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Trichosanthin alleviates streptozotocin-induced type 1 diabetes mellitus in mice by regulating the balance between bone marrow-derived IL6^{+} and IL10^{+} MDSCs

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A R T I C L E I N F O

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A B S T R A C T

Myeloid-derived suppressor cells (MDSCs) occupy a pivotal role in the intricate pathogenesis of the autoimmune disorder, Type 1 diabetes mellitus (T1DM). Since our previous work demonstrated that trichosanthin (TCS), an active compound of Chinese herb medicine Tian Hua Fen, regulated immune response, we aimed to clarify the efficacy and molecular mechanism of TCS in the treatment of T1DM. To this end, T1DM mouse model was established by streptozotocin (STZ) induction. The mice were randomly divided into normal control group (Ctl), T1DM group (STZ), TCS treated diabetic group (STZ + TCS) and insulin-treated diabetic group (STZ + insulin). Our comprehensive evaluation encompassed variables such as blood glucose, glycosylated hemoglobin, body weight, pertinent biochemical markers, pancreatic histopathology, and the distribution of immune cell populations. Furthermore, we meticulously isolated MDSCs from the bone marrow of T1DM mice, probing into the expressions of genes pertaining to the advanced glycation end product receptor (RAGE)/NF-κB signaling pathway through RT-qPCR. Evidently, TCS exhibited a substantial capacity to effectively counteract the T1DM-induced elevation in random blood glucose, glycosylated hemoglobin, and IL-6 levels in plasma. Pathological scrutiny underscored the ability of TCS to mitigate the damage incurred by islets. Intriguingly, TCS interventions engendered a reduction in the proportion of MDSCs within the bone marrow, particularly within the IL-6^{+} MDSC subset. In contrast, IL-10^{+} MDSCs exhibited an elevation following TCS treatment. Moreover, we observed a significant down-regulation of relative mRNA of pro-inflammatory genes, including arginase 1 (*Arg1*), inducible nitric oxide synthase (*iNOS*), *RAGE* and *NF-κB*, within MDSCs due to the influence of TCS. It decreases total MDSCs and regulates the balance between IL-6^{+} and IL-10^{+} MDSCs thus alleviating the symptoms of T1DM. TCS also down-regulates the RAGE/NF-κB signaling pathway, making it a promising alternative therapeutic treatment for T1DM. Collectively, our study offered novel insights into the underlying mechanism by which TCS serves as a promising therapeutic intervention for T1DM.

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1. Introduction

T1DM is an autoimmune disease [1], and early intervention may alleviate its progression [2]. A specific group of immune cells called myeloid-derived suppressor cells (MDSCs) has a significant association with the onset of autoimmune conditions like diabetes [3]. Conditional local infiltration of MDSCs may be the key to the development of T1DM in mice and human [4]. Recent research has revealed that MDSCs are notably present in various autoimmune disease models, exhibiting both tissue-specific and generalized enrichments, thereby influencing inflammatory immune responses [5]. The modulation of immune responses by MDSCs is primarily achieved through the secretion of bioactive molecules, including Arg, iNOS, and ROS [6–8]. Furthermore, a crucial contributor to the pathogenesis of diabetes is the receptor for advanced glycation end products (RAGE) [9]. In animal models of diabetes, RAGE expression is prominently heightened in regions where advanced glycation end products (AGEs) accumulate [10]. The interaction between RAGE and AGEs triggers the activation of NF-κB, subsequently resulting in oxidative stress and an elevated production of cytokines like IL-6. These events collectively give rise to inflammation within the islet β cells, ultimately culminating in a range of clinical manifestations [11].

Tian Hua Fen, derived from the root of *Trichosanthes kirilowii Maxim*, stands as a traditional Chinese herbal medicine. Over centuries, it has been employed for diabetes treatment, although its underlying mechanism largely remains an enigma [12]. Our previous work has demonstrated that TCS, one of the active compounds of Tian Hua Fen, regulated immune response through antigen presenting cells [12]. TCS is a 247-amino-acid-residue alkaline protein which belongs to the ribosome inactivating proteins and has been used to treat diseases including cancer and acquired immune deficiency syndrome [12,13]. However, the intricate details governing how TCS precisely modulates the pathogenesis and progression of diabetes remain elusive.

In the context of this investigation, we employed a mouse model of T1DM induced by intraperitoneal administration of STZ (25 mg/kg) to assess the therapeutic potential of TCS treatment for T1DM. We observed that TCS caused a remarkable relief of hyperglycemia and alleviated islet damage in T1DM mice. We also found that TCS treatment decreased MDSCs in bone marrow and spleen. Interestingly, TCS regulated the balance between IL-6^+ and IL-10^+ MDSCs by decreasing the IL-6^+ while increasing the IL-10^+ cell population. Mechanically, TCS functioned as a negative regulator of RAGE/NF-κB signaling pathway in MDSCs. In summary, our study provided insights into the underlying mechanism through which Tian Hua Fen, a Chinese herbal medicine, alleviates islet damage in T1DM. Notably, we demonstrated that its active component, TCS, potentially regulates the functional behavior and subtype equilibrium of MDSCs towards an anti-inflammatory phenotype via engagement with the RAGE/NF-κB pathway.

2. Materials and methods

2.1. Animals and reagents

All animal studies were approved by SLAC laboratory animal company (SLAC Laboratory Animal Company, Shanghai, China). Male C57BL/6J mice at the age of seven weeks were raised in the SPF grade animal room of Shanghai Jiao Tong University School of Medicine. Room temperature and humidity were maintained at 20 °C ± 2 °C and 55 % ± 5 %, respectively. Mice were placed in a room with a 12-h light/dark cycle and had unlimited access to food and water.

STZ was purchased from Sigma-Aldrich, USA. Glucometer and glucometer test paper were purchased from Terumo, Japan. Fluorescein isothiocyanate (FITC)-conjugated anti-rat Gr-1, Allophycocyanin (APC)-conjugated anti-rat CD11b, Phycoerythrin (PE)-conjugated anti-rat interferon-gamma (IFN-γ), Phycoerythrin (PE)-conjugated anti-rat IL-10, eBioscience™ Protein Transport Inhibitor Cocktail (500x) and eBioscience™ Foxp3/Transcription Factor Staining Buffer Set were purchased from eBioscience, USA. CD16/CD32 was purchased from Biolegend, USA. Myeloid-derived suppressor cell isolation kit, mouse was purchased from Miltenyi, Germany. RNAiso plus, SYBR® Premix Ex Taq™, and PrimeScript™ RT Reagent Kit were purchased from Takara, Japan. TCS is available as a crystalline water solution from Shanghai JinShan Pharmaceutical Co, Shanghai (Fig. S2). The stock TCS solution is diluted to 0.05 mg/mL with saline and sterilized with a 0.2 μm filter. Enzyme linked immunosorbent assay (ELISA) kit was purchased from Multi Sciences, China.

2.2. STZ-induced diabetic mice model

C57BL/6 mice were given STZ for 5 consecutive days as published methods [14,15]. Dark preparation and then injected intraperitoneally STZ (25 mg/kg/d) (Fig. S1) within 15 min of dissolution. Five days post STZ stimulation, mice with random fasting blood glucose ≥11.1 mmol/L were selected as T1DM mice. The mice were then divided into four groups: (1) Control (Ctl) (n = 6); (2) STZ (n = 7); (3) TCS treated (STZ + TCS) (n = 6); (4) insulin treated (STZ + insulin) (n = 7), and kept in separate cages. The dose of intraperitoneal injection of TCS was 50 μg/kg/d according to our previous protocol [16] and subcutaneous injection of insulin was 1 U/kg/d. From day 3 to day 5 of STZ injection, mice in the STZ + TCS group were intraperitoneally injected one side with STZ at 9 a.m. and intraperitoneally injected the other side with TCS at 4 p.m. Since this study observed the early intervention of diabetes, the use of STZ dose was small and the degree of fasting blood glucose rise was slow in mice. On the 18th day of the experiment, the rats in the STZ + insulin group were treated with intramuscular injection of insulin once their blood glucose exceeded 11.1 mmol/L. Once Mice in the control group and STZ group were given intraperitoneal injection of the same amount of sodium citrate buffer. On day 22, mice were used for ex vivo studies (Fig. 2A).

2.3. Blood glucose and body weight determinations

At 10 a.m., we conducted measurements of body weight and random blood glucose in each group on Day 0, Day 3, Day 8, Day 11, Day 16, Day 18, and Day 22. The mice were placed in a mice fixator to make the tail vein fully hyperemic, then the tail tip was cut off with scissors, and the first drop of blood was discarded. The blood glucose level of the mice was measured by electronic blood glucose meter.

2.4. Inflammatory cytokines determination

Take blood from inner canthus, blood was centrifuged to obtain plasma. Plasma of mice were collected after treatment and then frozen at −20 °C. Enzyme linked immunosorbent assay (ELISA) kit of IL-6 was used to measure the levels of plasmic IL-6.

2.5. H&E staining of pancreatic tissue and immunohistochemical detection of insulin in mice

Mice in each group were sacrificed at the end of TCS treatment. Pancreas of mice were stained with H&E, hematoxylin, eosin, and sealed with dehydration. Images were obtained with a microscope (Carl Zeiss Meditec, Dublin, CA) at a magnification of × 400 using identical acquisition settings for each section.

2.6. Flow cytometry

Murine femurs and tibiae were isolated. Cut the skin at the back and expose the legs. Cut the femur at the hip joint, then take out the whole leg. Separate the femur by cutting the knee joint. Separate the tibia from the paw. Cut off the epiphyses on both sides of the bone. Carefully insert a syringe filled with PBS and flush out bone marrow into a 50 mL falcon tube to collect bone marrow. The spleen was ground. Take blood from inner canthus, and the red blood cells were removed by using the red blood cell lysis buffer (sigma). Then the single cell suspension was prepared. For surface staining, Fc receptors were blocked with anti-mouse CD16/CD32 (Biolegend, CA, USA) and then stained with APC-CD3, PerCP-cy5.5-CD4, PE-cy7-CD8, FITC-CD45, APC-CD19, FITC-Gr-1, APC-CD11b, PerCP-cy5.5-Ly6C, PE-Ly6G, Alexa Fluor 700-CD3 and BV510-Live Dead (all from BD Biosciences, USA) and incubated for 30 min at 4 °C. The cells were analyzed using a FACs Fortessa Flow Cytometry system (BD Biosciences, USA). Gates were set based on the staining profile of the isotype controls.

2.7. Intracellular cytokine staining

Single cell suspension was added to eBioscience™ Protein Transport Inhibitor Cocktail (500x) and stimulated at 37 °C for 4 h, then eBioscience™ Foxp3/Transcription Factor Staining Buffer Set was added, and then stained with PE-IL-6, IL-10, and incubated for 45 min at 4 °C. The cells were analyzed using a FACs Fortessa Flow Cytometry system (BD Biosciences, USA). Gates were set based on the staining profile of the isotype controls.

2.8. Isolation of MDSCs

Murine MDSCs cells were magnetically isolated from the bone marrow of mice using myeloid-derived suppressor cell isolation kit. The purity of $CD11b^{+}Gr\text{-}1^{+}$ cells was more than 82 %.

2.9. Quantitative real-time PCR

To probe gene expression, RNA was extracted from MDSCs with Trizol reagent (Invitrogen Life Technologies), followed by reverse transcription with the PrimeScript™ RT reagent Kit (TaKaRa, Japan). Next, gene expression was determined by real-time RT-PCR with SYBR Premix Ex Taq (Tli RNaseH Plus) (TaKaRa, Japan). The $2^{-\Delta\Delta Ct}$ method was used to calculate fold changes [17]. The relative

Table 1
Real-time PCR primers used in this study.

Target/control gene	Primer sequences
Arg1	F 5′-CACGGCAGTGGCTTTAACCT-3′ R 5′-TGGCGCATTCACAGTCACTT-3′
iNOS	F 5′-CAGGGAGAACAGTACATGAACAC-3′ R 5′-TTGGATACACTGCTACAGGGA-3′
RAGE	F 5′-ACTACCGAGTCCGAGTCTACC-3′ R 5′-CCCACCTTATTAGGGACACTGG-3′
NF-κB	F 5′-TTCCCTCAGAGCCAGCCCAGG-3′ R 5′-TAGCGGAATCGCATGCCCCG-3′
β-actin	F 5′-GTGACGTTGACATCCGTAAAGA-3′ R 5′-GCCGGACTCATCGTACTCC-3′

expression levels were plotted as the proportion of the target gene versus the housekeeping gene β-actin. The primer sequences (Sangon) used in this study are listed in Table 1.

2.10. Statistical analysis

All results are presented as the mean ± SEM of data obtained from at least three independent experiments. Intergroup comparisons were made using one or two-way ANOVA or an unpaired Student's with GraphPad prism 6.0 (GraphPad Software, USA). A p-value<0.05 was considered statistically significant.

3. Results

3.1. TCS reduced blood glucose level in STZ-induced diabetic mice

To discover the underlying mechanism of TCS as a treatment for diabetes, we first obtained 15 active compounds of TCS from Traditional Chinese Medicine Systems Pharmacology Database and used PubChem database to identify 52 potential targets of these compounds (Fig. 1A). Next, we established a compound-target network using Cytoscape software to illustrate the network among these active compounds and their targets. Our network analysis generated 9 nodes which represented active compounds or their potential targets and 51 edges which indicated the interaction between the nodes (Fig. 1B). Meanwhile, we identified 82 type I diabetes associated genes from Genecards, TTD, OMIM, and PharmGKB databases. Our analyses revealed that IL-6 was not only involved in the pathogenesis of diabetes but also a target of TCS. Therefore, we speculated that IL6 may be critical in TCS treatment of diabetes.

Since TCS is one of the active components of Tian Hua Fen, and our previous work showed that it played immune regulatory roles in autoimmune diseases like T1DM, we aimed to evaluate the efficacy of TCS on T1DM in vivo. We observed that the T1DM mice exhibited markedly elevated fasting blood glucose and glycated hemoglobin (hemoglobin A1c, HbA1c) compared to control mice (Fig. 2C and D). In addition, the T1DM mice showed decreased body weight (Fig. 2B). Interestingly, treatments with TCS or insulin both significantly reduced the blood glucose ($P < 0.05$) and improved the loss of body weight in T1DM mice ($P < 0.05$ and 0.001, respectively) (Fig. 2B and D). We observed that the treatment of insulin in mice were more effective in weight gain compared to TCS. However, both results were statistically significant. Consistently, both treatments decrease glycated HbA1c in T1DM mice (Fig. 2C). Therefore, we conclude that TCS alleviated T1DM symptoms in STZ induced mouse model.

3.2. TCS attenuated insulitis in STZ-induced diabetic mice

We next compared the pathological morphology of the pancreases of T1DM mice to those of the control mice by H&E staining. Our result showed that the normal endocrine glands, namely the islets, were in a circular or orb-like state with regular and even distribution. Interestingly, the islet morphology of mice in TCS treated group was relatively normal and the degree of islet atrophy, sparse distribution and number reduction were all improved (Fig. 3A). Similarly, insulin treatment also ameliorated the morphological damage of islet in T1DM mice (Fig. 3A). Since pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α have been shown to be involved in the pathogenesis of T1DM [18], the plasma levels of IL-6 in T1DM mice were observed markedly increased IL-6 compared to normal control ($P < 0.01$). Consistently, both TCS and insulin treatments effectively suppressed this increase ($P < 0.05$, Fig. 3B). Taken together, TCS reduces peripheral IL-6 and inhibits the islet inflammation in STZ induced diabetic mice.

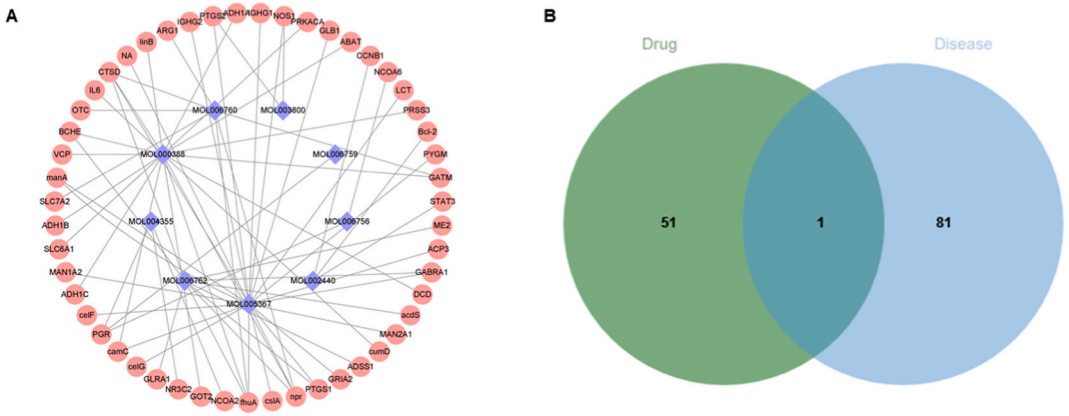

Fig. 1. Predicted network of targets of TCS-derived compounds.(A) Predicted network of targets of TCS-derived compounds using Traditional Chinese Medicine Systems Pharmacology Database. Orange nodes represent the active compounds, purple nodes the predicted targets, and edges indicate the interactions between compounds and targets. The size of nodes is proportional to the degree of interaction. (B) Venn diagram shows overlapping genes of predicted targets of mulberry compounds and genes down-regulated in restenosis tissue.

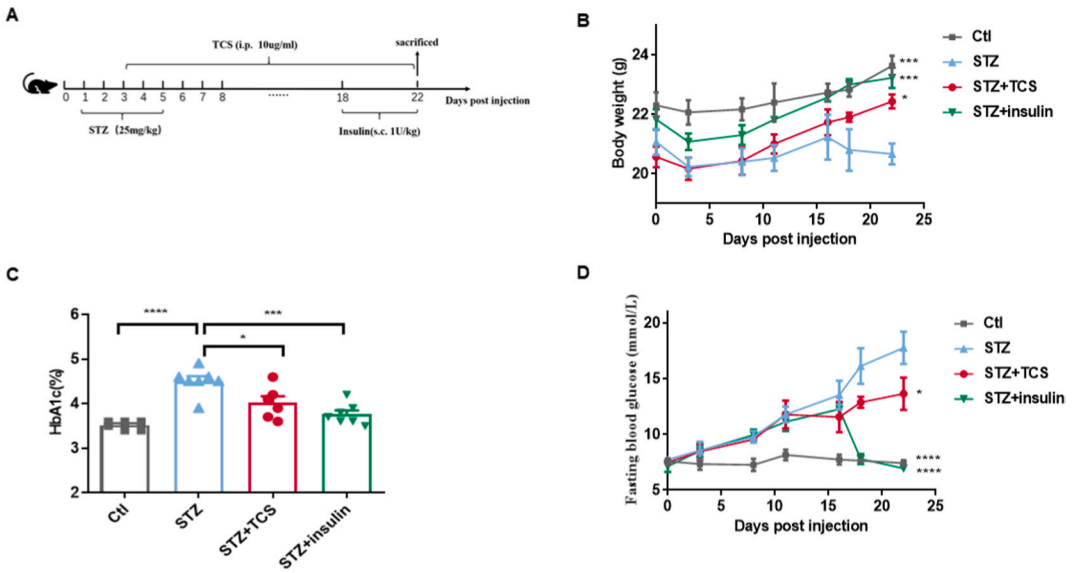

Fig. 2. Effects of TCS treatment in STZ-induced diabetic mice on fasting blood glucose, HbA1c and body weight. (A) C57BL/6 mice were stimulated with STZ (25 mg/kg/d, i. p.) for five consecutive days, and fasting blood glucose and body weight were monitored every three days until day 22, and then received TCS treatment (50 μg/kg/d, i. p.) for consecutive 20 days or insulin treatment (1U/kg/d, s. c.) for 5 days, finally the mice were used for in vivo and ex vivo studies. TCS treatment acutely reduced blood glucose (B), HbA1c (C) and increased body weight (D) on day 22. Data are presented as mean ± SEM, *P < 0.05, ***P < 0.001 vs STZ,****P < 0.0001 vs STZ.

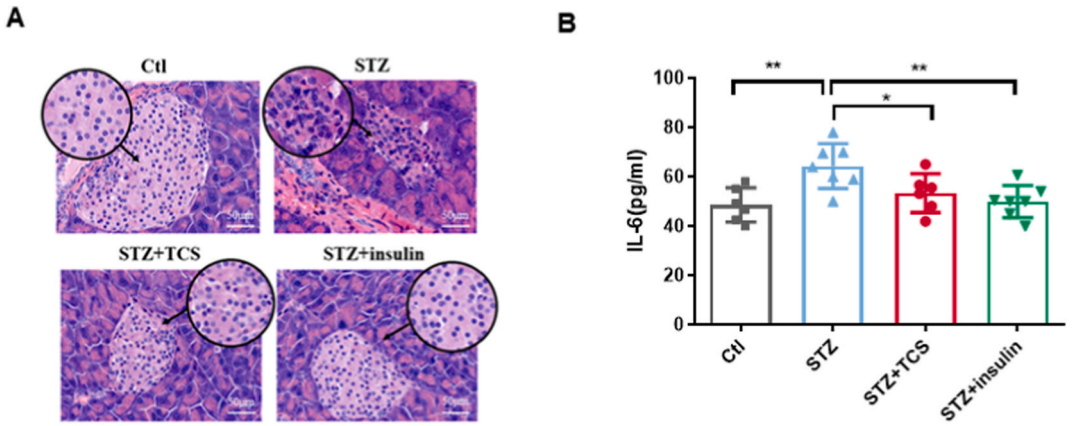

Fig. 3. Effect of TCS treatment in STZ-induced diabetic mice on insulitis in pancreatic islets (A) Representative images of the pancreas from different groups were created using H&E stain. All images were taken at × 400 magnification. Scale bars were 50 μm. (B) TCS reduced concentrations of IL-6 in plasma. Data are presented as mean ± SEM, *P < 0.05, **P < 0.01 vs STZ.

3.3. TCS decreased the proportion of MDSCs in bone marrow

Given that T1DM is an autoimmune disease, we aimed to explore how TCS affected the immune cell composition in different organs in T1DM mice. For this purpose, $CD4^+$, $CD8^+$ T cells and $CD19^+$ B cells were collected from spleen and peripheral blood and FACS analysis was used to measure their composition. The results showed that there were no significant differences of the $CD4^+$ T, $CD8^+$ T or $CD19^+$ B cell populations between T1DM and control mice in either spleen or peripheral blood (Fig. 4A, B, 4C and 4D). Additional treatments of TCS or insulin also did not cause any change in the composition of above immune cells.

We next examined the change of myeloid cells. In mice, myeloid-derived suppressor cells (MDSCs) are broadly defined as $CD11b^+$ $Gr1^+$ cells which carry out a variety of immune suppressor functions [19]. We isolated MDSCs from bonemarrow, spleenand peripheral blood of T1DM and control mice with or without treatments of TCS or insulin and measured their composition using FACS. We found markedly increased proportion of MDSCs in both bonemarrow and spleen in T1DM mice compared to healthy controls whereas MDSCs in peripheral blood showed no obvious change. Notably, TCS or insulin treatment significantly decreased the MDSCs in both bone marrow ($P < 0.001$) and spleen ($P < 0.001$) of T1DM mice (Fig. 4E and F).

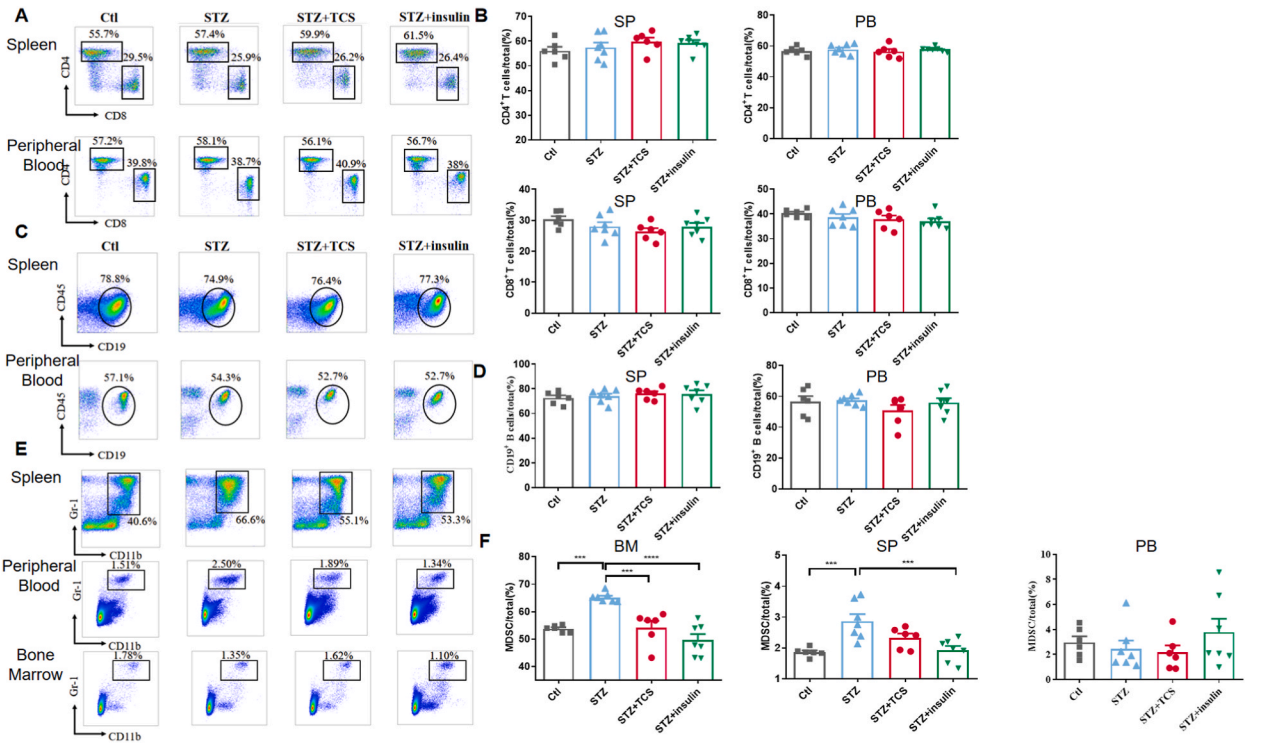

Fig. 4. Effects of TCS in bone marrow, spleen and peripheral blood derived MDSCs of STZ-induced diabetic mice. (A) Populations of T cells in the spleen and peripheral blood analyzed by flow cytometry. Cells in the spleen and peripheral blood were labeled with antibodies against CD4 and CD8. (B) Populations of B cells in the spleen and peripheral blood analyzed by flow cytometry. Cells in the spleen and peripheral blood were labeled with antibodies against CD19. There was no difference in $CD4^+$T cells (C), $CD8^+$T cells and $CD19^+$B cells (D) between STZ group and TCS or insulin treatment. MDSCs proportions ($CD11b^+$/$Gr\text{-}1^+$) in bone marrow, spleen, and blood of STZ-induced diabetic mice and TCS or insulin treatment mice compared. TCS decreased proportion of MDSCs in bone marrow, spleen and peripheral blood (E). But only MDSCs in the bone marrow significantly decreased after TCS treatment compared with STZ group (F). Data are presented as mean ± SEM, $^{**}P < 0.01$,$^{***}P < 0.001$, $^{****}P < 0.0001$ vs STZ.

MDSCs are a group of cells of heterogenous morphologies and functions [20,21]. Based on the expression of surface markers Ly6Gand Ly6C, MDSCs are categorized into granulocytic MDSCs (G-MDSCs) which are defined by $CD11b^+Ly6G^+Ly6C^-$ and monocytic MDSCs (M-MDSCs) which are defined by $CD11b^+Ly6G^-Ly6C^+$ [20,21]. G-MDSCs and M-MDSCs employ different mechanisms to suppress immune response by producing ROS and NO, respectively [20,21].

To further dissect the roles MDSCs played in T1DM, we checked the composition of its subgroups G-MDSCs and M-MDSCs in bone marrow, spleen and peripheral blood of T1DM and control mice. We found that G-MDSCs increased in spleen and peripheral blood but not in bone marrow in the T1DM mice. In contrast, M-MDSCs showed not obvious change in T1DM mice (Fig. 5A–F). These results suggested that elevated G-MDSCs correlated with the disease onset of T1DM. We also observed that upon TCS or insulin treatment, there were slight decreases of G-MDSCs in both spleen and peripheral blood. However, the difference was of no statistically significance. On the other hand, TCS or insulin treatment did not cause any change in the composition of M-MDSCs in all organs tested. In summary, G-MDSCs may play a moderate regulatory role in the pathogenesis of T1DM and TCS treatment may alleviate the disease by decrease the G-MDSCs in spleen and peripheral blood.

3.4. TCS treatment reduced IL-6$^+$ MDSCs while increased IL-10$^+$ MDSCs

Since we observed that the TCS reduced the proportion of MDSCs in T1DM mice whereas none of the subtypes G-MDSC or M-MDSC cells showed obvious change in either bone marrow, spleen or peripheral blood, we next investigated the MDSC subgroups by the cytokines they produced and the roles they played in regulating inflammatory and the pathogenesis of diabetes. Since our bioinformatic analysis revealed that IL-6 may play a critical role in TCS mediated T1D treatment, we first measured the IL-6 in T1DM mice and showed that they had increased IL-6 in peripheral blood (Fig. 3B). IL-6 is a pro-inflammatory cytokine implicated in the development of diabetes [20,21]. On the other hand, IL-10 is a cytokine involved in immuno-suppression [20,21]. The gating strategy was shown on Fig. 6A. Intriguingly, we found that IL-6^+ MDSCs in T1DM mice increased significantly compared to those in the control group (Fig. 6B and D). In contrast, the proportion of IL-10^+ MDSCs in T1DM mice was dramatically reduced (Fig. 6C and E). Importantly, upon treatments of TCS, the proportion of IL-6^+ MDSCs decreased significantly to level comparable to that of the control mice, whereas IL-10^+ MDSCs showed moderate increase (Fig. 6C and E). T1DM mice with insulin treatment showed similar changes of IL-6^+ and IL-10^+ MDSCs in bone marrow. Therefore, our results suggested that STZ-induced T1DM is accompanied by increased IL-6^+

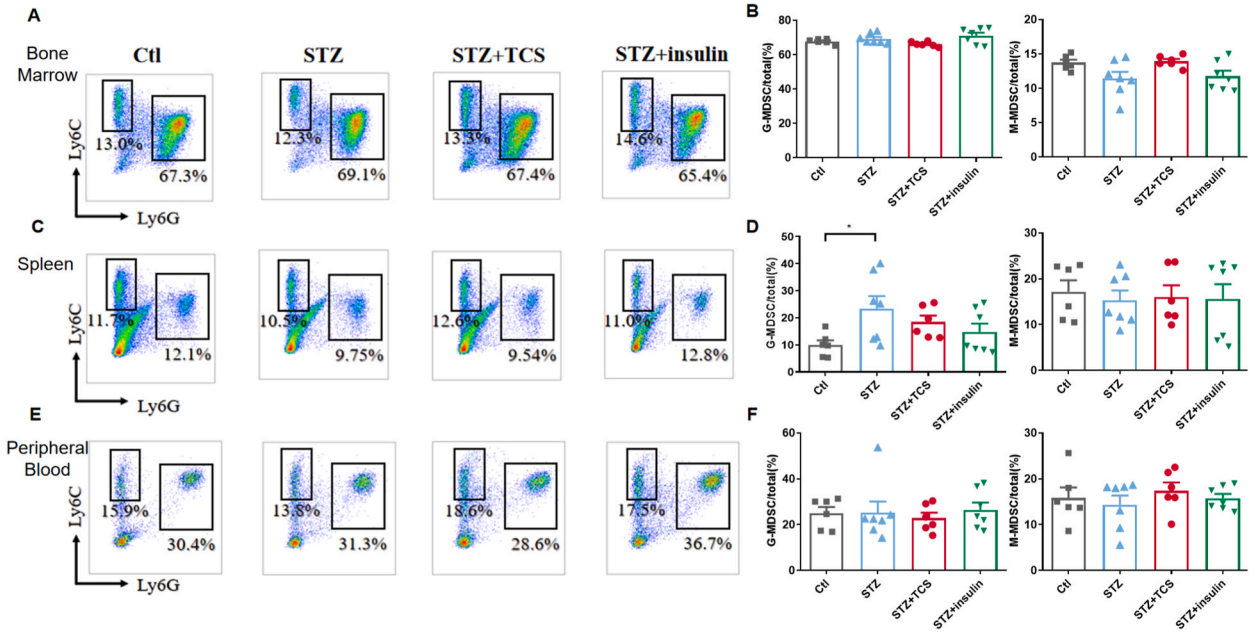

Fig. 5. Effects of TCS in bone marrow, spleen and peripheral blood derived G-MDSC and M-MDSC of STZ-induced diabetic mice. (A, C, E)Populations of G-MDSC and M-MDSC in the bone marrow, spleen and peripheral blood analyzed by flow cytometry. Splenocytes, bone marrow and peripheral blood cells were labeled with antibodies against Ly6C and Ly6G. G-MDSC proportions ($Ly6G^+/Ly6C^-$), M-MDSC proportions ($Ly6G^-/Ly6C^+$)in bone marrow, spleen, and peripheral blood of STZ-induced diabetic mice and TCS or insulin treatment mice compared. There was no difference in G-MDSC and M-MDSC (B, D, F) between STZ group and TCS or insulin treatment. Data are presented as mean ± SEM.

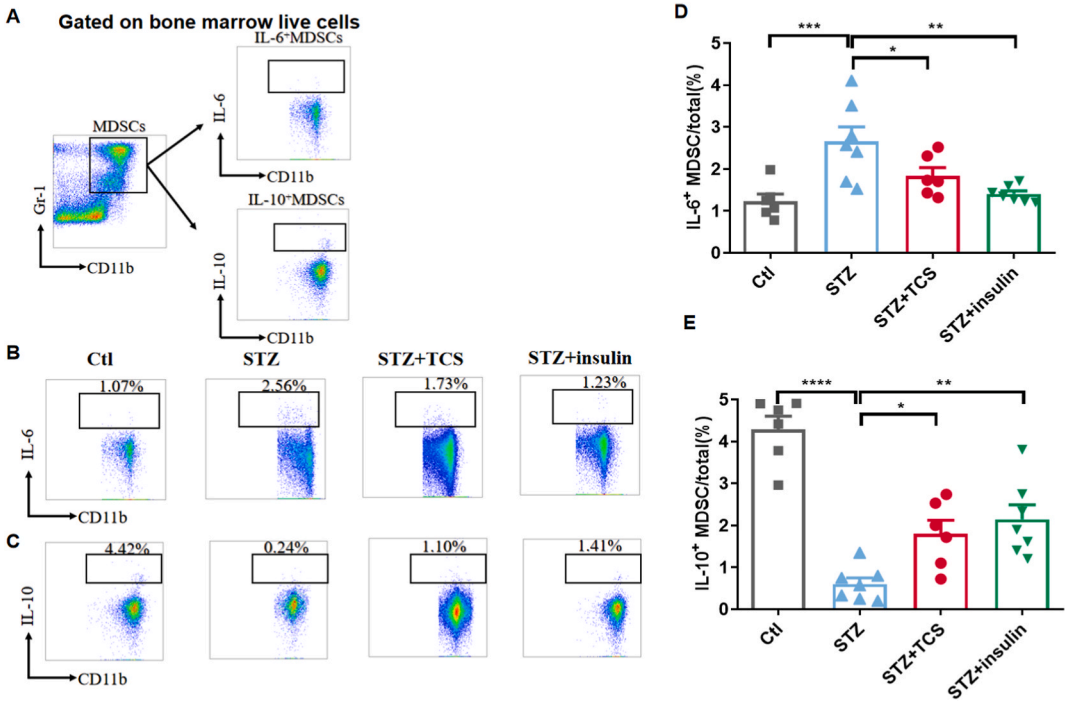

Fig. 6. Effects of TCS on the secretion levels of IL-6 and IL-10 in MDSCs derived from bone marrow of STZ-induced diabetic mice. (A)Flow gate strategy of $IL\text{-}6^+$ and $IL\text{-}10^+$ secreted by MDSCs in mice. Flow cytometry of $IL\text{-}6^+$MDSCs(B) and $IL\text{-}10^+$MDSCs(C) of mice in each group. TCS decreased proportion of $IL\text{-}6^+$MDSCs in bone marrow(D), and increased proportion of $IL\text{-}10^+$MDSCs in bone marrow(E). Data are presented as mean ± SEM, *P < 0.05, **P < 0.01,***P < 0.001, ****P < 0.0001 vs STZ.

and decreased IL-10^{+} MDSCs in bone marrow, indicating that IL-6^{+} may play a regulatory role in the pathogenesis of T1DM. Importantly, TCS as well as insulin treatments may alleviate T1DM by regulating the balance of IL-6^{+} and IL-10^{+} MDSCs in bone marrow. This finding is also supported by our bioinformatic analysis (Fig. 1).

3.5. TCS attenuated the mRNA expression of Arg1, iNOS, RAGE and NF-kB in MDSCs

Next we evaluated the effects of TCS on MDSCs in diabetes by examining the expression of functionally related genes. *Arg1* and *iNOS* are both critical for the immune suppressive functions of MDSCs [10]. On the other hand, the AGE-RAGE pathway plays an important role in diabetic complications. It has been shown that elevated expression of RAGE expression and accumulated AGE were found multiple tissues of diabetic animals [10]. This pathway also activates NF-κB seem signal and led to further oxidative stress and inflammation and promoted disease progression [20,21].

We examined the expression of *Arg1*, *iNOS*, *RAGE* and *NF-κB* of MDSCs by RT-qPCR using β-Actin as reference. The results showed that the expression all of above genes increased in diabetic mice compared to those in normal control mice ($P < 0.05$). Interestingly, TCS and insulin interventions caused significantly decrease of the mRNA levels of Arg1, iNOS, RAGE and NF-κB in MDSCs (Fig. 7). In summation, we conclude that TCS may alleviated T1DM by down-regulating the RAGE/NF-κB axis (Fig. 7), thereby positioning this axis as a critical target of TCS-mediated therapeutic effects.

4. Discussion

T1DM stands as an autoimmune disease characterized by the aberrant production of autoantibodies that meticulously target and impair pancreatic β cells, instigating hyperglycemia and an array of clinical manifestations [22]. To better understand the mechanism and efficacy of TCS on diabetes in vivo, we established a mouse disease model using multiple low-dose STZ injections to trigger autoimmune response to islet cells and induce diabetes. This model recapitulates the pathogenesis of human T1DM [23] and is a useful tool for drug development and assessment.

The aberrant accumulation of MDSCs within tissues has emerged as a potentially pivotal factor in the developmental trajectory of T1DM [24,25]. Notably, recent years have witnessed the discovery of enriched MDSC populations within the tissues of various murine models of autoimmune diseases, actively participating in inflammatory immune responses [4,18,26]. In this study, we found increased

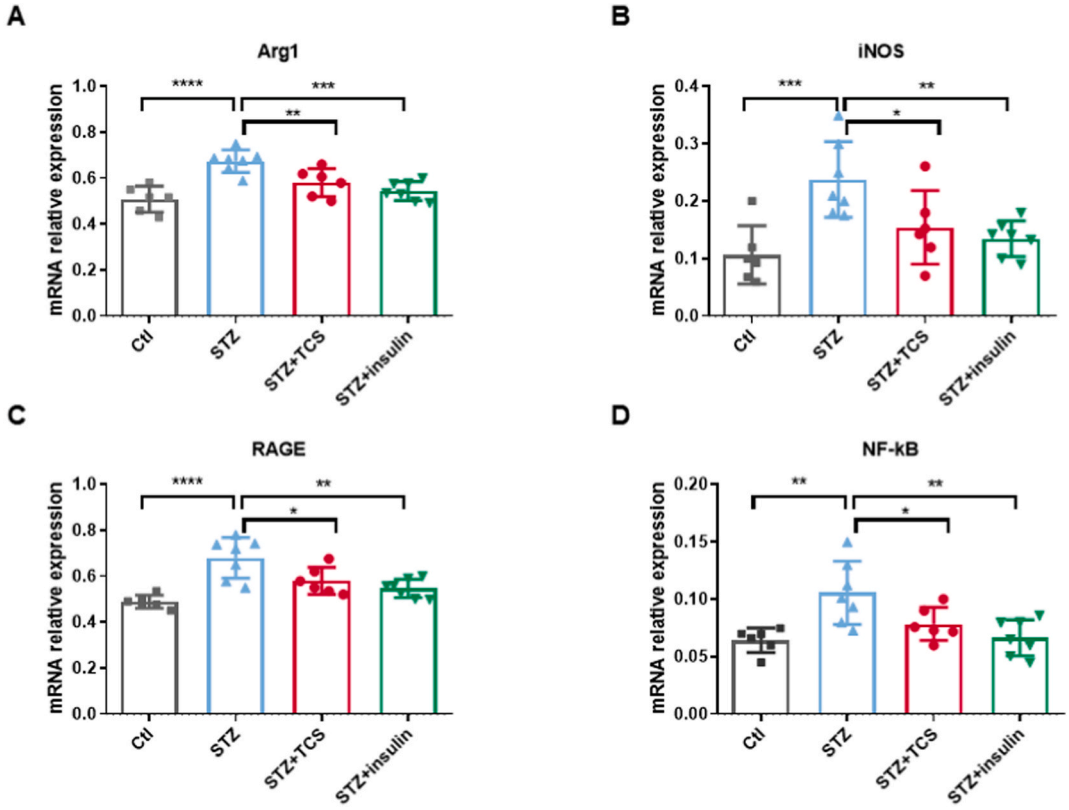

Fig. 7. Effects of TCS on the expression levels of Arg1, iNOS, RAGE, NF-κB mRNA in MDSCs derived from bone marrow of STZ-induced diabetic mice. BM-derived MDSCs was purified by Microbeads. Expression of Arg1(A), iNOS(B), RAGE(C), NF-κB(D) mRNA proportions from MDSCs determined using qPCR. Data are presented as mean ± SEM, *$P < 0.05$, **$P < 0.01$,***$P < 0.001$, ****$P < 0.0001$ vs STZ.

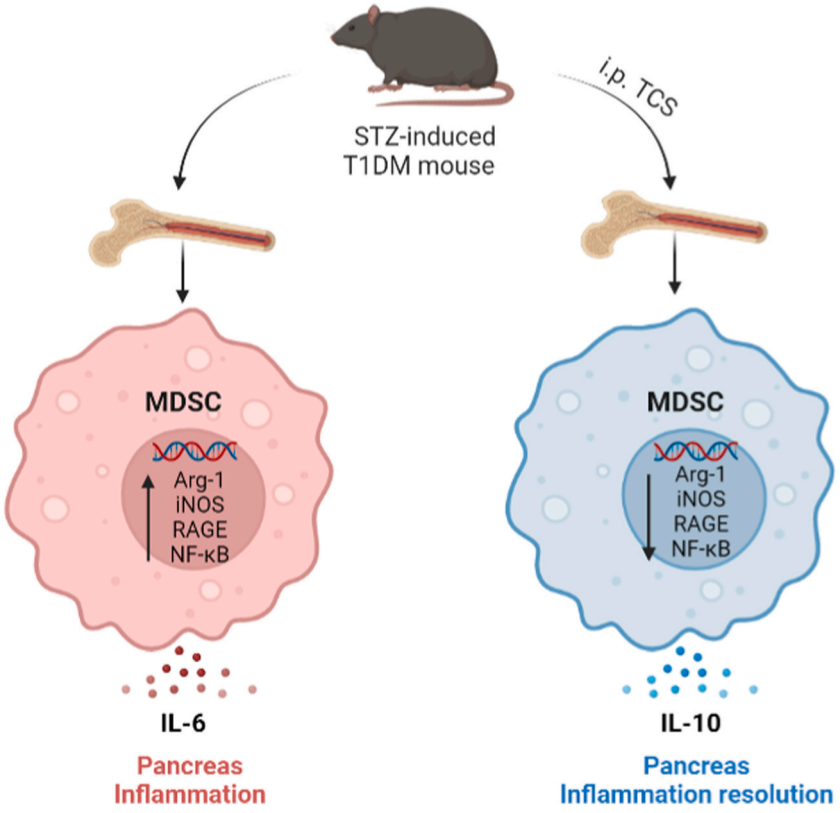

Fig. 8. TCS intervention reduced peripheral IL-6 and inhibits the islet inflammation in STZ induced diabetic mice. Also, TCS caused significantly decrease of the mRNA levels of Arg1, iNOS, RAGE and NF-κB in bone marrow derived-MDSCs. TCS may alleviate T1DM by down-regulating the RAGE/NF-κB axis.

MDSCs derived from bone marrow in STZ-induced T1DM mice compared to WT mice. In addition, these MDSCs increase the secretion of pro-inflammatory IL-6^{+} while decrease that of inhibitory IL-10^{+}, which may indicate their role in the pathogenesis of diabetes. Collectively, these findings underscore the potential involvement of MDSCs in the intricate pathogenesis of diabetes.

MDSCs additionally exert their influence on immune responses through the generation of active substances, including Arg, iNOS, and ROS. RAGE plays an important role in the pathogenesis of diabetes [10]. Notably, within animal models of diabetes, RAGE demonstrates heightened expression in regions characterized by AGE accumulation within tissues. The interaction between RAGE and AGE can be instigated via the NF-κB pathway, culminating in the initiation of oxidative stress and an ensuing inflammatory cascade. This, in turn, leads to the production of cytokines like IL-6, which triggers the inflammation of islet β cells, ultimately resulting in hyperglycemia and the constellation of clinical symptoms characteristic of diabetes.

The traditional Chinese herb medicine Tian Hua Fen has enjoyed longstanding use in the treatment of diabetes. Nonetheless, the intricate mechanisms underpinning its effects have largely remained elusive. This study brings to light a significant breakthrough, wherein we unveil that TCS, one of the bioactive constituents within Tian Hua Fen, exerts a remarkable ameliorative influence on diabetic symptoms within the STZ-induced T1DM mice model. These effects encompassed the mitigation of weight loss and the attenuation of islet β cell damage. TCS emerged as a promoter of an anti-inflammatory response, adeptly orchestrating a decrease in IL-6^{+} MDSCs and a concurrent increase in IL-10^{+} MDSCs populations. Furthermore, TCS exerted a counteractive influence on the RAGE/NF-κB signaling pathway within MDSCs, thereby presenting a multi-faceted approach to its efficacy. Our findings also revealed the molecular mechanisms of a traditional Chinese medicine (Fig. 8).

While the sample size employed within this study was not extensive, potentially contributing to the absence of statistically significant differences in MDSC proportions within the peripheral blood, the overarching trend of TCS reducing the MDSC percentage across bone marrow, spleen, and peripheral blood remains conspicuous. Notably, our observations thus far have centered on disparities at the mRNA level. In the ensuing phases, we anticipate augmenting our understanding by further investigating protein-level variations. Collectively, the data suggests that TCS could be very potential as functional drug to alleviates STZ-induced T1DM.

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Data availability statement

The data associated with our study has not been deposited into a publicly available repository. However, the data will be made available upon request.

CRediT authorship contribution statement

Jie Shu: Writing - original draft, Software, Methodology, Data curation. **Kefan Wang:** Writing - original draft, Software, Data curation. **Yuting Liu:** Software, Data curation. **Jie Zhang:** Methodology, Data curation. **Xuping Ding:** Software, Methodology. **Hanxiao Sun:** Software, Methodology. **Jiaoxiang Wu:** Software, Investigation. **Biao Huang:** Visualization, Resources. **Ju Qiu:** Software, Resources. **Huiming Sheng:** Writing - review & editing, Visualization, Funding acquisition, Formal analysis, Conceptualization. **Liming Lu:** Writing - review & editing, Visualization, Supervision, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e22907.

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