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Helminth-derived antigens induce an immunomodulatory response in autoimmune type 1 diabetes mellitus by promoting regulatory immune responses

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ABSTRACT

This study examined *Trichinella spiralis* muscle larvae (ML) antigens as preventive and therapeutic agents for type 1 diabetes mellitus (T1-DM) in mice. Sixty male albino mice were divided into six groups. Group 1 (Gp1) was left as a control non-diabetic group. Gp2, mice had injected with streptozotocin (STZ) at a dose of 40 mg/kg/day for 5 days intraperitoneally (i.p). Gp3 and Gp4 were the preventive groups; mice were received *T. spiralis* ML antigens at doses of 500 and 1000 µg/kg, respectively, i.p twice a week for 3 weeks. Then, they administered STZ at a dose of 40 mg/kg/day for 5 days i.p.. One week after that, they were again given *T. spiralis* ML antigens at the same doses twice a week for another three weeks. Gp5 and Gp6 were the therapeutic groups; mice were injected with STZ (40 mg/kg /day) for 5 days i.p.. After a week, they were inoculated with *T. spiralis* ML antigens at the same doses i.p., twice a week for 3 weeks. The immune responses in all groups were evaluated. *T. spiralis* antigens in STZ-induced T1-DM mice revealed that both preventive and therapeutic treatments altered the expressions of CD3+, CD4+, and CD8+ T cells, increased IgE, IgG, and IgM levels, boosted CD25+ T-reg cells, and elevated anti-inflammatory cytokines (IL-4, IL-10), while dropping the pro-inflammatory cytokine IFN-γ. This suggests a shift toward Th2-biased immunity, which improved glycemic control. To conclude, helminth antigens show promise as immunotherapeutic agents for T1-DM by modifying immune responses, suggesting their potential as drug candidates. More studies are needed to evaluate their effects in treating autoimmune diseases.

Keywords: Autoimmune diabetes, Larval antigens, Helminths, Immunotherapy, Inflammation.

1. Introduction

Type 1 diabetes mellitus (T1-DM) is an autoimmune complication impacting around one out of 400 children in Western countries, with a 4% annual rise in incidence in Europe and the U.S. in the past thirty years (Sharp Cinek et al., 2019). In autoimmune disease, the immune system targets insulin-producing β-cells, leading to hyperglycemia. The reasons for this immune failure are not fully understood and need further investigation (Purcell et al., 2019). Autoreactive T cells impact autoimmune targeting on β cells. CD8+ cytotoxic T lymphocytes directly target β cells by identifying precise antigens, while CD4+ helper T lymphocytes produce proinflammatory cytokines that recruit additional immune cells and sustain inflammation in pancreatic islets (Redondo et al., 2018). B lymphocytes contribute to autoimmune responses by generating autoantibodies against β-cell antigens like glutamic acid decarboxylase (GAD) and insulin, which are important for T1-DM diagnosis. Their precise role in β cell destruction remains uncertain, potentially aiding in autoantigen presentation to T cells or

activating damaging cell pathways (James et al., 2023). T1-DM is an autoimmune disorder destroying β cells via a Th1-type inflammatory response. Lymphocyte infiltration into the islets is a decisive early event in the disease's progress (Kreider et al., 2009; Alghanmi et al., 2024). Pro-inflammatory components such as interleukin-1 (IL-1), interferon-gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α) lead to β-cell damage, dysfunction, and apoptosis. Continuing targeting by these cytokines causes β-cell exhaustion, diminishing insulin discharge and rising cell death risk (Bender et al., 2021). Investigating how helminth antigens influence this localized inflammation could provide insights into their protective role against T1-DM (Koarada et al., 2002; Alghanmi et al., 2024).

Helminth antigens and products utilize various strategies to manipulate the host's immune system for their survival and reproduction. Helminth antigens induce significant changes in the innate and adaptive immune responses, which can help protect against autoimmune conditions (Redondo et al., 2018). Research on helminth-derived antigens showed potential for treating inflammatory and autoimmune diseases, including T1-DM. Helminth-derived antigens are being explored for their ability to prevent or alleviate T1-DM by regulating immune mechanisms to reduce autoimmune damage to insulin-producing β cells (Tang et al., 2019). These antigens may shift immune activity toward a Th2 profile while promoting regulatory T cells (T-reg) and anti-inflammatory cytokines such as IL-10 and tumor growth factor-β (TGF-β), which help inhibit the Th1-mediated autoimmune assault characteristic of T1-DM (Saunders et al., 2007; Camaya et al., 2023). Helminth-specific antigens can prevent the onset of T1-DM, even after the initial β-cell damage (insulitis) begins, possibly by enhancing the function and survival of both β cells and macrophages (Liu et al., 2009). Immunotherapies for T1-DM, such as immunosuppressants and immune modulators, show clinical promise (Ziegler et al., 2021). Helminth antigens, especially from *Trichinella spiralis*, may effectively treat T1-DM by stopping Th1-mediated autoimmune responses (Osada and Kanazawa, 2010). T1-DM, driven by Th1 cells, can be mitigated as *T. spiralis* antigens shift immune responses to Th2, inhibiting Th1 lymphocyte activity (Yazdanbakhsh et al., 2002). The increasing T1-DM incidence might relate to immune dysregulation from a lack of exposure to helminth parasites and antigens (Resende et al., 2007), while helminth-derived antigens like *T. spiralis*, *Heligmosomoides polygyrus*, or *Schistosoma mansoni* significantly lower T1-DM rates and reduce pancreatic islet lymphoid infiltration (Saunders et al., 2007).

Helminth-derived antigens enhance the function and expansion of T-reg, vital for suppressing autoimmunity and maintaining tolerance (Tang et al., 2019). They stimulate cytokine release (IL-4, IL-10, and TGF-β) that alleviates pancreatic inflammation (Saunders et al., 2007). Helminth antigens improve communication between macrophages and β cells, enhancing β cell survival. Helminth-driven antigens may curb T1-DM by blocking harmful CD8+ and CD4+ T lymphocytes or macrophage infiltration while improving the infiltration of regulatory immune cells that stop islet destruction (Gregori et al., 2003; Shimokawa et al., 2020). Combining helminth antigens with proinsulin therapy enhances T-reg levels and delays diabetes. Different helminthic antigens were found to protect against diabetes by inducing various immune responses.

These responses included heightened Th2 activity, increased T-reg production, IL-10 production by T and B cells, and decreased CD8+ T cell infiltration in the pancreas, all of which may help prevent the onset of T1-DM (Mughal et al., 2021). The mechanisms defining diabetes prevention after helminth antigen administration have not been completely clarified. Using specific helminth antigens and products may further clarify diabetes prevention mechanisms (Shimokawa et al., 2020). Accordingly, this research intended to discover the possible immunological modulatory, protective, and therapeutic effects of the *T. spiralis* soluble muscle larvae antigen *(T. spiralis* ML) on streptozotocin (STZ)-induced T1-DM in mice.

Materials and methods

Reagents

STZ was obtained from RPMI-1640 complete medium. Phosphate buffer saline (PBS), citrate buffer saline (CBS), RBC lysing buffer, antibiotic (penicillin/streptomycin), protease inhibitor cocktail, and lysis buffer were bought from eBiosciences (California, USA).

Antibodies

Monoclonal antibodies used in the study include anti-mouse CD3 (Catalogue No. 14-0032-82), anti-mouse CD4 (Catalogue No. 17-0043-82), anti-mouse CD8 (Catalogue No. MHCD0828), and anti-mouse CD25 (Catalogue No. 12-0251-82). All these antibodies were obtained from eBioscience (ScientificFisher, California, USA).

Mice

Sixty-week-old male Swiss albino mice, each weighing between 28 and 32 grams, were used in this study. These mice were obtained from the animal facility at the National Research Center in Giza, Egypt. They were kept in a controlled laboratory environment with a temperature of 25°C and a relative humidity of 55±10%. The lighting conditions followed a 12-hour light and 12-hour dark cycle.

Ethical consideration

All procedures involving laboratory animals were carried out in accordance with internationally accepted guidelines and received approval from the Research and Ethics Committee of the Faculty of Science, Tanta University (Approval No. IACUC-SCI-TU-0205).

Antigen preparation

The antigen derived from *T. spiralis* muscle larvae (ML) was prepared as outlined by Bień et al. (2013). In brief, the collected *T. spiralis* ML were purified multiple times using water through a series of sedimentation steps in cylinders. Following the last sedimentation step, the ML were placed into 1.5 ml tubes. The larval pellet was thoroughly washed three times in PBS containing antibiotics (50 µg/ml streptomycin, 50 U/ml penicillin). The *T. spiralis* ML were then stored at -70°C before protein extraction. After thawing, the ML was washed again three times in PBS and then suspended in a lysis buffer made up of 8 M urea, 4% CHAPS, and 40 mM Trizma base, along with a protease inhibitor cocktail. The protein extract was homogenized using a glass Potter-Elvehjem homogenizer and disrupted through sonication three times, each for 10 seconds. The lysate was then centrifuged at 14,000× g at 4°C for 15 minutes to clarify it. The supernatant containing the antigen was collected, lyophilized, and kept at -80°C until needed. The protein concentration of the antigens was assessed by means of a NanoDrop™ 2000 (NanoDrop Technologies, Wilmington, USA). The antigenic proteins were then frozen at -70°C for additional analysis.

Induction of type 1 diabetes mellitus

The induction of T1-DM in male Swiss albino mice was conducted following the protocols established by Cheng et al. (2013). Shortly, STZ was prepared by dissolving it in sodium citrate buffer. In summary, the induction of T1-DM in Swiss albino mice was accomplished through the injection of STZ intraperitoneal (i.p.) with a dose of 40 mg/kg/day for five consecutive days. To reduce the likelihood of hypoglycemia resulting from STZ treatment, the mice were given a 10% w/v glucose solution for overnight administration. Upon completion of the dosing regimen, the STZ-treated mice were maintained under standard laboratory conditions. Following the last STZ administration, postprandial glucose was regularly monitored utilizing a blood glucose monitor (OneTouch® Basic glucometer; LifeScan, Inc., Milpitas, CA, USA) until stable hyperglycemia was achieved. Mice with pronounced hyperglycemia (Fasting blood glucose > 250 mg/dl) were selected as diabetic animals for the study. The levels of blood glucose were regularly monitored throughout the experiments to ensure hyperglycemia in experimental mice (Baker et al., 2025).

In vivo treatment

The study was conducted in two main categories: preventive and therapeutic. Sixty Swiss albino mice were divided into six groups of ten. The first group (Gp1) was designated as

the non-diabetic control group. All the remaining groups were induced T1-DM by injection of STZ intraperitoneal (i.p) at a dose of 40 mg/kg daily for 5 days at different regimens. Gp2 was a control diabetic. The preventive groups, Gp3 and Gp4, mice received *T. spiralis* ML antigens i.p. at doses of 500 µg/kg and 1000 µg/kg, respectively, twice weekly for 3 weeks, followed by injection of STZ i.p (40 mg/kg/day) for 5 days. One week later post-last STZ dosing, they were given *T. spiralis* ML antigens i.p at 500 µg/kg and 1000 µg/kg, respectively. The therapeutic groups, Gp 5 and Gp 6, mice were administered STZ i.p at the same dosing regimen and timing as Gp 3 and Gp 4. Seven days post-STZ dosing, they received *T. spiralis* ML antigens i.p (500 µg/kg and 1000 µg/kg, respectively) twice weekly for 3 weeks.

Blood samples and sera collection

Two ml of blood was collected from the retro-orbital plexus into plain tubes and left to clot for three hours. After clotting, the samples were centrifuged (1500 rpm, 10 minutes, at 4°C) to separate the serum. The serum was stored at -80°C for later use in immunological and biochemical analysis.

Hematological analysis

Heparinized bloods were examined for hematological parameters using a hematology analyzer (Beckman Coulter Unicel DxH 690T, ScientificFisher, USA). The analysis included white blood cells (WBC), along with their differential relative percentage, specifically for neutrophils, lymphocytes, and monocytes.

Immunological analysis of the levels of antibodies and cytokines by ELISA

The levels of immunoglobulins; IgE, IgG, IgM, interleukins-4 (IL-4), IL-10, and interferon- γ (INF-γ) in the blood serum of the experimental mice were determined using ELISA. Specific kits were used for each substance: the Mouse IgE ELISA Kit, Mouse IgG ELISA Kit, Mouse IgM ELISA Kit, Mouse IL-4 ELISA Kit, Mouse IL-10 ELISA Kit, and Mouse INF-γ ELISA Kit. These kits were used according to the manufacturer's guidelines (ThermoFisher, CA, USA). The absorbance was identified at 450 nm.

Flowcytometric analysis

Fresh splenocytes were collected, and a total of 1×10^6 cells were incubated on ice for 30 min in PBS containing 2% BSA and 0.02% sodium azide, along with fluorescein isothiocyanate (FITC) or phycoerythrin (PE) conjugated monoclonal antibodies (mAb), or with control irrelevant isotype-matched mAb. The monoclonal antibodies used were anti-mouse CD3, anti-mouse CD4, anti-mouse CD8, and anti-mouse CD25. Following the incubation period, the cells were washed two times with PBS. Then, they were fixed using 500 µL of 2% paraformaldehyde in PBS. The fixed cells were stored on ice in the dark until they were analyzed using flow cytometry. Flow cytometry data were collected from 10,000 events using a FACS Calibur (Becton Dickinson), and the results were analyzed using WinList software (Verity Software House, Topsham, ME).

Cell suspension preparation

Splenocyte single cell suspension was prepared as previously described by Nassef (2017). Mice were compassionately euthanized through cervical dislocation. The spleens were rinsed with phosphate-buffered saline (PBS) and then pressed with glass slides to create a suspension of single cells. The splenocyte suspension was then rinsed twice with PBS and centrifuged (500 rpm, 5 minutes, 4°C). To remove red blood cells, the resulting pellet was rinsed in red blood cell lysis buffer for 5 mins and then diluted with PBS. The splenocyte suspension was then spun again (2000 rpm, 5 minutes). The pellet was then diluted in an appropriate buffer and filtered through a cell strainer. The number of solenocytes was determined.

Biochemical assessments

The activities of Serum alanine and aspartate transaminases (ALT, AST), the levels of urea and creatinine were evaluated using calorimetric techniques with standard, ready-to-use kits (MBH, Germany) on a fully automated Pentra C400 automated clinical chemistry analyzer (Block Scientific, USA).

The manufacturer's instructions were strictly followed.

Statistics

The data were coded and inputted utilizing the statistical software SPSS ver. 27 (IBM, USA). The analysis of the data was conducted using the mean and standard error. Group comparisons were performed through analysis of variance (ANOVA), followed by *post hoc* tests and Dunnett's test for multiple comparisons. A *P*-value of less than 0.05 was recognized as statistically significant.

3. Results

Impacts of *T. spiralis* ML antigens on immune-related leucocytes

The properties of administering *T. spiralis* ML antigens on the overall leucocyte count and the relative proportions of their differentials are presented in Table 1. In comparison to the values observed in diabetic mice treated with CBS (control diabetic), the total leucocyte count exhibited significant increases ($p < 0.05$) in low antigen pre-treated diabetic mice, amounting to $6725\pm335\times10^3/\mu l$. Conversely, the total leucocyte counts were significantly reduced ($p < 0.05$) in low antigen pre-treated diabetic mice, recorded at $5375\pm1110\times10^3/\mu l$, and in low antigen post-treated diabetic mice, which measured $3600\pm208\times10^3/\mu l$ (Table 1). When compared to the values in diabetic mice receiving CBS (control diabetic), the relative neutrophil count demonstrated significant decreases ($p < 0.05$) in low antigen pre-treated diabetic mice by 3.50±1.61%, in high antigen pre-treated diabetic mice by 2.00±0.45%, in low antigen post-treated STZ-induced diabetic mice by 2.58±0.62%, and in high antigen post-treated diabetic mice by 2.40±0.51% (Table 1). In contrast to the values in diabetic mice treated with CBS (control diabetic), the relative lymphocyte count showed significant increases ($p < 0.05$) in low antigen pre-treated diabetic mice by 85.25±3.96%, in high antigen pre-treated diabetic mice by 90.75±3.06%, in low antigen post-treated diabetic mice by 89.75±1.93%, and in high antigen post-treated diabetic mice by 87.66±3.17% (Table 1). In comparison to their values in diabetic mice receiving CBS (control diabetic), the relative count of monocytes exhibited significant reductions in low antigen pre-treated diabetic mice by 11.00±2.12%, in high antigen pre-treated diabetic mice by 7.50±2.59%, in low antigen pre-treated diabetic mice by 8.00±1.08%, and in high antigen post-treated diabetic mice by 7.33±0.88%. These increases in the relative count of monocytes were notably significant in high antigen pre-treated diabetic mice by 7.50±2.59% and in high antigen post-treated diabetic mice by 7.33±0.88%. Conversely, the decreases in the relative count of monocytes were intriguingly heightened in high antigen post-treated diabetic mice by 28.67±2.18% (Table 1).

Table 1. Impacts of *T. spiralis* ML antigens on the immune-related leucocytes in diabetic mice

Treated groups		Leucocyte count ($10^{3/\mu l}$)	Differential count (%)		
			Neutrophils	Lymphocytes	Monocytes
Nondiabetic		8073±1200	12.80±5.25	60.33±9.02	15.00±2.08
Cont. Diabetic		6400±600	5.67±0.88	73.00±2.64	21.33±2.67
Preventive	Diabetic/Antigen. low	6725±335	3.50±1.61$^{\#}$	85.25±3.96	11.00±2.12
	Diabetic/Antigen. high	5375±1110	2.00±0.45$^{\#}$	90.75±3.06$^{\#}$	7.50±2.59$^{\$}$
Therapeutic	Diabetic/Antigen. low	5900±385	2.58±0.62$^{\#}$	89.75±1.93$^{\#}$	8.00±1.08$^{\$}$
	Diabetic/Antigen. high	3600±208$^{\#}$	2.40±0.51$^{\#}$	87.66±3.17$^{\#}$	7.33±0.88$^{\$}$

Data were evaluated as mean ± standard error (n=10). Differences between groups were identified as statistically significant at $p < 0.05$. $^{\#}$: statistically significant vs. nondiabetic mice receiving PBS (nondiabetic control), $^{\$}$: statistically significant vs. diabetic mice receiving CBS alone (diabetic control).

Potentials of *T. spiralis* ML antigens on the levels of antibodies

The potentials of administering *T. spiralis* ML antigen on serum IgE levels are illustrated in Fig. (1). In general, serum IgE levels were significantly elevated in low antigen pre-treated diabetic mice at 28.97±0.23 ng/ml, in high antigen pre-treated diabetic mice by 27.10±1.21 ng/ml, in low antigen post-treated diabetic mice by 27.63±1.10 ng/ml, and in high antigen post-treated diabetic mice at 26.27±1.12 ng/ml, when compared to nondiabetic receiving PBS (Fig. 1). Conversely, these notable increases were significantly reduced in STZ-induced diabetic mice that were administered CBS (control diabetic) at 7.77±0.55 ng/ml compared to the values in nondiabetic mice receiving PBS (Figure 1). When compared to their levels in diabetic mice receiving CBS (control diabetic), serum IgE levels were significantly elevated in low antigen pre-treated diabetic mice by 28.97±0.23 ng/ml, in high antigen pre-treated diabetic mice at 27.10±1.21 ng/ml, in low antigen post-treated diabetic mice at 27.63±1.10 ng/ml, and in high antigen post-treated diabetic mice at 26.27±1.12 ng/ml (Fig. 1).

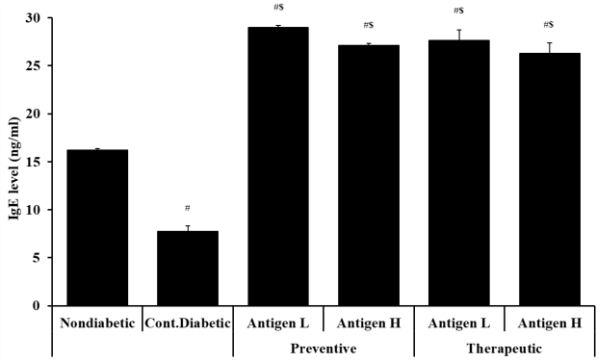

Fig. 1. Potentials of *T. spiralis* ML antigen on the serum levels of antibody IgE in diabetic mice. Data were evaluated as mean ± standard error. Differences between groups were identified as statistically significant at $p < 0.05$. [#]: statistically significant vs. nondiabetic mice receiving PBS (nondiabetic control), [$]: statistically significant vs. diabetic mice receiving CBS alone (diabetic control).

The influences of antigens on serum IgG levels are illustrated in Fig. (2). Generally, serum IgG levels were significantly elevated in low antigen post-treated diabetic mice by 743±7.80 ng/ml and in high antigen post-treated diabetic mice by 805±13.90 ng/ml when compared to nondiabetic mice receiving PBS (Fig. 2). In contrast, these notable increases were significantly reduced in diabetic mice that were administered CBS (control diabetic) by 65±2.70 ng/ml in comparison to nondiabetic mice receiving PBS (Fig. 2). When compared to their values in diabetic mice receiving CBS, serum IgG levels were significantly elevated in low antigen post-treated diabetic mice by 743±7.80 ng/ml and in high antigen post-treated diabetic mice by 805±13.90 ng/ml, relative to those in nondiabetic mice receiving PBS (Fig. 2).

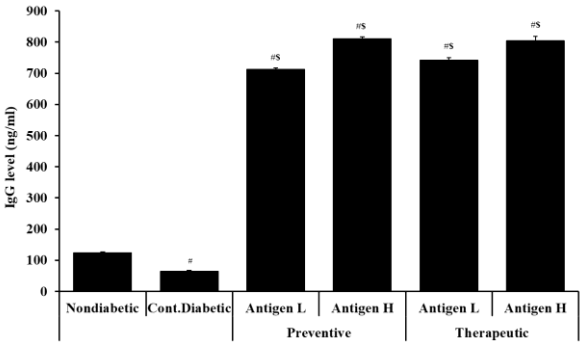

Fig. 2. Potentials of *T. spiralis* ML antigen on the serum levels of antibody IgG in diabetic mice. Data were evaluated as mean ± standard error. Differences between groups were identified as statistically significant at $P < 0.05$. [#]: statistically significant vs. nondiabetic mice receiving PBS (nondiabetic control), [$]: statistically significant vs. diabetic mice receiving CBS alone (diabetic control).

The impact of managing *T. spiralis* ML antigens on serum IgM levels is illustrated in Fig. (3). Typically, serum IgM levels were markedly elevated in diabetic mice treated with CBS by 18.33±0.88 ng/ml, in low antigen pre-treated diabetic mice by 14.66±0.88 ng/ml, in high antigen pre-treated diabetic mice by 12.33±0.88 ng/ml, in low antigen post-treated diabetic mice by 13.33±0.88 ng/ml, and in high antigen post-treated diabetic mice by 11.33±0.88 ng/ml, when compared to nondiabetic mice receiving PBS (Fig. 3). In comparison to the values observed in diabetic mice receiving CBS (control diabetic), serum IgM levels were significantly reduced in low antigen pre-treated diabetic mice by 14.66±0.88 ng/ml, in high antigen pre-treated diabetic mice by 12.33±0.88 ng/ml, in low antigen post-treated diabetic mice by 13.33±0.88 ng/ml, and in high antigen post-

treated diabetic mice by 11.33±0.88 ng/ml (Fig. 3).

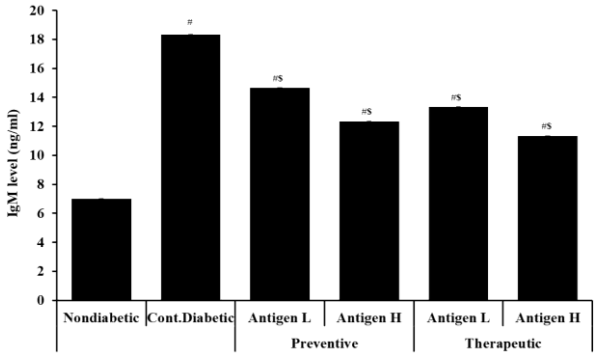

Fig. 3. Potentials of *T. spiralis* ML antigen on the serum levels of antibody IgM in diabetic mice. Data were evaluated as mean ± standard error. Differences between groups were identified as statistically significant at $P < 0.05$. [#]: statistically significant vs. nondiabetic mice receiving PBS (nondiabetic control), [$]: statistically significant vs. diabetic mice receiving CBS alone (diabetic control).

Effectiveness of *T. spiralis* ML antigens on the level of cytokines

The potentials of managing *T. spiralis* larval antigens on serum IL-4 levels are illustrated in Fig. (4). Overall, serum IL-4 levels were markedly elevated in low antigen pre-treated diabetic mice at 759.00±5.51 pg/ml, in high antigen pre-treated diabetic mice by 817.00±3.61 pg/ml, in low antigen post-treated diabetic mice by 763.33±6.77 pg/ml, and in high antigen post-treated diabetic mice by 819.67±2.73 pg/ml, when compared to nondiabetic mice that did not receive any antigen treatment. In contrast, serum IL-4 levels were significantly reduced in diabetic mice treated with CBS by 23.17±1.34 pg/ml (Fig. 4). When compared to the values in diabetic mice receiving CBS, serum IL-4 levels were significantly elevated in low antigen pre-treated STZ-induced diabetic mice by 759.00±5.51 pg/ml, in high antigen pre-treated diabetic mice by 817.00±3.61 pg/ml, in low antigen post-treated diabetic mice by 763.33±6.77 pg/ml, and in high antigen post-treated diabetic mice by 819.67±2.73 pg/ml (Fig. 4).

The effectiveness of *T. spiralis* ML antigens inoculation on serum IL-10 levels is demonstrated in Fig. (5). Typically, serum IL-10 levels were markedly elevated in low antigen pre-treated diabetic mice, measuring 1811±5.86 pg/ml; in high antigen pre-treated diabetic mice at 1910.33±6.10 pg/ml; in low antigen post-treated diabetic mice at 1861.00±6.25 pg/ml; and in high antigen post-treated diabetic mice at 1850.33±9.13 pg/ml.

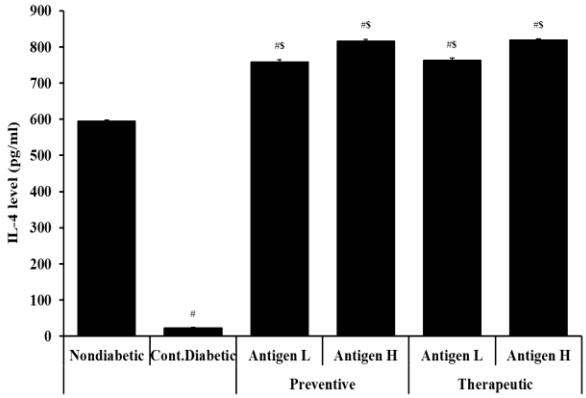

Fig.4. Effectiveness of *T. spiralis* ML antigen on the serum level of cytokine IL-4 in diabetic mice. Data were evaluated as mean ± standard error. Differences between groups were identified as statistically significant at $p < 0.05$. [#]: statistically significant vs. nondiabetic mice receiving PBS (nondiabetic control), [$]: statistically significant vs. diabetic mice receiving CBS alone (diabetic control).

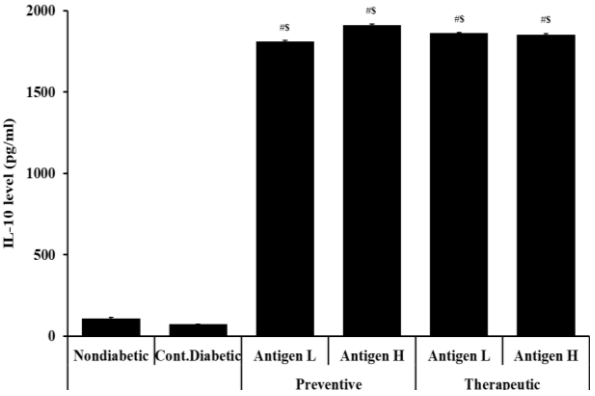

Fig. 5. Effectiveness of *T. spiralis* ML antigen on the serum level of cytokine IL-10 in diabetic mice. Data were evaluated as mean ± standard error. Differences between groups were identified as statistically significant at $p < 0.05$. [#]: statistically significant vs. nondiabetic mice receiving PBS (nondiabetic control), [$]: statistically significant vs. diabetic mice receiving CBS alone (diabetic control).

In contrast, serum IL-10 levels were significantly reduced in diabetic mice administered CBS, recording 73.30±1.42 pg/ml, when compared to nondiabetic mice that did not receive any antigen treatment (Fig. 5). When compared to the values in diabetic mice receiving CBS, serum IL-10 levels were significantly elevated in low antigen pre-treated diabetic mice at 1811±5.86 pg/ml, in high antigen pre-treated diabetic mice at

1910.33±6.10 pg/ml, in low antigen post-treated diabetic mice at 1861.00±6.25 pg/ml, and in high antigen post-treated diabetic mice at 1850.33±9.13 pg/ml (Fig. 5).

The efficacy of administering *T. spiralis* ML antigens on serum INF-γ levels is illustrated in Fig. (6). Typically, serum INF-γ levels were significantly elevated in low antigen pre-treated diabetic mice by 91.77±1.72 pg/ml, in low antigen post-treated diabetic mice by 95.50 pg/ml, and in high antigen post-treated diabetic mice by 101.73±3.65 pg/ml; in contrast, these elevations were significantly reduced in diabetic mice when compared to nondiabetic mice that were administered PBS. In comparison to the values observed in diabetic mice receiving CBS, serum INF-γ levels were markedly increased in low antigen pre-treated diabetic mice by 91.77±1.72 pg/ml, in high antigen pre-treated diabetic mice by 90.10±1.49 pg/ml, in low antigen post-treated diabetic mice by 95.50±2.47 pg/ml, and in high antigen post-treated diabetic mice by 101.73±3.6 pg/ml (Fig. 6).

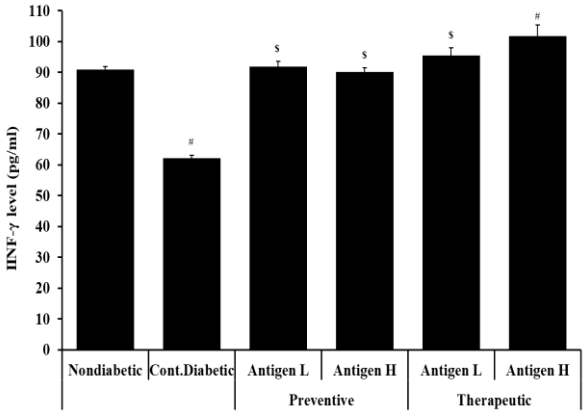

Fig. 6. Effectiveness of *T. spiralis* ML antigen on the serum level of cytokine INF-γ in STZ-induced diabetic mice. Data were evaluated as mean ± standard error. Differences between groups were identified as statistically significant at $P < 0.05$. #: statistically significant vs. nondiabetic mice receiving PBS (nondiabetic control), $: statistically significant vs. diabetic mice receiving CBS alone (diabetic control).

Efficiency of *T. spiralis* ML antigens on the phenotypic expression of lymphocytes

The impact of inoculation with *T. spiralis* larval antigens on the phenotypic expression of CD3+ T lymphocytes is illustrated in Figs. 7 and 8. Typically, the phenotypic expression of CD3+ T lymphocytes was markedly elevated in diabetic mice that did not receive any treatment by 16.88±1.67%, in low antigen pre-treated diabetic mice by 52.43±2.19%, in high antigen pre-treated diabetic mice by 25.55±1.32%, in low antigen post-treated diabetic mice by 55.35±1.00%, and in high antigen post-treated diabetic mice by 23.98±0.21%, when compared to nondiabetic mice that were administered PBS (Figs. 7, 8). In comparison to their values in diabetic mice receiving CBS (control diabetic), the phenotypic expression of CD3+ T lymphocytes was significantly reduced in low antigen pre-treated diabetic mice by 52.43±2.19%, in high antigen pre-treated diabetic mice by 25.55±1.32 %, in low antigen post-treated diabetic mice by 55.35±1.00%, and in high antigen post-treated STZ-induced diabetic mice by 23.98±0.21% (Figs. 7, 8).

The influence of the injection of *T. spiralis* larval antigens on the phenotypic expression of CD4+ T lymphocytes is illustrated in Figs. 9 and 10. Generally, the phenotypic expression of CD4+ T lymphocytes was significantly elevated in diabetic mice receiving no treatment (control diabetic) by 18.50±1.58%, in low antigen pre-treated diabetic mice by 33.87±1.30%, in high antigen pre-treated diabetic mice by 22.53±0.67%, in low antigen post-treated diabetic mice by 36.53±0.40%, and in high antigen post-treated diabetic mice by 21.90±0.20%, when compared to nondiabetic mice receiving PBS (Figs. 9, 10). In comparison to their values in diabetic mice receiving CBS (control diabetic), the phenotypic expression of CD4+ T lymphocytes was significantly enhanced in low antigen pre-treated diabetic mice by 33.87±1.30%, in high antigen pre-treated diabetic mice by 22.53±0.67%, in low antigen post-treated diabetic mice by 36.53±0.40%, and in high antigen post-treated STZ-induced diabetic mice by 21.90±0.20% (Figs. 9, 10).

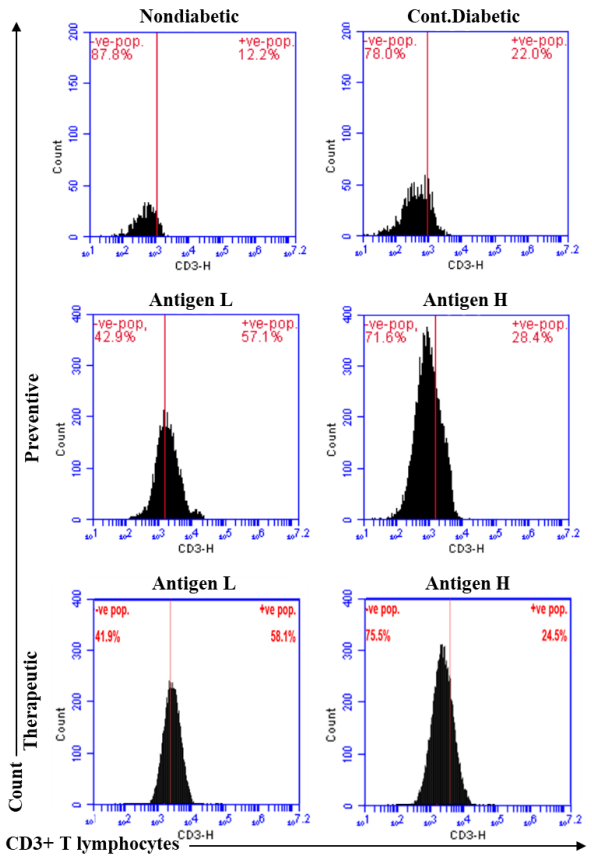

Fig. 7. Representative flow cytometry analysis showing the phenotypic frequency rate of CD3+ T lymphocytes in diabetic mice preventatively or therapeutically i.p. administered with *T. spiralis* ML antigens.

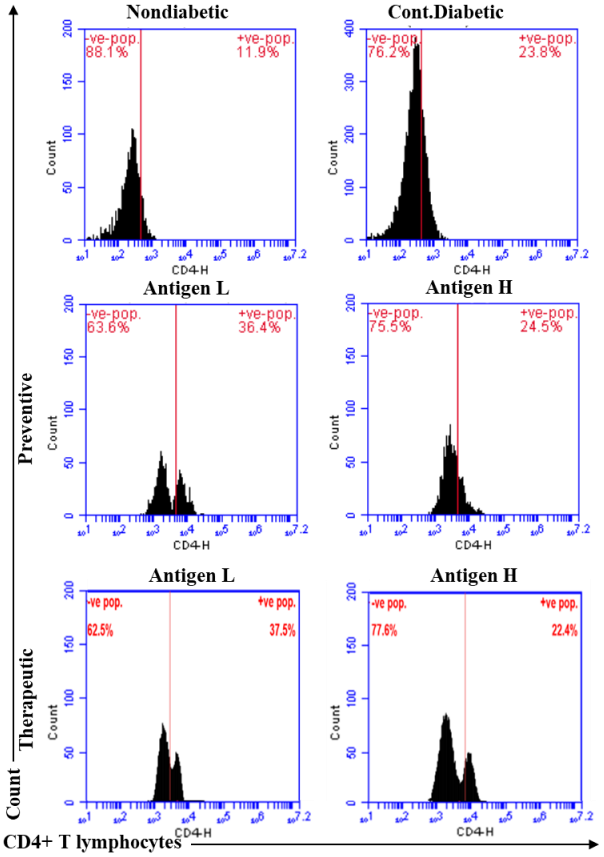

Fig. 9. Representative flow cytometry analysis showing the phenotypic frequency rate of CD4+ T lymphocytes in diabetic mice preventatively or therapeutically i.p. administered with *T. spiralis* ML antigens.

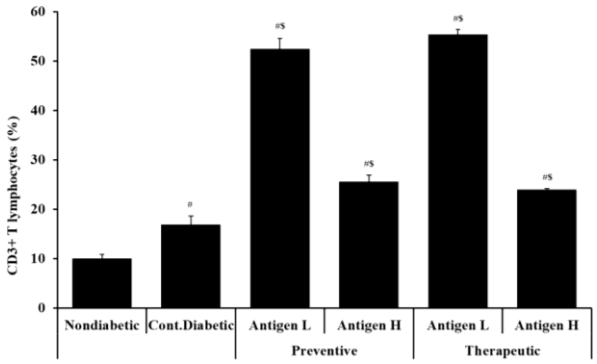

Fig. 8. Flow cytometry analysis showing the relative number of CD3+ T lymphocytes in diabetic mice preventatively or therapeutically i.p. administered with *T. spiralis* ML antigens. Data were evaluated as mean ± standard error. Differences between groups were identified as statistically significant at $P < 0.05$. $^{\#}$: statistically significant vs. nondiabetic mice receiving PBS (nondiabetic control), $^{\$}$: statistically significant vs. diabetic mice receiving CBS alone (diabetic control).

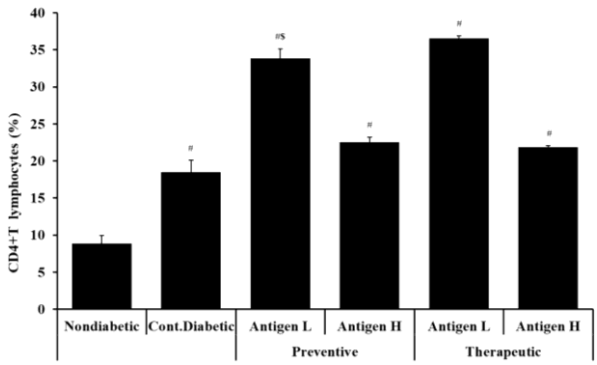

Fig. 10. Flow cytometry analysis showing the relative number of CD4+ T lymphocytes in diabetic mice preventatively or therapeutically i.p. administered with *T. spiralis* ML antigens. Data were evaluated as mean ± standard error. Differences between groups were identified as statistically significant at $P < 0.05$. $^{\#}$: statistically significant vs. nondiabetic mice receiving PBS (nondiabetic control), $^{\$}$: statistically significant vs. diabetic mice receiving CBS alone (diabetic control).

The impact of inoculating *T. spiralis* ML antigens on the phenotypic expression of CD8+ T lymphocytes is illustrated in Figs. 11 and 12. Typically, the expression rate of CD8+ T lymphocytes was markedly elevated in

diabetic mice that were administered PBS by 17.68±1.32%, in low antigen pre-treated diabetic mice by 47.80±1.38%, in high antigen pre-treated diabetic mice by 35.30±0.33%, in low antigen post-treated diabetic mice by 47.17±0.89%, and in high antigen post-treated diabetic mice by 35.37±0.71% when compared to nondiabetic mice receiving PBS (Figures 11, 12). In comparison to their values in diabetic mice that did not receive antigen treatment, the phenotypic expression of CD8+ T lymphocytes was significantly enhanced in low antigen pre-treated diabetic mice by 47.80±1.38%, in high antigen pre-treated diabetic mice by 35.30±0.33%, in low antigen post-treated diabetic mice by 47.17±0.89%, and in high antigen post-treated diabetic mice by 35.37±0.71% (Figes. 11, 12).

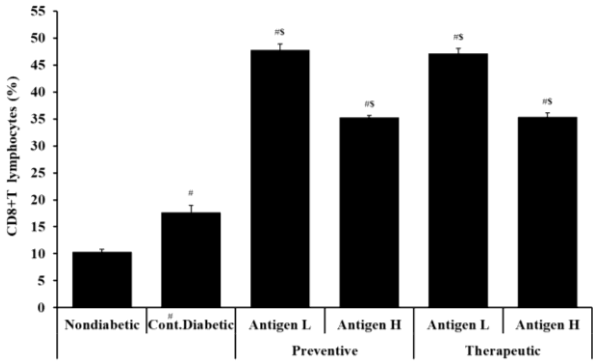

Fig. 12. Flow cytometry analysis showing the relative number of CD8+ T lymphocytes in diabetic mice preventatively or therapeutically i.p. administered with *T. spiralis* ML antigens. Data were evaluated as mean ± standard error. Differences between groups were identified as statistically significant at $p < 0.05$. #: statistically significant vs. nondiabetic mice receiving PBS (nondiabetic control), $: statistically significant vs. diabetic mice receiving CBS alone (diabetic control).

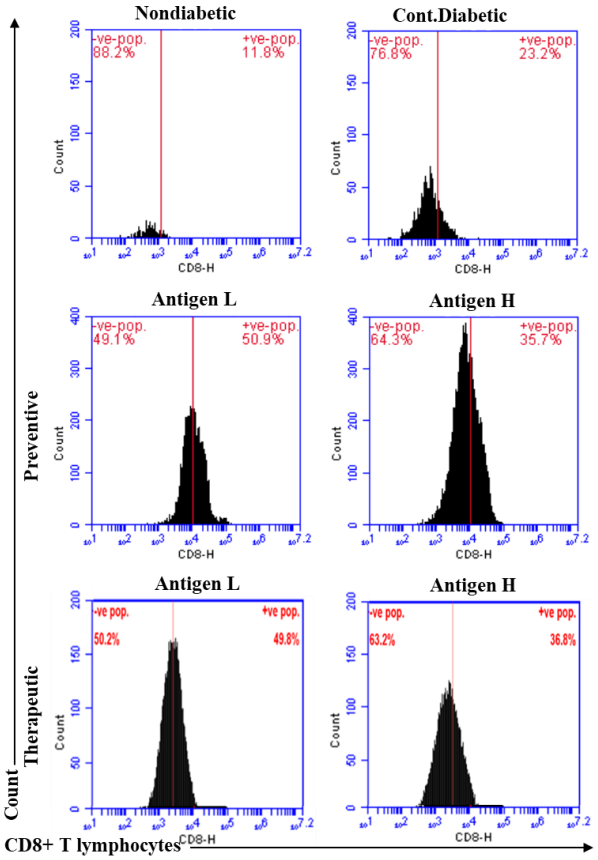

Fig. 11. Representative flow cytometry analysis showing the phenotypic frequency rate of CD8+ T lymphocytes in diabetic mice preventatively or therapeutically i.p. administered with *T. spiralis* ML antigens.

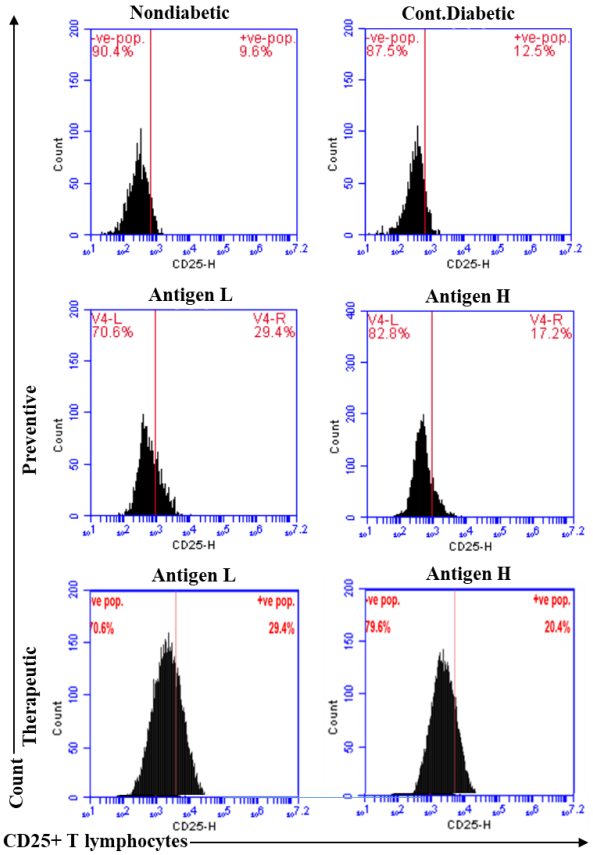

Fig. 13. Representative flow cytometry analysis showing the phenotypic frequency rate of CD25+ T lymphocytes in diabetic mice preventatively or therapeutically i.p. administered with *T. spiralis* ML antigens.

The effectiveness of *T. spiralis* ML antigens on the phenotypic expression of CD25+ T lymphocytes is illustrated in Figures 13 and 14. Typically, the phenotypic expression of CD25+ T lymphocytes was markedly elevated in diabetic mice that did not receive any antigen treatment by 10.47±0.70%, in low antigen pre-treated diabetic mice by 27.85±0.93%, in high antigen pre-treated diabetic mice by 17.25±1.16%, in low antigen post-treated diabetic mice by 27.73±0.57%, and in high antigen post-treated diabetic mice by 18.03±0.92% when compared to nondiabetic mice that were administered PBS (Figs. 13, 14). In comparison to their values in diabetic mice receiving CBS, the phenotypic expression of CD25+ T lymphocytes was significantly enhanced in low antigen pre-treated diabetic mice by 27.85±0.93%, in high antigen pre-treated diabetic mice by 17.25±1.16%, in low antigen post-treated diabetic mice by 27.73±0.57%, and in high antigen post-treated diabetic mice by 18.03±0.92% (Figs. 13, 14).

Potentials of *T. spiralis ML* antigens on liver and kidney functions

The properties of *T. spiralis* larval antigens on the levels of ALT, AST, urea, and creatinine are illustrated in Table 2. In comparison to the values observed in diabetic mice receiving CBS (control diabetic), serum ALT levels were significantly reduced in low antigen pre-treated diabetic mice, high antigen pre-treated diabetic mice, low antigen post-treated diabetic mice, and high antigen post-treated diabetic mice (Table 2).

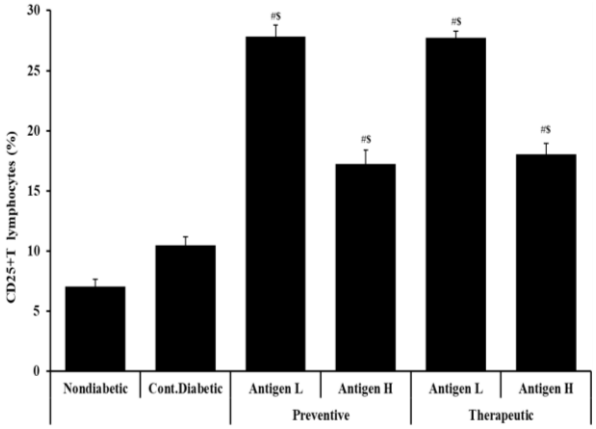

Fig. 14. Flow cytometry analysis showing the relative number (B) of CD25+ T lymphocytes in diabetic mice preventatively or therapeutically i.p. administered with *T. spiralis* ML antigens. Data were evaluated as mean ± standard error. Differences between groups were identified as statistically significant at $P < 0.05$. $^{\#}$: statistically significant vs. nondiabetic mice receiving PBS (nondiabetic control), $^{\$}$: statistically significant vs. diabetic mice receiving CBS alone (diabetic control).

Furthermore, when compared to the values in diabetic mice receiving CBS (control diabetic), serum AST levels were significantly lower in high antigen pre-treated diabetic mice, low antigen post-treated diabetic mice, and high antigen post-treated diabetic mice (Table 2).

Table 2. Potentials of *T. spiralis* ML antigens on liver and kidney functions in diabetic mice

Treated groups		ALT (ng/ml)	AST (ng/ml)	Urea (mg/dl)	Creatinine (mg/dl)
Nondiabetic		45.00±2.30	52.33±1.85	202.033±4.26	0.49±0.08
Cont. Diabetic		67.00±3.60$^{\#}$	70.33±3.17$^{\#}$	671.40±6.08$^{\#}$	2.10±0.15$^{\#}$
Preventive	Diabetic/Antigen. low	17.66±1.45$^{\#\$}$	185.33±5.23$^{\#\$}$	301.40±7.91$^{\#\$}$	0.92±0.05$^{\$}$
	Diabetic/Antigen. high	49.66±0.88$^{\$}$	50.33±0.88$^{\$}$	213.33±4.09b	0.92±0.03$^{\$}$
Therapeutic	Diabetic/Antigen. low	35.66±1.20$^{\$}$	59.33±1.45	241.33±3.17$^{\#\$}$	1.26±0.08$^{\#\$}$
	Diabetic/Antigen. high	37.66±2.96$^{\$}$	49.00±1.15$^{\$}$	178.33±6.06$^{\#\$}$	0.92±0.04$^{\$}$

Data were evaluated as mean ± standard error (n=10). Differences between groups were identified as statistically significant at $P < 0.05$. $^{\#}$: statistically significant vs. nondiabetic mice receiving PBS (nondiabetic control), $^{\$}$: statistically significant vs. STZ-induced diabetic mice receiving CBS alone (diabetic control).

Additionally, relative to the values in diabetic mice treated with CBS (control diabetic), serum urea levels were significantly diminished in low antigen pre-treated diabetic mice, high antigen pre-treated diabetic mice, low antigen post-treated diabetic mice, and high antigen post-treated STZ-induced diabetic mice (Table 2). Likewise, serum creatinine levels were significantly reduced in low antigen pre-treated diabetic mice, high antigen pre-treated diabetic mice, low antigen post-treated diabetic mice, and high antigen post-treated diabetic mice compared to diabetic mice receiving CBS (control diabetic) (Table 2).

4. DISCUSSION

T1-DM is an autoimmune disease where the immune system mistakenly attacks and destroys insulin-producing beta cells, resulting in minimal to no insulin production (Purcell et al., 2019). Autoreactive T cells are crucial in attacking beta cells in autoimmune diseases. CD8+ cytotoxic T lymphocytes specifically target these cells, while CD4+ helper T cells produce inflammatory molecules that recruit more immune cells and sustain inflammation in pancreatic islets (Redondo et al., 2018). Pro-inflammatory molecules IL-1, TNF-α, and IFN-γ are key players in the destruction of beta cells. Helminth antigens and products utilize various strategies to manipulate the host's immune system for their survival and reproduction (Fidler et al., 2009). Helminth antigens induce significant changes in innate and adaptive immune responses, which can help protect against autoimmune diseases. Research on helminth-derived antigens shows potential for treating inflammatory and autoimmune diseases, including T1-DM. Helminth antigens show prophylactic and therapeutic potential for diabetes by immunomodulating the host immune system to reduce inflammation and insulin resistance (Redondo et al., 2018).

The current data indicate that, in comparison to diabetic mice treated with CBS (control diabetic), both total and differential leucocyte counts remarkably changed in diabetic mice that were pre- and post-treated with low antigen, as well as in those that were pre- and post-treated with high antigen. Significant increases in monocyte relative numbers were noted specifically in diabetic mice that were pre- and post-treated with high antigen. In diabetic mice, antigens derived from helminths can influence both the overall leukocyte counts and the relative proportions of neutrophils, lymphocytes, and monocytes, frequently in contradictory manners. While hyperglycemia associated with diabetes usually results in elevated leukocyte counts and increased relative numbers of neutrophils, lymphocytes, and monocytes, helminth-derived antigens have the capacity to modulate these elevations, potentially diminishing inflammation and modifying immune responses (Fidler et al., 2009). Typically, diabetic mice display heightened levels of circulating leukocytes, neutrophils, lymphocytes, and monocytes. This phenomenon is often correlated with the inflammatory condition linked to diabetes (Adane et al., 2002). Furthermore, antigens derived from helminths can mitigate inflammation and modify the equilibrium of immune responses, which may influence the levels of total leukocytes, neutrophils, lymphocytes, and monocytes (Rajamanickam and Babu, 2024).

Our data indicated that, in comparison to the values observed in control diabetic mice, the serum levels of IgE and IgG were markedly elevated in diabetic mice receiving both pre- and post-treatment with low antigen, as well as in those subjected to pre- and post-treatment with high antigen. Helminth-derived antigens increase total serum IgE and IgG levels in diabetic mice by inducing a Th2-biased immune response, which promotes the production of specific cytokines like IL-4, IL-5, and IL-13 and B cell class switching (Saunders et al., 2007). Antigens derived from helminths have been shown to modulate the immune response and potentially delay or prevent the onset of T1-DM, potentially by shifting the immune balance towards a Th2 response, increasing the number or function of regulatory T cells (Treg), and changing the production of antibody IgE and insulin-specific IgG (Reynolds et al., 2012). Although T1-DM is primarily driven by Th1 cells and autoimmune responses, there is a notable

connection with IgE-mediated allergies linked to Th2 cells. Elevated IgE levels might result from immune dysregulation where both Th1 and Th2 responses are activated, potentially involving mast cells in pancreatic inflammation characteristic of T1-DM (Reynolds et al., 2012; Alghanmi et al., 2024). The cross-linking of IgE on mast cells by allergens triggers the release of inflammatory mediators, potentially contributing to the autoimmune process in T1-DM (Betto et al., 2017). IgE and IgG play a complex role in diabetic mice, influencing insulin resistance through specific interactions while potentially enhancing insulin sensitivity at high doses. Understanding these mechanisms is vital for developing diabetes treatments (Chadha et al., 2015).

The present study indicated that, in comparison to the values observed in control diabetic mice, the serum levels of IgM were significantly dropped in diabetic mice receiving both pre- and post-treatment with low antigen, as well as in those subjected to pre- and post-treatment with high antigen. Helminth antigens in diabetic mice may lead to decreased levels of IgM antibodies, but this isn't consistent and is often associated with a shift towards a Th2 immune response. Helminth antigens can modulate the immune system, potentially offering protection against autoimmune diabetes by inducing a Th2 response and reducing inflammation. While IgM levels might decrease, this is often accompanied by an increase in other antibody isotypes like IgE and IgG1, which are associated with Th2 responses (Alghanmi et al., 2024). IgM antibodies appear to be more effective at restoring immune balance and reversing diabetes, suggesting that a deficiency or dysregulation of IgM might contribute to the development of T1-DM. Furthermore, in diabetic mice, IgM plays a protective role by modulating the immune response and potentially preventing the onset and progression of T1-DM. Specifically, IgM immunotherapy can restore immune homeostasis, expand Treg populations, and even reverse hyperglycemia in new-onset diabetic mice (Chhabra et al., 2018).

The present study indicated that, in comparison to the values observed in control diabetic mice, the levels of IL-4 and IL-10 were significantly elevated in diabetic mice receiving both pre- and post-treatment with low antigen, as well as in those subjected to pre- and post-treatment with high antigen. Research shows that helminth antigens can stimulate a type 2 immune response in diabetic mice, leading to increased IL-4 levels. This cytokine helps protect against T1-DM by suppressing Th1-driven inflammation that harms pancreatic beta cells (Hübner et al., 2012). In diabetic mice, IL-4 can prevent and delay autoimmune T1-DM by promoting the function of regulatory T helper-2 (Th2), Treg, and dendritic cells (DCs). Additionally, IL-4 improves insulin signalling in muscle and liver cells, which aids glucose uptake and reduces insulin resistance (Mueller et al., 1997; Camaya et al., 2023). In diabetic mice treated with helminth antigen, there is an increase in the anti-inflammatory cytokine IL-10, which modulates the immune response to a Th2-dominant profile and helps reduce inflammation in T1-DM. The helminth antigen also promotes the expansion of Treg, enhancing the suppression of autoimmune responses (Hübner et al., 2012). Helminth antigens trigger a significant immune response in T1-DM, skewing the immune response towards Th2, which can suppress the harmful Th1 response, characterized by excessive production of cytokines like IL-4 and IL-10. IL-10 plays a crucial role in curbing excessive inflammation and tissue damage during infection (Mishra et al., 2013). Increased IL-10 levels can suppress the inflammatory response in the pancreas, limiting the damage to insulin-producing cells. Additionally, IL-10 can promote the development of regulatory T cells, which are essential for maintaining immune tolerance and preventing autoimmunity (Hübner et al., 2012).

The present study indicated that, in comparison to the values observed in control diabetic mice, the serum levels of IFN-γ were significantly increased in diabetic mice receiving both pre- and post-treatment with low antigen, as well as in those subjected to pre- and post-treatment with high antigen. In

diabetic mice with T1-DM, helminth antigens cause a shift in the immune response, decreasing IFN-γ and increasing IL-10, effectively suppressing the type 1 diabetes-associated Th1 response. This shift is primarily driven by helminth-induced type 2 immunity, which involves increased levels of IL-4, IL-13, and IL-10 (Liu et al., 2009; Shimokawa et al., 2020). IFN-γ has a complex role in diabetes, especially T1-DM. While it's typically known for its pro-inflammatory effects and involvement in the destruction of insulin-producing beta cells, its absence doesn't halt the disease, and it also has regulatory immune functions (De George et al., 2023). IFN-γ has a dual role in diabetes, particularly T1-DM, where it can promote both inflammation and beta-cell destruction, as well as regulate immune responses to mitigate damage. Understanding its mechanisms at different stages is vital for developing effective therapeutic strategies (Driver et al., 2017).

The current data indicated that, in comparison to the values observed in control diabetic mice, the expressions of CD3+ T lymphocytes, CD4+ T lymphocytes, CD8+ T lymphocytes, and CD25+ T lymphocytes were significantly increased in STZ-induced T1-DM mice receiving both pre- and post-treatment with low antigen, as well as in those subjected to pre- and post-treatment with high antigen. CD3+ T lymphocytes have dual roles in T1-DM and helminth antigen administration. In T1-DM, they contribute to the autoimmune attack on beta cells, while in helminth antigen inoculation, they can help protect against T1-DM by promoting Th2 responses and Treg. Their activation status is linked to inflammation and insulin resistance, affecting disease progression (Huang et al., 2022). CD3+ T lymphocytes are involved in inflammatory processes related to insulin resistance and disease progression, particularly in T1-DM. They contribute to the destruction of insulin-producing beta cells in the pancreas, highlighting their role in the disease's inflammatory mechanisms (Huang et al., 2022).

Targeting CD3+ T lymphocytes with strategies like oral CD3-specific monoclonal antibodies and helminth-derived antigens has shown potential in preclinical models of T1-DM. These approaches may help modulate T lymphocyte responses and enhance immune tolerance, offering promising avenues for both prevention and treatment of diabetes (Xia et al., 2017). CD4+ T lymphocytes have a dual role in T1-DM. CD4+ T lymphocytes, specifically autoreactive ones, contribute to the destruction of insulin-producing beta cells, while regulatory CD4+ T cells (Treg) can suppress this immune response, offering potential protection against T1-DM. This protective effect is believed to be mediated by T-reg through the production of IL-10 and other mechanisms that reduce inflammation, driving beta cell destruction, highlighting the dual functions of CD4+ T cells in this autoimmune condition (Espinosa-Carrasco et al., 2018). Helminth-derived antigens significantly influence the immune system, especially CD4+ T lymphocytes, which may contribute to autoimmune diseases like T1-DM. Their interaction is complex, often triggering a Th2-type immune response while also modulating Treg populations, leading to both protective and harmful outcomes (Aravindhan and Anand, 2017).

Helminth-derived antigens often activate a Th2-immune response, leading to the release of cytokines IL-5 and IL-4, affecting the differentiation and function of CD4+ T lymphocytes (Zaccone and Cooke, 2013). Helminth antigens can induce a Th2-type immune response, resulting in cytokine production (like IL-4 and IL-10) that suppresses Th1 inflammation. This modulation may influence insulin sensitivity and the progression of T1-DM (Huang et al., 2022). Helminth-derived antigens can affect T1-DM development and progression by modulating the immune system, particularly by inducing Treg responses that suppress inflammation and may offer protection against T1-DM (Shimokawa et al., 2020). Helminth antigens can impair the differentiation of effector CD8 T lymphocytes and decrease their IFN-γ production. They might also promote the growth of virtual memory CD8 T cells (Shimokawa et al., 2020).

Helminth antigens may influence the immune response in T1-DM by promoting the

formation of CD25+ T lymphocytes, or regulatory T cells (T-reg), which can help suppress inflammation and potentially protect against the disease, including T1-DM (Zaccone et al., 2013). Helminth antigens from intestinal nematodes may impact T1-DM by enhancing T-reg activity, which helps mitigate the autoimmune response against insulin-producing pancreatic beta cells (Zhu et al., 2024). Helminth antigens can influence the host's immune system, often triggering a Th2 immune response and enhancing Treg activity. This modulation can lead to a reduction in disease severity or even prevention, largely due to the increased T-reg numbers (Liu et al., 2009; Huang et al., 2022). In a diabetic mouse model of T1-DM, treatment with helminth antigens decreased the infiltration of autoreactive immune cells, including CD4+ T lymphocytes, CD8+ T lymphocytes, and B lymphocytes, into pancreatic islets, which is known as insulitis—a key feature of T1-DM, resulting in fighting against T1-DM (Liu et al., 2022).

Our results indicated that, in diabetic mice, both low and high antigen pre-treatment and post-treatment led to significantly decreased serum levels of ALT, AST, urea, and creatinine compared to diabetic mice receiving CBS. In diabetic mice, helminth *T. spiralis* antigens can influence liver enzyme activity through immune-mediated mechanisms by promoting a type 2 immune response. This response leads to increased cytokines such as IL-4, IL-10, and IL-13, which may enhance liver metabolism and help reduce diabetes-related inflammation. A shift from a Th1 to a Th2 immune response can influence liver enzyme activity, including ALT and AST levels (Yang et al., 2024). Helminth-derived antigens in diabetic mice may enhance kidney function by improving serum urea and creatinine levels. They achieve this by modulating antioxidant enzymes and shifting the immune response from a pro-inflammatory (Th1) to an anti-inflammatory (Th2) state, potentially reducing inflammation linked to diabetic nephropathy (El-Kady et al., 2025).

Conclusion

Our findings show that *T. spiralis* antigens in STZ-induced type 1 diabetic mice modulate immune responses by increasing Treg cells and anti-inflammatory cytokines (IL-4, IL-10) while reducing IFN-γ. This Th2 immune shift improved glycemic control, indicating the potential of helminth antigens as immunotherapeutic candidates for T1-DM. Further research is needed to evaluate their clinical applicability in autoimmune disease management.

Declaration and Conflicts of interest

The authors have no conflicts of interest.

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