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Mesenchymal Stem Cells Attenuate Renal Microscopic Alterations in Induced Diabetic Nephropathy in Rats through Suppression of Oxidative Stress, Inflammation, Apoptosis and Upregulation of Nrf2/PPAR-γ Inflammatory Signaling Pathway

Las Células Madre Mesenquimales Atenúan las Alteraciones Microscópicas Renales en la Nefropatía Diabética Inducida en Ratas a Través de la Supresión del Estrés Oxidativo, la Inflamación, la Apoptosis y la Regulación Positiva de la Vía de Señalización Inflamatoria Nrf2/PPAR-γ

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SUMMARY: Diabetic nephropathy (DN) is a prevalent complication of diabetes, necessitating the development of effective therapies targeting the mechanisms by which type 2 diabetes mellitus (T2DM) induces renal tissue damage. In this study, DN was induced in rats using a high-fat diet for 13 weeks combined with streptozotocin to assess the effects of mesenchymal stem cells (MSCs) injection on renal tissues and function. Histological, immunohistochemistry, and biochemical analysis were employed to evaluate inflammation, oxidative stress, apoptosis, and histological architecture. The results demonstrated that MSCs improved metabolic derangement (glucose and lipid profile) and kidney function (urea and creatinine) associated with a significant reduction in inflammatory biomarkers: high sensitivity CRP (hs-CRP), tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), as well as oxidative stress biomarkers :reactive: oxygen species (ROS) and superoxide dismutase (SOD). Additionally, MSCs administration upregulates peroxisome proliferator-activated receptor gamma (PPARγ) and nuclear factor erythroid 2-related factor 2 (Nrf2) expression levels. MSCs also show improved histological architecture associated with decreased CD45 (marker of inflammation) and caspase-3 (marker of apoptosis) immunostaining in renal tissues of the treated diabetic group. In conclusion, MSCs improved diabetic-induced nephropathy through suppression of oxidative stress, inflammation, apoptosis and upregulation of the Nrf2/PPAR-γ inflammatory signaling pathway.

KEY WORDS: Mesenchymal stem cells; Diabetic nephropathy; Oxidative stress; Nrf2/PPAR-γ signaling pathway.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease in which glucose, fat, and protein metabolism induces serious injuries in the renal tissue (Hua, 2020).

End-stage kidney disease (ESKD) is primarily caused by diabetic kidney disease (DKD). DN is regarded as a microvascular consequence of both type 1 diabetes mellitus (T1DM) and T2DM. A progressive drop in the rate of glomerular filtration and recurring albuminuria are the disorder's initial symptoms. Early treatment can reverse or decrease the progression of the illness (Wandile, 2023).

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Chronic hyperglycemia caused by insulin deficiency (type 1 DM) or insulin resistance (type 2 DM) is a significant risk factor for renal damage and glomerular dysfunction (Wada & Makino, 2013; Nagib *et al*., 2019). Due to the progressive and irreversible nature of DN, it is pivotal to find therapeutic agents to slow the progression of kidney damage by targeting the oxidative stress, hemodynamic, genetic, inflammatory, and metabolic pathways, which are known as the main driving factors in the pathogenesis of DN (Barutta *et al*., 2019; Vodosek Hojs *et al*., 2020).

It has been shown that the Nrf2 transcription factor is a crucial molecular component in the regulation of adaptive cellular connections in response to a broad range of extracellular or intracellular cellular inflammation and oxidative stress and is also linked to the improvement of diabetic nephropathy (Li *et al*., 2012).

Nrf2 deficiency augments kidney dysfunction by increasing the susceptibility to ischemic and nephrotoxic acute kidney injury; hence, this transcription factor is identified as a potential therapeutic target in DN (Ayers *et al*., 2015).

The Nrf2 transcription factor has been identified as a key molecular player in controlling the oxidative stress associated with DM and orchestrating adaptive cellular interactions following a wide spectrum of cellular conditions that could be either extracellular or intracellular (Hashemi *et al*., 2023).

The PPARγ, constitutively expressed in the kidney, is potentially crucial in preserving renal function. It achieves this by improving insulin sensitivity, hyperglycemia, and blood pressure while suppressing inflammation and oxidative stress. Studies have reaffirmed the potential of PPARg as an effective target for preventing and treating DN (Abdel-Rahman *et al*., 2012; Kuo *et al*., 2015).

The therapeutic role of stem cells in diabetic kidney diseases is an attractive field of research (Zheng *et al*., 2023). Some researchers believe that bone marrow-mesenchymal cells (BM-MSCs) may protect tissues from inflammation. The beneficial effects of MSCs are ascribed to their anti-inflammatory releasing factors and antioxidant paracrine-secreted mediators (Han *et al*., 2022).

This study aimed to investigate the therapeutic effect of MSCs on diabetic-induced renal ultrastructural alterations through suppression of oxidative stress, inflammation, apoptosis, and upregulation of the Nrf2/PPAR-γ inflammatory signaling pathway in diabetic nephropathy rats.

MATERIALS AND METHOD

Animals

The study protocol (HAP-01-R-059) received approval from the research ethics committee at Princess Nourah Bint Abdulrahman University. This approval was in accordance with the guidelines set forth in the Guide for the Care and Use of Laboratory Animals, a publication by the US National Institutes of Health (NIH publication No. 85-23, revised 1996). Male Wistar rats, aged 8-9 weeks and weighing between 180-200 g, were used for the study. During the adaptation period, the rats were kept in a sanitary environment with alternating 12-hour light and dark cycles. They were fed standard pellets and had unlimited access to water.

Animal Groups and Procedures of Experimentation

In this research, a total of 24 rats were used. After a week-long adaption period, they were blindly allocated to three groups (n=8): Group 1: Control group fed on laboratory diet for 13 weeks, group 2: Type 2 diabetes (T2DM) model in which rats received high-fat diet for three weeks, then single streptozotocin injection (Reed *et al*., 2000), group 3: Diabetic rats received MSCs (MSCs+T2DM): Eight weeks after receiving (STZ) injections, the rats were administered a single intravenous dose of bone marrow-derived mesenchymal stem cells (BM-MSCs). Each rat received 5 ¥ 106 BM-MSCs through this method (A-Elgadir *et al*., 2024).

Establishing T2DM model. Before administering streptozotocin (STZ) from Sigma-Aldrich (St Louis, MO, USA), the rats were provided with a high-fat diet (HFD) for three weeks, with 40 % of their caloric intake coming from fat. The STZ powder was dissolved in a sterile sodium citrate buffer and adjusted to a pH between 5 and 6. Following preparation, the solution was promptly administered intraperitoneally (i.p.) at a dosage of 40 mg/kg (A-Elgadir *et al*., 2024). In the control group, a vehicle consisting of an equivalent dosage of the sterile prepared buffer was injected.

One week following the STZ injection, diabetes in the model group was confirmed using a method to measure fasting blood glucose levels. This verification process employed a Randox reagent kit (Randox Laboratories Ltd., Crumlin, UK) and considered blood glucose readings exceeding 200 mg/dL as indicative of diabetes.

Mesenchymal stem cells (MSCs) preparation: MSCs were isolated using the femurs of male Wistar rats following

previously documented research (ShamsEldeen *et al*., 2022). Cells were identified using surface markers and flow cytometry. Before transplantation, the enriched cells underwent flow cytometry analysis to confirm their purity and verify the presence of CD73 and CD90 markers, while ensuring the absence of CD45 and CD34 phenotypic markers (Fig. 1). The cells were then stained using the PKH26 Red Fluorescent Cell Linker Kit from Sigma Aldrich (Fig. 2) before being administered via the rat's tail vein.

Specimens collection. By the end of week 13, the rats were anaesthetized with sodium phenobarbital anaesthesia at 40 mg/kg body weight before sacrifice (Alshahrani *et al*., 2023). Blood was collected from the tail vein of rats and placed into Eppendorf tubes with a 10-millilitre capacity. Following centrifugation of the collected blood samples, serum was separated and subsequently utilized for additional biochemical analyses.

A high dosage of sodium phenobarbital was administered to euthanize the animals, after which both kidneys were surgically extracted. After being cleaned with cold phosphate buffer saline (PBS) at a pH of 7.4, the kidneys were left to dry on filter paper. The left kidney transverse cut was fixed in 10 % phosphate-buffered formalin (PBF) for immunohistochemistry and histopathology analysis. The left kidney was processed and homogenised for biochemical measurement and stored at -80 °C for ELISA and further biochemical analysis. Using a fluorescence microscope, researchers examined unstained tissue sections to verify the presence of stem cells in kidney specimens. This approach confirmed the identification of stem cells within the renal tissues.

Biochemical Analysis

Evaluation of Serum-Fasting Glucose and Lipid Profile. The serum glucose level was determined using the Rat Glucose Assay Kit (Catalog #81693), which employs a multi-step reaction mechanism. The resultant dye's absorbance, measured at 505nm, directly corresponds to the glucose concentration in the rat sample. Serum levels of total cholesterol (CHO), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C)

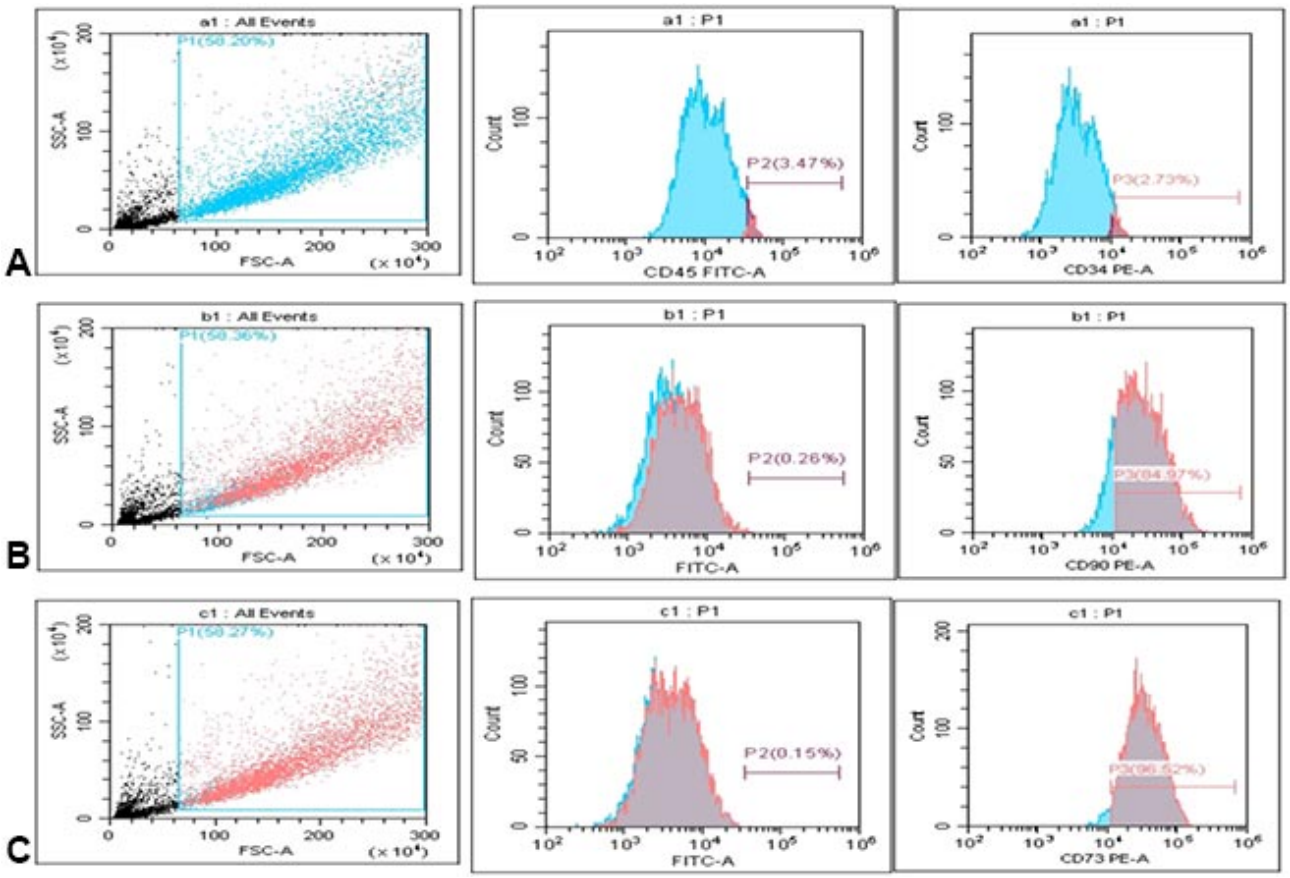

Fig. 1. Identification and immunological characteristics of the isolated BM-MSCs. The cells exhibited positive expression of CD90 and CD73 markers while demonstrating a lack of CD45 and CD34 expression.

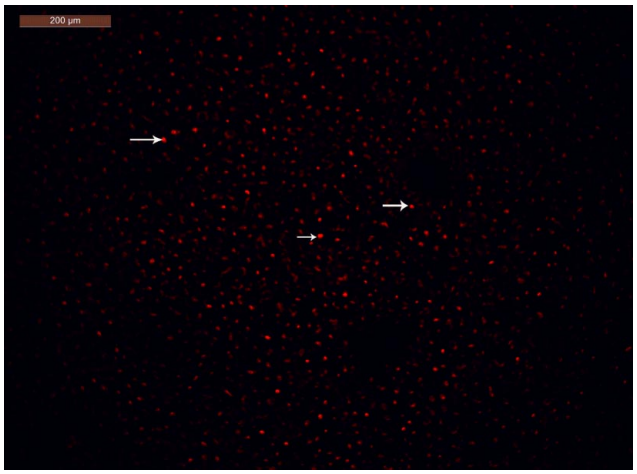

Fig. 2. Identification of BM-MSC characteristics in the renal tissue using a fluorescence microscope. Cells marked with PKH26 fluorescent dye were identifiable by their red fluorescence (homing properties).

were assessed through an enzyme-linked immunosorbent assay. Specifically, the Total cholesterol (Catalog No. ABIN772507), HDL-C, and LDL-C rat ELISA Kit (Catalog No: MBS266554) were employed. The intensity of colour was quantified spectrophotometrically using a microplate reader.

Evaluation of Renal Functions. Using commercially available colourimetric kits, serum was used to determine the amounts of creatinine (#ab204537) and urea (#E-BC-K329-S), as reported by earlier studies (El-Sherbiny *et al.*, 2022).

Evaluation of Inflammation, Oxidative Stress and Fibrosis. The measurement of inflammatory biomarkers, specifically highly sensitive C-reactive protein (hsCRP), TNF-α, and IL-6, was conducted using ELISA kits. The hsCRP levels were determined using kit #ERC1021-1 from ASSAYPRO (St. Charles, MO, US). For TNF-α quantification, kit #R6365 from BIOTA NG INC (MA, US) was employed. Lastly, IL-6 concentrations were assessed using kit #ELR-IL6-001 from RayBio (GA, US). Oxidative stress biomarkers [Superoxide dismutase (SOD), Reactive oxygen species (ROS) were also measured in the serum using the following ELISA kits: #706002 (Cayman Chemical Company, Ann Arbor, MI, US), #MBS039665 (MyBioSource, San Diego, US) respectively.

RNA isolation, Reverse transcription, and real-time quantitative Polymerase chain reaction (PCR). The extraction of total RNA from renal tissue homogenate was conducted following the protocol provided in the RNeasy Mini Kit (Qiagen Pty, Victoria, Australia). Subsequently, cDNA was synthesized using a reverse transcription kit (Takara Biomedical Technology, Dalian, Liaoning, China), followed by PCR amplification performed on the Agilent-Stratagene Mx3000P Q-PCR System (Agilent Technologies Inc, Santa Clara County, CA, USA). The obtained data were standardized against the b-actin reference gene. The specific primer sequences employed for PCR amplification are presented in Table I.

Histopathological and Immunohistochemical assessments. Renal tissues treated in 10 % formalin for 24 hours were embedded in paraffin after processing in increasing alcohol grades for dehydration. The deparaffinized 5 µm sections were stained with hematoxylin and eosin (H&E) using documented procedures (Elsherbini & Ebrahim, 2020). Renal morphological and structural alterations were examined using light microscopy with a "Leica Qwin 500 C" image analyzer (Cambridge, UK). To quantify the percentage area of fibrosis as well as evaluate the extent of fibrosis, the sections underwent a staining process with Masson's trichrome (Sigma-Aldrich, Gillingham, Dorset, UK), which stains parenchymal cells red and fibrotic areas blue.

For immunohistochemical analysis, the sections were rehydrated after deparaffinization and submerged in an EDTA solution (pH 8) to retrieve antigens. Subsequently, the sections underwent treatment with 3 % hydrogen peroxide and a protein block. This was followed by an overnight incubation at 4 °C with Caspase-3 and CD45 antibodies. Meyer's hematoxylin served as a counterstain for the sections. The proportions of CD45 and Caspase-3 immunostaining were then quantified.

Statistical analysis. The data was presented using the mean ± standard deviation. Statistical analysis was conducted using GraphPad Prism V 6 software (GraphPad Software Inc., San Diego, USA). Data distribution was evaluated to determine the appropriate statistical tests, either parametric or non-parametric. For normally distributed variables, the assessment of statistical significance among groups was carried out by one-way ANOVA followed by Tukey's post hoc test. Statistical significance was defined as a p-value below 0.05.

Table I. Primer Sequences.

Gene	Sense (forward)	Antisense (Reverse)
Nrf2	5'CACATCCAGACAGACACCAGT 3'	5'CTACAAATGGGAATGTCTCTGC3'
PPAR-γ	5'ATTCTGGCCCACCAACTTCGG-3'	5'TGGAAGCCTGATGCTTTATCCCCA3'
β-actin	5'GGTCGGTGTGAACGGATTTGG 3'	5'ATGTAGGCCATGAGGTCCACC3'

RESULTS

MSCs Treatment Improved Metabolic Alterations in Diabetic Rats. In comparison to the control group, the T2DM group exhibited a considerable increase in fasting blood glucose levels **(Fig. 3A; P** < 0.0001. In contrast to the T2DM group, MSC treatment effectively lowered the fasting blood glucose level of the MSCs+T2DM group (P<0.0001). Moreover, the lipid profile showed significant improvement in the MSCs+T2DM group (Fig. 3 B-F; P<0.0001) compared to the T2DM group with lower FFA, CHO, TG, LDL-C, and higher HDL-C levels; however, these changes did not ultimately return to control levels (Fig. 3).

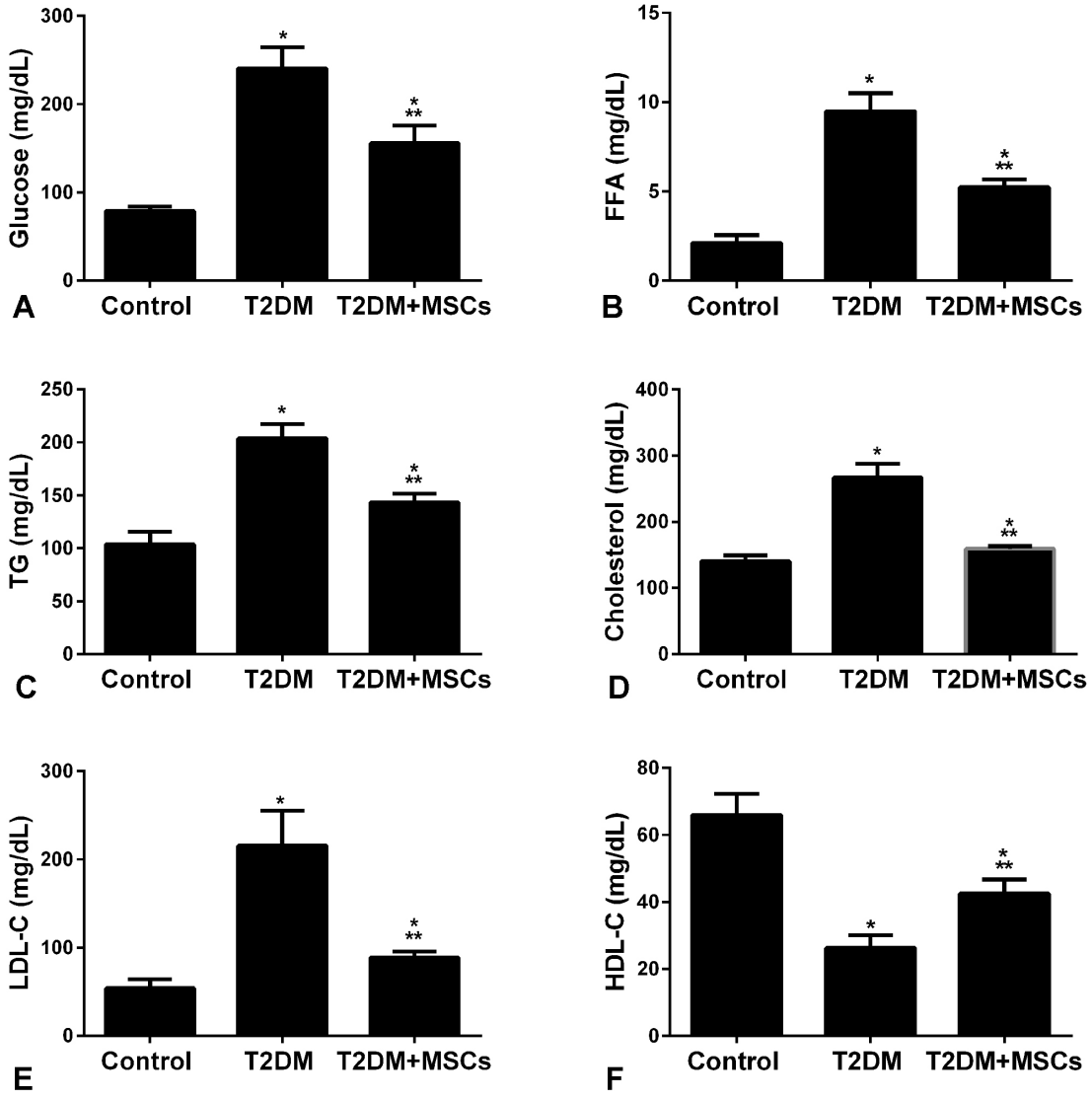

Fig. 3. A, B, C, D, E, & F Changes in the glucose and lipid profile among experimental groups. The results are presented as mean ± SD. * Indicates statistical significance compared to the corresponding value in the control group (n=8; P<0.0001). ** Indicates statistical significance relative to the corresponding value in the T2DM group (n=8; P<0.0001).

MSCs Treatment Enhanced Renal Functions in Diabetic Rats. The urea and creatinine levels were significantly increased in T2DM compared to the corresponding values in the control group. Administration of MSCs attenuated kidney injury, which was observed by a significant decrease in urea and creatinine levels compared to the diabetic group elaborating the improvement in the renal functions of MSCs+T2DM rats (Fig. 4; P<0.0001).

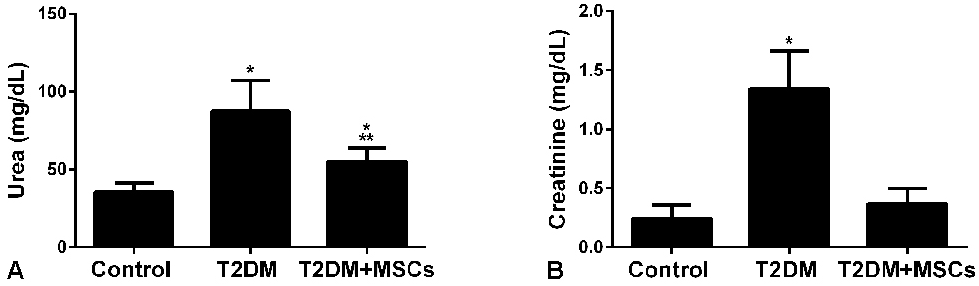

Fig. 4. A & B Changes in the renal functions (urea and creatinine) among experimental groups. The results are presented as mean ± SD. * Indicates statistical significance compared to the corresponding value in the control group (n=8; P<0.0001). ** Indicates statistical significance relative to the corresponding value in the T2DM group (n=8; P<0.0001)

MSCs Treatment Ameliorated Oxidative Damage in Renal Tissue of Diabetic Rats. The Critical link between diabetes and high oxidative stress is implicated in the pathophysiology of diabetic nephropathy (Cao *et al.*, 2016). In light of our finding of diabetes-induced dysregulation of the ROS/ SOD axis with a significant increase in the ROS level in the renal tissue of the diabetic group associated with considerable depression of the SOD serum levels compared to the control group (Fig. 5; P<0.0001). The MSC treatment attenuated oxidative damage by induction of SOD level in renal tissue which significantly reduced the ROS level (Fig. 5; P<0.0001).

MSCs Treatment Combat Inflammation in Renal Tissue of Diabetic Rats. Organ damage caused by diabetes mellitus is mainly caused by inflammation. Accordingly, the diabetic rats in this research experienced significant elevations in hs-CRP, TNF-a and IL6 levels in the serum (Fig. 6); P<0.0001.

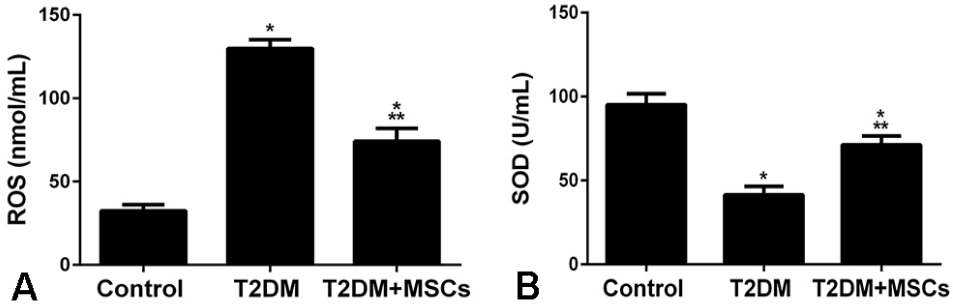

Fig. 5. A and B: Changes in the ROS and SOD serum levels among experimental groups. The data is shown as mean ± SD. * Indicates statistical significance compared to the corresponding value in the control group (n=8; P<0.0001). ** Indicates statistical significance relative to the corresponding value in the T2DM group (n=8; P<0.0001).

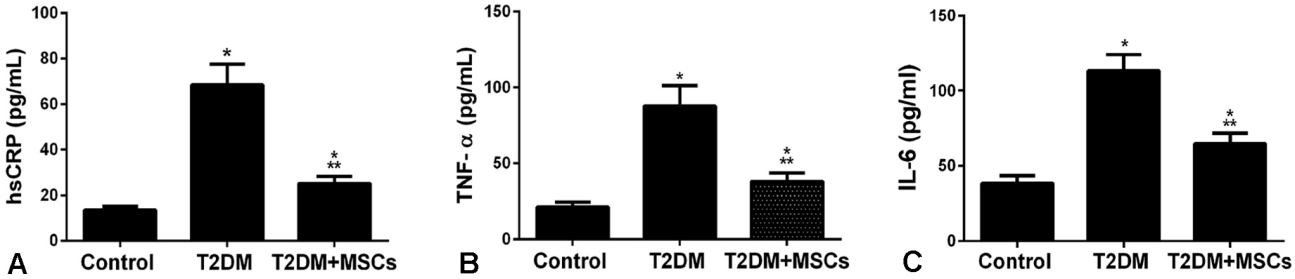

Fig. 6. A, B, and C: Changes in the serum levels of inflammatory indicators among experimental groups. The data is shown as mean ± SD. * Indicates statistical significance compared to the corresponding value in the control group (n=8; P<0.0001). ** Indicates statistical significance relative to the corresponding value in the T2DM group (n=8; P<0.0001).

Moreover, At the renal tissue level, the CD45 immunostaining, representing inflammatory cell infiltration, revealed few positive CD45 immunostained cells in between the tubules in the control group. The mean area % of CD45 immunostaining was significantly increased in the T2DM group in comparison with the control group. In contrast, this inflammatory milieu was significantly countered by MSCs in diabetic rats as shown in the marked decrease in tissue levels of inflammatory markers with reduced mean area % of CD45 immunostaining in renal tissue specimens of T2DM+MSCs group (Fig. 7).

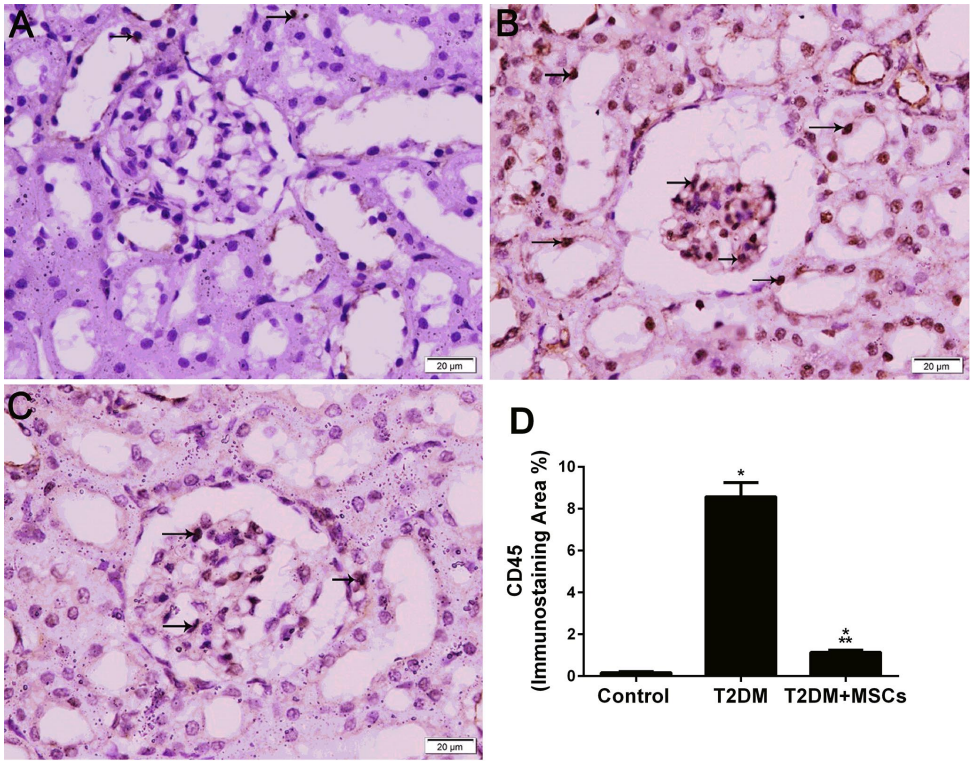

Fig. 7. Photomicrographs of renal cortical sections immunostained by CD45. A, Control group showing few positive immunostained cells (arrow) in-between the tubules. B, T2DM group revealing many widespread immunopositive inflammatory cells (arrow) inside the glomerulus and in-between the tubules. C, T2DM+MSCs group demonstrating minimal immunopositive cells (arrow) inside the glomerulus and in-between the tubules. D, Histogram showing Mean area % of CD45 immunostaining of the studied groups. * Indicates statistical significance compared to the corresponding value in the control group (n=8; P<0.0001). ** Indicates statistical significance relative to the corresponding value in the T2DM group (n=8; P<0.0001).

MSCs Treatment Combat inflammation through upregulation of expression of PPARg /Nrf2 pathway in Renal Tissue of Diabetic Rats. As shown in Figure 8, compared to the control group, the expression of Nrf2 was significantly downregulated in diabetic renal tissue (Fig. 8B; P<0.0001), associated with suppression of PPAR-g (Fig. 8A; P<0.0001). In contrast, MSC treatment significantly upregulated the expression of PPAR-g and Nrf2 in renal tissue of the T2DM+MSCs group rats (Figs. 8A-B; P<0.0001).

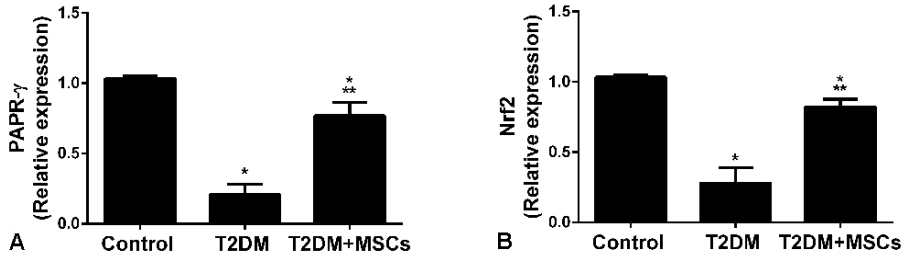

Fig. 8. Changes in the tissue expression levels of PPAR-g /Nrf2 expression among experimental groups. The data is shown as mean ± SD. * Indicates statistical significance compared to the corresponding value in the control group (n=8; P<0.0001). ** Indicates statistical significance relative to the corresponding value in the T2DM group (n=8; P<0.0001).

MSCs Treatment Attenuated Cellular Damage in Renal Tissue of Diabetic Rats. Renal tissue sections stained with H&E among experimental groups are displayed in Figure 9. The control group (Fig. 9A) showed Malpighian renal corpuscles formed of glomeruli surrounded by glomerular space (Bowman's space). Proximal convoluted tubules were lined by pyramidal cells having pale vesicular nuclei and a prominent brush margin. Distal convoluted tubules were lined with simple cubical cells having pale vesicular nuclei and ill-defined brush margins. The diabetic group specimens revealed significant renal damage as evidenced by dilated degenerated tubules with desquamated cells in their lumen; some tubular cells with vacuolated cytoplasm, and some pyknotic; shrunken glomeruli, a widening of the capsular space, and some congested glomeruli (Fig. 9B). Nevertheless, MSC treatment restored the normal histological architecture of renal tissue in the MSCs+T2DM group; glomeruli with a normal tuft of capillaries surrounded by glomerular space. Proximal, distal convoluted tubules and collecting ducts lined with cells having pale vesicular nuclei apart from a few cells revealed dark nuclei (Fig. 9C).

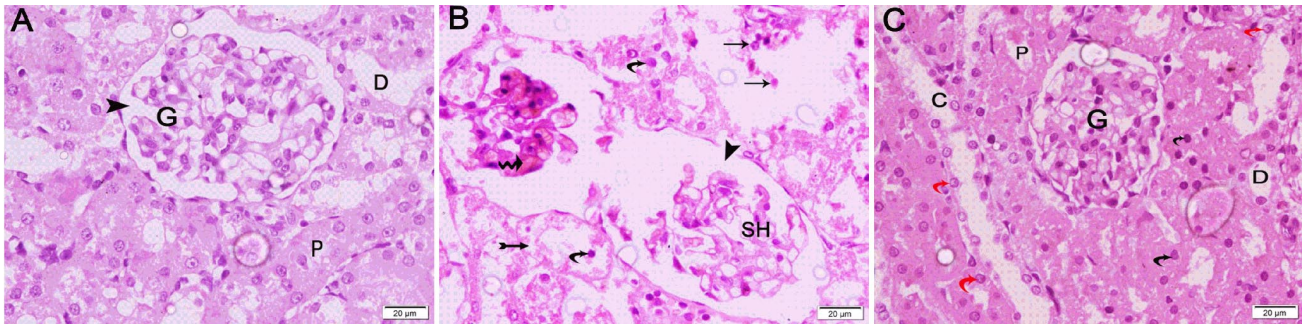

Fig. 9. Photomicrographs of H&E-stained sections of the renal tissue. A Control group: Malpighian renal corpuscles are formed of a glomerulus (G) surrounded by glomerular space (arrowheads). Proximal convoluted tubules (P) are lined by pyramidal cells having pale vesicular nuclei and a prominent brush margin. Distal convoluted tubules (D) are lined with simple cubical cells having pale vesicular nuclei and ill-defined brush margins. B, T2DM group: Some glomeruli are shrunken (SH) while others are congested (wavy arrows) with widened glomerular space (arrowheads). Tubules are degenerated with desquamated cells (arrows); some with vacuolated cytoplasm (bifid arrow) and dark pyknotic nuclei (curved arrow). C, T2DM+MSCs group: The glomerulus (G) is formed of a tuft of capillaries surrounded by glomerular space (arrowheads). Proximal (P), Distal (D) convoluted tubules, and collecting ducts (C) are lined with cells exhibiting pale vesicular nuclei apart from a few cells revealing dark nuclei. (Magnification x400).

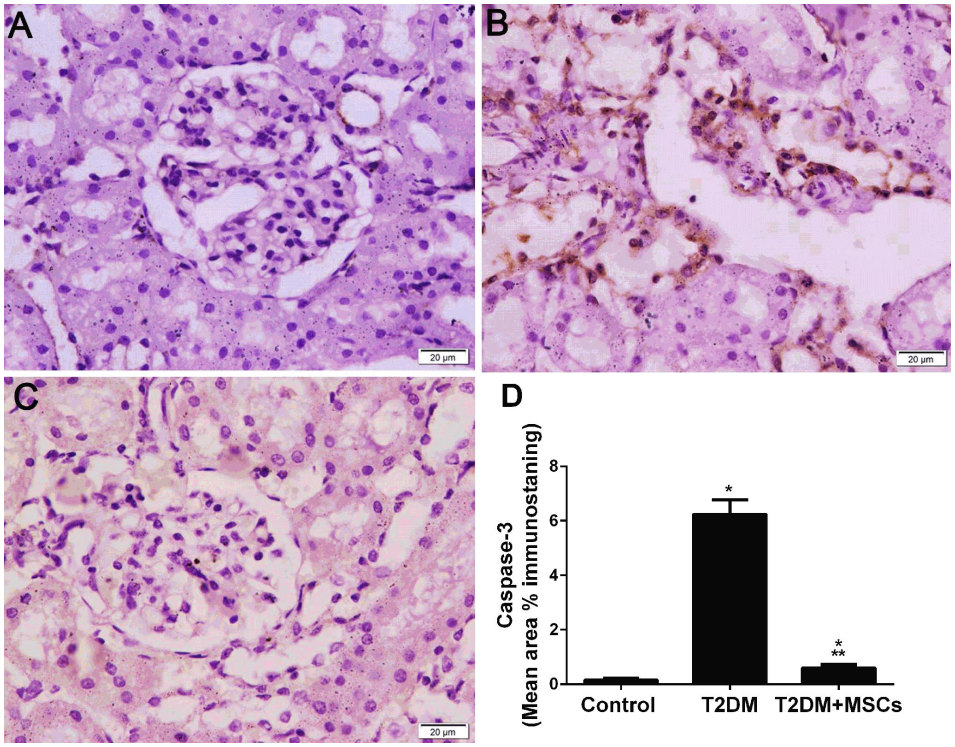

Fig. 10. Photomicrographs of renal cortical sections immunostained by Caspase-3. A, Control group revealing negative caspase-3 immunostaining. B, T2DM group exhibiting marked widespread cytoplasmic and nuclear caspase-3 immunostaining. C, T2DM+MSCs group showing faint cytoplasmic caspase-3 immunostaining. D, Histogram showing Mean area % of Caspase-3 immunostaining of the studied groups. * Indicates statistical significance compared to the corresponding value in the control group (n=8; P<0.0001). ** Indicates statistical significance relative to the corresponding value in the T2DM group (n=8; P<0.0001). (Magnification x400)

MSCs Treatment Attenuated Apoptosis in Renal Tissue of Diabetic Rats. Figure 10 indicated that the renal tissue of the diabetic control rats experienced significant apoptosis, as evidenced by a considerable increase in cytoplasmic and nuclear caspase-3 immunostaining compared to the control group, which exhibited negative caspase-3 immunostaining. On the other hand, administration of MSCs to diabetic rats significantly decreased the immunostaining for caspase-3 with a prominent reduction in mean area % of caspase-3 immunostaining among renal tissue specimens of the T2DM+MSCs group compared to the T2DM group.

DISCUSSION

Approximately one-third of individuals with diabetes develop diabetic nephropathy, a chronic complication of diabetes that progresses to end-stage renal disease and necessitates dialysis (Samsu, 2021). The unclear pathogenesis of diabetic nephropathy renders standard therapy, which targets blood glucose and blood pressure control, insufficient in halting the progression of the disease to end-stage renal failure (Arora & Singh, 2013).

In the present study, we aimed to identify a therapeutic approach that targets kidney repair and protection and to evaluate its effectiveness in restoring kidney function by investigating the effect of MSCs treatment in the TDM model with diabetic nephropathy.

T2DM-induced renal function impairment was evidenced by our findings, which showed a significant dysregulation of metabolic parameters and elevation of serum urea and creatinine levels. Additionally, structural damage was observed in the histopathological examination, revealing shrunken and congested glomeruli with widened glomerular space. The tubules exhibited marked degeneration with desquamated cells, vacuolated cytoplasm, and dark pyknotic nuclei.

Hyperglycemia and dyslipidemia in our diabetic nephropathy model associated with reduced kidney function led to a significant increase in serum levels of ROS and SOD accompanied by a marked reduction in Nrf2 expression in renal tissues. This was also associated with elevated serum inflammatory markers, including hsCRP, TNF, and IL-6, and a significant decrease in PPAR-g expression was observed in the diabetic group, contributing to renal damage and dysfunction.

In our study, we targeted kidney tissue repair and normal function, protecting the undamaged and newly repaired tissue from being affected by the metabolic derangement that initially caused the insult. At this stage of renal damage, the only goal of the classic treatment is to slow the progression to end-stage renal failure.

BM-MSCs were injected into the diabetic rats eight weeks after the induction of diabetes, following the expected timeframe for the development of diabetic nephropathy. As noted by previous studies, nephropathy typically develops in this model within 3-8 weeks after the administration of STZ (Noshahr *et al*., 2020).

According to our findings, injection of MSCs in DN-induced rats achieved a significant decrease in serum urea and creatine compared to the untreated diabetic rats, assuring improvement of the renal functions, which was further documented structurally after histological analysis of renal tissue of T2DM+MSCs group rats. This can be explained by the ability of the injected stem cells to largely engraft the kidney and integrate into damaged tubules, as evidenced by the fluorescence tracing of the MSCs.

To investigate the underlying mechanisms that could protect the engrafted MSCs in the chronic diabetic milieu, we examined the antioxidative properties of the newly injected MSCs by assessing the effectiveness of the MSCs on serum ROS, SOD, and Nrf2 in renal tissues.

Our results demonstrated a substantial increase in Nrf2 expression and a marked elevation in tissue SOD levels, accompanied by a significant decrease in tissue ROS levels in the T2DM+MSCs group compared to the T2DM group. These findings are in agreement with previous studies that documented increased expression of Nrf2 as a paracrine activity of MSCs, highlighting elevated Nrf2 expression in undifferentiated human stem cells compared to differentiated cells (Jang *et al*., 2014; Han *et al*., 2022).

SOD and other antioxidant enzymes are among the cytoprotective genes whose transcription is enhanced by Nrf2's nuclear translocation and activation, as previously demonstrated concerning oxidative stress (Tang *et al*., 2021). Moreover, Nrf2 plays a pivotal role in regulating stem cell homeostasis, mitigates the effects of diabetes, and enhances cellular proliferation and differentiation, mitochondrial function, and protein quality control of the differentiating MSCs (Bigarella *et al*., 2014; Holmström *et al*., 2016).

Recent data showed that oxidative stress and inflammation are fundamental in developing DN (Mistry *et al*., 2020). They reported elevated levels of TNF-α and IL1-a, alongside a marked reduction in SOD levels, in patients with DM and DN. This aligns with our findings, which revealed a significant reduction in serum hs-CRP, TNF-α, and IL-6, along with a remarkable decrease in CD45

immunostaining in the renal tissue of MSC-treated rats compared to the untreated diabetic group.

Our findings underscore the significant role of MSC treatment in reducing oxidative stress and inflammation and activating the expression of PPAR-γ.PPARγ is particularly interesting among the PPARs due to its significant role in regulating numerous physiological processes, including glucose and lipid metabolism, cell proliferation, and inflammation. PPAR-γ is constitutively expressed in the kidney and has long been recognized as a therapeutic target for DN due to its potential to enhance insulin sensitivity, reduce hyperglycemia, regulate blood pressure, and inhibit inflammation and oxidative stress (Yang *et al*., 2012).

Our findings align with those of Kökény *et al*. (2021), who reported that MSCs express transcription factors involved in differentiation processes, including PPAR=γ. Consistent with our results, data showed that Nrf2-regulates PPAR-γ expression is crucial to protect against acute lung injury in mice (Cho *et al*., 2010). This confirms our hypothesis that MSCs upregulate the Nrf2/ PPAR-g inflammatory signaling pathway.

Our study also showed an association between the expression of PPAR-γ in renal tissue and markers of apoptosis (caspase-3). The untreated diabetic group exhibited a marked reduction in PPARγ expression, consistent with recent research (Liu *et al*., 2022), and a significant increase in caspase-3 immunostaining. In contrast, the MSCs-treated diabetic group showed considerable upregulation of PPAR-γ, accompanied by a marked reduction in both caspase-3 immunostaining.

CONCLUSION

MSC injection improved renal structure and function, offering a promising treatment approach. This method involves engrafting and replacing damaged tissue and maintains a homeostatic environment through paracrine activity, supporting the survival and optimal function of differentiated cells. The underlying protective mechanisms are complex, involving the activation of Nrf2 expression, which provides antioxidant protection, reduces ROS-induced injury, and modulates the inflammatory response. Furthermore, MSC treatment reduced apoptosis by upregulating Nrf2/ PPAR-g expression, which confer protection to kidney tissues.

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RESUMEN: La nefropatía diabética (ND) es una complicación frecuente de la diabetes, que requiere el desarrollo de terapias efectivas dirigidas a los mecanismos por los cuales la diabetes mellitus tipo 2 (DM2) induce daño tisular renal. En este estudio, se indujo ND en ratas utilizando una dieta rica en grasas durante 13 semanas combinada con estreptozotocina para evaluar los efectos de la inyección de células madre mesenquimales (CMM) en los tejidos y la función renal. Se realizaron análisis histológicos, inmunohistoquímicos y bioquímicos para evaluar la inflamación, el estrés oxidativo, la apoptosis y la arquitectura histológica. Los resultados demostraron que las CMM mejoraron el desequilibrio metabólico (perfil de glucosa y lípidos) y la función renal (urea y creatinina) asociada con una reducción significativa de los biomarcadores inflamatorios: PCR de alta sensibilidad (hs-CRP), factor de necrosis tumoral alfa (TNF-α) e interleucina-6 (IL-6), así como los biomarcadores de estrés oxidativo, especies reactivas de oxígeno (ROS) y superóxido dismutasa (SOD). Además, la administración de CMM aumenta los niveles de expresión del receptor activado por el proliferador de peroxisomas gamma (PPARγ) y del factor nuclear eritroide 2 relacionado con el factor 2 (Nrf2). Las CMM también muestran una arquitectura histológica mejorada asociada con una disminución de la inmunotinción de CD45 (marcador de inflamación) y caspasa-3 (marcador de apoptosis) en los tejidos renales del grupo diabético tratado. En conclusión, las células madre mesenquimales mejoraron la nefropatía inducida por la diabetes mediante la supresión del estrés oxidativo, la inflamación, la apoptosis y la regulación positiva de la vía de señalización inflamatoria Nrf2/PPAR-γ.

PALABRAS CLAVE: Células madre mesenquimales; Nefropatía diabética; Estrés oxidativo; Vía de señalización Nrf2/PPAR-γ

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