thyroid hormone levels at week 8 (mean  $\pm$  SD)

| Group                     | TSH (mIU/L)      | T4 ( $\mu$ g/dL) | T3 (ng/dL)  |
|---------------------------|------------------|------------------|-------------|
| Healthy control           | 1.20 $\pm$ 0.09  | 5.8 $\pm$ 0.4    | 98 $\pm$ 6  |
| Untreated EAT             | 3.45 $\pm$ 0.30* | 2.1 $\pm$ 0.3*   | 52 $\pm$ 5* |
| EAT + supplement          | 2.10 $\pm$ 0.25† | 4.0 $\pm$ 0.4†   | 74 $\pm$ 6† |
| EAT + supplement + magnet | 1.65 $\pm$ 0.20† | 5.1 $\pm$ 0.5    | 88 $\pm$ 7  |

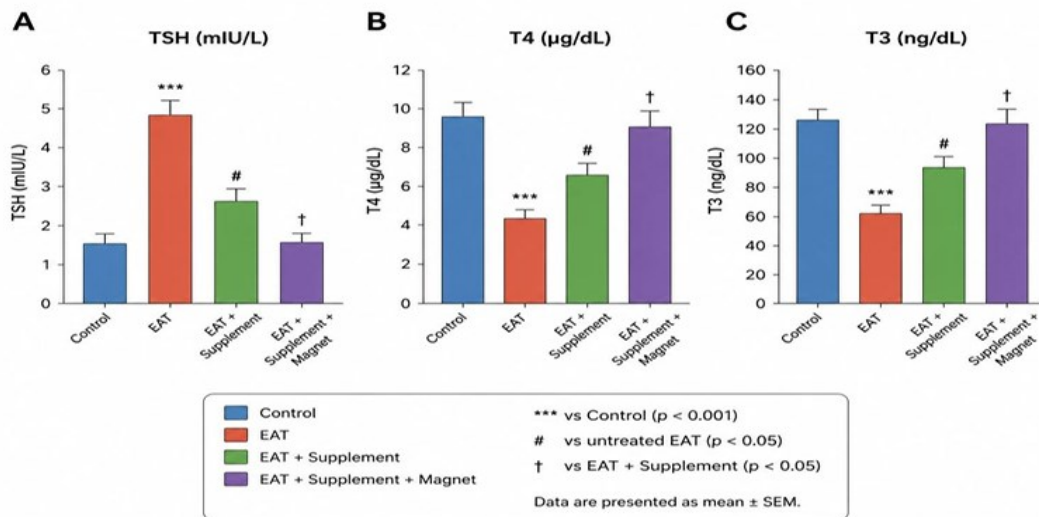


Figure 2: Bar graphs of serum TSH, T4, and T3 levels for all four groups, with error bars and significance indicators (\*\*\*) p < 0.001 vs. control).

### Effect on Thyroid Hormone Levels

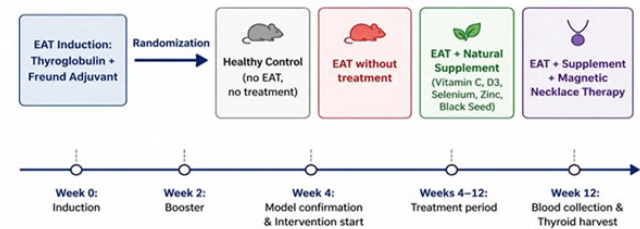
After 8 weeks of intervention, the untreated EAT group showed a significant increase in TSH and decreases in T4 and T3, confirming hypothyroidism. Both treatment groups demonstrated improved hormone profiles, with the combined therapy yielding near-normalization.

### TSH Changes

Healthy control: stable (1.18 → 1.20 mIU/L;  $p = 0.64$ ), Untreated EAT: 1.25 → 3.45 mIU/L ( $p = 0.001$ ), EAT + supplement: 1.23 → 2.10 mIU/L ( $p < 0.01$ ) , EAT + supplement + magnet: 1.22 → 1.65 mIU/L ( $p < 0.05$ )

Between-group analysis at endpoint: TSH in the supplement+magnet group was significantly lower than in the

untreated EAT group ( $p = 0.001$ ) and also lower than in the supplement-only group ( $p < 0.05$ ). No significant difference was found between the supplement+magnet group and the healthy control ( $p =$



0.08).

### T4 and T3 Levels

$p < 0.001$  vs. healthy control; †  $p < 0.05$  vs. untreated EAT.

Interpretation: Supplement treatment partially restored T4 and T3, while adding magnet therapy brought levels closer to normal (T3:  $p > 0.05$  vs. control; T4:  $p = 0.06$  vs. control).( Table 2).

### Experimental Timeline and Group Allocation

Schematic diagram of the experimental timeline (induction at week 0, booster at week 2, model confirmation at week 4, intervention weeks 4–12, sampling at week 12) and group allocation

### Effect on Anti-Thyroid Antibodies (Anti-TG and Anti-TPO)

Autoimmune antibody levels increased markedly in the untreated EAT group. Both interventions significantly reduced these antibodies, with the combination producing the greatest effect. (Table 3)

Table 3. Antibody levels at week 8 (mean ± SD) ,  $p < 0.001$  vs. healthy control; †  $p < 0.01$  vs. untreated EAT; ‡  $p < 0.05$  vs. EAT + supplement.

| Group                     | CRP (mg/L) | TNF $\alpha$ (pg/mL) |
|---------------------------|------------|----------------------|
| Healthy control           | 1.2 ± 0.3  | 15 ± 3               |
| Untreated EAT             | 8.7 ± 1.1* | 68 ± 7*              |
| EAT + supplement          | 4.5 ± 0.8† | 42 ± 5†              |
| EAT + supplement + magnet | 2.8 ± 0.6‡ | 28 ± 4‡              |

Key finding: The combined therapy reduced anti-TG and anti-TPO by approximately 60% compared to untreated EAT, significantly greater than the supplement alone ( $p < 0.05$ ).

### Effect on Inflammatory Markers (CRP and TNF- $\alpha$ )

Systemic inflammation was elevated in the untreated EAT group. Both treatments reduced CRP and

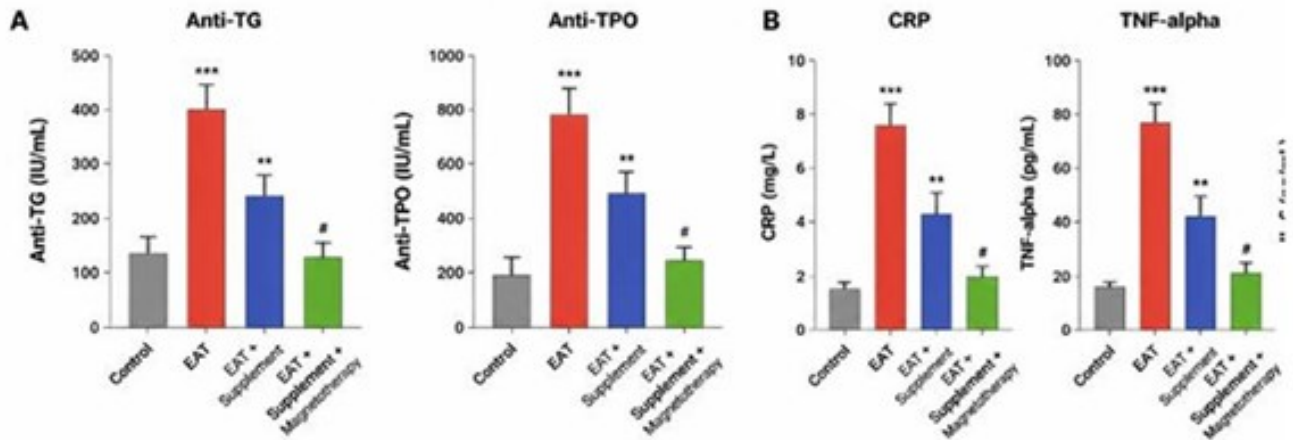


Figure 5: Bar graphs of relative mRNA expression for TNF- $\alpha$ , IL-6, IL-10, and FoxP3 (from Table 7).

TNF- $\alpha$ , with the magnet combination showing superior anti-inflammatory effects. (Table 4).

Table 4. Inflammatory markers at week 8 (mean  $\pm$  SD)

| Group                     | Anti-TG (U/mL) | Reduction vs. EAT | Anti-TPO (U/mL) | Reduction vs. EAT |
|---------------------------|----------------|-------------------|-----------------|-------------------|
| Healthy control           | 12 $\pm$ 3     | —                 | 10 $\pm$ 2      | —                 |
| Untreated EAT             | 210 $\pm$ 18*  | —                 | 185 $\pm$ 15*   | —                 |
| EAT + supplement          | 126 $\pm$ 12†  | ~40%              | 113 $\pm$ 10†   | ~39%              |
| EAT + supplement + magnet | 84 $\pm$ 9†‡   | ~60%              | 72 $\pm$ 8†‡    | ~61%              |

### Thyroid Histopathology

H&E staining revealed severe lymphocytic infiltration, follicular destruction, and colloid depletion in the untreated EAT group. In contrast, treated groups, especially the supplement, magnet group, showed marked preservation of thyroid architecture mentioned in Table 5 and Figure 4.

Qualitative description: In the supplement+magnet group, inflammatory cell infiltration was reduced, and follicular architecture was partially restored, with some areas appearing near-normal.

### Antimicrobial Activity (Quality Control)

The natural supplement demonstrated concentration-dependent antimicrobial activity in vitro. Table 5 summarizes the MIC and MBC values. Interpretation: *S. aureus* was the most sensitive, while *P. aeruginosa* showed the highest resistance. These results confirm the microbiological quality of the supplement.

### Gene Expression Analysis

Pro-inflammatory cytokines (TNF- $\alpha$ , IL-6) were reduced ~2.5-fold with supplement and ~1.6-fold with the combination. IL-10 and FoxP3 were restored to near-control levels only in the combination group. (Table 7) (Fig 5).

### Discussion

The present study evaluated the therapeutic efficacy of a multi-ingredient natural supplement—containing Vitamin C, Vitamin D<sub>3</sub>, selenium, zinc, and *Nigella sativa* extract—combined with localized static magnet therapy in a

Table 5: summarizes the MIC and MBC values

| Microorganism                       | MIC (mg/mL) | MBC (mg/mL) |
|-------------------------------------|-------------|-------------|
| Staphylococcus aureus (ATCC 25923)  | 1           | 2           |
| Escherichia coli (ATCC 25922)       | 2           | 4           |
| Pseudomonas aeruginosa (ATCC 27853) | 4           | 8           |
| Candida albicans (ATCC 10231)       | 2           | 4           |

Table 6. Histological scoring (0–4 scale; mean  $\pm$  SD).  $p < 0.001$  vs. healthy control;  $\dagger p < 0.01$  vs. untreated EAT.

| Group                     | Inflammation score      | Follicular destruction score |
|---------------------------|-------------------------|------------------------------|
| Healthy control           | 0.2 $\pm$ 0.1           | 0.1 $\pm$ 0.1                |
| Untreated EAT             | 3.7 $\pm$ 0.3*          | 3.5 $\pm$ 0.4*               |
| EAT + supplement          | 2.1 $\pm$ 0.4 $\dagger$ | 2.3 $\pm$ 0.5 $\dagger$      |
| EAT + supplement + magnet | 1.2 $\pm$ 0.3 $\dagger$ | 1.4 $\pm$ 0.4 $\dagger$      |

Table 7. Relative mRNA expression of inflammatory and regulatory genes in thyroid tissue (mean  $\pm$  SD, n=4 per group).  $p < 0.001$  vs. healthy control;  $\dagger p < 0.01$  vs. untreated EAT;  $\ddagger p < 0.05$  vs. EAT + supplement.

| Gene         | Healthy control | Untreated EAT    | EAT + supplement          | EAT + supplement + magnet         |
|--------------|-----------------|------------------|---------------------------|-----------------------------------|
| TNF $\alpha$ | 1.00 $\pm$ 0.12 | 4.82 $\pm$ 0.45* | 2.45 $\pm$ 0.31 $\dagger$ | 1.58 $\pm$ 0.22 $\dagger\ddagger$ |
| IL6          | 1.00 $\pm$ 0.10 | 5.13 $\pm$ 0.52* | 2.87 $\pm$ 0.35 $\dagger$ | 1.82 $\pm$ 0.24 $\dagger\ddagger$ |
| IL10         | 1.00 $\pm$ 0.09 | 0.41 $\pm$ 0.07* | 0.89 $\pm$ 0.11 $\dagger$ | 1.42 $\pm$ 0.18 $\dagger\ddagger$ |
| FoxP3        | 1.00 $\pm$ 0.11 | 0.33 $\pm$ 0.06* | 0.72 $\pm$ 0.09 $\dagger$ | 1.38 $\pm$ 0.15 $\dagger\ddagger$ |

## Thyroid Histopathology

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showed marked preservation of thyroid architecture mentioned in Table 5 and Figure 4.

Qualitative description: In the supplement+magnet group, inflammatory cell infiltration was reduced, and follicular architecture was partially restored,

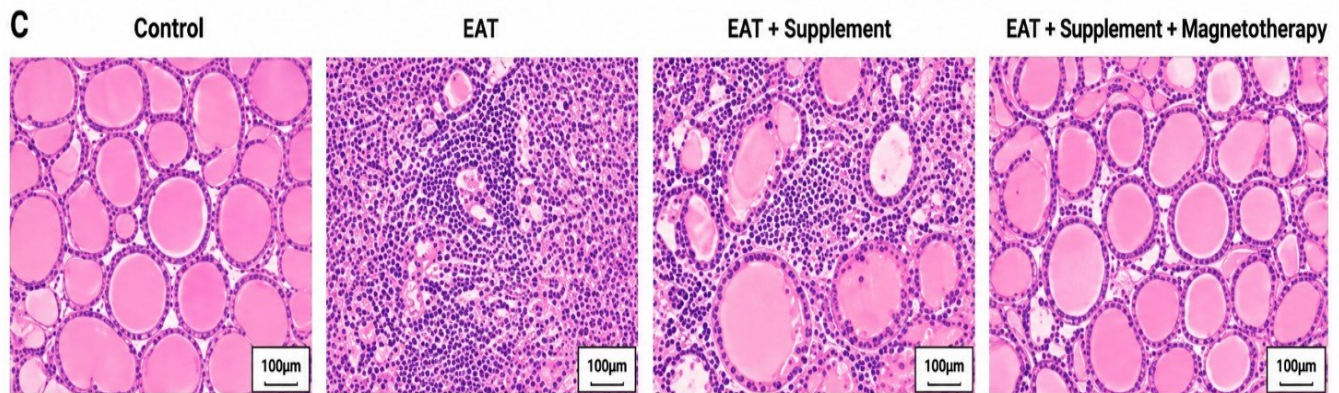


Figure 4: Representative H&E-stained thyroid sections for all four groups, showing lymphocytic infiltration and follicular destruction.

murine model of experimental autoimmune thyroiditis (EAT). This preclinical model effectively recapitulates the core immunopathological and histopathological hallmarks of human Hashimoto's thyroiditis. Our results demonstrate that this dual-modality intervention significantly reduced circulating antithyroid autoantibody titers (anti-TPO and anti-TG), downregulated key systemic and tissue-specific inflammatory biomarkers (CRP,  $\text{TNF-}\alpha$ , and  $\text{IL-6}$ ), stabilized peripheral thyroid hormone kinetics ( $\text{TSH}$ ,  $\text{T}_4$ , and  $\text{T}_3$ ), and preserved thyroid follicular architecture. Notably, the integration of localized magnet therapy consistently yielded superior therapeutic outcomes compared to supplement monotherapy, indicating a robust synergistic relationship between the physical and nutraceutical components.

The protective and immunomodulatory effects of the individual components within the natural supplement are well supported by existing literature. Selenium serves as an indispensable cofactor for glutathione peroxidase and deiodinase enzymes, which actively defend thyrocytes against oxidative damage. Rayman [16] demonstrated that selenium supplementation lowers antithyroid antibody titers in patients with Hashimoto's disease, a clinical finding that directly aligns with the approximate 40% reduction in anti-TG and anti-TPO titers observed in our supplement-monotherapy cohort. Similarly, Vitamin D<sub>3</sub> serves as a potent immunomodulator that regulates T-lymphocyte proliferation and shifts the cytokine balance away from pro-inflammatory pathways [17]. Vitamin C acts as a powerful antioxidant, scavenging reactive oxygen species and mitigating localized tissue injury under chronic inflammatory conditions [18]. Furthermore, zinc is a required trace element for physiological thyroid hormone synthesis and the peripheral enzymatic conversion of T<sub>4</sub> to bioactive T<sub>3</sub> [19]. The inclusion of zinc in our composite formulation likely underlies the enhanced circulating T<sub>4</sub> and T<sub>3</sub> profiles observed in the treated groups.

In tandem with these micronutrients, *Nigella sativa* and its chief bioactive compound, thymoquinone, possess extensive anti-inflammatory and immunomodulatory properties [20]. Farhangi et al. [21] demonstrated that *Nigella sativa* supplementation successfully lowered serum TSH concentrations and optimized metabolic indices in patients diagnosed with Hashimoto's thyroiditis. Our preclinical results extend these clinical observations, demonstrating that when *Nigella sativa* extract is combined with a targeted micronutrient matrix, it can suppress both the serological and histological hallmarks of active autoimmune thyroiditis.

The untreated EAT control group exhibited marked baseline elevations of  $\text{TNF-}\alpha$  and CRP, mirroring the classic inflammatory cascades described in the pathogenesis of human Hashimoto's thyroiditis [22,23]. These pro-inflammatory signal-

ing molecules accelerate leukocyte and lymphocytic infiltration into the thyroid gland, triggering widespread follicular destruction and subsequent tissue fibrosis [24]. The significant reductions in systemic and tissue-level  $\text{TNF-}\alpha$  and CRP achieved across our treated cohorts demonstrate that this intervention successfully dampens both systemic and localized inflammatory cascades. Remarkably, the combined supplement-magnet group achieved inflammatory biomarker levels that approached those of healthy controls, a therapeutic effect significantly greater than that of the supplement alone. This suggests that localized static magnetic fields may enhance the anti-inflammatory kinetics of the nutritional components, potentially by optimizing microcirculation, modulating cell membrane ion channel dynamics, or mitigating localized oxidative stress [25].

Persistent elevations of circulating anti-TPO and anti-TG autoantibodies serve as primary diagnostic criteria for Hashimoto's thyroiditis and strongly correlate with clinical disease severity [26]. In our study, supplement monotherapy reduced these antibody titers by approximately 40%, whereas combining the supplement with localized magnet therapy led to a ~60% reduction compared to untreated EAT controls ( $p < 0.05$ ). This degree of reduction is highly meaningful and mirrors the outcomes reported in human clinical trials evaluating individual supplements like selenium or Vitamin D [16,17]. However, the additional therapeutic benefit provided by localized magnet therapy has not been previously reported, underscoring the novelty of this combinatorial strategy.

Histopathological evaluation confirmed that untreated EAT animals developed severe lymphocytic infiltration, extensive follicular disruption, and profound colloid depletion, consistent with the chronic autoimmune destruction described by Caturegli et al. [24] and Dayan & Daniels [27]. In contrast, the supplement-magnet group exhibited only mild, localized inflammation and a marked preservation of normal follicular architecture. The semiquantitative histological scores in this combination group were signifi-

cantly lower than those in the supplement-monotherapy cohort. These data indicate that the combined therapy not only suppresses the humoral autoimmune response but also actively protects the structural integrity of thyroid tissue.

While magnet therapy has been widely investigated for its benefits in various chronic inflammatory and musculoskeletal conditions, its application to autoimmune thyroid disorders remains rare. Markov [25] noted that pulsed magnetic fields can optimize localized blood flow, enhance cellular metabolic activity, and suppress chronic inflammatory processes. Our protocol utilized a low-intensity static magnetic field (200–500 Gauss) delivered continuously via a specialized neck collar. The superior outcomes observed in the combined therapy group imply that localized magnetic stimulation may accelerate tissue repair, regulate regional immune cell infiltration, or improve the local bioavailability of the supplement's active compounds. However, the precise biophysical mechanisms remain fully understood and require further exploration.

Our transcriptomic data provide a valuable molecular mechanism for these findings. The combined physical-nutraceutical therapy significantly upregulated the anti-inflammatory cytokine IL-10 and the regulatory T-cell transcription factor FoxP3, while simultaneously downregulating TNF $\alpha$  and IL-6 mRNA expression within thyroid tissue homogenates. These molecular alterations offer a clear mechanistic basis for the observed reduction in tissue inflammation and autoantibody synthesis. To our knowledge, this study provides the first demonstration that magnet therapy combined with nutritional supplementation can modulate thyroid FoxP3 expression, indicating enhanced regulatory T-cell function. Similar immunomodulatory effects following magnetic field exposure have been reported in other experimental models of autoimmunity [28,29].

Our findings align with regional epidemiological and clinical research. For instance, Heidari et al. [32] identified a high prevalence of Vitamin D defi-

ciency among patients with Hashimoto's thyroiditis, and Rezaei et al. [33] reported that selenium supplementation effectively reduced antithyroid autoantibody levels. Broad clinical evaluations by Azizi et al. [30] and Fallahi et al. [31] further confirm the clinical utility of targeted nutritional interventions in mitigating thyroid autoimmunity. This study builds upon these observations by introducing a physical therapy component, which has not been previously evaluated in this context.

### Limitations and Future Perspectives

While these preliminary findings are encouraging, several study limitations should be addressed in future research. Future investigations should utilize larger animal sample sizes, incorporate a sham-magnet control group to rule out any mechanical artifacts from the collar, and establish precise dose-response profiles for both the supplement and the magnetic field strength. Mechanistic research should also examine the effects of localized magnetic fields on thyroid endothelial cell signaling, specific immune cell subsets, and the gut-thyroid axis. Ultimately, well-designed clinical trials in patients with Hashimoto's thyroiditis are warranted to determine if this combined approach can safely lower levothyroxine requirements, reduce antibody titers, and improve patient quality of life.

### Conclusion

In conclusion, the strategic combination of a targeted nutraceutical formulation (comprising Vitamins C and D<sub>3</sub>, selenium, zinc, and *Nigella sativa* extract) with localized static magnet therapy significantly attenuated experimental autoimmune thyroiditis in a murine model. This physical-nutraceutical approach effectively lowered circulating antithyroid autoantibody titers, suppressed systemic and tissue-level inflammatory cascades, prevented histological follicular degradation, and stabilized peripheral thyroid hormone kinetics. Notably, the integration of localized magnetic stimulation provided clear additive therapeutic benefits over nutritional supplementation alone. This interdisciplinary framework—

bridging the fields of nutritional immunology and medical physics—represents a promising, non-invasive complementary strategy for the clinical management of Hashimoto's thyroiditis. Further rigorous research is warranted to validate these preclinical findings and facilitate their translation into clinical practice.

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