RESEARCH PAPER

Approaches for Diabetic Salivary Gland Dysfunction: L-Arginine versus Bone Marrow- Mesenchymal Stem Cells

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Abstract

Background: Diabetes mellitus remains a major global public health concern, with a steadily increasing prevalence across both developed and developing countries despite declining incidence in some regions. The global burden is expected to rise substantially in the coming decades, affecting both adults and younger populations, highlighting the urgent need for effective prevention and management strategies.

Objective: The present study evaluated and compared the effects of L-arginine and bone marrow-derived mesenchymal stem cells (BM-MSCs) on diabetes-induced inflammatory and histopathological alterations in the submandibular salivary glands of streptozotocin (STZ)-induced diabetic rats.

Material and Methods: 17 albino rats were randomly allocated into four experimental groups: control group, diabetic group, L-arginine group, and bone marrow-derived mesenchymal stem cell (BM-MSC) group. Diabetes was induced by a single injection of streptozotocin (STZ), and all animals were sacrificed on day 28 for tissue collection. The effects were evaluated through histopathological examination of the submandibular glands using hematoxylin and eosin (H&E) and immunohistochemical assessment of nuclear factor kappa B (NF-κB) expression, and enzyme linked immunosorbent assay (ELISA) quantification of interleukin-6 (IL-6) levels to assess inflammatory changes.

Results: diabetic rats exhibited marked histopathological deterioration of the submandibular glands, whereas both L-arginine and BM-MSCs administrations significantly improved glandular architecture and reduced inflammatory changes. BM-MSCs produced the greatest histological recovery, accompanied by the lowest NF-κB expression and IL-6 levels, indicating superior anti-inflammatory and regenerative effects.

Conclusion: Both L-arginine and BM-MSCs administrations improved diabetes-induced salivary gland injury; however, BM-MSCs produced greater regenerative and anti-inflammatory effects, highlighting their promise as a long-term therapeutic approach.

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Introduction:

Diabetes mellitus (DM) is a heterogeneous chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin action, or both. As one of the leading global health challenges, the number of affected individuals is projected to rise to approximately 693 million worldwide by 2045, highlighting the growing burden of the disease and the need for improved therapeutic approaches (1-3). L-arginine is a conditionally essential amino acid belonging to the arginine family, which also includes glutamine, glutamate, ornithine, proline, and citrulline. These amino acids are metabolically interconnected through shared biosynthetic and catabolic pathways (4). Larginine is involved in numerous physiological processes, serving as a substrate for protein synthesis and supporting cell proliferation, tissue repair, wound healing, hormone secretion, and normal metabolic function (4, 5). It is metabolized by several key enzymes, including nitric oxide synthase (NOS), arginase (ARG), arginine decarboxylase (ADC), and arginine amidinotransferase (AGAT) (6), generating biologically active metabolites such as nitric oxide (NO), creatine, ornithine, polyamines, agmatine, and

glutamate (4, 5, 7). Evidence suggests that L-arginine may improve glucose metabolism and enhance insulin sensitivity in diabetic patients (7). The therapeutic potential of L-arginine is largely attributed to its antioxidant, anti-inflammatory, and antidiabetic properties, which are primarily mediated through enhanced NO production. As the physiological precursor of NO, L-arginine supports endothelial function, maintains vascular homeostasis, and improves tissue perfusion, thereby reducing oxidative stress and inflammatory responses (6). In addition, L-arginine modulates immune activity by suppressing the production of pro-inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), while promoting tissue repair and cellular regeneration (8). Its antioxidant activity is further mediated through activation of endothelial nitric oxide synthase (eNOS) and the nuclear factor erythroid -related factor 2 (Nrf2) signaling pathway, leading to increased expression of endogenous antioxidant enzymes and enhanced scavenging of reactive oxygen species (ROS)(9).

Experimental and clinical studies have demonstrated that L-arginine supplementation decreases oxidative stress biomarkers, such as malondialdehyde (MDA) and thiobarbituric acid reactive substances (TBARS), restores antioxidant defenses, improves endothelial function and insulin sensitivity, and may delay the progression of diabetes and its associated complication (7, 10, 11). Clinically, supplementation with 6.4 g/day of L-arginine for 18 months in individuals with impaired glycemic regulation has been shown to reduce the incidence of diabetes over long-term follow-up compared with controls (12). Furthermore, histochemical and immunohistochemical studies have demonstrated that L-arginine exerts beneficial effects on hyperglycemia control in experimental diabetes, along with antiplatelet aggregation properties in both animal and human studies (13). Additionally, Gram D.Y. A. et al. reported a significant upregulation of vascular endothelial growth factor (VEGF) expression following L-arginine administration (14).

On the other hand, Mesenchymal stem cells (MSCs) are multipotent stromal cells characterized by self-renewal capacity, multilineage differentiation potential, and immunomodulatory properties (15). According to the International Society for Cell & Gene Therapy (ISCT), MSCs are identified by the expression of CD73, CD90, and CD105, absence of hematopoietic markers, and the ability to differentiate into osteogenic, chondrogenic, and adipogenic lineages (16). Among different MSC sources, bone marrow-derived mesenchymal stem cells (BM-MSCs) are widely investigated due to their high proliferative capacity, paracrine activity, and regenerative potential (17-19). BM-MSCs exert therapeutic effects in diabetes through multiple mechanisms, including enhancement of pancreatic β-cell regeneration, improvement of insulin secretion, modulation of immune responses, suppression of pro-inflammatory cytokines, reduction of insulin resistance and direct intercellular connection by recipient cells (20-22). These effects are mediated by their secretion of bioactive molecules, induction of regulatory T-cell activity, and polarization of macrophages from the pro-inflammatory M1 phenotype toward the anti-inflammatory M2 phenotype, resulting in attenuation of chronic inflammation and promotion of tissue repair (23, 24).

Several experimental studies have demonstrated the beneficial effects of BM-MSCs on salivary gland damage associated with diabetes and other injuries. **Denewar and Amin (2020)** reported that systemic administration of BM-MSCs in streptozotocin (STZ)-induced diabetic rats preserved parotid gland architecture, reduced cytoplasmic vacuolation, and increased aquaporin-5 expression, suggesting improved glandular secretory function (25). Similarly, **Rasheed et al. (2022)** demonstrated that BM-MSCs treatment alleviated cadmium-induced submandibular gland injury, restoring glandular morphology, reducing oxidative stress markers, and decreasing metallothionein gene expression, indicating cytoprotective and antioxidant effects (26).

Furthermore, **a 2023 study** using histological, immunohistochemical, and qRT-PCR analyses in STZ-induced diabetic submandibular glands showed that BM-MSCs administration improved tissue architecture and modulated diabetesassociated molecular alterations, including the reduction of inducible nitric oxide synthase (iNOS) and regulation of heme oxygenase-1 (HO-1) expression (27). Additionally, a 2025 investigation revealed that BM-MSCs enhanced regeneration of irradiated submandibular glands by increasing angiogenesis, demonstrated by elevated CD34positive micro vessel density, and stimulating cellular proliferation through increased Ki-67 expression (28).

Materials and Methods:

Animals:

The study was conducted on 17 male albino rats aged 6 weeks and weighing 200–250 g. The animals were obtained from and housed in the Animal House of the Faculty of Medicine, Al-Azhar University, Cairo, Egypt, under standard laboratory conditions.

Chemicals:

Diabetogenic agent:

Streptozotocin (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) is a glucosaminenitrosourea derivative with potent alkylating properties. It is commonly used in experimental studies to induce diabetes in animals through selective destruction of pancreatic β-cells (29).

Drug L-arginine:

L-arginine powder (Loba Chemie, India) was precisely measured and transferred into sterile glass containers, followed by sterilization with diethyl ether for 24 hours. The sterilized compound was then dissolved in 100 mL of sterile 0.85% normal saline to obtain an 8% stock solution. The prepared solution was further sterilized through filtration using a 0.45 µm membrane filter and administered to the experimental animals at a dose of 1 g/kg/day for seven successive days (30).

Bone marrow mesenchymal stem cells (BM-MSCs):

Isolation and culture of BM-MSCs:

Bone marrow-derived mesenchymal stem cells (BM-MSCs) were isolated from the tibiae of albino rats (150–200 g). Bone marrow cells were harvested by flushing the tibial cavities with phosphate-buffered saline (PBS). The collected cell suspension was subjected to density gradient centrifugation using Ficoll-Paque to isolate the mononuclear cell fraction. The recovered mononuclear cells were collected from the interphase, washed with PBS supplemented with ethylenediaminetetraacetic acid (EDTA), and centrifuged to remove residual components. Subsequently, the isolated cells were seeded into culture flasks containing RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 0.5% penicillin–streptomycin. Cells were incubated under controlled conditions (37°C, 5% CO_2) until reaching 80–90% confluence after approximately 7 days (31).

Characterization of BM-MSCs:

The identity of cultured BM-MSCs was confirmed through morphological assessment and immunophenotypic characterization using fluorescence-activated cell sorting (FACS) analysis. The cells demonstrated the characteristic MSC surface marker profile, showing positive expression of CD105 and CD90, while lacking hematopoietic markers CD34 and CD45, confirming their mesenchymal stem cell phenotype (32).

Experimental design:

The study involved four groups of rats:

Group I (Control GP) included 2 healthy un-diabetic rats. After 28 days the animals were euthanized by intra peritoneal injection of ketamine 100 mg/kg (33). Then, the submandibular salivary glands were dissected. **Group II (Diabetic GP)** included 5 Albion rats with STZ-induced diabetes (35-40 mg/kg body weight) (34), confirmed by blood glucose >250 mg/dl, also euthanized at 28th day. **Group III (L-arginine GP)** consisted 5 STZ-diabetic rats received L-arginine injection of (1 g/kg/day) (30) for 7 days before euthanasia, also euthanized 28 days for salivary gland analysis. **Group IV (BM-MSCs group)** included 5 STZ-diabetic rats given a single dose of BM-MSCs (0.5ml containing 2 × 106 cells/rat)(35) via tail vein injection, followed by euthanasia at 28 days for salivary gland analysis.

In all experimental groups, submandibular gland specimens from one side were fixed and processed for histological examination using Hematoxylin and Eosin (H&E) staining, and immunohistochemical detection of Nuclear Factor Kappa Beta (NF-κB). The contralateral gland specimens underwent enzyme-linked immunosorbent assay (ELISA), a technique that quantifies antigen presence by the specific binding of antibodies to their targets. This assay was utilized to evaluate inflammation by detecting interleukin-6 (IL-6) expression.

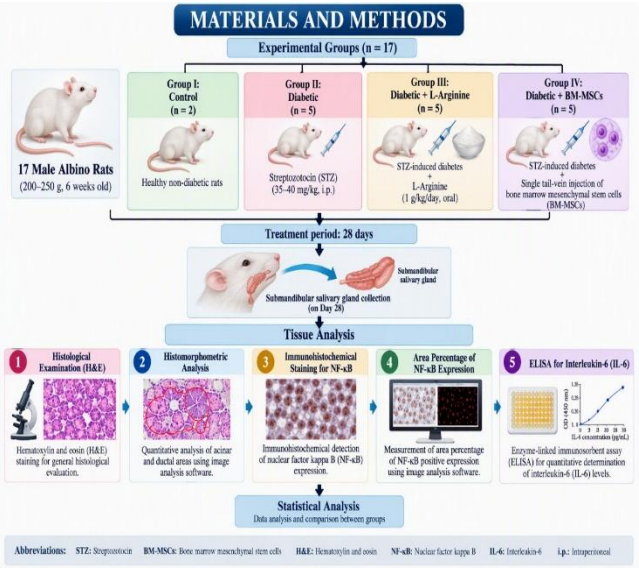


Diagram (1): showing, the summary of the methodology.

Results:

1-Histopathological results:

Histological examination of the submandibular salivary glands in the control group demonstrated normal lobular architecture, with well-organized serous acini, granular convoluted tubules (GCTs), striated ducts, and excretory ducts. In contrast, the diabetic group exhibited marked degenerative changes, including disruption of lobular organization, acinar atrophy and disorganization, cytoplasmic vacuolation, fatty degeneration, pyknotic nuclei, widened peri-acinar and peri-ductal spaces, increased adipocyte infiltration, and degenerative alterations affecting both GCTs and ductal elements. Besides, reflecting severe impairment of glandular organization, features indicative of microangiopathic. L-arginine group resulted in partial restoration of glandular architecture, as evidenced by improved acinar organization, reduced cytoplasmic vacuolation, recovery of basal striations in striated ducts, and near-normal epithelial lining of excretory ducts; however, mild residual vacuolar and vascular changes persisted. Moreover, extravasated erythrocytes and congested blood vessels were observed. Conversely, BM-MSCs administration produced more pronounced histological improvement, with near-complete restoration of the normal glandular architecture, preservation of acinar and ductal morphology, well-defined GCTs with prominent secretory granules, and minimal cytoplasmic vacuolation, although focal erythrocyte extravasation remained evident around some acini and ducts **(Fig. 1)**

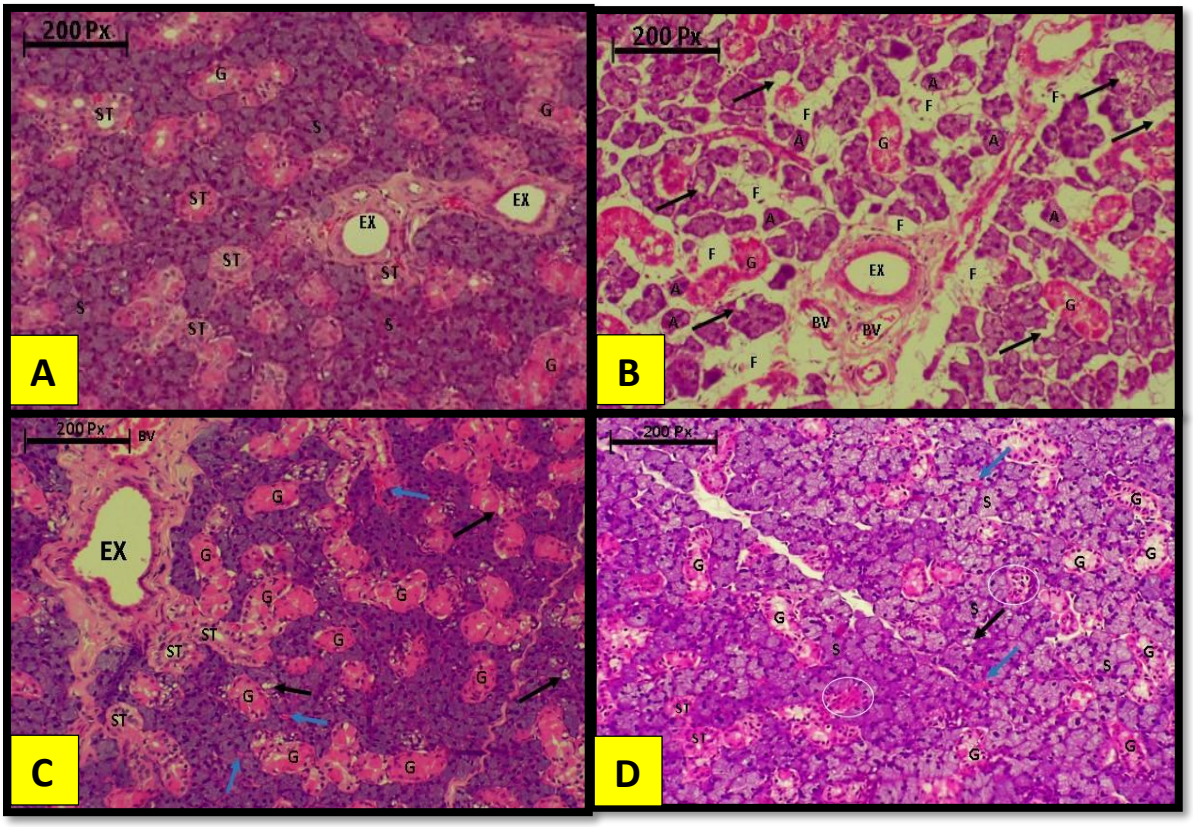


Fig. (1): A photomicrograph of rat submandibular salivary gland at day 28, **(A):** group I (control group) showing, normal histology of the gland with serous acini (S), GCT (G), striated ducts (ST) and excretory ducts (EX). **(B):** group II (diabetic group) showing, apparent loss of lobe infirmity, acinar architectural disruption, and significant fat cell infiltration between acini with apparent spacing between the acini and the ducts (F), large intracytoplasmic vacuolization (black arrows) and acinar atrophy (A). **(C):** group III (L-arginine group) showing, the lobular architecture appeared homogeneous with a uniform organization. Certain serous acini and ducts showed intracytoplasmic vacuolization (black arrows). Extravasated red blood cells were also observed (blue arrows), along with a congested blood vessel. Striated ducts (ST), GCT (G) and excretory duct (EX). **(D):** group IV (BM-MSCs-group) showing, a more compact glandular architecture. Striated duct (ST), GCT (G), serous acini (S) appeared almost

normal. A few serous acini demonstrated intracytoplasmic vacuolization (black arrows) and extravasated RBCs, (blue arrows) with a proliferative area was noted (circle). **(H&E, Orig. Mag. X200)**

- Histomorphometric examination

- The number of acini/µm² at day 28

The greatest mean value the number of acini/µm² was recorded in group I (control group), whereas the least value was recorded in group II (diabetic group).

Analysis of variance (ANOVA) test demonstrated a statistically significant difference in histomorphometric scores across the experimental conditions (F = 25.94, *p* < 0.001). The Diabetic group exhibited markedly impaired tissue architecture (mean score = 73.0), while both intervention groups demonstrated substantial recovery. The L-arginine group showed significant improvement (mean score = 98.0), and BM-MSCs group achieved the highest morphological preservation (mean score = 109.0), closely approaching the non-diabetic reference level (126.0). (Table 1, Fig.2)

Table (1): Mean values and Standard deviation (Mean ± SD) of the number of acini/µm² in the studied groups.

Parameter	Control Group	Diabetic Group	L-arginine Group	Stem Cell Group
Mean	126	73	98	109
Std. Dev.	25	15	20	22
P value*	0.000123*			

***ANOVA test** * significant

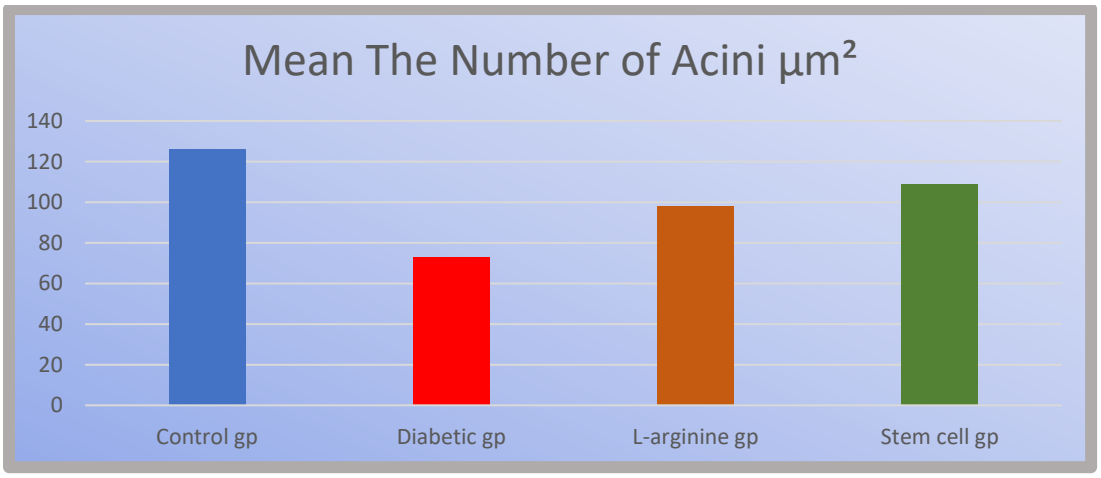


Fig. (2): Column chart representing mean the number of acini/µm² in different groups at day 28.

2-Immunohistochemical results:

Immunohistochemical analysis of NF-κB expression revealed distinct differences among the experimental groups. The control group exhibited weak NF-κB immunoreactivity throughout the gland, with acinar cells showing negative to minimal cytoplasmic staining and only weak expression in the

ductal system, connective tissue, and blood vessels. In contrast, the diabetic group demonstrated marked upregulation of NF-κB, characterized by intense cytoplasmic immunostaining in both acinar and ductal cells, accompanied by evident nuclear translocation, indicating activation of the NF-κB signaling pathway. Both L-arginine and BM-MSCs markedly attenuated NF-κB expression compared with the diabetic group. Immunoreactivity was reduced to weak or mild levels in the acinar and ductal components, connective tissue, and blood vessels, with a complete absence of nuclear translocation. Overall, BM-MSCs group exhibited an immunostaining pattern closely resembling that of the control group (Fig.3).

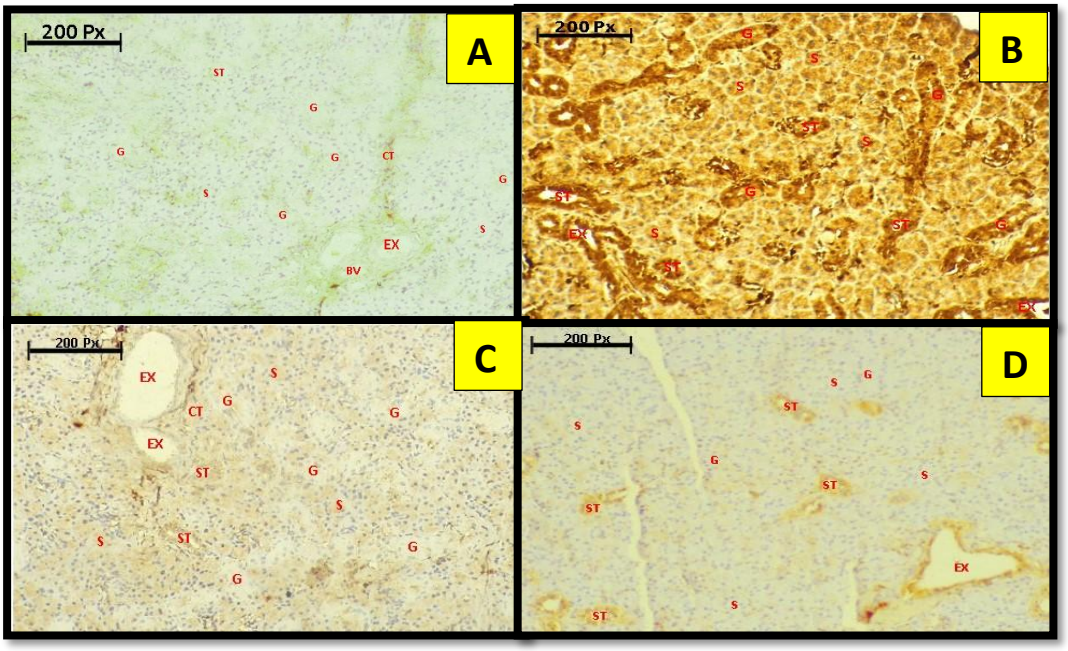


Fig. (3): A photomicrograph of rat submandibular salivary gland at day 28, **(A):** group I (control group) showing, negative immunoreactivity for NF-κB in acinar cells (S), weak cytoplasmic immunoreaction in GCT (G), striated ducts (ST) and excretory duct (ex). Weak immunoreaction in connective tissue septa (CT). Blood vessel (BV). **(B):** group II (diabetic group) showing, a strong cytoplasmic NF-κB immunostaining in the acinar cells (S). Strong cytoplasmic immunoreactivity for NF-κB in GCTs (g), striated ducts (ST) and excretory ducts. **(C):** group III (L-arginine group) showing, a weak cytoplasmic NF-κB immunostaining in the most of acinar cells (S). A mild cytoplasmic immunoreactivity for NF-κB in striated ducts (ST), GCT (G) and excretory ducts (EX). Connective tissue (CT) with mild immunoreaction. **(D):** group IV (BM-MSCs group) showing, a weak cytoplasmic NF-κB immunostaining in the most of acinar cells (S). A mild cytoplasmic immunoreactivity for NF-κB in striated ducts (ST), GCT (G) and excretory ducts (EX). Connective tissue (CT) with mild immunoreaction. **(DAB, Orig. Mag. X400)**

-Examination of area percent:

-Area percent of NF-κB expression at day 28:

The greatest mean value of area percentage of NF-κB immunoexpression was recorded in group II (diabetic group), whereas the least value was recorded in group I (control group). Analysis of variance (ANOVA) test demonstrated significant effects of Time, Group, and their interaction (all *p* < 0.001). (Table, 2 &3, Fig.4)

NF-κB expression was significantly elevated in the Diabetic group (61.64) compared to the Control (11.18; *p* < 0.001, One-Way ANOVA). Both interventions significantly downregulated NFKB (L-Arginine: 29.72; Stem Cell: 21.27; *p* < 0.001), with the Stem Cell group showing a more significant reduction than the L-Arginine group (*p* < 0.01). This

confirms that both interventions effectively inhibit the central NFKB-mediated inflammatory pathway. (Table, 2 &3 Fig.4)

Tukey's post-hoc test for multiple 2- group comparison revealed that, the difference in the mean value was significant between all different groups (Table, 3)

Table (2): Mean values and Standard deviation (Mean ± SD) of the area percent of NF-κB immunoexpression in the studied groups at day 28.

Parameter	Control GP	Diabetic GP	L-arginine GP	Stem cell GP
Mean	11.1775	61.6375	29.72	21.2675
Std. Dev.	0.472712	0.803859	1.586794	1.465261
P value *	≤0.05*			

ANOVA test * significant

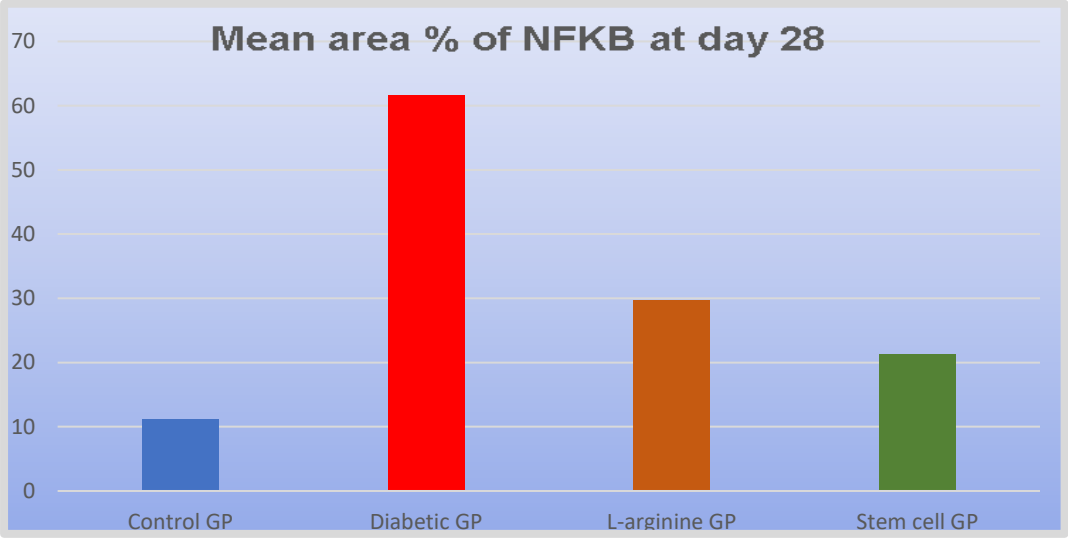


Fig. (4): Column chart representing mean area percent of NF-κB immunoexpression at day 28.

Table (3): P-values from pair wise comparison using Tukey's post-hoc test.

group 1	*group 2*	*p-value*
Control GP	Diabetic GP	4.13E-12
Control GP	L-arginine GP	5.77E-07
Control GP	Stem cell GP	0.000284
Diabetic GP	L-arginine GP	1.19E-09
Diabetic GP	Stem cell GP	8.74E-11
L-arginine GP	Stem cell GP	0.001331

3-IL-6 protein expressions in ELISA test:

The greatest mean value of area percentage of IL-6 level was recorded in group II (diabetic group), whereas the least value was recorded in group I (control group).

Analysis of variance (ANOVA) test demonstrated significant effects of time, group, and their interaction (all *p* < 0.001).

Table (4): Mean values and Standard deviation (Mean ± SD) of IL-6 protein expression presents across different groups.

Parameter	Time Point	Control GP	Diabetic GP	L-arginine GP	Stem Cell GP
IL-6(pg/mg tissue)	Day 28	7.25 ± 0.65	73.60 ± 7.78	20.04 ± 3.12	10.85 ± 1.07

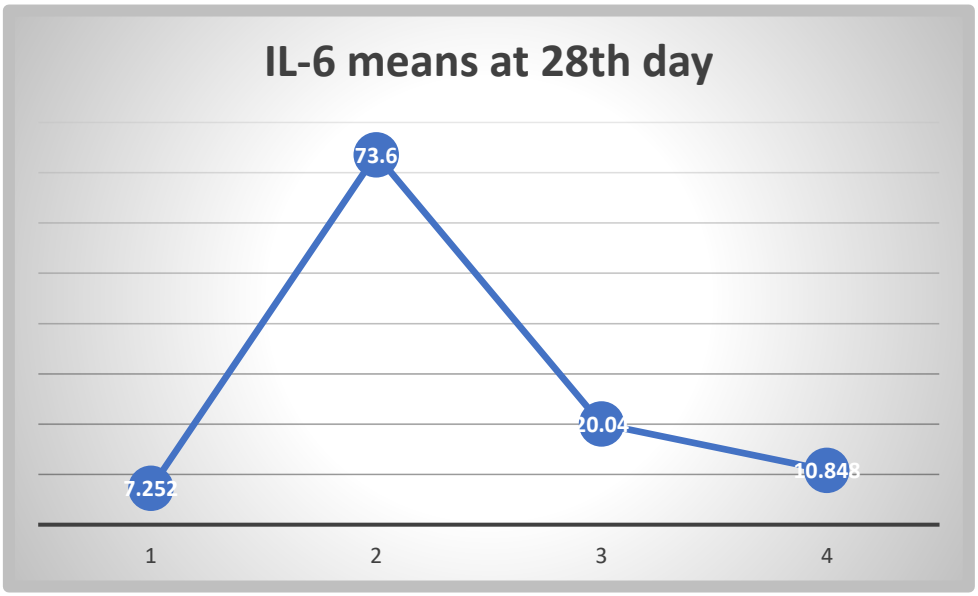


Fig. (5): Column chart representing IL-6 means for all group at 28th day.

Table (5): summarizes the results of the Two-Way ANOVA and Post-Hoc Tukey HSD tests of IL-6, highlighting significant differences between the intervention groups and the diabetic control.

Parameter	Time Point	Comparison (vs. Diabetic Group)	P-value	Significance
IL-6	Day 28	L-arginine	*p* < 0.001	Significant
		Stem Cell	*p* < 0.001	Significant

***ANOVA test** * significant.

Table (6): indicates the distribution properties of the data of IL-6, which informs the choice of parametric or non-parametric tests.

Parameter	Group	Shapiro-Wilk Test	Normality
IL-6	All Groups	W-stat, *p* > 0.05	Normal (Parametric)

Discussion:

Diabetes mellitus is associated with persistent hyperglycemia, oxidative stress, vascular dysfunction, and chronic inflammation, all of which may contribute to structural and functional impairment of salivary gland tissue (27, 36). The present study demonstrated that, at day 28, both L-arginine and bone marrow-derived mesenchymal stem cells (BM-MSCs) improved diabetes-associated damage in the submandibular salivary gland, with BM-MSCs producing the greater degree of structural recovery. Histomorphometric analysis showed a statistically significant difference among the experimental groups ($F = 25.94$, $p < 0.001$). The diabetic group exhibited pronounced architectural disruption, whereas L-arginine improved tissue organization and BM-MSCs produced the highest degree of preservation, approaching the architecture of the control gland.

The superior structural recovery observed with BM-MSCs, which the glands exhibited close to normal histology compared to the control and the result is consistent with experimental findings by **Denewar and Amin (2020),** who reported preservation of salivary gland architecture following BM-MSC administration in streptozotocin-induced diabetic rats(25). Similarly, **Abd Elwahab et al.** reported improved submandibular gland architecture following BM-MSC administration (27). These findings support the regenerative potential of BM-MSCs in diabetic salivary tissue.

The greater regenerative effect of BM-MSCs may be explained by their paracrine and immunomodulatory actions. BM-MSCs release trophic factors and extracellular vesicles containing bioactive proteins and regulatory microRNAs that can enhance angiogenesis, cell survival, and tissue remodeling (37). In particular, BM-MSC-derived extracellular vesicles have been reported to promote endothelial activation and neovascularization, while **Wu et al. (2024)** demonstrated an angiogenic effect mediated through miR-150-5p (38). Beyond paracrine signaling, MSCs can directly rescue injured cells by donating functional mitochondria through tunneling nanotubes (TNTs)(22). Studies show BM-MSCs transfer mitochondria to stressed nucleus pulposus cells (39)and diabetic podocytes via TNTs (40), restoring respiration and lowering ROS and apoptosis (39, 40). Thus, TNT-mediated mitochondrial transfer is a key regenerative mechanism by which BM-MSCs revive metabolically compromised cells (22, 39, 40).

L-arginine demonstrated that tissue architecture becomes organized and mature, as L-arginine supports sustained collagen remodeling, extracellular matrix regulation, and improved structural alignment. This effect may be related to its role as a substrate for nitric oxide synthase, increasing nitric oxide availability and thereby improving microvascular perfusion and tissue oxygenation(14). The resulting vascular support, together with the antioxidant and anti-inflammatory effects described in the original study, may facilitate cellular survival and tissue remodeling. Experimental studies cited have similarly reported beneficial effects of L-arginine in diabetic experimental models (41).

The difference between the two interventions may therefore reflect their distinct mechanisms of action. L-arginine mainly provides metabolic and vascular support through nitric oxide-dependent pathways, whereas BM-MSCs act through a broader combination of paracrine signaling, angiogenesis, immunomodulation, and tissue regeneration through TNTs. This wider biological activity may explain the superior structural restoration observed with BM-MSCs.

The molecular findings further support this interpretation. NF-κB immunohistochemistry demonstrated marked inflammatory activation in the diabetic gland, whereas both intervention groups reduced NF-κB expression, with the most pronounced suppression observed following BM-MSC administration. The NF-κB value decreased from 61.64 ± 1.58 in the diabetic group to 21.27 ± 3.07 in the BM-MSC group, indicating substantial inhibition of inflammatory signaling. The anti-inflammatory action of BM-MSCs may involve regulation of NF-κB through microRNA-mediated mechanisms and secretion of TSG-6, which can inhibit the TLR2/MyD88/NF-κB pathway, as reported by **Wang et al. (2022) and Yang et al. (2020)(42, 43)**.

This anti-inflammatory effect is also compatible with the interaction between NF-κB and IL-6, where NF-κB activation promotes IL-6 expression, while sustained IL-6 signaling can maintain inflammatory activity (44, 45). **Ahmad et al. (2024)** similarly reported increased NF-κB together with IL-6, IL-1β, and TNF-α in the submandibular glands of streptozotocin-induced diabetic rats (46). BM-MSC-mediated modulation of this inflammatory environment, including promotion of reparative M2 macrophage polarization and anti-inflammatory signaling, may therefore contribute to the structural recovery observed in the present study (47, 48).

Overall, the day 28 findings suggest that both L-arginine and BM-MSCs have therapeutic potential against diabetes-associated submandibular gland injury, but BM-MSCs produced the more pronounced

restoration. The difference is likely related to the broader regenerative mechanisms of BM-MSCs, involving paracrine signaling, angiogenesis, immunomodulation and direct intercellular connection by TNTs, compared with the predominantly NO-dependent vascular and metabolic actions of L-arginine. These findings support BM-MSCs as a promising regenerative approach for diabetic salivary gland injury.

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