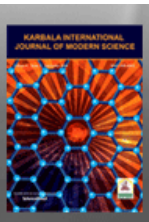

Karbala International Journal of Modern Science

Volume 12 | Issue 2 Article 3

Anti-Inflammatory and Insulin-Modulating Effects of Tithonia-Curcuma-Moringa (TCM) Formulation in Type 2 Diabetes: An Experimental Study in Mice

Rif'atul Hawani Burhan
Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, 65145 Malang, Indonesia;

Nabila Shafa Yumna Salsabila
Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, 65145 Malang, Indonesia

Devita Zulfa Pratama
Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, 65145 Malang, Indonesia;

La Tazkia Aulia Wibowo
Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, 65145 Malang, Indonesia;

Yulian Ervin Maulana
Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, 65145 Malang, Indonesia

See next page for additional authors

Recommended Citation

Burhan, Rif'atul Hawani; Salsabila, Nabila Shafa Yumna; Pratama, Devita Zulfa; Wibowo, La Tazkia Aulia; Maulana, Yulian Ervin; Dliyauddin, Moh; Lestari, Noviana Dwi; Marhendra, Agung Pramana Warih; Soewondo, Aris; Tsuboi, Hideo; and Rifa'i, Muhaimin (2026) "Anti-Inflammatory and Insulin-Modulating Effects of Tithonia-Curcuma-Moringa (TCM) Formulation in Type 2 Diabetes: An Experimental Study in Mice," *Karbala International Journal of Modern Science*: Vol. 12 : Iss. 2 , Article 3.
Available at: https://doi.org/10.33640/2405-609X.3455

Anti-Inflammatory and Insulin-Modulating Effects of Tithonia-Curcuma-Moringa (TCM) Formulation in Type 2 Diabetes: An Experimental Study in Mice

Abstract

Type 2 diabetes is a chronic metabolic disorder characterized by hyperglycemia and chronic inflammation. This study investigated the anti-inflammatory and insulin-modulating effects of *Tithonia-Curcuma-Moringa* (TCM) formulation in type 2 diabetes model. Male BALB/c mice were divided into six groups: normal control, type 2 diabetes control, metformin treatment, and three TCM formulation treatment groups with three different dose combinations. Type 2 diabetes was induced using a high-fat diet combined with streptozotocin injection, supported by sucrose administration. After 21 days of treatment, the spleen and pancreas were isolated for flow cytometry analysis. The results showed that the expression levels of NF-κB, TNF-α, and IFN-γ in CD4 T helper cells were significantly higher in diabetic mice compared to normal mice. Combination 1 (*T. diversifolia* 500 mg/kg BW+*C. longa* 200 mg/kg BW+*M. oleifera* 300 mg/kg BW) exhibited the most potent anti-inflammatory effect, significantly reducing the expression of NF-κB, TNF-α, and IFN-γ to levels comparable to those observed with metformin treatment. The proportion of T cells $CD4^{+}CD25^{+}$ was significantly increased in diabetic mice, indicating T cell activation, and then significantly decreased after administration of metformin and combination 3; insignificantly combinations 1 and 2. Insulin expression was significantly decreased in diabetic mice and then insignificantly increased by metformin and two combinations (1 and 2). In conclusion, the combination 1 TCM formulation demonstrated strong anti-inflammatory and suggested a potential insulin-modulating effect comparable to metformin in the type 2 diabetic mouse model, showing its role as an alternative treatment for managing chronic inflammation associated with type 2 diabetes.

Keywords

T. diversifolia, C. longa, M. oleifera, anti-inflammatory, insulin, type 2 diabetes

Authors

Rif'atul Hawani Burhan, Nabila Shafa Yumna Salsabila, Devita Zulfa Pratama, La Tazkia Aulia Wibowo, Yulian Ervin Maulana, Moh Dliyauddin, Noviana Dwi Lestari, Agung Pramana Warih Marhendra, Aris Soewondo, Hideo Tsuboi, and Muhaimin Rifa'i

RESEARCH PAPER

Anti-Inflammatory and Insulin-Modulating Effects of *Tithonia-Curcuma-Moringa* (TCM) Formulation in Type 2 Diabetes: An Experimental Study in Mice

Rif'atul H. Burhan [a], Nabila S.Y. Salsabila [a], Devita Z. Pratama [a], La T.A. Wibowo [a], Yulian E. Maulana [a], Moh Dliyauddin [b], Noviana D. Lestari [c], Agung P.W. Marhendra [a], Aris Soewondo [a], Hideo Tsuboi [d], Muhaimin Rifa'i [a,*]

[a] Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, 65145, Malang, Indonesia
[b] Study Program of Biology Education, Faculty of Education and Teacher Training, Jember University, 68121, Jember, Indonesia
[c] Study Program of Medical Education, Faculty of Medicine, Muhammadiyah Malang University, 65145, Malang, Indonesia
[d] Department of Immunology, Graduate School of Medicine, Nagoya University, 466-8550, Showa-ku, Nagoya, Japan

Abstract

Type 2 diabetes is a chronic metabolic disorder characterized by hyperglycemia and chronic inflammation. This study investigated the anti-inflammatory and insulin-modulating effects of *Tithonia-Curcuma-Moringa* (TCM) formulation in type 2 diabetes model. Male BALB/c mice were divided into six groups: normal control, type 2 diabetes control, metformin treatment, and three TCM formulation treatment groups with three different dose combinations. Type 2 diabetes was induced using a high-fat diet combined with streptozotocin injection, supported by sucrose administration. After 21 days of treatment, the spleen and pancreas were isolated for flow cytometry analysis. The results showed that the expression levels of NF-κB, TNF-α, and IFN-γ in CD4 T helper cells were significantly higher in diabetic mice compared to normal mice. Combination 1 (*T. diversifolia* 500 mg/kg BW + *C. longa* 200 mg/kg BW + *M. oleifera* 300 mg/kg BW) exhibited the most potent anti-inflammatory effect, significantly reducing the expression of NF-κB, TNF-α, and IFN-γ to levels comparable to those observed with metformin treatment. The proportion of T cells $CD4^{+}CD25^{+}$ was significantly increased in diabetic mice, indicating T cell activation, and then significantly decreased after administration of metformin and combination 3; insignificantly combinations 1 and 2. Insulin expression was significantly decreased in diabetic mice and then insignificantly increased by metformin and two combinations (1 and 2). In conclusion, the combination 1 TCM formulation demonstrated strong anti-inflammatory and suggested a potential insulin-modulating effect comparable to metformin in the type 2 diabetic mouse model, showing its role as an alternative treatment for managing chronic inflammation associated with type 2 diabetes.

Keywords: *T. diversifolia*, *C. longa*, *M. oleifera*, Anti-inflammatory, Insulin, Type 2 diabetes

1. Introduction

Diabetes is a serious health problem and the eighth leading cause of death worldwide [1]. This persistent metabolic condition is characterized by hyperglycemia [2] and, over time, leads to organ damage and various vascular complications [3]. Diabetes is categorized into several types, such as type 1, type 2, and gestational diabetes [4]. More than 90 % of individuals with diabetes are affected by type 2 diabetes, which occurs across all age groups, from youth to old age. Those diagnosed at a younger age often experience greater challenges in maintaining optimal glycemic control and face an increased risk of developing complications. Type 2 diabetes is often associated with unhealthy eating patterns and low physical activity levels [2,3].

Received 11 November 2025; revised 18 February 2026; accepted 26 February 2026.
Available online 2 April 2026

* Corresponding author.
E-mail addresses: rifa123@ub.ac.id, immunobiology@ub.ac.id (M. Rifa'i).

https://doi.org/10.33640/2405-609X.3455

The consumption of foods high in fat and sugar, without balanced physical activity, affects immunological mechanisms, which in turn impact the body's metabolic processes. The higher the fat level, the greater the production of free fatty acids [5]. In contrast, high blood sugar levels encourage the formation of advanced glycation end products (AGEs) [6]. Free fatty acids and AGEs can worsen chronic inflammation through the NF-κB signal transduction pathway [6,7]. NF-κB activation triggers the expression of various pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6, IL-17, and IFN-γ [8,9].

Chronic inflammation is associated with the pathogenesis of type 2 diabetes, which is associated with metabolic dysregulation. TNF-α, a primary pro-inflammatory cytokine, serves as an early biomarker for detecting type 2 diabetes. Patients with type 2 diabetes experience increased TNF-α levels, which can disrupt insulin signaling pathways in pancreatic β cells [10]. Simultaneously, IFN-γ levels increase during the early stages of inflammation, inhibiting insulin expression by damaging pancreatic β cells. These pro-inflammatory cytokines trigger uncontrolled increases in blood glucose levels, further worsening type 2 diabetes [11]. In the context of the immune system, regulatory T (Treg) cells, a subpopulation of CD4 T helper cells, play a role in suppressing overactive and uncontrolled immune responses to inhibit the expansion of adaptive and innate immunity [12]. $CD4^{+}CD25^{+}$ regulatory T cells suppress chronic inflammation [13]. However, in patients with type 2 diabetes, Treg cells are reduced, disrupting the mechanisms that regulate inflammation and further damaging immunometabolic homeostasis [14].

To date, conventional therapies used in clinical treatment have primarily focused on achieving glycemic control and improving metabolic function. The oral pharmacotherapies such as metformin, finerenone, and sulfonylureas have anti-inflammatory effects but do not directly target inflammation through the NF-κB signal transduction pathway in CD4 T helper cells [15–17]. The NF-κB signaling pathway in CD4 T helper cells mediates pro-inflammatory activity that links immune and metabolic dysregulation [10]. Herbal medicines have emerged as alternative treatment options for type 2 diabetes. Herbal medicines have pleiotropic effects that can target multiple biological pathways simultaneously to provide antioxidant, anti-inflammatory, immunomodulatory, and metabolic effects [18,19], while synthetic drugs tend to be more specific.

Several plants believed to have the potential to improve chronic inflammation include *Tithonia diversifolia* (Hemsl.) A.Gray, *Curcuma longa* L., and *Moringa oleifera* Lam. These three plants are rich in bioactive compounds from the phenolic group, such as phenolic acids and flavonoids, which are found abundantly in the leaves of *T. diversifolia* and *M. oleifera* [20,21] and curcuminoids found in the rhizome of *C. longa* [22]. Studies on the simultaneous intervention of this three-herb combination in inhibiting the NF-κB signal transduction pathway in CD4 T helper cells have not yet been systematically explored. Therefore, three plant parts were combined as TCM formulation to assess their potential efficacy as alternative medicines for managing chronic inflammation in patients with type 2 diabetes.

2. Methods

2.1. Ethical clearance

This study was approved by the Research Ethics Committee (Animal Care and Use Committee) of Brawijaya University (approval number 141-KEP-UB-2024), based on the ARRIVE guidelines for animal use in study [23] and Directive 2010/63/EU of the European Parliament and the Council for animal use in study.

2.2. Extraction of TCM formulation

The TCM formulation was obtained from a combination of *T. diversivolia* leaves, *C. longa* rhizomes, and *M. oleifera* leaves. Each plant part was processed into powder at Materia Medica Batu, East Java, Indonesia. The extraction procedure was performed as described in a previous study [24], with modifications. A total of 100 g of each powder was brewed into 1000 mL of distilled water (1:10), boiled at 95 °C, and dissolved until a color change occurred for 10 min. Subsequently, the solution was filtered in two stages using cloth and filter paper with the assistance of a Buchner funnel filtration. Each liquid extract was then frozen at −20 °C for 24 h before being freeze-dried to obtain a dry extract.

2.3. Experimental design

Male BALB/c mice (*Mus musculus*) were obtained from Kemuning, Central Java, Indonesia. BALB/c mice were used due to their relatively moderate inflammatory response in the pathogenesis [25], making them suitable for modeling type 2 diabetes, which is characterized by low-grade chronic

inflammation [26]. The mice were in normal, healthy, and active condition at 7–8 weeks of age. The design used in this study was a completely randomized one. A total of 24 mice were used in this study and were divided into six groups (each group = 4 mice) consisting of two control groups: normal (K-) and type 2 diabetes (K+), as well as four treatment groups confirmed with type 2 diabetes: metformin (MO), TCM formulation combinations 1 (C1), 2 (C2), and 3 (C3). Each mouse was individually placed in a cage measuring 31 x 22 × 9.5 cm^3, lined with the wood shavings, and covered with a wire lid at a temperature of 26 °C and relative humidity of 40–70 %. The cages were cleaned every three days by replacing the wood shavings.

2.4. Type 2 diabetes animal model

Type 2 diabetes modeling in mice started with the administration of a high-fat diet and water daily from the acclimatization period. The acclimatization period for the mice was two weeks. At 9–10 weeks of age, the mice received intraperitoneal (i.p.) injections of streptozotocin solution for four consecutive days at a multiple dose of 4 × 50 mg/kg body weight [27,28]. The streptozotocin powder obtained from BioWORLD (Dublin, Ohio, USA) was dissolved in 0.1 M citrate buffer, which is a mixture of sodium citrate dihydrate (294.10 g/mol) and citric acid (192.12 g/mol) at pH 4.5 [29]. The streptozotocin solution was injected within 15–20 min after preparation because it degrades rapidly [30]. The blood glucose levels of the mice were checked before the injection and on the fifth day after the injection of streptozotocin [31]. Mice were then administered a 10 % sucrose solution to maintain their blood glucose levels [32]. After a week, the mice's blood glucose levels were checked again, and those confirmed to have diabetes were shown by blood glucose levels of ≥200 mg/dL [33]. At this stage, all mice that reached blood glucose levels ≥200 mg/dL met the eligibility criteria, and no mice were excluded for the randomization (Fig. 1). Before drugs administration, the average body weight of mice in each group was 30.5 ± 1 g (K−), 28.8 ± 2.1 g (K+), 32.8 ± 0.5 g (MO), 26 ± 3.6 g (C1), 26.3 ± 2.2 g (C2), and 32.8 ± 1.5 g (C3).

2.5. Treatment of metformin and TCM formulation

The treatment was carried out for 21 days, during which mice that had been confirmed to have type 2 diabetes were treated with metformin and the TCM formulations. Mice in the MO group were administered metformin at a dose of 150 mg/kg BW [33]. Mice in groups C1, C2, and C3 were administered the TCM formulations of three different dose combinations. The dose was determined according to OECD guidelines regarding the total daily oral dose limit of an herbal formulation for rodents, which is 1000 mg/kg BW [34], thus the dose of each herbal component in a combination was determined based on a 2:3:5 ratio. The combination 1

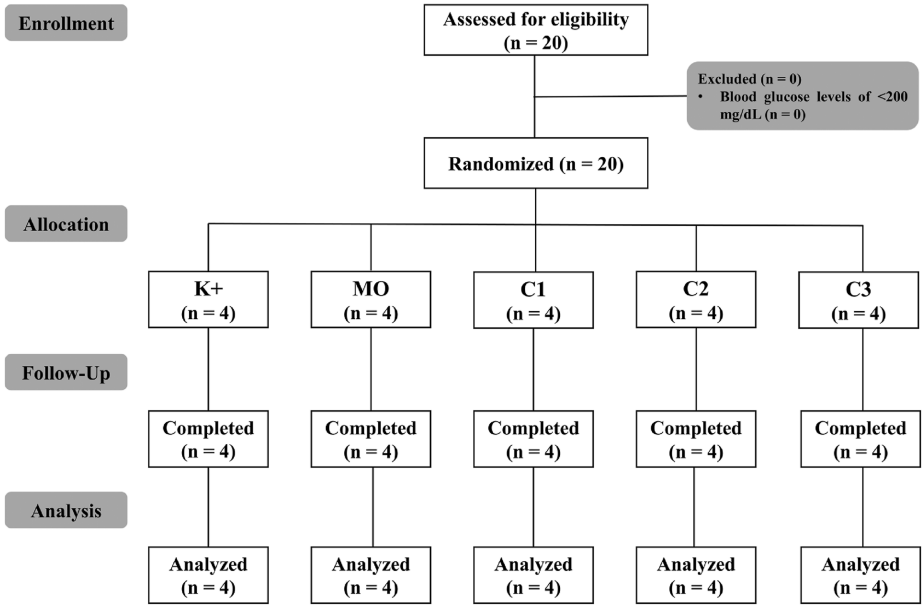

Fig. 1. CONSORT flow diagram. K+, type 2 diabetes control; MO, metformin treatment; C1, combination 1 TCM formulation; C2, combination 2 TCM formulation; C3, combination 3 TCM formulation.

TCM formulation (*T. diversifolia* 500 mg/kg BW + *C. longa* 200 mg/kg BW + *M. oleifera* 300 mg/kg BW) was administered to the mice in group C1. The combination 2 TCM formulation (*T. diversifolia* 200 mg/kg BW + *C. longa* 300 mg/kg BW + *M. oleifera* 500 mg/kg BW) was administered to the mice in group C2. The combination 3 TCM formulation (*T. diversifolia* 300 mg/kg BW + *C. longa* 500 mg/kg BW + *M. oleifera* 200 mg/kg BW) was administered to the mice in group C3. The mice were treated via oral administration.

2.6. Pancreas and spleen isolation

The mice were anesthetized using ketamine, and the abdomens were surgically opened to collect the pancreas and spleen. The pancreas and spleen were isolated from each group for further analysis. Each organ was placed in a petri dish containing 3 mL PBS and then macerated. The macerated samples were transferred into polypropylene tubes and centrifuged (2500 rpm, 10 °C) for 5 min. The pellet was separated from the supernatant and resuspended in 1 mL PBS. Furthermore, the samples were homogenized using a vortex and transferred into 50 μL microtubes [35].

2.7. Antibody staining for flow cytometry

For extracellular staining, cells from the spleen were incubated with FITC anti-mouse CD4 and PE anti-mouse CD25 (BioLegend, San Diego, CA, USA) at 4 °C for 20 min. Subsequently, the homogenate was resuspended in 400 μL PBS and transferred into a cuvette. For both extracellular and intracellular staining, cells from the spleen were incubated with FITC anti-mouse CD4 (BioLegend, San Diego, CA, USA) at 4 °C for 20 min. Subsequently, the homogenate was resuspended in 50 μL Cytofix and incubated at 4 °C for 20 min. Next, the homogenate was incubated with 500 μL washperm at 4 °C for 5 min, followed by centrifugation (2500 rpm, 10 °C) for 5 min. Each pellet separated from the supernatant was incubated with PE/Cy5 anti-mouse NF-κB, PE anti-mouse TNF-α, and PE/Cy5 anti-mouse IFN-γ (BioLegend, San Diego, CA, USA) at 4 °C for 20 min. Finally, the homogenate was resuspended in 400 μL PBS and transferred into a cuvette. For intracellular staining, cells from the pancreatic organ were resuspended in 50 μL Cytofix and incubated at 4 °C for 20 min. The homogenate was then incubated with 500 μL washperm at 4 °C for 5 min and centrifuged (2500 rpm, 10 °C) for 5 min. The pellet separated from the supernatant was incubated with APC anti-mouse insulin (R&D Systems, Minneapolis, MN, USA) at 4 °C for 20 min. The homogenate was then resuspended in 400 μL PBS and transferred into a cuvette. All procedures were performed under minimal light. Samples were analyzed using BD Cellquest Pro™ Software (BD Biosciences, San Jose, CA, USA) [35].

2.8. Data analysis

Raw data were analyzed using the FlowJo™ Software (FlowJo LLC, Ashland, OR, USA). Statistical analysis was performed using one-way ANOVA and subsequent post-hoc tests with a significance level of 0.05 using SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Effect of TCM formulation on $CD4^{+}NF\text{-}\kappa B^{+}$

The results showed that NF-κB expression in CD4 T helper cells increased significantly in diabetic mice to 20 ± 4 % compared to 4.74 ± 1.42 % in normal mice ($p \leq 0.05$). This surge in transcription factor expression in diabetic mice was significantly reduced after the administration of metformin and the combination 1 and 2 TCM formulations ($p \leq 0.05$). Metformin, as the standard drug, decreased NF-κB expression by 3.81 ± 0.57 %. The combination 1 TCM formulation had the best effect among the three combinations in suppressing NF-κB expression, reducing it by 9.64 ± 2.49 % (Fig. 2). In contrast, a reduction in NF-κB expression was also observed with the combination 3 TCM formulation, although it was not statistically significant compared to diabetic conditions ($p \geq 0.05$).

3.2. Effect of TCM formulation on $CD4^{+}TNF\text{-}\alpha^{+}$

TNF-α expression increased significantly by 23.12 ± 9.38 % in diabetic mice compared to 9.42 ± 3.21 % in normal mice ($p \leq 0.05$). This increase in TNF-α expression was significantly reduced after administration of metformin and the combination 1 TCM formulation ($p \leq 0.05$). Metformin reduced TNF-α expression by 4.26 ± 0.41 % in diabetic mice, which was even lower than that in normal conditions. The combination 1 TCM formulation exhibited the same anti-inflammatory effect of metformin in diabetic mice by reducing TNF-α expression by 7.65 % ± 2.99 %. In contrast, the TCM formulations of the other two combinations showed varying anti-inflammatory effect on TNF-α and not statistically significant compared to diabetic conditions ($p \geq 0.05$) (Fig. 3a–b).

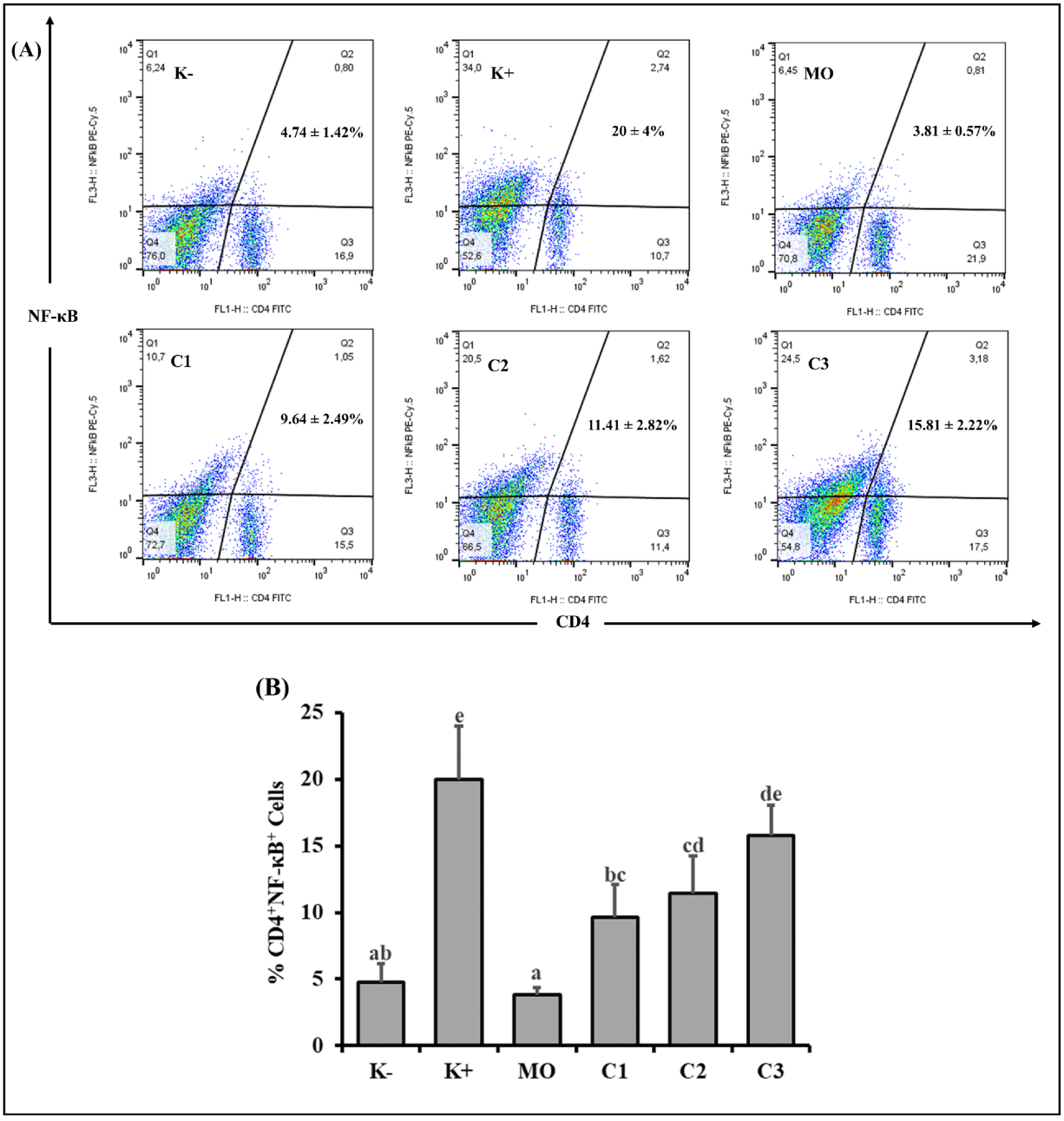

Fig. 2. TCM formulation led to a reduction in the expression of transcription factor NF-κB in CD4 T helper cells. A) Dot plot for flow cytometry analysis representing six separate groups. B) Statistical analysis conducted using one-way ANOVA followed by Tukey post-hoc test. K-, normal control; K+, type 2 diabetes control; MO, metformin treatment; C1, combination 1 TCM formulation; C2, combination 2 TCM formulation; C3, combination 3 TCM formulation.

3.3. Effect of TCM formulation on $CD4^{+}IFN\text{-}\gamma^{+}$

IFN-γ expression increased significantly in diabetic mice by 30.96 ± 10.65 % compared to normal mice at 11.19 ± 2.73 % ($p \leq 0.05$ %). This pro-inflammatory cytokine was significantly reduced ($p \leq 0.05$) following the administration of the metformin and the combination 1 TCM formulation in diabetic mice. Metformin produced the highest anti-inflammatory effect at 4.99 ± 2.09 %. The combination 1 TCM formulation also suppressed IFN-γ expression by 8.02 ± 1.56 %, equal to the anti-inflammatory effect of metformin. However, the combination 2 and 3 TCM formulations did not show any anti-inflammatory effect related to IFN-γ, as they resulted in slightly higher IFN-γ expression than that in diabetic mice ($p \geq 0.05$) (Fig. 3c–d).

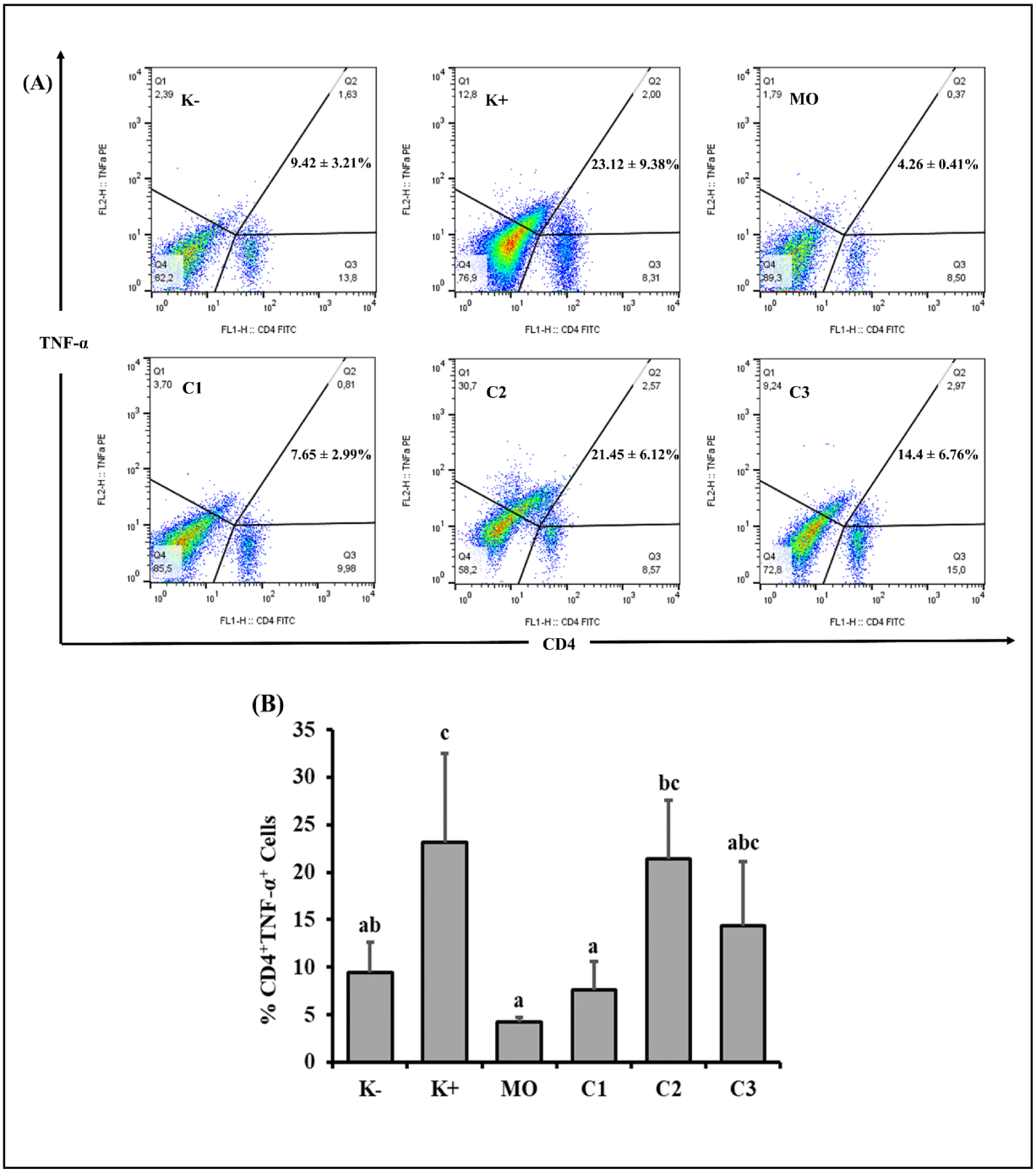

Fig. 3 TCM formulation showed its potential as an anti-inflammatory agent. (A–B) TNF-α expression in CD4 T helper cells, showing a dot plot from flow cytometry analysis and a statistical test using one-way ANOVA followed by a Tukey post-hoc test. (C–D) IFN-γ expression in CD4 T helper cells, showing a dot plot from flow cytometry analysis and a statistical test using one-way ANOVA followed by a Tukey post-hoc test. K-, normal control; K+, type 2 diabetes control; MO, metformin treatment; C1, combination 1 TCM formulation; C2, combination 2 TCM formulation; C3, combination 3 TCM formulation.

3.4. Effect of TCM formulation on $CD4^+CD25^+$ cells

Diabetic mice had a significant increase in the proportion of T cells $CD4^+CD25^+$, reaching 15.32 ± 6.32 % compared to a normal 5.98 ± 1.37 % ($p \leq 0.05$). Administration of metformin and the TCM formulations of three different dose combinations to diabetic mice gradually reduced the proportion of T cells $CD4^+CD25^+$ (Fig. 4). The TCM formulation that most significantly reduced the proportion of T cells $CD4^+CD25^+$ was combination 3, reaching 6.21 ± 2.35 % ($p \leq 0.05$). This combination showed only a

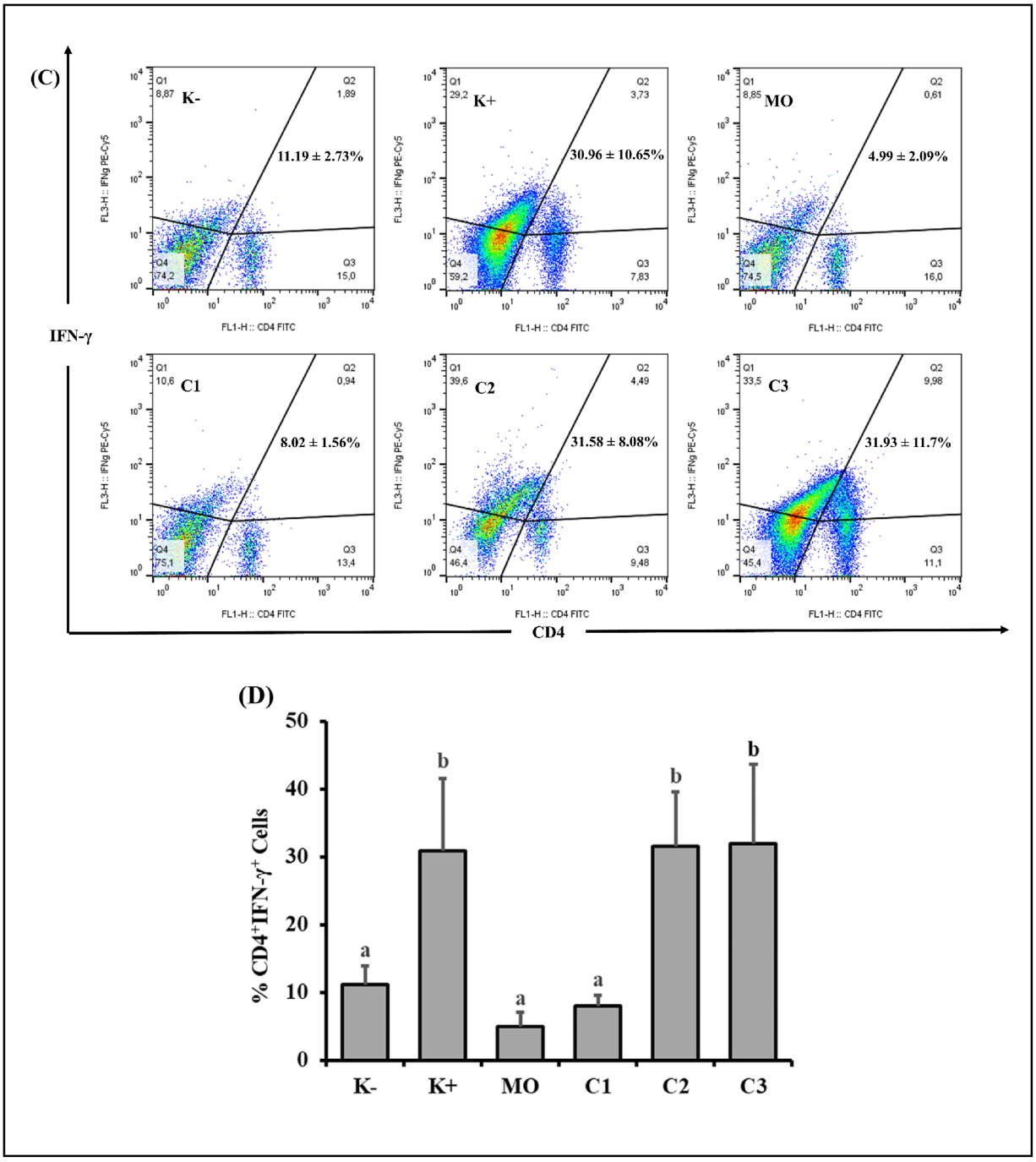

Fig. 3. (continued).

small difference compared to the decreasing effect of metformin. Metformin significantly decreased the proportion of T cells $CD4^{+}CD25^{+}$ at 5.76 % ± 2.32 % in diabetic mice ($p \leq 0.05$). In contrast, combination 1 produced the highest proportion of T cells $CD4^{+}CD25^{+}$ (9.14 % ± 3.98 %) among the three combinations, although it was still the lower compared to diabetic conditions but the difference was not statistically significant ($p \geq 0.05$).

3.5. Effect of TCM formulation on insulin expression

The results showed that insulin expression decreased significantly by 17.16 ± 5.41 % in diabetic mice compared to 45.98 ± 15.57 % in the normal group ($p \leq 0.05$). Insulin expression showed not statistically significant increase in diabetic mice after treatment with metformin and the combination 1 and 2 TCM formulations, which also

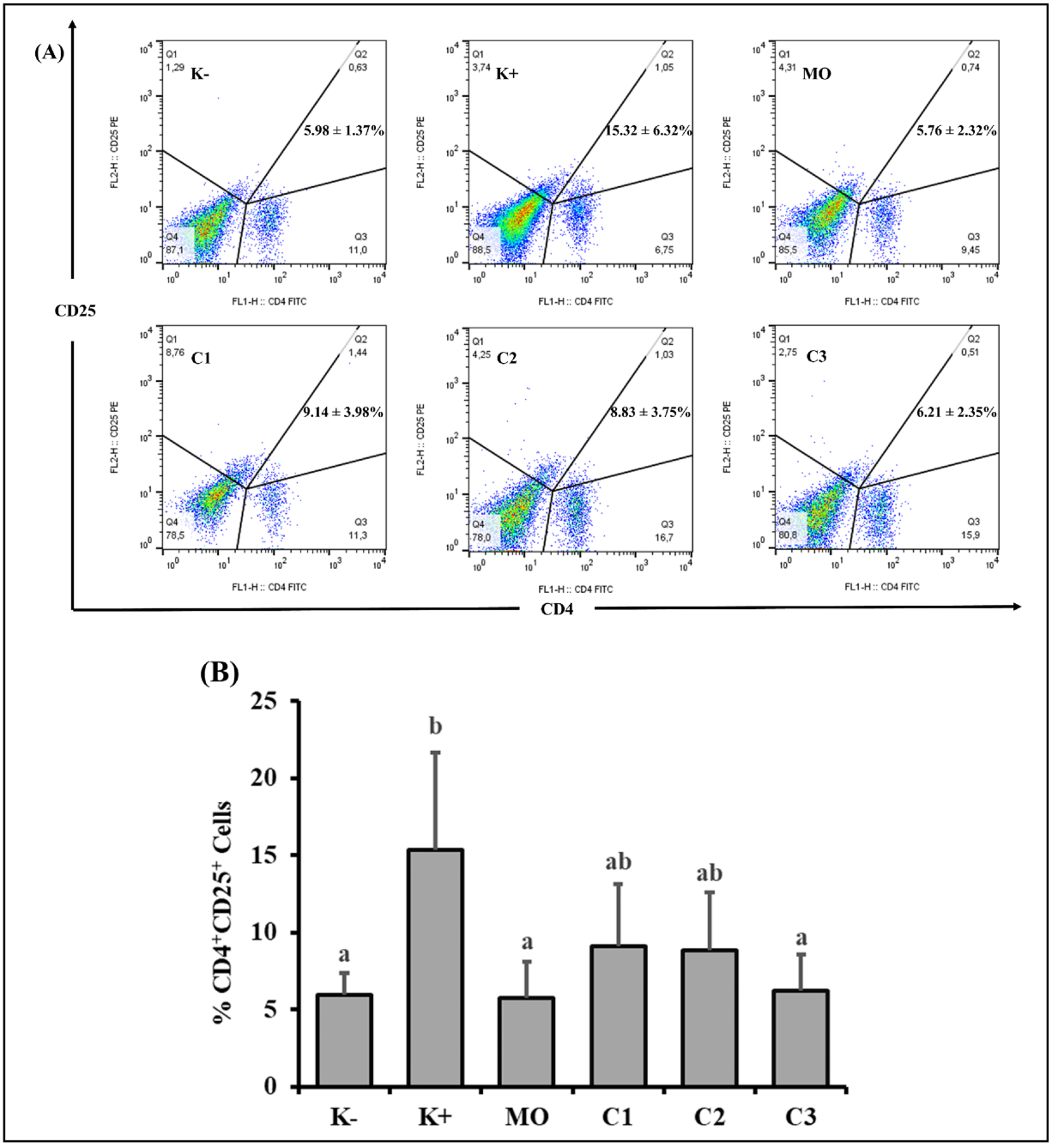

Fig. 4. TCM formulation decreased the proportion of T cells $CD4^{+}CD25^{+}$. A) Dot plot for flow cytometry analysis representing six separate groups. B) Statistical analysis conducted using one-way ANOVA followed by Tukey post-hoc test. K-, normal control; K+, type 2 diabetes control; MO, metformin treatment; C1, combination 1 TCM formulation; C2, combination 2 TCM formulation; C3, combination 3 TCM formulation.

approached normal conditions ($p \geq 0.05$). The combination 1 TCM formulation produced the highest among the three combinations, with insulin expression increasing by 30.4 ± 7.62 % (Fig. 5). The effect produced by this combination was almost equal to the increase in insulin expression by metformin, the standard drug, which reached 35.21 ± 5.1 %. In contrast, combination 3 resulted in the lowest insulin expression (17.39 ± 2.06 %), which was comparable to the diabetic condition ($p \geq 0.05$).

4. Discussion

Diabetes is a chronic metabolic disorder characterized by low-grade chronic inflammation [26]. Hyper-inflammatory responses worsen the progression of

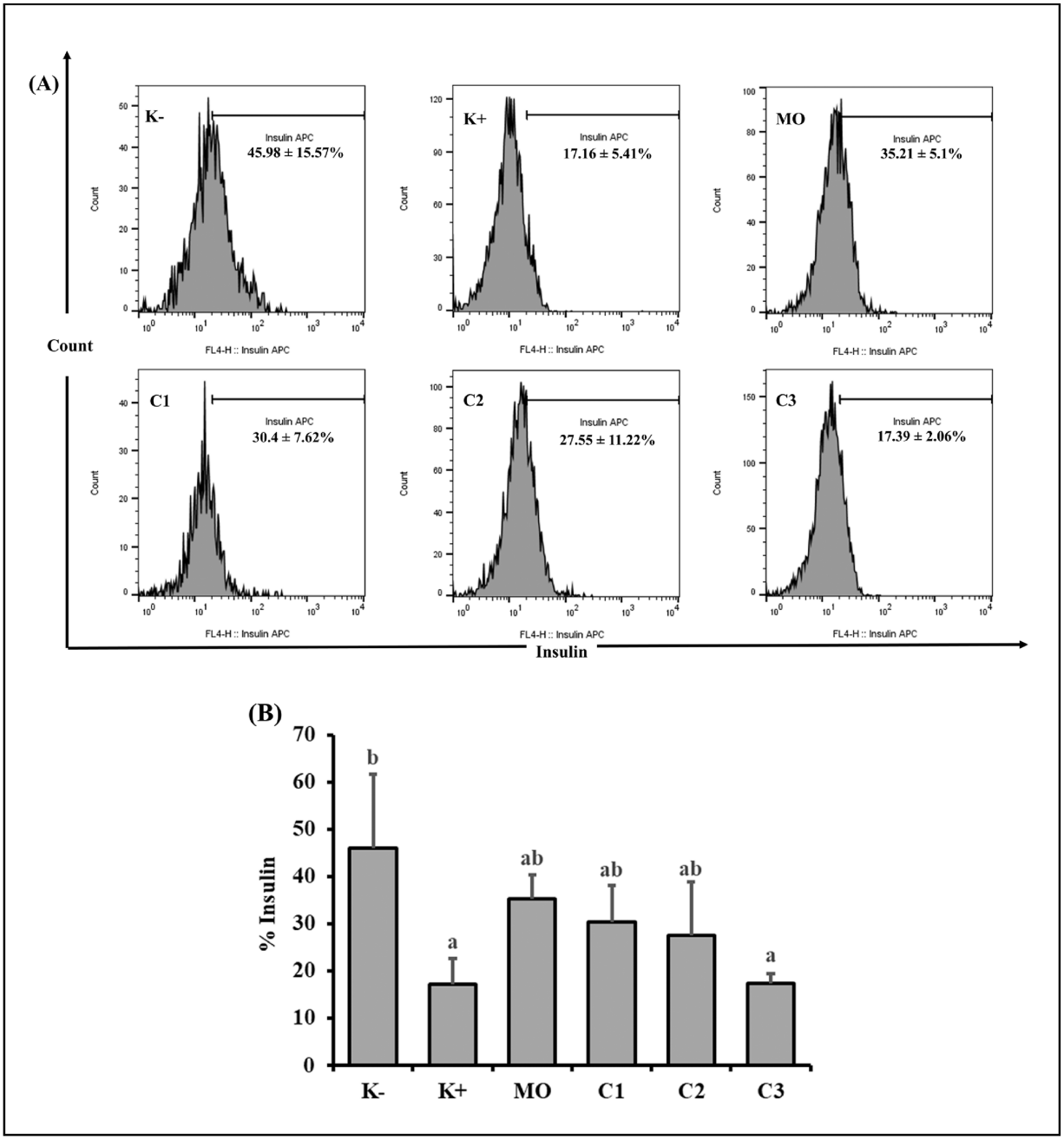

Fig. 5. TCM formulation modulated insulin expression. A) Histogram for flow cytometry analysis representing six separate groups. B) Statistical analysis conducted using one-way ANOVA followed by Tukey post-hoc test. K-, normal control; K+, type 2 diabetes control; MO, metformin treatment; C1, combination 1 TCM formulation; C2, combination 2 TCM formulation; C3, combination 3 TCM formulation.

diabetes, leading to organ damage and vascular complications [3]. This inflammation needs to be reduced through the exploration of the TCM formulations of three different dose combinations that can target the NF-κB signaling cascade pathway, thus enabling improved metabolic regulation. In this study, NF-κB expression modulated the inflammation expressed by CD4 T helper cells in type 2 diabetes (Fig. 2). Nuclear factor kappa-B (NF-κB), a transcription factor, is involved in the pathogenesis of various disorders. NF-κB centrally modulates pro-inflammatory genes, resulting in the expression of various cytokines and chemokines [36]. In the inflammatory response mechanism occurring in type 2 diabetes, activation of NF-κB predominantly occurs through the canonical signaling pathway [37]. This signaling pathway begins with the phosphorylation of the multi-subunit IκB complex (IKK) by TGF-β-

activated kinase 1 (TAK1). This phosphorylation activates IKKβ, which in turn phosphorylates IκBα. Subsequently, IκBα becomes unstable and undergoes proteasomal degradation. This condition causes IκBα to dissociate from the NF-κB. Consequently, NF-κB becomes free to translocate to the nucleus from the cytoplasm [36]. Together with AP-1, NF-κB promotes the expression of various pro-inflammatory genes, such as TNF-α, IL-1β, IL-6, IL-17, and IFN-γ [8,9,38]. The increased expression of NF-κB in type 2 diabetic mice is consistent with the increased expression of the two pro-inflammatory cytokines observed, TNF-α and IFN-γ. These two pro-inflammatory cytokines are among the main biomarkers indicating the pathogenesis of type 2 diabetes [10,11]. Tumor necrosis factor-alpha (TNF-α) expressed in CD4 T helper cells is a key contributor to metabolic inflammation [39]. In addition to TNF-α, interferon-gamma (IFN-γ), which is also expressed in CD4 T helper cells, has the potential to contribute to the death of pancreatic β-islet cells when secreted [40].

Our findings indicate that type 2 diabetic mice treated with the combination 1 TCM formulation consistently exhibited anti-inflammatory effects compared to those treated with combinations 2 and 3. Combination 1 successfully suppressed the expression of the NF-κB transcription factor and reduced the expression of TNF-α and IFN-γ (Fig. 3). This combination produced improvements similar to those produced by metformin alone. The effectiveness of the combination 1 TCM formulation cannot be separated from the dosage proportions of its three herbal components. *T. diversifolia* had the largest dosage proportion, followed by *M. oleifera* and *C. longa*. In contrast, the herbal components in combination 2 had the largest dosage proportion in *M. oleifera* and the smallest in *T. diversifolia*. Combination 3 had the largest dosage proportion in *C. longa* and the smallest in *M. oleifera*. *T. diversifolia* has the largest dosage proportion in combination 1, which may strengthen the anti-inflammatory effect of this combination. *T. diversifolia*, also known as paitan, contains the highest levels of flavonoids (apigenin) and phenolic acids (gallic, chlorogenic, caffeic, and p-coumaric acids) in its leaves [41]. The leaves of this plant are also known for their very strong bitter taste, which is stronger than that of *M. oleifera* leaves and *C. longa* rhizomes. This indicates that *T. diversifolia* is rich in antidiabetic metabolic compounds, such as chlorogenic acid [42,43], which contributes to its bitterness [43,44].

5-caffeoylquinic acid (5-CQA), a compound from the chlorogenic acid group, has good solubility in hot water during extraction [45]. Chlorogenic acid has strong anti-inflammatory effects, which work by inhibiting IKK phosphorylation, rendering IKKβ inactive. Consequently, IKKβ is unable to phosphorylate IκBα, which supports proteasomal degradation. Consequently, the translocation of NF-κB from the cytoplasm to the nucleus is hindered. In contrast, chlorogenic acid blocks the MAPK signaling pathway by inhibiting the phosphorylation of the kinase trio (p38, ERK1/2, and JNK) [46]. This condition suppresses the translocation of NF-κB to the nucleus and reduces the transcription of pro-inflammatory genes [47]. Another compound that dissolves in water is gallic acid [48]. Similar to the anti-inflammatory effects of chlorogenic acid, gallic acid works similarly. Gallic acid is also known for its high antioxidant activity. This compound has been reported to reduce the production of reactive oxygen species (ROS) [49], thereby preventing the phosphorylation of p38 and JNK, inhibiting NF-κB activation, and ultimately suppressing the transcription of pro-inflammatory genes [50]. In contrast, *M. oleifera* has anti-inflammatory and antidiabetic effects due to the rich content of bioactive compounds in its leaves from the phenolic acid group [21]. The compounds in *M. oleifera* are believed to regulate the immune system and metabolites under diabetic conditions [51]. The effectiveness of this combination is further complemented by the presence of curcumin, which dominates the rhizome of *C. longa*, with its potential as an anti-inflammatory and antidiabetic agent [22].

An increase in the expression of pro-inflammatory cytokines in CD4 T helper cells should correspond to a decrease in the proportion of Treg cells [14]. Interestingly, we found that the proportion of T cells $CD4^+CD25^+$ increased in type 2 diabetes. First, this condition can be explained as a form of compensation in the immune response, where the increased proportion of T cells $CD4^+CD25^+$ aims to suppress the expression of TNF-α and IFN-γ. In contrast, a higher expansion of T cells $CD4^+CD25^+$ in type 2 diabetes does not correspond to an increase in their suppressive function. Under these conditions, the proportion of T cells $CD4^+CD25^+$ increases, but their ability to suppress effector T cell activation decreases. Previous studies have reported that the increase in T cells $CD4^+CD25^+$ during the pathogenesis of type 2 diabetes indicates the activation and proliferation of CD4 T cells [52], rather than indicating Treg cells with the $CD4^+CD25^+$ marker. CD4 T cells require the CD25 molecule to bind with IL-2 to support the clonal expansion of activated T cells [52]. Consequently, T cells can express pro-inflammatory cytokines, such as IFN-γ [53]. This fact is further supported by the decrease in the

proportion of T cells $CD4^+CD25^+$ following the administration of metformin and the TCM formulations of three different dose combinations in mice with type 2 diabetes. Although combination 1 exhibited the least reduction effect compared to the other two combinations even metformin and showed a decreasing trend compared to diabetic conditions, it consistently suppressed T cell activation and reduced their expression of pro-inflammatory cytokines (Fig. 4).

In type 2 diabetes, pro-inflammatory activity can reduce the expression of insulin produced by pancreatic β-cells. The decrease in insulin expression is caused by damage to β-cells in a pro-inflammatory environment. IFN-γ, together with TNF-α, induces apoptosis in β-cells, resulting in decreased insulin expression [54]. IFN-γ binds to the IFNGR, which activates JAK1/JAK2. This is followed by the phosphorylation of STAT1, which leads to the translocation of STAT1 dimers into the cell nucleus [55]. STAT1 triggers transcription of the IRF-1 target gene. IRF-1 expression makes pancreatic β-cells more susceptible to caspase-dependent apoptotic signals induced by TNF-α [56]. Therefore, when IFN-γ and TNF-α expression decreases, pancreatic β-cells are not susceptible to receiving apoptotic signals, which subsequently impacts the reduction of insulin expression. The decline in insulin expression observed in type 2 diabetes showed an improvement trend following treatment with metformin and the combination 1 and 2 TCM formulations. Combination 1, in particular, showed an increasing trend in insulin expression to a level comparable to that achieved with metformin (Fig. 5).

Taken together, these findings are in line with the previous study reporting that herbal compounds inhibit the inflammatory response through the NF-κB signaling cascade pathway, which can reduce pro-inflammatory cytokines and improve insulin regulation [57]. Although this study led to potential findings for type 2 diabetes treatment, there are several limitations to this research. First, the study was conducted in vivo using an animal model. Therefore, herbal therapy using the TCM formulation cannot yet be implemented in humans, as it must first undergo various stages of further evaluation. Second, this study has not yet progressed to toxicity evaluation for the TCM formulation as a newly formulated drug, making toxicity evaluation an important direction for future studies. Third, the treatment period was relatively limited, thus future studies with extended durations and complementary mechanistic analyses are needed to further validate these findings.

5. Conclusion

In conclusion, the TCM formulation has been shown to have significant therapeutic potential in intervening in the pathogenesis of type 2 diabetes. The efficacy of the TCM formulation depends on the dosage proportion of each herbal component, where combination 1, characterized by a high dose of *T. diversifolia*, exhibited the strongest and most consistent anti-inflammatory effect, comparable to that of metformin. This combination effectively mitigates chronic inflammation by suppressing the NF-κB transcription factor in CD4 T helper cells, similar to the mechanisms of chlorogenic and gallic acids. This, in turn, leads to a reduction in the expression of key pro-inflammatory cytokines, TNF-α and IFN-γ, which play a significant role in the apoptosis of pancreatic β-cells, where insulin is produced. The combination 1 TCM formulation not only exerts an anti-inflammatory effect but also shows a restoring trend toward insulin expression in type 2 diabetes models, to levels nearly equivalent to the effects of metformin. This combination minimizes the pro-inflammatory environment and prevents pancreatic β-cells from undergoing apoptosis, allowing β-cells to maintain their function in producing insulin. This study provides strong evidence that the TCM formulation enriched with *T. diversifolia* acts as a potent agent against inflammation and suggests that the formulation has a potential modulatory role in insulin regulation, found in type 2 diabetes.

Ethical statement

This study was approved by the Research Ethics Committee (Animal Care and Use Committee) of Brawijaya University (approval number 141 -KEP -UB -2024), based on the ARRIVE guidelines for animal use in study and Directive 2010/63/EU of the European Parliament and the Council for animal use in study.

Disclaimer

The authors bear responsibility for this study.

Funding disclosure

The authors received no external funding related to this study.

Conflicts of interest

The authors declare no conflicts of interest related to this study.

Acknowledgements

The authors appreciate and express gratitude for the contributions made by the laboratory staff of the Laboratory of Physiology, Structure, and Animal Development, University of Brawijaya, during this study.

References

[1] M. Wahidin, A. Achadi, B. Besral, S. Kosen, M. Nadjib, A. Nurwahyuni, S. Ronoatmodjo, E. Rahajeng, M. Pane, D. Kusuma, Projection of diabetes morbidity and mortality till 2045 in Indonesia based on risk factors and NCD prevention and control programs, Sci. Rep. 14 (2024) 1–17, https://doi.org/10.1038/s41598-024-54563-2.

[2] U. Galicia-Garcia, A. Benito-Vicente, S. Jebari, A. Larrea-Sebal, H. Siddiqi, K.B. Uribe, H. Ostolaza, C. Martin, Pathophysiology of type 2 diabetes mellitus, Int. J. Mol. Sci. 21 (2020) 1–34, https://doi.org/10.3390/ijms21176275.

[3] J.Y. Kim, J. Lee, J.H. Moon, S.E. Park, S.H. Ko, S.H. Choi, N. H. Kim, Prevalence, incidence, and metabolic characteristics of young adults with type 2 diabetes mellitus in South Korea (2010–2020), Diabetes Metab. J. 49 (2025) 172–182, https://doi.org/10.4093/dmj.2024.0826.

[4] S.A. Antar, N.A. Ashour, M. Sharaky, M. Khattab, N.A. Ashour, R.T. Zaid, E.J. Roh, A. Elkamhawy, A.A. Al-Karmalawy, Diabetes mellitus: classification mediators, and complications; a gate to identify potential targets for the development of new effective treatments, Biomed. Pharmacother. 168 (2023) 1–25, https://doi.org/10.1016/j.biopha.2023.115734.

[5] L. Olsen, E. Thum, N. Rohner, Lipid metabolism in adaptation to extreme nutritional challenges, Dev. Cell. 56 (2021) 1417–1429, https://doi.org/10.1016/j.devcel.2021.02.024.

[6] Y. Zhang, Z. Zhang, C. Tu, X. Chen, R. He, Advanced glycation end products in disease development and potential interventions, Antioxidants (Basel) 14 (2024) 1–40, https://doi.org/10.3390/antiox14040492.

[7] G. Boden, P. She, M. Mozzoli, P. Cheung, K. Gumireddy, P. Reddy, X. Xiang, Z. Luo, N. Ruderman, Free fatty acids produce insulin resistance and activate the proinflammatory nuclear factor-kappa B pathway in rat liver, Diabetes 54 (2005) 3458–3465, https://doi.org/10.2337/diabetes.54.12.3458.

[8] S. Borzouei, H. Moghimi, A. Zamani, M. Behzad, Changes in T helper cell-related factors in patients with type 2 diabetes mellitus after empagliflozin therapy, Hum. Immunol. 82 (2021) 422–428, https://doi.org/10.1016/j.humimm.2021.03.004.

[9] M.Y. Zamanian, H.O. Alsaab, M. Golmohammadi, A. Yumashev, A.M. Jabba, M.K. Abid, A. Joshi, A.H. Alawadi, N. S. Jafer, F. Kianifar, S.B. Obakiro, NF-κB pathway as a molecular target for curcumin in diabetes mellitus treatment: focusing on oxidative stress and inflammation, Cell Biochem. Funct. 42 (2024) 1–24, https://doi.org/10.1002/cbf.4030.

[10] H. Bashir, S.A. Bhat, S. Majid, R. Hamid, R.K. Koul, M.U. Rehman, I. Din, J.A. Bhat, J. Qadir, A. Masood, Role of inflammatory mediators (TNF-α, IL-6, CRP), biochemical and hematological parameters in type 2 diabetes mellitus patients of Kashmir, India, Med. J. Islam. Repub. Iran. 34 (2020) 1–7, https://doi.org/10.47176/mjiri.34.5.

[11] B.Y. Li, W. Tan, J.L. Zou, Y. He, S. Yoshida, B. Jiang, Y.D. Zhou, Role of interferons in diabetic retinopathy, World. J. Diabetes 12 (2021) 939–953, https://doi.org/10.4239/wjd.v12.i7.939.

[12] F. Bayati, M. Mohammadi, M. Valadi, S. Jamshidi, A.M. Foma, E. Sharif-Paghaleh, The therapeutic potential of regulatory T cells: challenges and opportunities, Front. Immunol. 11 (2021) 1–25, https://doi.org/10.3389/fimmu.2020.585819.

[13] W. Qin, L. Sun, M. Dong, G. An, K. Zhang, C. Zhang, X. Meng, Regulatory T cells and diabetes mellitus, Hum. Gene. Ther. 32 (2021) 875–881, https://doi.org/10.1089/hum.2021.024.

[14] R. Liu, J. Bao, Y. Tang, D. Xu, L. Shen, H. Qin, Changes in Treg cells and cytokines in the peripheral blood of patients with coronary artery disease combined with type 2 diabetes mellitus, Heart Lung 69 (2025) 147–154, https://doi.org/10.1016/j.hrtlng.2024.10.004.

[15] L.P. Bharath, B.S. Nikojczyk, The intersection of metformin and inflammation, Am. J. Physiol. Cell Physiol. 320 (2021) 873–879, https://doi.org/10.1152/ajpcell.00604.2020.

[16] A. Verma, A.B. Patel, Finerenone: a non-steroidal mineralocorticoid receptor blocker for diabetic kidney disease, Trends Endocrinol. Metab. 32 (2021) 261–263, https://doi.org/10.1016/j.tem.2021.02.002.

[17] V. Kothari, J.A. Galdo, S.T. Mathews, Hypoglycemic agents and potential anti-inflammatory activity, J. Inflamm. Res. 9 (2016) 27–38, https://doi.org/10.2147/JIR.S86917.

[18] G.M. Pang, F.X. Li, Y. Yan, Y. Zhang, L.L. Kong, P. Zhu, K.F. Wang, F. Zhang, B. Liu, C. Lu, Herbal medicine in the treatment of patients with type 2 diabetes mellitus, Chin. Med. J. (Engl.). 132 (2019) 78–85, https://doi.org/10.1097/CM9.0000000000000006.

[19] S.A. Adha, N.N. Afifah, I.R. Latarissa, G.N. Iftinan, A.S.W. Kusuma, R.M. Febriyanti, M.I. Barliana, K. Lestari, Herbal medicines as complementary therapy for managing complications in COVID-19 patients with diabetes mellitus, Diabetes Metab. Syndr. Obes. 18 (2025) 135–146, https://doi.org/10.2147/DMSO.S498774.

[20] A.A. Ajao, A.N. Moteetee, *Tithonia diversifolia* (Hemsl) A. Gray. (Asteraceae: Heliantheae), an invasive plant of significant ethnopharmacological importance: a review, S. Afr. J. Bot. 113 (2017) 396–403, https://doi.org/10.1016/j.sajb.2017.09.017.

[21] M. Klimek-Szczykutowicz, K. Gawel-Beben, A. Rutka, E. Blicharska, M. Tatarczak-Michalewska, K. Kulik-Siarek, W. Kukula-Koch, M.A. Malinowska, A. Szopa, *Moringa oleifera* (drumstick tree)—nutraceutical, cosmetological, and medicinal importance: a review, Front. Pharmacol. 15 (2024) 1–44, https://doi.org/10.3389/fphar.2024.1288382.

[22] S. Fuloria, J. Mehta, A. Chandel, M. Sekar, N.N.I.M. Rani, M. Y. Begum, V. Subramaniyan, K. Chidambaram, L. Thangavelu, R. Nordin, Y.S. Wu, K.V. Sathasivam, P.T. Lum, D.U. Meenakshi, V. Kumarasamy, A.K. Azad, N.K. Fuloria, A comprehensive review on the therapeutic potential of *Curcuma longa* Linn. in relation to its major active constituent curcumin, Front. Pharmacol. 13 (2022) 1–27, https://doi.org/10.3389/fphar.2022.820806.

[23] N. Percie du Sert, V. Hurst, A. Ahluwalia, S. Alam, M.T. Avey, M. Baker, W.J. Browne, A. Clark, I.C. Cuthill, U. Dirnagl, M. Emerson, P. Garner, S.T. Holgate, D.W. Howells, N.A. Karp, S.E. Lazic, K. Lidster, C.J. MacCallum, M. Macleod, E.J. Pearl, O.H. Petersen, F. Rawle, P. Reynolds, K. Rooney, E.S. Sena, S.D. Silberberg, T. Steckler, H. Würbel, The ARRIVE guidelines 2.0: updated guidelines for reporting animal research, BMC Vet. Res. 16 (2020) 1–7, https://doi.org/10.1186/s12917-020-02451-y.

[24] M.J. Tourkey, Effects of Moringa oleifera aqueous leaf extract in alloxan induced diabetic mice, Interv. Med. Appl. Sci. 8 (2016) 109–117, https://doi.org/10.1556/1646.8.2016.3.7.

[25] P.S. Sankaran, High-fat-diet induced obesity and diabetes mellitus in Th1 and Th2 biased mice strains: a brief overview and hypothesis, Chronic Dis. Transl. Med. 9 (2023) 14–19, https://doi.org/10.1002/cdt3.57.

[26] R. Zhang, Q. Xie, X. Lu, R. Fan, N. Tong, Research advances in the anti-inflammatory effects of SGLT inhibitors in type 2 diabetes mellitus, Diabetol. Metab. Syndr. 16 (2024) 1–15, https://doi.org/10.1186/s13098-024-01325-9.

[27] K. Wang, Z. Tang, Z. Zheng, P. Cao, W. Shui, Q. Li, Y. Zhang, Protective effects of *Angelica sinensis* polysaccharide against hyperglycemia and liver injury in multiple low-dose streptozotocin-induced type 2 diabetic BALB/c mice, Food Funct 7 (2016) 4889–4897, https://doi.org/10.1039/c6fo01196a.

[28] A. Zeinhom, S.A. Fadallah, M. Mahmoud, Human mesenchymal stem/stromal cell-based therapy in diabetes

mellitus: experimental and clinical perspectives, Stem Cell Res. Ther. 15 (2024) 1–32, https://doi.org/10.1186/s13287-024-03974-z.
[29] V.K. Bayrasheva, A.Y. Babenko, V.A. Dobronravov, Y.V. Dmitriev, S.G. Chefu, I.Y. Pchelin, A.N. Ivanova, A.A. Bairamov, N.P. Alexeyeva, I.S. Shatalov, E.N. Grineva, Uninephrectomized high-fat-fed nicotinamide-streptozotocin-induced diabetic rats: a model for the investigation of diabetic nephropathy in type 2 diabetes, J. Diabetes Res. (2016) 1–18, https://doi.org/10.1155/2016/8317850.
[30] B.L. Furman, Streptozotocin-induced diabetic models in mice and rats, Curr. Protoc. Pharmacol. 70 (2015) 1–20, https://doi.org/10.1002/0471141755.ph0547s70.
[31] L. Chunudom, M. Thongsom, N. Karim, M.A. Rahman, M. N. Rana, J. Tangpong, *Tithonia diversifolia* aqueous fraction plays a protective role against alloxan-induced diabetic mice via modulating GLUT2 expression, S. Afr. J. Bot. 133 (2020) 118–123, https://doi.org/10.1016/j.sajb.2020.07.007.
[32] M.M. Ramzy, A.A.K. El-Sheikh, M.Y. Kamel, S.A. Abdelwahab, M.A. Morsy, Mechanism of testicular protection of carvedilol in streptozotocin-induced diabetic rats, Indian J. Pharmacol. 46 (2014) 161–165, https://doi.org/10.4103/0253-7613.129307.
[33] R.A.H. Al-Sarray, S.J.J. Al-Shaeli, Metformin and bee venom: a comparative detection of histological alteration of the pancreas and systemic inflammatory markers in diabetic mice, Arch. Razi Inst. 77 (2022) 2335–2343, https://doi.org/10.22092/ARI.2022.358619.2269.
[34] A.A. Balkrishna, S. Haldar, A. Varshney, OECD-407 driven 28-day-repeated-dose non-clinical safety evaluation of *Tinospora cordifolia* (Giloy) stem aqueous extract in sprague-dawley rats under GLP-compliance, Front. Pharmacol. 14 (2023) 1–23, https://doi.org/10.3389/fphar.2023.1095083.
[35] N.D. Lestari, W.I. Adharini, Widodo, S. Rahayu, H. Tsuboi, Y.D. Jatmiko, M. Rifa'i, Anti-inflammatory evaluation of *Moringa*-Albumin combination in inhibiting IFN-γ and TNF-α expression in diabetic mouse model, Res J. Pharm. Technol. 15 (2022) 628–632, https://doi.org/10.52711/0974-360X.2022.00103.
[36] T. Liu, L. Zhang, D. Joo, S.C. Sun, NF-κB signaling in inflammation, Signal Transduct. Target. Ther. 2 (2025) 1–9, https://doi.org/10.1038/sigtrans.2017.23.
[37] M.V. Singh, T. Wong, S. Moorjani, A.M. Mani, A.O. Dokun, Novel components in the nuclear factor-kappa B (NF-κB) signaling pathways of endothelial cells under hyperglycemic-ischemic conditions, Front. Cardiovasc. Med. 11 (2024) 1–14, https://doi.org/10.3389/fcvm.2024.1345421.
[38] S. Fujioka, J. Niu, C. Schmidt, G.M. Sclabas, B. Peng, T. Uwagawa, Z. Li, D.B. Evans, J.L. Abbruzzese, P.J. Chiao, NF-kappa B and AP-1 connection: mechanism of NF-kappa B-dependent regulation of AP-1 activity, Mol. Cell. Biol. 24 (2004) 7806–7819, https://doi.org/10.1128/MCB.24.17.7806-7819.2004.
[39] S.K. Shaw, S. Sengupta, R. Jha, C. Pattanik, H. Behera, P.K. Barik, D. Meher, R. Sarangi, S. Devadas, Meta-inflammation in type 2 diabetes mellitus: unveiling the role of aberrant $CD4^+$ T cells and pro-inflammatory cytokine networks, Front. Immunol. 16 (2025) 1–15, https://doi.org/10.3389/fimmu.2025.1603484.
[40] K.C. Osum, A.L. Barack, T. Martinov, N.L. Sahli, J.S. Mitchell, C.G. Tucker, K.E. Pauken, K. Papas, B. Appakalai, J.A. Spanier, B.T. Fife, Interferon-gamma drives programmed death-ligand 1 expression on islet β cells to limit T cell function during autoimmune diabetes, Sci. Rep. 8 (2018) 1–12, https://doi.org/10.1038/s41598-018-26471-9.
[41] O.A. Ojo, A.B. Ojo, B.O. Ajiboye, O. Olaiya, M.A. Okesola, A.A. Boligon, M.M.A. de Campos, B.E. Oyinloye, A.P. Kappo, HPLC-DAD fingerprinting analysis, antioxidant activities of *Tithonia diversifolia* (Hemsl.) A. Gray leaves and its inhibition of key enzymes linked to Alzheimer's disease, Toxicol. Rep. 5 (2018) 582–592, https://doi.org/10.1016/j.toxrep.2018.05.003.
[42] F.D. Passoni, R.B. Oliveira, D.A. Chagas-Paula, L. Gobbo-Neto, F.B. da Costa, Repeated-dose toxicological studies of *Tithonia diversifolia* (Hemsl.) A. Gray and identification of the toxic compounds, J. Ethnopharmacol. 147 (2013) 389–394, https://doi.org/10.1016/j.jep.2013.03.024.
[43] K. Ferrare, L.P.R. Bidel, A. Awwad, P. Poucheret, G. Cazals, F. Lazennec, J. Azay-Milhau, M. Tournier, A.D. Lajoix, D. Tousch, Increase in insulin sensitivity by the association of chiocoric acid and chlorogenic acid contained in a natural chicoric acid extract (NCRAE) of chicory (*Cichorium intybus* L.) for an antidiabetic effect, J. Ethnopharmacol. 215 (2018) 241–248, https://doi.org/10.1016/j.jep.2017.12.035.
[44] S. Soares, S. Kohl, S. Thalmann, N. Mateus, W. Meyerhof, V. de Freitas, Different phenolic compounds activate distinct human bitter taste receptors, J. Agric. Food Chem. 61 (2013) 1525–1533, https://doi.org/10.1021/jf304198k.
[45] S. Behne, H. Franke, S. Schwarz, D.W. Lachenmeier, Risk assessment of chlorogenic and isochlorogenic acids in coffee by-products, Molecules 28 (2023) 1–35, https://doi.org/10.3390/molecules28145540.
[46] J. Huang, M. Xie, L. He, X. Song, T. Cao, Chlorogenic acid: a review on its mechanisms of anti-inflammation, disease treatment, and related delivery systems, Front. Pharmacol. 14 (2023) 1–10, https://doi.org/10.3389/fphar.2023.1218015.
[47] R. Zhong, L. Miao, H. Zhang, L. Tan, Y. Zhao, Y. Tu, M.A. Prieto, J. Simal-Gandara, L. Chen, C. He, H. Cao, Anti-inflammatory activity of flavonols *via* inhibiting MAPK and NF-κB signaling pathways in RAW264.7 macrophages, Curr. Res. Food Sci. 5 (2020) 1176–1184, https://doi.org/10.1016/j.crfs.2022.07.007.
[48] Y.R. Park, B.S. Lee, Solubility of gallic acid in single and mixed solvents, Separations 11 (2024) 1–16, https://doi.org/10.3390/separations11010036.
[49] M. Rahimifard, M. Baeeri, H. Bahadar, S. Moini-Nodeh, M. Khalid, H. Haghi-Aminjan, H. Mohammadian, M. Abdollahi, RETRACTED: therapeutic effects of gallic acid in regulating senescence and diabetes; an in vitro study, Molecules 25 (2020) 1–15, https://doi.org/10.3390/molecules25245875.
[50] Y. Xu, G. Tang, C. Zhang, N. Wang, Y. Feng, Gallic acid and diabetes mellitus: its association with oxidative stress, Molecules 26 (2021) 1–15, https://doi.org/10.3390/molecules26237115.
[51] C.S. Tripathy, A. Mishra, S.K. Behera, S. Parida, Ethnomedicinal uses of *Moringa oleifera* Lam. and the phytochemicals in regulating type 2 diabetes and inflammation, Plant Sci. Today 11 (2024) 197–206, https://doi.org/10.14719/pst.3549.
[52] M. Rifa'i, N. Widodo, Significance of propolis administration for homeostasis of $CD4^+CD25^+$ immunoregulatory T cells controlling hyperglycemia, SpringerPlus 3 (2014) 1–8, https://doi.org/10.1186/2193-1801-3-526.
[53] E.E. Contreras-Castillo, V.Y. García-Rasilla, M.G. García-Patiño, P. Licona-Limón, Stability and plasticity of regulatory T cells in health and disease, J. Leukoc. Biol. 116 (2024) 33–53, https://doi.org/10.1093/jleuko/qiae049.
[54] D.J. De George, T. Ge, B. Krishnamurthy, T.W.H. Kay, H.E. Thomas, Inflammation versus regulation: how interferon-gamma contributes to type 1 diabetes pathogenesis, Front. Cell. Dev. Biol. 11 (2023) 1–8, https://doi.org/10.3389/fcell.2023.1205590.
[55] J.E.Jr. Darnell, I.M. Kerr, G.R. Stark, Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins, Science 264 (1994) 1415–1421, https://doi.org/10.1126/science.8197455.
[56] K. Suk, S. Kim, Y.H. Kim, K.A. Kim, I. Chang, H. Yagita, M. S. Lee, IFN-gamma/TNF-alpha synergism as the final effector in autoimmune diabetes: a key role for STAT1/IFN regulatory factor-1 pathway in pancreatic beta cell death, J. Immunol. 166 (2001) 4481–4489, https://doi.org/10.4049/jimmunol.166.7.4481.
[57] H. Song, W. Jia, Beneficial effects of food-derived polyphenols on type 2 diabetes: mechanistic insights based on gut microbiota alterations and anti-inflammatory responses, Food Sci. Anim. Prod. 1 (2023) 1–14, https://doi.org/10.26599/FSAP.2023.9240043.