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Synergistic Effects of Hypoxia-Preconditioned Mesenchymal Stem Cells Secretome and Alkaline Water in Alleviating Oxidative Stress and Inflammation in Type 2 Diabetic Rats

Irni Nasution[1], Agung Putra[2,3], Eko Setiawan[4,5]

[1]Student of Postgraduate Biomedical Science, Universitas Islam Sultan Agung, Semarang, Indonesia

[2]Stem Cell and Cancer Research (SCCR) Laboratory, Semarang, Indonesia

[3]Department of Doctoral Biomedical Science, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang, Indonesia

[4]Department of Postgraduate Biomedical Sciences, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang Indonesia

[5]Department of Surgery, Faculty of Medicine, Universitas Islam Sultan Agung,

Semarang, Indonesia

Corresponding author: Irni Savitri Nasution, dr., Biomedical Science, Universitas Islam Sultan Agung. Address: Jl. Kaligawe Raya No.Km.4, Terboyo Kulon, Kec. Genuk, Kota Semarang, Jawa Tengah 50112, Indonesia. Phone: +6281299079700, E-mail: irnisavitri.isn@gmail.com, ORCID ID: https://orcid.org/0009-0009-4764-662X.

ABSTRACT

Background: Chronic inflammation and oxidative stress are central to the pathophysiology of Type 2 Diabetes Mellitus (T2DM), contributing to the progression of metabolic dysfunction and related complications. **Objective:** The aim of this study was to explore the therapeutic potential of combining hypoxia-preconditioned mesenchymal stem cell SH-MSC with alkaline water in a T2DM rat model. **Methods:** T2DM was induced in Wistar rats through a high-fat diet (HFD) followed by streptozotocin (STZ) administration. A total of 30 healthy male Wistar rats were randomly assigned to five groups: healthy control, T2DM, T2DM + Metformin, T2DM + SH-MSC, and T2DM + SH-MSC + alkaline water. **Results:** The combination of SH-MSC and alkaline water significantly reduced malondialdehyde (MDA) levels, a key indicator of lipid peroxidation, and suppressed the expression of p65 mRNA, a crucial component of the NF-κB signaling pathway. Notably, the most pronounced reduction in p65 mRNA expression was observed in the group receiving both SH-MSC and alkaline water, suggesting a synergistic effect in mitigating oxidative stress and inflammation. **Conclusion:** These findings highlight the potential of SH-MSC and alkaline water as a novel therapeutic strategy for alleviating T2DM.

Keywords: Alkaline water, MDA, SH-MSC, T2DM.

1. BACKGROUND

Type 2 diabetes mellitus (T2DM) is the most prevalent form of diabetes, accounting for approximately 90–95% of all cases worldwide (1). The primary pathophysiology of T2DM is characterized by impaired glucose homeostasis, insulin resistance, insufficient insulin secretion, and progressive β-cell dysfunction (2). Oxidative stress, primarily driven by excessive reactive oxygen species (ROS), plays a crucial role in β-cell damage and insulin resistance (3). Elevated malondialdehyde (MDA) as a key biomarker of cellular oxidative damage have been consistently observed in T2DM animal models, which further exacerbates insulin resistance and impairs pancreatic β-cell function (4). Simultaneously, inflammation that regulated by nuclear factor kappa B (NF-κB) pathway acts as a central pathological factor in T2DM (5).

The p65 is one of NF-κB subunit and is known to be upregulated in insulin-resistant tissues, leading to the production of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) (6). Current therapeutic interventions, such as insulin sensitizers and exogenous insulin administration, provide temporary glycemic control yet do not effectively counteract the underlying proinflammatory and cellular oxidative damage, which contribute to persistent insulin resistance and progressive β-cell dysfunction (7). Consequently, alternative treatment strategies are urgently needed.

Recent study highlighted the potential of mesenchymal stem cells (MSCs) in regen-

erative medicine due to their ability to secrete a wide range of bioactive molecules, collectively referred to as the secretome (8). The MSC-derived secretome exerts therapeutic effects through its immunomodulatory, anti-inflammatory, and regenerative properties (9). Notably, preconditioning MSCs under hypoxic conditions enhances the secretion of growth factors and cytokines such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and interleukin-10 (IL-10) (10). Secretome of hypoxia-preconditioned MSCs (SH-MSCs), has demonstrated significant potential in mitigating insulin resistance and improving pancreatic function. In addition to its role in β-cell preservation, SH-MSCs modulate oxidative stress by enhancing antioxidant enzyme activity, such as superoxide dismutase (SOD), while suppressing inflammatory mediators like interleukin-6 (IL-6) (11). Through these mechanisms, SH-MSCs may play a crucial role in reducing MDA levels and inhibiting NF-κB/p65 activation, ultimately alleviating oxidative stress and inflammation in T2DM (12).

Alongside stem cell-based therapies, alkaline water has emerged as a complementary approach for managing metabolic disorders. Alkaline water, characterized by its negative oxidation-reduction potential (ORP) and alkalizing effects, has been reported to reduce oxidative stress by neutralizing ROS and improving metabolic homeostasis (13). Previous studies suggest that alkaline water may enhance glucose uptake in insulin-resistant cells, modulate inflammatory pathways, and restore antioxidant balance (14). The combined application of SH-MSCs and alkaline water may offer a synergistic therapeutic effect by concurrently addressing oxidative stress, inflammation, and metabolic dysregulation in T2DM.

Previous studies have demonstrated that MSC-based therapies effectively improve insulin resistance and hyperglycemia in experimental models of diabetes (15). MSCs have been shown to regulate inflammatory signaling pathways, enhance β-cell function, and restore metabolic balance (16). SH-MSCs have exhibited superior therapeutic potential due to their enriched anti-inflammatory and regenerative secretome (11). However, while SH-MSCs have been shown to modulate oxidative stress and inflammation, their precise effects and therapeutic combination potential with alkaline water in T2DM remain largely unexplored.

2. OBJECTIVE

In this study, we investigated the therapeutic effects of SH-MSCs, alkaline water, and their combination on oxidative stress and inflammatory signalling in a T2DM rat model.

3. MATERIAL AND METHODS

Animals

The present study was conducted following ethical approval from the Institutional Animal Care and Use Committee at Universitas Islam Sultan Agung (Reference No. 16/I/2025/Komisi Bioetik). A total of 30 male Sprague-Dawley rats, aged eight weeks and weighing 225 ± 25 g, were utilized in this experiment. Prior to the study, the animals underwent a one-week acclimatization period under controlled environmental conditions, maintained at a temperature of 24 ± 2°C with a 12-hour light/dark cycle. The rats were housed in standard laboratory cages, ensuring optimal welfare conditions, and were provided unrestricted access to food and water throughout the study duration.

Type 2 diabetes melitus induction

Following the acclimatization period, the healthy rats was maintained on a standard pellet diet, while the experimental groups were fed a high-fat diet (HFD) for eight weeks to induce metabolic disturbances. The composition of the HFD included 50% ground standard pellet, 25% wheat flour, 10% mutton fat, 5% egg yolk, 1% coconut oil, and 10% sodium chloride (NaCl). Body weight measurements were recorded weekly to monitor metabolic changes. To induce type 2 diabetes mellitus (T2DM), nicotinamide (120 mg/kg body weight; Sigma-Aldrich, MO, USA) was administered intraperitoneally, followed by streptozotocin (STZ) (60 mg/kg body weight; Santa Cruz Biotechnology, TX, USA) after a 15-minute interval. The STZ solution was freshly prepared and utilized within five minutes to ensure stability. Healthy rats received intraperitoneal injections of phosphate-buffered saline (PBS, Elabscience, USA) and 0.05 M sodium citrate buffer (pH 4.5) as vehicle controls in place of nicotinamide and STZ.

Seven days post-STZ administration, all rats underwent a six-hour fasting period before measuring fasting blood glucose, insulin levels and HOMA-IR to confirm diabetes onset. Rats were classified as diabetic if fasting blood glucose levels exceeded 15 mmol/L and HOMA-IR exceeded 2.4. The diabetic rats (n = 6 per group) were then randomly assigned to different treatment groups, and therapeutic interventions were conducted over 21 days. Except for the healthy rats, all diabetic rats continued the HFD throughout the study period. The positive control group received metformin at a dose of 45 mg/kg body weight, administered once a week for three weeks. Treatment 1 group received 500 µL of SH-MSC via intravenous injection once a week for three weeks. Treatment 2 group received the same SH-MSC regimen as Treatment 1, with the addition of alkaline water (SCCR, Semarang, Indonesia) at dose 5 mL per day administered orally for three weeks.

UC-MSCs isolation and characterization

Umbilical cords (UCs) were harvested from 19-day-pregnant Wistar rats. The isolation of UC-derived mesenchymal stem cells (UC-MSCs) was performed following our previously established protocol11. The isolated UC-MSCs were cultured in Dulbecco's Modified Eagle Medium (DMEM; Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS; Gibco, Invitrogen, NY, USA), 1.5% penicillin/streptomycin (Gibco), and 0.25% amphotericin B (Gibco). The cultures were maintained at 37°C in a humidified incubator with 5% CO_2, and the medium was refreshed every three days. Once

the cells reached 80% confluency, they were passage into new culture flasks. UC-MSCs at passage 5 were utilized for subsequent experiments.

To characterize UC-MSCs, flow cytometry analysis was conducted at passage 3 following the manufacturer's protocol. Briefly, cells were stained with rat anti-CD90-FITC, CD29-PE, CD31-PerCP, and CD45-APC antibodies (BD Biosciences, San Jose, CA, USA) and incubated for 20 minutes in the dark at room temperature. Following incubation, the stained cells were analysed using a BD FACS Lyric, and data interpretation was performed using BD FACS Suite (BD Biosciences).

The multilineage differentiation potential of UC-MSCs at passage 5 was further evaluated by inducing adipogenic and osteogenic differentiation. Cells were cultured in standard growth media until they reached 95% confluency, after which the medium was replaced with Rat MesenCult™ adipogenic and osteogenic differentiation basal media (Stem Cell Technologies, Singapore) supplemented with their respective differentiation supplements, 1% L-glutamine, 1% penicillin, and 0.25% amphotericin B (Gibco). Media changes were performed every three days, and after 21 days of induction, adipogenic and osteogenic differentiation was confirmed by staining lipid and calcium deposits with Oil Red O and Alizarin Red, respectively (Sigma-Aldrich).

Hypoxia-preconditioned MSCs induction

Upon reaching 80% confluence, MSCs were subjected to hypoxic conditions using a hypoxic chamber (Stem Cell Technologies) set to 5% O_2. The oxygen partial pressure (pO_2) was continuously monitored and validated using an oxygen controller (BioSpherix, Lacona, NY, USA) to ensure precise environmental regulation. The cells were incubated under these conditions for 24 hours at 37°C with 5% CO_2 to enhance hypoxia-induced cellular responses. Following incubation, the conditioned culture medium was carefully collected for further analysis.

SH-MSCs Preparation

The conditioned medium (CM) from hypoxia-preconditioned UC-MSCs was collected and subjected to centrifugation at 13,000 × g for 10 minutes at 4°C to remove cellular debris. The isolation of the secretome from hypoxia-preconditioned MSCs (SH-MSCs) was performed using the tangential flow filtration (TFF) system (Formulatrix, MA, USA), as described in our previous study (11). The filtration process employed a series of molecular weight cut-off (MWCO) filter cassettes, including 10–100 kDa membranes (Formulatrix), allowing for the fractionation of bioactive molecules. The purified SH-MSCs were subsequently stored at −80°C for further analyses, including enzyme-linked immunosorbent assays (ELISA), and utilized in downstream experiments.

SH-MSCs profilling

The levels of cytokines and growth factors in the SH-MSCs were quantified using enzyme-linked immunosorbent assays (ELISAs) according to the manufacturer's protocol (Invitrogen, CA, USA). The concentrations of vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and stromal cell-derived factor-1 (SDF-1) were assessed at room temperature. Absorbance was measured at a wavelength of 450 nm using a microplate reader (Bio-Rad, CA, USA), and the data were analyzed to determine the secretory profile of SH-MSCs.

Blood glucose analysis

Blood glucose levels were measured one week after the final treatment intervention. Following an overnight fasting period, blood samples were collected, and fasting blood glucose levels were assessed using an Accu-Chek Glucometer (Roche, Germany) to evaluate the glycemic response to the treatments.

Serum parameter analysis

One week after the final treatment, whole blood samples were collected to assess MDA and insulin levels in fasting serum using an ELISA following the manufacturer's instructions (Elabscience). The degree of insulin resistance was quantified using the homeostatic model assessment of insulin resistance (HOMA-IR) index, calculated with the formula (1).

$$HOMA\text{-}IR = (fasting\ blood\ glucose\ (mmol/L) \times fasting\ serum\ insulin\ (\mu U/mL))/22.5 \quad (1)$$

p65 mRNA analysis

Total RNA was isolated from 50 mg of dorsal skin tissue using a commercially available TRI Reagent (Sigma-Aldrich) following the manufacturer's protocol. Synthesis of complementary DNA (cDNA) was performed using the Enhanced Avian First Strand cDNA Synthesis Kit (Sigma-Aldrich) with oligo d(T) primers. The reverse transcription was carried out by incubating the samples at 70°C for 10 minutes to denature RNA secondary structures, followed by cDNA synthesis at 45°C for 15 minutes. Quantitative real-time PCR (qPCR) was performed on an Eco Real-Time PCR System (Illumina, San Diego, CA, USA) using KAPA SYBR® FAST Universal Kit (Sigma-Aldrich) with reaction conditions optimized according to the manufacturer's guidelines. Gene-specific primers for p65 (forward: 5′-AACACTGCCGAGCTCAAGAT-3′; reverse: 5′-CATCGGCTTGAGAAAAGGAG-3′) and the reference gene β-actin (forward: 5′-GCCTTCCTTCCTGGGTATG-3′; reverse: 5′-AGGAGCCAGGGCAGTAATC-3′) were designed to amplify 100–150 bp products. Thermocycling parameters included an initial denaturation at 95°C for 3 min, followed by 40 cycles of 95°C for 10 sec (denaturation) and 60°C for 30 sec (annealing/extension). Relative quantification of p65 mRNA expression was calculated using the 2−ΔΔCt method, normalized to β-actin as an internal control. All reactions were performed in technical triplicates to ensure reproducibility.

Data analysis

The results are presented as the mean ± standard deviation (SD). Statistical analyses were performed using SPSS version 26.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was determined using one-way ANOVA and Kruskal-Wallis tests, followed by post hoc analysis with the least significant difference (LSD) and

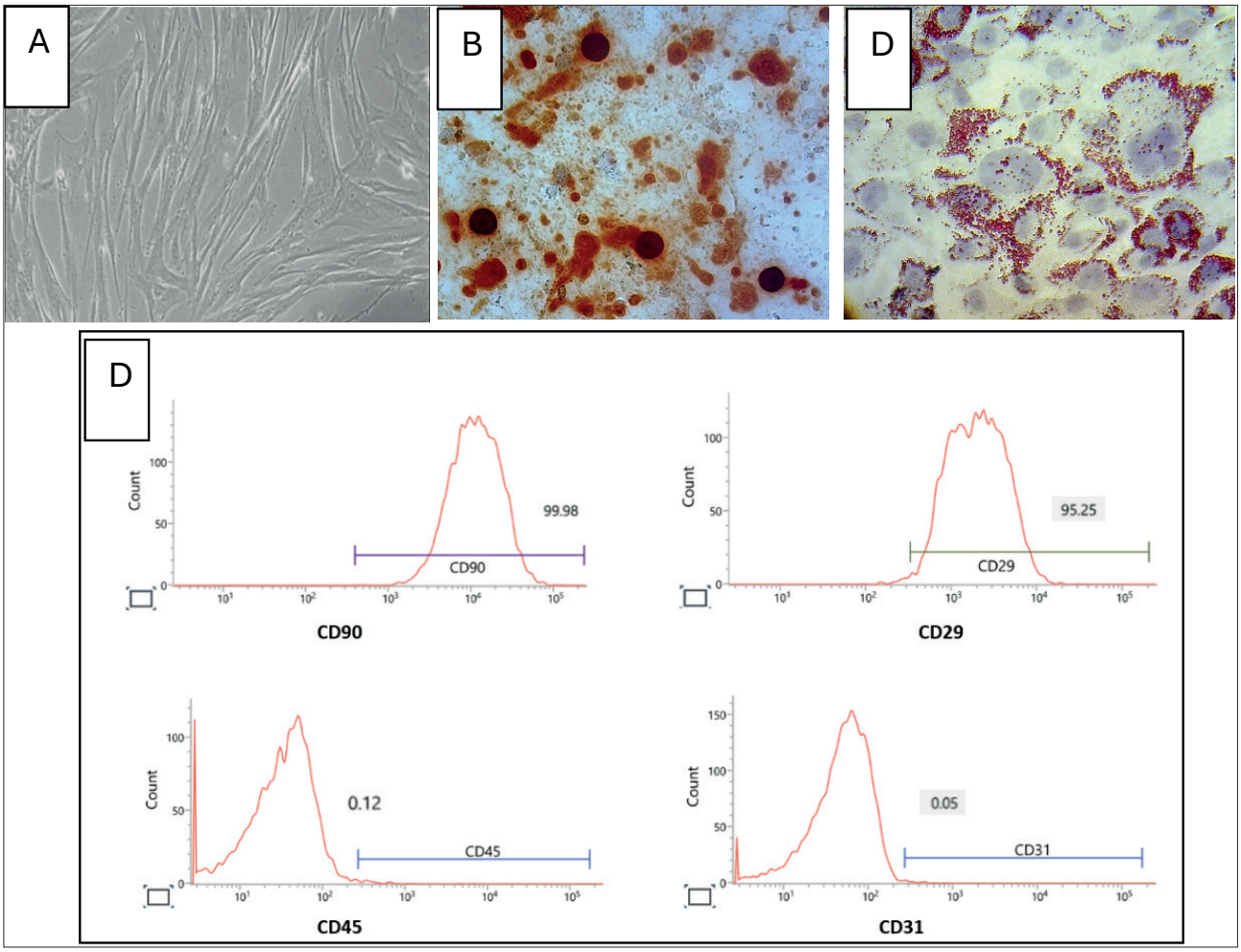

Figure 1. At passage 5, H-MSCs exhibited a spindle-shaped, fibroblast-like morphology, adhering to the plastic surface (A). Their multilineage differentiation potential was confirmed through adipogenic (B) and osteogenic (C) differentiation, evidenced by lipid droplet accumulation stained with Oil Red O and calcium deposition visualized with Alizarin Red staining (red coloration). Images were captured at 200× magnification. (D) Flow cytometry analysis demonstrated a high expression of mesenchymal markers CD90.1 (99.9%) and CD29 (95.3%), with minimal expression of hematopoietic and endothelial markers CD45 (0.1%) and CD31 (0.1%), further validating their mesenchymal phenotype.

Mann-Whitney tests. A p-value of less than 0.05 was considered statistically significant (*p<0.05, **p<0.01, ***p<0.001).

4. RESULTS

The characteristics of MSCs

MSCs were successfully isolated from the umbilical cords of pregnant mice and cultured in vitro, where they exhibited a spindle-shaped, fibroblast-like morphology. The MSCs adhered to the plastic flask surface and reached approximately 80% confluence (Figure 1A). The differentiation potential of these MSCs was assessed through adipogenic and osteogenic induction. Osteogenic differentiation was confirmed by the formation of calcium deposits, which stained red with Alizarin Red (Figure 1B). In contrast, adipogenic differentiation was evidenced by the accumulation of lipid droplets, visualized using Oil Red O staining (Figure 1C). Flow cytometric analysis further validated the mesenchymal nature of the isolated cells, revealing high expression of the mesenchymal markers CD90.1 and CD29, and low levels of the hematopoietic markers CD45 and CD31 (Figure 1D). These results collectively confirm the successful isolation, culture, and characterization of MSCs from rat umbilical cord tissue, demonstrating their multipotent differentiation capacity and mesenchymal origin.

The level of several molecules contained in SH-MSCs

Conditioned medium (CM) was collected from H-MSCs after 24 hours of incubation in a hypoxic environment. To isolate pure secretome-derived SH-MSCs, we separated the cytokines and growth factors present in the H-MSC-CM using a filtration strategy based on molecular weight cut-off categories, as described in our previous study16. Specifically, we employed tangential flow filtration (TFF) with 10-100 kDa filter cassettes to selectively isolate the soluble molecules. Following filtration, the levels of cytokines and growth factors in the SH-MSCs were quantified using ELISA. The concentration of each soluble molecule identified in SH-MSCs is summarized in Table 1.

Parameter	Results
VEGF	1064.74 pg/mL
SDF	7374.94 pg/mL
IL-10	523.23 pg/mL

Table 1. SH-MSCs validation using ELISA.

Figure 1. At passage 5, H-MSCs exhibited a spindle-shaped, fibroblast-like morphology, adhering to the

Groups	Blood glucose level	HOMA-IR
Healthy	105.000±0.189	0.770±0.045
T2DM	481.833±3.791***	20.913±3.03***

Table 2. Validation of T2DM rats. Significance level = ***p<0.001

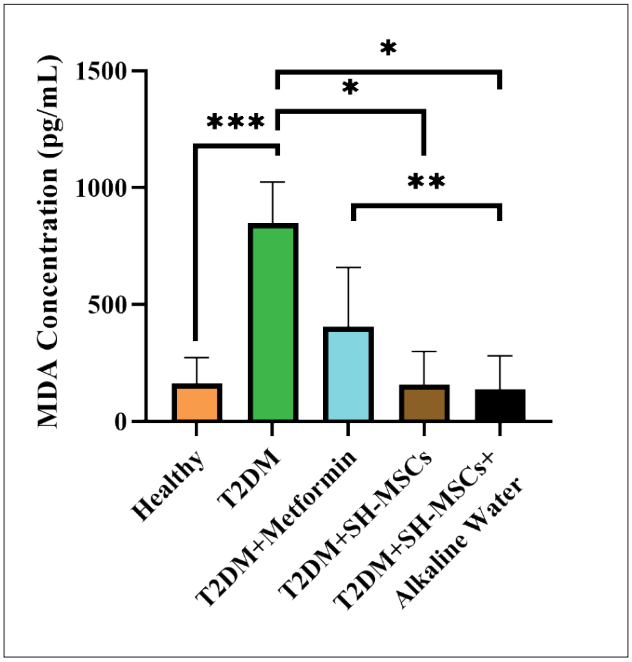

Figure 2. Effects of SMSCs and alkaline water on MDA levels in T2DM rats. SH-MSC and its combination with alkaline water exhibited a significant reduction in MDA levels compared to T2DM rats. Moreover, the combination of SH-MSC and alkaline water further decreased MDA levels compared to the metformin-treated T2DM. (*p < 0.05; **p < 0.01; ***p < 0.001).

plastic surface (A). Their multilineage differentiation potential was confirmed through adipogenic (B) and osteogenic (C) differentiation, evidenced by lipid droplet accumulation stained with Oil Red O and calcium deposition visualized with Alizarin Red staining (red coloration). Images were captured at 200× magnification. (D) Flow cytometry analysis demonstrated a high expression of mesenchymal markers CD90.1 (99.9%) and CD29 (95.3%), with minimal expression of hematopoietic and endothelial markers CD45 (0.1%) and CD31 (0.1%), further validating their mesenchymal phenotype.

Characteristics of T2DM rat model

Blood glucose levels and HOMA-IR were measured to confirm the successful induction of hyperglycemia in the type 2 diabetes mellitus (T2DM) rat model following high-fat diet (HFD) and streptozotocin (STZ) treatment (Table 2). One week post-STZ injection, blood glucose levels in the STZ-treated rats were significantly elevated compared to untreated controls ($p<0.001$). Additionally, the HOMA-IR index was significantly higher in the STZ-treated rats, further corroborating the insulin resistance induced by STZ, as compared to the untreated rats ($p<0.001$).

Combination of SH-MSC and alkaline water ameliorate MDA level

The level of malondialdehyde (MDA), a key marker of oxidative stress, was quantified using ELISA after

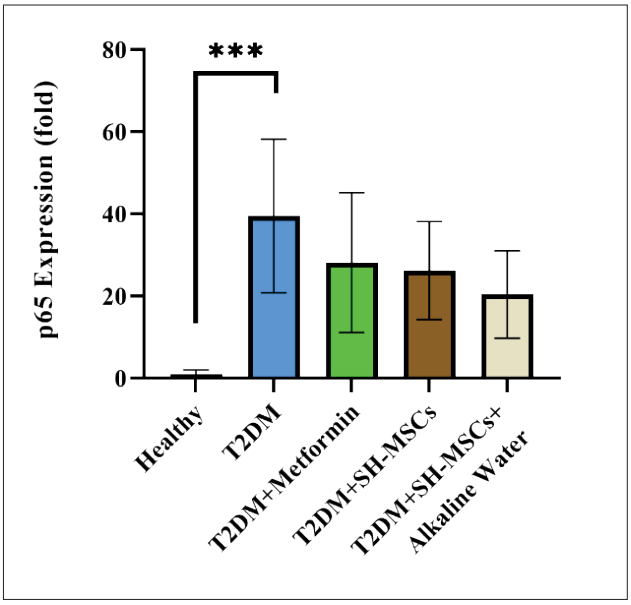

Figure 3. Effects of SH-MSC and alkaline water on p65 expression in T2DM rats. SH-MSC and its combination with alkaline water exhibited a reduction in p65 expression compared to the T2DM rats. (***p ≤ 0.001).

21 days of treatment (Figure 2). T2DM rats exhibited significantly elevated MDA levels compared to healthy rats ($p<0.001$), indicating heightened lipid peroxidation. Treatment with Metformin led to a mild reduction in MDA levels. In contrast, administration of 500 µL SH-MSCs resulted in a significant decrease in MDA levels ($p<0.05$), highlighting its potential in reducing oxidative stress. Notably, the combination of SH-MSCs and alkaline water induced the most pronounced and optimal reduction in MDA levels ($p<0.05$). Moreover, combination of SH-MSCs and alkaline water showed significant lower of MDA level than Metformin ($p<0.01$), suggesting a synergistic and more optimum effect in mitigating oxidative damage than standard treatment in T2DM.

Combination of SH-MSC and alkaline water inhibit p65 mRNA expression

The expression of p65 mRNA in pancreatic tissue was assessed using quantitative PCR (qPCR) after 21 days of treatment (Figure 3). T2DM rats exhibited significantly elevated p65 expression compared to healthy rats ($p<0.001$), indicating increased NF-κB pathway activation. Treatment with Metformin led to a modest reduction in p65 expression. However, administration of 500 µL SH-MSCs resulted in a more substantial suppression of p65 levels, demonstrating a stronger anti-inflammatory effect. The most pronounced decrease in p65 expression was observed in the group receiving a combination of 500 µL SH-MSCs and alkaline water, suggesting a synergistic effect in mitigating inflammation in T2DM.

5. DISCUSSION

Inflammation is a complex biological response to various stimuli, including pathogen infections, damaged cells, or harmful chemicals, with the primary goal of eliminating the cause of injury, clearing dam-

aged cells, and initiating tissue repair (17). In Type 2 Diabetes Mellitus (T2DM), inflammation is triggered by hyperglycaemia, which leads to increased oxidative stress and inflammation, both of which play critical roles in the progression of disease complications (18). Hyperglycemia in T2DM induces the formation of reactive oxygen species (ROS), which, in turn, initiates lipid peroxidation, a chain reaction where free radicals attack cell membrane lipids, generating additional free radicals and ultimately causing further cellular damage (3). This process contributes not only to β-cell dysfunction but also exacerbates insulin resistance, a hallmark of T2DM (4). The elevated levels of malondialdehyde (MDA), a byproduct of lipid peroxidation, play a key role in the induction of pro-inflammatory molecules such as IL-6, IL-8, and ICAM-1, which further enhance systemic inflammation (19). NF-κB, a major transcription factor, is activated in M1 macrophages and induces the secretion of pro-inflammatory cytokines, including IL-6, IL-8, IL-12, and TNF-α (20).

In this study, the combination of SH-MSC and alkaline water was shown to effectively reduce MDA levels in the T2DM rat model, with the lowest MDA concentration observed in the combined SH-MSC and alkaline water treatment group (Figure 2). This suggests that alkaline water enhances the effectiveness of SH-MSC in mitigating inflammation, primarily by reducing oxidative stress, which contributes to the activation of the NF-κB signaling pathway. The reduction in inflammation, as indicated by lower MDA levels, is likely associated with the presence of anti-inflammatory cytokines in SH-MSC, including IL-10 and TGF-β.21 IL-10, present in SH-MSC, inhibits NF-κB activation via the IL-10/STAT3 pathway. IL-10 binds to IL-10R, activating JAK1, which subsequently induces STAT3 phosphorylation. The phosphorylated STAT3 enters the nucleus and activates SOCS3 mRNA sequences, which are expressed intracellularly and suppress NF-κB signaling, thereby reducing pro-inflammatory cytokine expression (21).

In addition to IL-10, TGF-β is known to inhibit NF-κB activation through multiple signaling pathways, including the Smad pathway, TAK1 inhibition, and the upregulation of IκBα (22). A previous study have shown that SH-MSC can reduce pro-inflammatory cytokines such as IL-18 and TNF-α in diabetic rat models (23). Notably, the combination of SH-MSC with alkaline water demonstrated a more pronounced effect in suppressing inflammation, as evidenced by the significant reduction in MDA levels. This aligns with previous research, which has shown that alkaline water can decrease pro-inflammatory cytokines in human kidney cells (HK-2) through the JAK/STAT pathway (24). Similarly, alkaline water has been reported to lower MDA levels in the liver of T2DM rats (25). MDA levels in diabetic patients are a known biomarker of oxidative stress, and the reduction in MDA levels in this study suggests that the combination of SH-MSC and alkaline water significantly mitigates oxidative stress, contributing to the improvement of both inflammatory and metabolic conditions in T2DM (26).

Furthermore, the increased expression of the p65 subunit of NF-κB contributes to the regulation of various pro-inflammatory cytokines, exacerbating inflammation and accelerating the progression of T2DM (6). Our findings indicate that both single-dose SH-MSC and its combination with alkaline water effectively reduced p65 levels in the T2DM rat model (Figure 5.5). This effect is likely due to the anti-inflammatory cytokines within SH-MSC, such as TGF-β, which interact with p65. TGF-β binds to TGF-β receptors II and III, activating the Smad2/3 pathway (27). The phosphorylated Smad2/3 forms a complex with Smad4, translocates to the nucleus, and inhibits p65 transcription, thus suppressing NF-κB activation (28). TGF-β also inhibits TAK1, maintaining p65 in its inactive state by binding to IκB, and promotes the expression of IκBα, preventing NF-κB from translocating to the nucleus (20). The combination of SH-MSC and alkaline water resulted in the lowest expression of p65, highlighting the ability of alkaline water to enhance the anti-inflammatory effects of SH-MSC.

Combination of SH-MSC and alkaline water holds potential as an alternative treatment for T2DM, particularly in reducing complications associated with chronic oxidative stress and inflammation. By reducing p65 expression, this therapy may help prevent T2DM complications such as diabetic retinopathy, nephropathy, and neuropathy. Future research should focus on optimizing the dosage and exploring the underlying cellular mechanisms of this therapeutic approach. Long-term evaluations of the effects of SMSCs and alkaline water on pancreatic regeneration and glucose homeostasis are also necessary to fully understand the therapeutic potential of this combination.

6. CONCLUSION

Current study underscores the therapeutic potential of combining SH-MSC with alkaline water as an alternative approach to mitigate inflammation and oxidative stress in T2DM. The combination therapy demonstrated reduction in MDA levels and p65 expression, key markers of oxidative damage and pro-inflammatory pathways. This suggests that SMSCs, enhanced by alkaline water, can effectively attenuate the inflammatory processes associated with T2DM, contributing to improved metabolic conditions and potentially reducing the risk of T2DM-related complications. Furthermore, the anti-inflammatory and antioxidative mechanisms underlying the observed therapeutic effects highlight the promise of this combination strategy for the management of T2DM. Future studies focusing on optimal dosing, long-term efficacy, and in-depth mechanistic insights are warranted to fully establish the clinical applicability of this novel therapeutic modality.

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