Effect of Pulsed Electromagnetic Field Stimulation on Splenomegaly and IgE Levels in an DNCB-Induced Atopic Dermatitis Mouse Model

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Article

Effect of Pulsed Electromagnetic Field Stimulation on Splenomegaly and IgE Levels in an DNCB-Induced Atopic Dermatitis Mouse Model

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Abstract: Effects of pulsed electromagnetic fields (PEMF) on immunological factors in a 2,4-dinitrochlorobenzene (DNCB)-induced atopic dermatitis (AD) model were investigated. Hairless mice were randomly assigned to control, acetone and olive oil solution-treated (AOO), PEMF 15 Hz, PEMF 75 Hz, and sham groups (n=5 each). AOO solution was used to dissolve DNCB. Both PEMF and sham groups were exposed to similar DNCB doses, causing similar AD symptoms. After AD induction for five weeks, only the PEMF group was exposed to PEMF stimulations (15 Hz, 75 Hz, and 15 mT) for two weeks inside the solenoid coil. In both groups, splenomegaly was observed, as AD was induced by hyperimmune reactions caused by DNCB sensitization. However, splenomegaly did not occur in the PEMF-exposed groups, and spleen weight decreased similarly to that of the control. Hence, the total splenocytes in the PEMF group were similar to those in the control group, whereas the sham group showed thrice the number of splenocytes compared to the PEMF group. The serum immunoglobulin E levels did not significantly change in the PEMF group; however, they increased more than fourfold in the sham group. These results demonstrate that PEMF stimulation ameliorates the abnormal symptoms caused by hyperimmune reactions.

Keywords: atopic dermatitis; DNCB; LLLF_PEMF; hairless mouse; ELISA; splenocyte; IgE

1. Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin condition caused by a delayed hypersensitivity reaction to repeated allergen exposures [1,2]. This condition is characterized by various dermatological abnormalities and the accumulation of dead skin cells [3,4]. The increasing global incidence of AD has severely impacted the quality of life of affected individuals, emphasizing the urgent need for efficacious treatment strategies [5,6]. Patients with AD primarily exhibit Type I allergic hypersensitivity reactions [7]. These reactions are characterized by immunoglobulin E (IgE) production in the bloodstream, propelled by an intensified immune response [8,9], which releases histamine through antigen-antibody interactions, thereby causing symptoms such as pruritus and inflammation [10–12].

The increase in IgE concentration in the bloodstream is intricately linked to T helper 2 cell activation, which subsequently stimulates basophils and mast cells, leading to the production of interleukin-4, a pivotal cytokine in the allergic response [13–15]. This cascade of immunological reactions significantly affects the spleen, a key secondary lymphatic organ that is crucial in mediating the immune response of the body to circulating antigens [16–18]. Therefore, a thorough investigation of the splenic response to these immune challenges constitutes a fundamental aspect of AD research. Studies have demonstrated that between 70–80% individuals with AD exhibit the IgE-mediated

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exogenous subtype, whereas 20–30% manifest symptoms associated with the IgE-non-mediated endogenous subtype [19,20]. AD encompasses a multifaceted interplay of immunological components and is further complicated by compromised skin barrier function [21,22]. This impairment increases the skin's permeability to various irritants, including allergens and bacteria, thereby causing inflammation and subsequent skin tissue degradation. Hence, interrupting these detrimental cycles and rectifying the underlying skin abnormalities is an important strategy for effective AD management.

In conventional drug therapies for AD, achieving complete remission remains elusive and frequently results in several adverse effects [23–26]. The absence of viable alternatives to pharmacological interventions necessitates variability in treatment outcomes, including both therapeutic benefits and undesirable reactions. Because of this predicament, new treatment methodologies that can circumvent the limitations and side effects associated with current practices are necessary. Numerous studies have substantiated the diverse physiological benefits of Pulsed Electromagnetic Fields (PEMF), including anti-inflammatory effects, enhancement of tissue regeneration, acceleration of wound healing, and improvement of blood circulation [27–31]. These attributes have propelled PEMF as a notable candidate in our investigation, particularly in exploring its potential as a non-invasive treatment method within the context of DNCB-induced atopic dermatitis models. This study delves into how the physiological effects of PEMF can contribute to alleviating symptoms of atopic dermatitis by reducing tissue inflammation, promoting regeneration processes, facilitating quicker wound recovery, and ultimately enhancing blood flow. This novel therapeutic approach presents a promising avenue for overcoming the challenges and constraints associated with traditional pharmacological interventions. This novel therapeutic approach presents a promising avenue for overcoming the challenges and constraints associated with traditional pharmacological interventions.

In this context, PEMF application in AD treatment, particularly in models characterized by hyperimmune responses, is an unexplored frontier. This innovative approach harnesses the noninvasive nature of magnetic fields to modulate immune reactions without eliciting discomfort, paving the way for a novel therapeutic strategy for managing dermatological conditions. Capitalizing on these benefits, the non-perceptual nature of PEMF allows for conducting more objective and controlled experiments, minimizing stress-related variables that could skew the results. Histological analyses from prior research confirmed that PEMF stimulation had discernible anti-inflammatory and tissue regenerative effects in 2,4-dinitrochlorobenzene (DNCB)-induced AD models, emphasizing its therapeutic potential [32]. This evidence suggests that PEMF is not only a viable treatment modality for AD but also a catalyst for advancing our understanding of its underlying mechanisms and therapeutic pathways.

This study involved a detailed examination of the effects of PEMF on AD, focusing on analyzing changes in serum IgE levels, splenomegaly, and spleen size as primary indicators of immunological responses. These specific parameters were quantified to uncover the extent to which PEMF influenced the role of the immune system in AD, potentially reducing IgE levels and alleviating the symptoms associated with splenomegaly. To achieve these objectives, a structured experimental design was adopted in which hairless mice were systematically exposed to PEMF of varying intensities and durations. This approach enables the discovery of the nuanced relationship between PEMF exposure and its subsequent effects on immunological markers of interest, specifically serum IgE levels, splenomegaly, and splenocyte counts. These immunological outcomes were correlated with PEMF parameters to establish a foundational understanding of the therapeutic mechanisms of PEMF in mitigating allergic and inflammatory responses in the AD model.

In conclusion, this study aimed to demonstrate the beneficial effects of PEMF stimulation in mitigating the symptoms associated with hyperimmune reactions, along with its anti-inflammatory and tissue-regenerative capabilities, in DNCB-induced AD models. These outcomes would corroborate the preliminary hypotheses derived from our initial pilot study, offering substantive evidence of the therapeutic advantages of PEMF in AD management. This research intends to expand

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our understanding of the role of PEMF in AD treatment and establish a scientific foundation for its broader application in dermatological therapy.

2. Materials and Methods

2.1. PEMF

2.1.1. PEMF System Design

To assess the improvement in abnormal symptoms and anti-inflammatory effects of PEMF stimulation in DNCB-induced AD, a coil-optimized PEMF stimulation system was designed. The voltage and current applied to the core were controlled in multiple stages to adjust the varying magnetization levels of the magnetic field-generating core within the range of 10–30 mT. The hardware and firmware were designed to vary and control the stimulation parameters according to the frequency. Additionally, to address the heat generation problem of the magnetized field-generating core, the duty ratio was designed to vary from 10 to 50% via pulse width modulation, while the frequency was set within the range of 1–100 Hz. This system was designed to perform PEMF stimulation using a solenoid coil with up to two channels simultaneously (Figure 1).

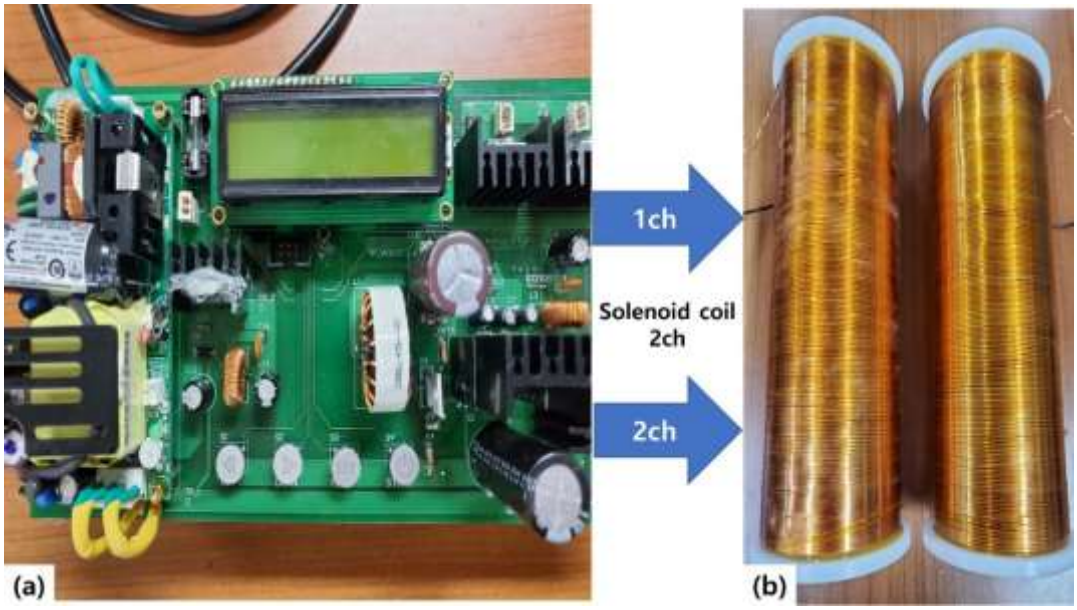

Figure 1. (a) LLLF_PEMF generation and control system with (b) two-channel solenoid cores. The PEMF system is designed to vary the frequency (1–100 Hz), pulse width (1%–50%), and magnetic intensity (10–30 mT). The solenoid core size is 80 mm (diameter) × 200 mm (height).

2.1.2. PEMF Generation Cores and Experimental Parameters

The solenoid coil used in this experiment was fabricated using a 3-dimensional printer with a core composed of a copolymer consisting of acrylonitrile, butadiene, and styrene. The dimensions of the device, which included 80 mm external diameter, 60 mm internal diameter, and 200 mm height, were customized to fit those of the animal cage dimensions. The coil was wound uniformly around the core and designed to adjust the pulsed voltage and current applied to the coil according to the parameters for the PEMF stimulation used in the experiment: 15 Hz, 75 Hz, duty rate = 30%, and Avg = 15 mT.

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2.2. Experimental Materials

2.2.1. Experimental Animals

The experimental cohort consisted of 25 six-week-old male hairless mice (SKH1), (Junbiotech, Gyeonggi-do, Korea), weighing between 26 and 27 g. The animals were divided into five groups: control, acetone and olive oil solution (AOO), sham, PEMF 15 Hz, and PEMF 75 Hz (n = 5 each). Randomized allocation ensured unbiased distribution across groups. Throughout the study, all animals had ad libitum access to standard solid feed and water to ensure that their nutritional needs were met autonomously. The animals were housed in a specific pathogen-free environment, and optimal conditions conducive to their well-being were maintained. The facility operated on a 12-h light-dark cycle, while indoor temperature (24 ± 2 ℃) and humidity (55 ± 5%) were meticulously regulated using a thermo-hygrostat.

2.2.2. Experimental Reagents

To prepare a DNCB solution to induce AD through drug contact hypersensitivity, DNCB (Sigma-Aldrich, MO, USA), 100% acetone (Sigma-Aldrich), and olive oil (Sigma-Aldrich) were used. Trypan blue (Sigma-Aldrich) was used for splenocyte cell counting. An enzyme-linked immunosorbent assay (ELISA) kit (Abcam, Cambridge, UK) was used to analyze serum IgE concentrations.

2.2.3. DNCB Solution Preparation and AD Induction Methods

DNCB crystals were diluted in an AOO solution (olive oil: 100% acetone = 1:3) to produce a DNCB solution. The solution concentration (0.1–1%) was adjusted according to sensitization, and abnormal symptoms observed in the sham, PEMF 15 Hz, and 75 Hz groups. Topical application of 200 μl of the solution was performed on the mice using a micropipette every 3–4 days to induce AD of moderate severity or higher. The AOO group was subjected to topical application of AOO solution alone that did not contain DNCB, whereas the control group was not administered any treatment throughout the experiment.

2.3. Experimental Process

All experimental animals were raised for the same duration. Following a one-week adaptation period, AD was induced via DNCB sensitization for five weeks. From weeks 6 to 8, the PEMF 15 Hz and 75 Hz groups were exposed to PEMF at a magnetic flux density of 15 mT for 1 h per day over two weeks inside the solenoid coil. In the sham group, the animals were placed inside the solenoid coil for 1 h per day, similar to the PEMF group; however, no PEMF stimulation was administered. In contrast, the control and AOO groups did not undergo solenoid coil insertion and thus did not receive PEMF stimulation. On the conclusion of the eight-week experimental period, all subjects were humanely euthanized via cervical dislocation. The experimental procedure is represented in Figure 2. All methods and processes were approved by the Institutional Animal Care and Use Committee (YWCI–202106-011-01, IACUC, Yonsei University).

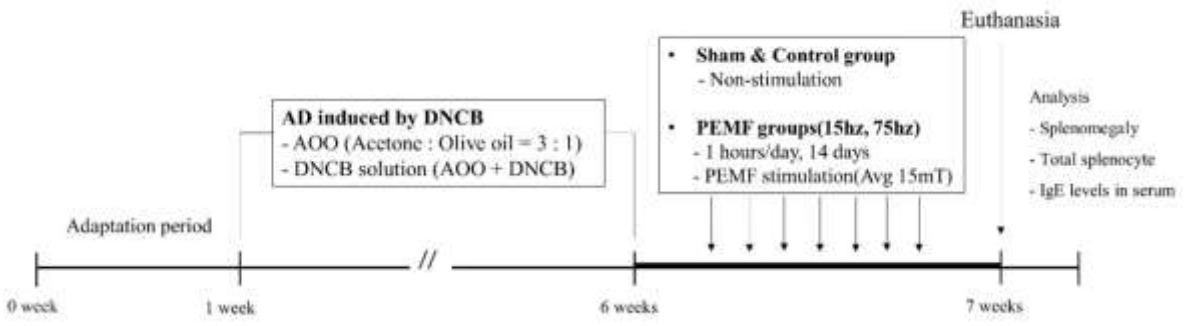

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Figure 2. Overview of the experimental procedure used to assess the effects of Pulsed Electromagnetic Field (PEMF) stimulation on DNCB-induced atopic dermatitis in hairless mice. This schematic provides a comprehensive guide to the research methodology, facilitating understanding of the experimental design and analytic approach.

2.3.1. Measurement of Spleen Weight

The spleen, from which the fascia and surrounding adipose tissue were removed during dissection, was stored in a microtube containing 1 mL of phosphate-buffered saline (PBS) solution. The weight of the microtubes containing 1 mL of PBS was adjusted to 0 g using an experimental electronic scale (OHAUS, NJ, USA), and the average spleen weight of each individual in each group was measured and statistically analyzed. The spleen weight of each individual subject was then measured and recorded. This process was repeated for all the experimental subjects to ensure data reliability and consistency.

2.3.2. Splenocyte Counting

Splenocytes were isolated after removing the spleen membrane using PBS solution and a cell strainer. All cell counting processes were completed within 12 h of extraction to maintain the maximum possible splenocyte viability. RBC lysis buffer (Sigma-Aldrich) was added and allowed to react for 3 min to remove any residual red blood cells from the splenocyte suspension. Each individual splenocyte sample was stained with Trypan blue (Sigma-Aldrich), loaded into a hemocytometer, and observed under an optical microscope.

Cells stained because of cell membrane injury were classified as dead cells, whereas unstained cells were classified as living cells. Cell counting was performed in four of the nine regions of the hemocytometer, and the total number of cells in the suspension was estimated via calculating the grid volume using the depth (0.1 mm) and area (0.025 mm^2) of the hemocytometer. The total number of cells estimated for each individual was statistically analyzed via calculating the average value for each group. Additionally, the precision of the cell counting process was ensured via repeating the procedure thrice for each sample and calculating the mean value (Figure 3).

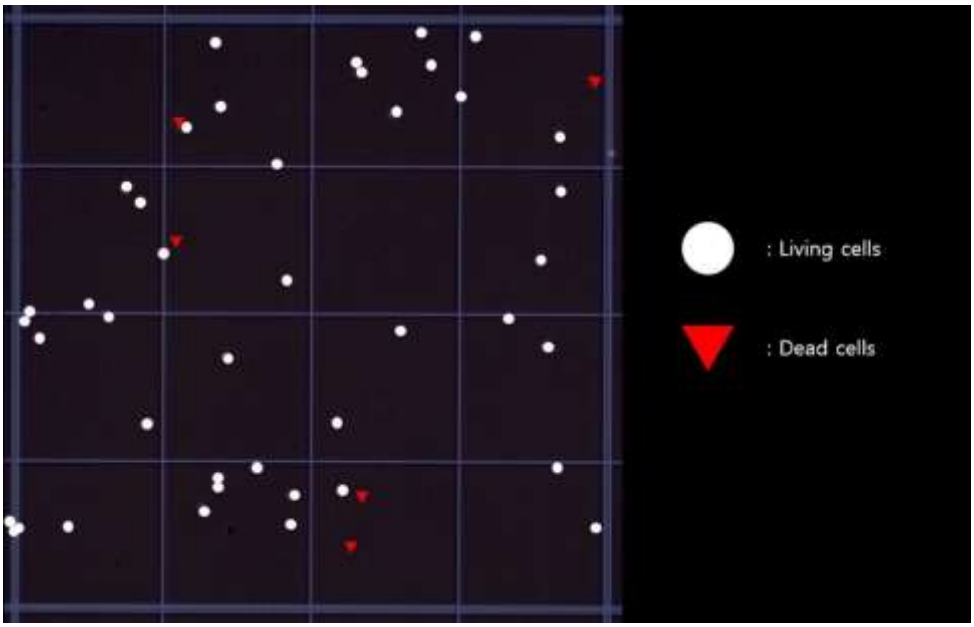

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Figure 3. Living and dead cells of splenocytes dyed with a trypan blue solution inside the hemocytometer.

2.3.3. Measurement of Serum IgE Levels Using ELISA

Blood collected from the abdominal aorta in a volume of 0.4–0.6 cc was allowed to coagulate at room temperature (26 ± 2 ℃) for 30 min. The coagulated blood was then centrifuged (1200 rpm, 5 min), and the separated serum was aliquoted into 20 μl portions and stored in an ultra-low temperature freezer. In this study, a sandwich ELISA technique was employed. Each serum sample was immobilized onto the target protein using a capture antibody-coated 96-well plate. A sandwich structure was formed via attaching a biotin-labeled detection antibody, and conjugating streptavidin-horseradish peroxidase enzyme. The optical density value for serum IgE concentration was obtained at a wavelength of 450 nm.

To determine the appropriate dilution ratio corresponding to the serum IgE concentration, three pilot tests were conducted using serum samples from all subjects to estimate the average IgE concentration in each group. For each experiment, the standard curve was duplicated to verify reliability, and the IgE concentration corresponding to the optical density value was determined using the log-log curve fitting method. After the pilot test, the average IgE concentration of each group was measured in three independent experiments to ensure objectivity and statistical reliability. The serum utilized in the experiment was not subjected to repeated freeze-thaw cycles, and ELISA was performed as per the manufacturer's instructions.

2.4. Statistical Analysis

The data derived from this study were processed and analyzed using GraphPad Prism (version 5.02, GraphPad Software, CA, USA). Results are presented as means ± standard errors of the mean (SEM). Comparative analyses across the study groups were performed employing a contrast test via one-way analysis of variance (ANOVA), supplemented with Tukey's Multiple Comparisons Test for post-hoc analysis. Significance thresholds were set at $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ to discern statistically significant differences.

3. Results

3.1. Results of Splenomegaly Comparison Analysis

In the splenomegaly analysis, both the DNCB-treated PEMF and sham groups displayed pronounced splenic enlargement caused by hyperimmune responses. The average spleen weights were as follows: control group, 144.6 mg; AOO group, 181 mg; PEMF 15 Hz group, 172.8 mg; PEMF 75 Hz group, 169.8 mg; sham group, 367.8 mg. Spleen enlargement in the sham group was approximately 2.5-fold greater than that in the control group, which was not treated with DNCB or AOO, indicating the most substantial discrepancy observed ($^{***}p < 0.001$). A significant disparity was evident in spleen weight between the sham and remaining experimental groups, as determined by a one-way ANOVA comparing the mean weights ($^{***}p < 0.001$).

While the control group showed the least spleen enlargement, the spleen weight did not significantly differ from that of the AOO and PEMF treatment groups ($p > 0.05$). In contrast, although no significant differences in spleen weight were detected between the PEMF groups (indicating no frequency-dependent variation within these groups, $^{*}p < 0.05$), a significant difference was apparent when compared with the sham group. Similar to the sham group, both PEMF groups showed comparable severity in skin abnormalities. Overall, significant reductions in spleen weight, indicative of less pronounced splenomegaly, were observed in the 15 Hz and 75 Hz groups relative to the sham group ($^{***}p < 0.001$) (Figure 4).

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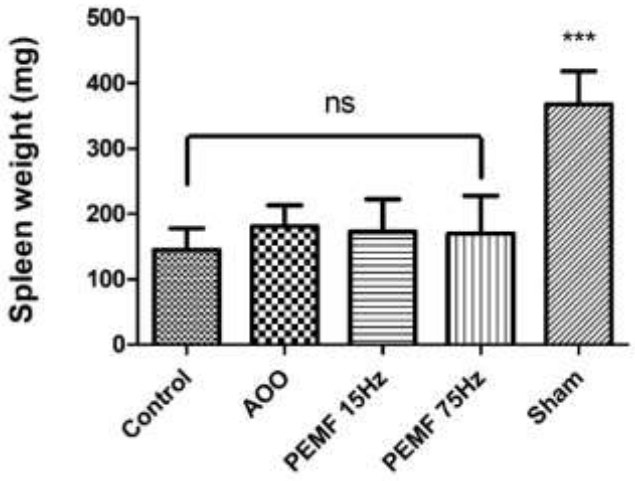

Figure 4. Comparative analysis of spleen weights in each group. All groups were statistically based on the Control group. Data points reflect mean spleen weights ± standard deviation, highlighting significant differences in splenomegaly across the groups(***$p < 0.001$).

3.2. Splenocyte Counting Results

To examine the differentiation of T and B lymphocytes in response to splenomegaly, splenocytes were isolated and counted following red blood cell depletion. The average splenocyte counts were as follows: control group, 1.03×10^8; AOO group, 1.44×10^8; PEMF 15 Hz group, 1.48×10^8; PEMF 75 Hz group, 1.35×10^8; and sham group, 2.96×10^8. The Sham group displayed a 2.9-fold increase in splenocyte count compared with the control group, which was the largest observed difference. Moreover, this was an approximately 2.1-fold increase compared to the PEMF-treated groups.

A subsequent one-way ANOVA with the control group as a reference, showed no significant enhancement in splenocyte differentiation among the experimental groups, excluding the sham group ($p > 0.05$). Similarly, comparisons within the PEMF groups revealed no significant differences in splenocyte counts between the 15 Hz and 75 Hz frequencies ($p > 0.05$). In contrast, a marked decrease in splenocyte count was observed between the PEMF and sham groups, indicating a significant 2.1-fold reduction caused by PEMF exposure (***$p < 0.001$) (Figure 5).

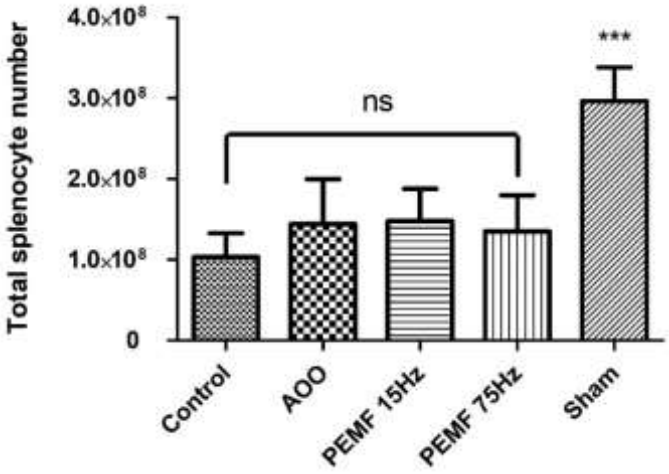

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Figure 5. Comparative analysis of the total splenocyte number of the spleen in each group. All groups were statistically based on the Control group. Each bar represents the average number of splenocytes ± standard deviation, emphasizing the observable reduction in splenocyte counts in the PEMF groups compared to the sham group (***p < 0.001).

3.3. IgE Level Analysis in Serum (ELISA)

Serum IgE levels were quantified using ELISA after inducing a hyperimmune reaction through repeated DNCB exposure. This analysis revealed distinct differences in IgE levels between the experimental groups. The control group had an average IgE level of 66.63 ng/mL, whereas the AOO group had a level of 136.65 ng/mL. PEMF stimulation at 15 Hz and 75 Hz resulted in average IgE levels of 307.09 ng/mL and 553.69 ng/mL, respectively, illustrating a frequency-dependent increase in IgE secretion. The most pronounced elevation was observed in the sham group, with an average level of 1942.32 ng/mL, signifying a substantial increase in IgE secretion, approximately 29.2 times that of the control group (**p < 0.01).

Upon detailed comparison, although the 15 Hz and 75 Hz PEMF groups showed elevated IgE levels, indicating a 1.8-fold increase with frequency, this difference was not statistically significant (p > 0.05). When comparing the PEMF and sham groups, which exhibited severe skin abnormalities caused by DNCB, a significant reduction in IgE levels was observed. The PEMF 75 Hz group showed a 3.51-fold decrease, whereas the PEMF 15 Hz group displayed a more substantial reduction, with a 6.32-fold difference in IgE concentration, both achieving significant decreases (**p < 0.01) (Figure 6).

These findings underscore the role of PEMF frequency in modulating serum IgE levels after DNCB exposure, suggesting the frequency-dependent efficacy of PEMF stimulation in altering immune responses.

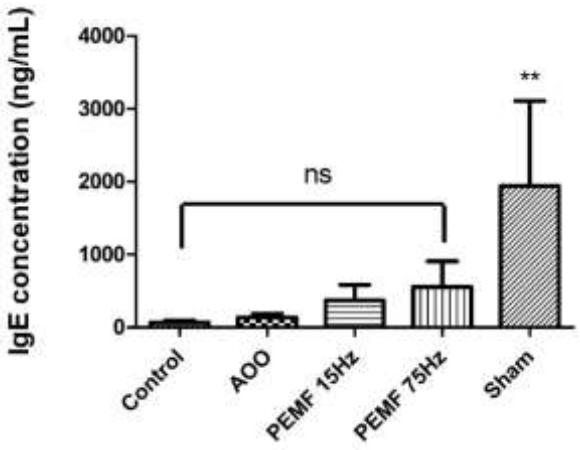

Figure 6. Comparative analysis of IgE concentration (ng/mL) in each group. All groups were statistically based on the Control group. Each bar represents the mean IgE level ± standard deviation for each group, showcasing a significant decrease in serum IgE levels in the PEMF-treated groups relative to the sham group (**p < 0.01).

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4. Discussion

This study examined the anti-inflammatory and immunological ramifications of PEMF stimulation in DNCB-induced allergic reactions and the inflammatory skin abnormalities resulting from hyperimmune responses. Building on our previous research that highlighted the histological influence of PEMF on skin tissues and nonspecific lesions, the current investigation focuses on the immunological foundations associated with AD. This includes a detailed analysis of serum IgE levels, splenomegaly, and total splenocyte count with the aim of integrating histological insights with immunological data. This comprehensive approach offers a more nuanced understanding of AD, illuminating the immunomodulatory capacity of PEMF stimulation via assessing the changes in serum IgE concentration, spleen weight, and splenocyte counts in DNCB-exposed hairless mice. This endeavor not only deepens our understanding of the therapeutic potential of PEMF for AD but also bridges the existing knowledge gap between histological findings and immunological pathways.

In the previous research, skin sections were stained with hematoxylin and eosin to observe the inflammation induced by leukocyte infiltration accompanied by keratinization [32]. Similar to the current findings, both the 15Hz and 75Hz PEMF groups exhibited a reduction in inflammation and lesion size comparable to the control group, while the Sham group displayed significantly greater inflammation thickness and larger lesions. This continuity in results across studies not only reaffirms the anti-inflammatory effects of PEMF treatment but also provides a deeper insight into its mechanism of action. Specifically, the consistent decrease in inflammation and lesion size across different frequencies of PEMF stimulation highlights its potential as a versatile and effective therapeutic tool for managing atopic dermatitis. Furthermore, integrating these histological findings with the current study's immunological data, including serum IgE levels, splenomegaly, and total splenocyte count, offers a more comprehensive understanding of the multifaceted role PEMF therapy plays in modulating the immune response in atopic dermatitis. This synthesis of histological and immunological perspectives paves the way for a holistic approach to treating atopic dermatitis, underscoring the importance of targeting both the visible manifestations of the disease and its underlying immunological mechanisms.

Considering the critical function of the spleen within the lymphatic system, the observed differences in splenomegaly attributable to PEMF stimulation imply an elevation in T- and B-cell activities, which are crucial for immune responses triggered by DNCB sensitization. This suggests that PEMF stimulation may modulate these heightened immune reactions, potentially offering a therapeutic approach for managing excessive immune responses. The findings of this study did not reveal a significant increase in spleen weight or total splenocyte count attributable to splenomegaly in the PEMF group compared to the control, suggesting a potential mitigation of immune dysregulation, which is typically associated with drug-induced hypersensitivity reactions. Conversely, the sham group exhibited a significant enhancement in both spleen size and splenocyte population compared to the other groups, highlighting a pronounced hyperimmune condition in response to allergenic provocations. These observations suggest that PEMF stimulation may be an effective strategy for modulating systemic anomalies in hematopoietic organs or managing inflammatory infectious conditions via potentially restoring immune imbalances.

The serum IgE levels, while highlighting variations between the PEMF 15 Hz and 75 Hz groups, did not reveal statistically significant differences. However, the observable trend towards lower IgE levels in the 15 Hz group indicates the nuanced impact of PEMF frequencies on IgE regulation. Despite the lack of statistical significance, this insight emphasizes the potential role of frequency in fine-tuning immunological responses, marking a promising direction for further research. To delve deeper into these frequency-dependent effects, subsequent studies should investigate the influence of PEMF on specific immune markers, such as targeted proteins or inflammatory cytokines, to more precisely determine the relationship between PEMF frequency and immune modulation.

Overall, integrating histological findings from preliminary research with the immunological insights gained from the current study, including detailed analyses of serum IgE levels, splenomegaly, and total splenocyte counts, suggests that extended PEMF exposure may offer dual benefits: promoting the regeneration of damaged skin tissue and alleviating symptoms related to

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hyperimmune reactions. This dual capacity of PEMF indicates its potential as a multifaceted therapeutic tool in AD management. Therefore, the influence of PEMF on the histological and immunological aspects of this condition must be further explored. The observed effects on spleen size and splenocyte proliferation further suggest that PEMF can modulate the key components of the immune system, enhancing its therapeutic potential in AD management. Although establishing a direct correlation between PEMF application and comprehensive AD management presents challenges, especially in understanding the mechanisms underlying splenomegaly and splenocyte modulation, our findings lay a strong foundation for future research. Investigating the mechanisms underlying PEMF affecting IgE levels, splenocyte dynamics, and spleen size is vital for a more in-depth understanding of its multifaceted role in AD treatment, opening avenues for novel approaches to modulate immune responses under atopic conditions.

Despite the encouraging outcomes of this study, acknowledging its limitations is crucial for guiding future research. This analysis primarily assessed the short-term effects of PEMF therapy; longitudinal studies are required to determine the long-term efficacy and sustainability of PEMF treatment, especially in relation to AD recurrence rates. Additionally, the PEMF operational parameters, such as frequency, intensity, and duration, require further refinement for the complete optimization of therapeutic benefits. Currently, our research has focused on the immunological effects of PEMF; the investigation must be broadened to include the entire spectrum of its physiological effects and examine potential synergies with existing treatment methods.

By addressing these gaps, we can further elucidate the complex mechanisms driving the therapeutic benefits of PEMF and facilitate its adoption as an effective, noninvasive treatment option for AD in clinical settings.

5. Conclusions

The experimental findings of this study demonstrated that skin abnormalities were prevalent among the subjects in both the PEMF and sham groups, which were consistently exposed to DNCB. Specifically, PEMF stimulation resulted in observable variations in splenomegaly, underscoring its potential to mitigate the adverse symptoms induced by hyperimmune reactions. These outcomes not only corroborate the therapeutic efficacy of PEMF in modulating immune responses but also align with previous research, which had demonstrated the histological benefits of PEMF on skin tissues and lesions. Such consistency in results across studies reinforces the anti-inflammatory and immunomodulatory properties of PEMF therapy, providing a solid foundation for its application in managing AD.

Advancing the optimization and thorough evaluation of PEMF therapy emerges as a pivotal next step. By doing so, we can significantly enhance AD management strategies, offering patients innovative, safe, and effective treatment options. This endeavor will not only contribute to the existing body of knowledge but also pave the way for future research aimed at fine-tuning PEMF parameters for maximized therapeutic outcomes. Incorporating insights from both histological and immunological studies, our comprehensive approach fosters a more nuanced understanding of AD, advocating for a multifaceted treatment paradigm that addresses the complex interplay between histopathological findings and immune mechanisms.

Author Contributions: Conceptualization, J.Y. and Y.H.; methodology, J.Y.; formal analysis, J.Y.; investigation, J.Y., J.E. and S.H.; resources, J.Y. and Y.H.; data curation, J.Y.; writing—original draft preparation, J.Y.; writing—review and editing, J.Y.; visualization, J.Y.; supervision, K.J., Y.S. and Y.H.; project administration, J.Y.; funding acquisition, J.Y. and Y.H. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The animal study protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of YONSEI UNIVERSITY (YWCI–202106-011-01).

Informed Consent Statement: Not applicable.

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Data Availability Statement: Owing to the nature of this research, the participants of this study did not consent to having their data would be shared publicly;, and therefore, supporting data are not available.

Conflicts of Interest: The authors declare no conflicts of interest.

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