Original Article

The Therapeutic Effect of Honey Bee Venom versus Mesenchymal Stem Cells on Submandibular Salivary Glands of Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Introduction: Diabetes mellitus is a serious disease of worldwide concern. Therapeutic bee venom (BV) and bone marrow mesenchymal stem cells (BMSCs) were widely applied in various diseases.

Aim of the Work: This study was conducted to compare the effect of BV and BMSCs on the submandibular salivary glands of streptozotocin (STZ)-induced diabetic rats.

Materials and Methods: In the ongoing study, 40 male rats were used. Five rats were utilized for BMSCs isolation and culture, 5 rats served as the control group, however the remaining 30 rats were distributed as follow: untreated diabetic group; received a single intraperitoneal (IP) dose of STZ, BV treated diabetic group; diabetic rats received a daily IP dose of 0.5 mg/kg BV for 4 weeks, and BMSCs treated diabetic group; diabetic rats received a single IV injection of BMSCs. All animals were euthanized after a month, the submandibular salivary glands were dissected and evaluated by histological, immunohistochemical and quantitative Real-Time Polymerase Chain Reaction (qRT- PCR) examinations. Finally, all obtained data were statistically analyzed.

Results: Regarding control group the salivary gland architecture was normal, while the diabetic group revealed degenerative glandular changes. Both diabetic treated groups showed improved histological pictures, however; improvement was more obvious in the BMSCs treated group in comparison to the BV treated one. Regarding the diabetic group, a significantly increased inducible nitric oxide synthase
(iNOS) immunoexpression and Hemeoxygenase-1 (HO-1) expression were noticed. However, following BV and BMSCs treatments, a significantly decreased iNOS immunoreactivity and overexpression of HO-1 gene were noticed.

Conclusion: Both BMSCs and BV treatments ameliorated degenerative effects of diabetes, yet; BMSCs exhibited a more significant therapeutic effect than BV.

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Key Words: Bee venom, heme oxygenase-1 gene, inducible nitric oxide synthase, stem cells, streptozotocin.

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia due to defect in insulin action, insulin secretion, or both[1]. In Egypt, diabetes is a rapidly developing health concern that has a considerable impact on mortality, mortality, and health-care resources[2]. Oral complications of DM include numerous long-term problems such as burning mouth syndrome, increased incidence of infection, delayed wound healing, dry mouth, tooth decay, gingivitis, periodontal disease, altered taste and salivary gland dysfunction, in addition to other neurosensory diseases[3].

The live bee stings could be utilized as a therapeutic bee.venom (BV) in a variety of diseases, including multiple sclerosis, rheumatoid arthritis, lupus, sciatica and low back pain[4]. The main active components of BV has been classified in three major groups: (a) proteins (melittin: MEL, adolapin, and apamin: APA), (b) enzymes (hyaluronidase and phospholipase A2: PLA2), and (c) non-peptides[5]. The most potent anti-inflammatory effect of MEL might be returned to its ablility to inhibit cytokines, mainly interleukin-6 (IL-6), IL-8, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ)[6]. However; when high dosages of MEL are given, it causes local pain, itching, and inflammation[7].

Interestingly, the potentiality of stem cell to differentiate into diversity of cells implicated its clinical application in several medical fields, such as diabetes and cancer. This might return to its ability to regenerate and restore damaged tissues in different body organs. In diabetes treatment, the stimulation of stem cells and consequent production of vital hormones, as insulin could be highly effecient[8].

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Utilizing the previously mentioned data, the ongoing study was designed to assess BV therapy versus bone marrow mesenchymal stem cells (BMSCs) on streptozotocin (STZ)-induced salivary gland alterations, in an attempt to evaluate the potential therapeutic applications.

MATERIALS.AND.METHODS

Animals

40 male albino rats have been used with an average weight of about 200 gms at the animal house, Faculty of Medicine, Cairo University. They were conventionally fed by rat chow besides distilled water and housed with the technician's help in stainless steel cages under a controlled environment (temperature 25±2° and 12-hour dark/light cycles). The esthical approval was obtained from the Institutional Animal Care and Use Committee (IACUC) - Cairo University. (Approval code: CU III F 74 18).

Drugs and chemicals

BV

This collection experiment was carried out on Italian and Craniolian hybrid in the summer corn season in July at the National Research Center, 2019. BV was collected from Apis mellifera bees' colonies by a BV collector device.

BMSCs

Five rats were utilized for BMSCs isolation and culture according to previously published protocols, at the Biochemistry and Microbiology Unit, Faculty of Medicine, Cairo University[9]. Cultured BMSCs were examined regarding their adhesiveness and fusiform shape. In addition, Florescent Analysis Cell Sorting (FACS) revealed that these stem cells expressed CD29 and CD90 marker but didn't express CD45 marker[10]. The ability of BMSCs to differentiate into three cell types (osteocytes, chondrocytes and adipocytes) was evaluated in *vitro*[11].

Labelling of BMSCs with PKH26 dye

BMSCs cells were obtained during the 4th passage and were labelled with PKH26 Red Fluorescent Cell Linker Kit (Sigma-Aldrich) in accordance with manufacturer's instructions. The final concentration of cells labelled with PKH26 dye was 2 x 10-6 M PKH26 dye and 1 X 107cells/ml in a 2 ml total volume (Sigma-Aldrich, Saint Louis, USA)[12]. Detection of injected cells homing in the rat's submandibular salivary gland: At the study's conclusion, the rat's submandibular salivary gland sections were examined with a phase-contrast fluorescent microscope to detect cells stained with PKH26 dye to ensure injected cells engraftment into the gland.

Streptozotocin

STZ was purchased from Sigma-Aldrich: Louis, MO, USA. Diabetes mellitus was induced via STZ dissolved in 0.1 M freshly prepared citrate buffer (dose: 60 mg/kg body weight, route: IP injection)[13].

Experimental Procedures

Five rats were left untreated (control group), while the other rats injected with STZ to induce DM[13]. Then, DM was evaluated as a random blood glucose reading of >300 mg/dl after 72 hours of STZ injection[14].

Diabetic rats were separated into three groups when DM was confirmed:

- Untreated diabetic group (STZ): diabetic rats were left untreated.
- BV treated diabetic group (BV+STZ): diabetic rats were IP injected each with a daily dose of 0.5 mg/kg BV for 4 weeks[15].
- Stem cells treated diabetic group (BMSCs+STZ): A single IV injection of previously cultured BMSCs at a concentration of 1 million cells/ml in phosphate-buffered saline (PBS) was administrated by diabetic rats[16].

One month later, both treated and untreated rats were eventually euthanized. The submandibular salivary glands were dissected, fixed in 10% neutral buffered formalin, dehydrated by ethanol (70%, 80%, 90%, 95%, two absolute changes of ethanol), cleared in xylene, impregnated and embedded in melting paraffin wax. Sections with a thickness of 4-5 µm were obtained and subjected to the following examinations:

Histological Examination

Using hematoxylin;and eosin stain (H&E) according to the conventional method of Bancroft and Gamble[17].

Immunohistochemical Examination using anti-iNOS antibody:

Cat. # RB-9242-R7 (7.0 ml) (Ready-to-Use for immunohistochemistry).

(Thermo Fisher Scientific, Anatomical Pathology, Tudor Road, Manor Park, Runcorn, Cheshire WA7 1TA, UK).

The reagents in this kit constituted a labeled streptavidin-biotin immune-enzymatic antigen detection system. This technique involves the sequential incubation of the specimen with an unconjugated primary antibody specific to the target antigen. Then, biotinylated secondary antibody reacts with the primary antibody, enzyme-labeled streptavidin and chromogen, which is diaminobenzidine (DAB).

Procedures of Staining[18]

Three cycles incubation in xylene deparaffinized the sections, 5 minutes each. Then slides were rehydrated in a series of descending ethanol, washed with distilled water and by immersing the slides in 3 percent hydrogen peroxide for 10-15 minutes at room temperature, endogenous peroxidase was blocked, then washed two times with PBS. Afterwards, slides were dipped in antigen reagent and

were heated in a microwave oven at 92ºC for 20 minutes, then it was rinsed in PBS three times. One or two drops of the ready to use anti-iNOS primary antibody were added to each section, then slides were incubated for thirty minutes in a humid chamber. Afterwards, the slides were rinsed 3 times in PBS, three minutes each. The secondary antibody, biotinylated goat anti-mouse immunoglobulin G (IgG) was added and incubated (10-15 minutes) at room temperature then rinsed with PBS three times, 3 minutes and Streptavidin peroxidase was added, incubated (10-15 minutes) at room. temperature, then rinsed with PBS 3 times. Finally, the DAB substrate chromogen was applied and incubated at room. temperature for 10 minutes before being washed in PBS for 3 minutes. All sections were counterstained with hematoxylin, washed, dried in air, then mounted in Canada balsam.

Histomorphometric Analysis of Immunohistochemical Sections

The immunostained sections were examined using Leica Quin 500 analyzer computer system. Immunostaining was measured as area percent in 10 fields per group using magnification (x400) by light microscopy transferred to the screen. Mean values and standard deviation were obtained for each specimen.

qRT- PCR detection of HO-1 gene expression

Ribonucleic acid (RNA) extraction was provided by Thermo Fisher Scientific Inc. Germany (GeneJET, Kit, #K0731). The yield of total RNA obtained was determined spectrophotometrically at 260 nm. In real-time PCR, the prepared reaction mix samples were used (Step One Applied Biosystem, Foster city, USA). Three-step cycling was performed with ViPrime PLUS One Step Taq RT-qPCR Green Master Mix I with ROX Kit. Relative expression of studied genes was calculated by the comparative cycle threshold (Ct). The PCR datasheet includes Ct values of the assessed gene (HO-1) and the housekeeping gene (β-actin) as a control .gene. The relative quantification (RQ) of each target gene is quantified according to the calculation of delta-delta Ct (ΔΔCt). Primer sequences for each gene were demonstrated in (Table 1).

Table 1: Primers sequence of all studied genes

Gene symbol	Primer sequence from 5′- 3′ F: Forward primer, R: Reverse primer
HO-1	F: CAGTCGCCTCCAGAGTTTCC R: GTACAAGGAGGCCATCACCAGA
β-actin	F: TCCGTCGCCGGTCCACACCC R: TCACCAACTGGGACGATATG

Statistical Analysis

Statistical analysis was then performed using a commercially available software program (SPSS version 22). Values were existing as mean ± standard deviation (SD). Data were explored for normality using Kolmogorov-Smirnov test of normality. Since data were parametric, a one-way analysis of variance (ANOVA) test was conducted to compare all groups. When ANOVA revealed a significant difference, Tukey's post hoc test was used. The significance level was set at $P < 0.05$.

RESULTS

Characterization and homing of BMSCSs

The adhesiveness and fusiform shape of BMSCs in culture were observed. In addition, Florescent Analysis Cell Sorting (FACS) revealed that the stem cells expressed CD29 and CD90 marker but didn't express CD45 marker[19].

Detection of homing of the injected cells in the rat's submandibular salivary gland:

Examining rat's gland tissue under a fluorescent microscope revealed the engraftment of PKH26 labelled BMSCs in the studied tissues. (Figure 1).

Histological Results

Control Group

Examining the rat's submandibular salivary gland of this group revealed roughly circular acinar portions lined with pyramidal cells having spherical basally located nuclei. The granular convoluted tubule (GCTs) were lined with large columnar cells with basal nuclei and apical eosinophilic granules. The striated ducts were lined with columnar cells having rounded central nuclei and prominent basal striations (Figure 2a). The C.T. septa also have excretory ducts with pseudostratified columnar epithelial lining and medium-sized blood vessels. (Figure 3a).

Untreated Diabetic Group (STZ group)

Following STZ administration, the gland showed massive intracytoplasmic vacuolizations and reduced cytoplasmic basophilia of acinar portion. Areas of degeneration with loss of gland architecture were also observed. Regarding the duct portion, GCTs showed vacuolizations and reduction in their eosinophilic granules. Besides, the striated ducts presented vacuolations and indistinct basal striations (Figure 2b). Some excretory ducts were dilated with retained secretion. Intense chronic inflammatory cells infiltration was noticed in the C.T septa as well (Figure 3b).

Bee Venom Treated Diabetic Group (BV + STZ group)

The diabetic gland of the rat was histologically studied, following BV administration, revealed an enhancement in the general glandular architecture comparing to the STZ group. Acinar boundaries were mostly more defined, while in few areas, homogenization and damage of gland architecture were detected. Obviously reduced intracytoplasmic vacuolizations and some mitotic figures were displayed among the acinar cell. As regards the duct portion, the GCT displayed intensely increased, clumped eosinophilic content and they showed fewer vacuoles and fewer areas of degeneration (Figure 2c). The striated

duct showed an almost normal cell lining with rarely degenerated areas (Figure 2c). Between the gland's lobules were found dilated excretory ducts, some of which had metaplastic changes in the shape of flattened linings. The C.T. septa rarely displayed chronic inflammatory cells (Figure 3c).

Stem Cells Treated Diabetic Group (BMSCs +STZ) Group

Following BMSCs treatment, the rat's diabetic submandibular salivary gland showed the followings (compared to the STZ group): the whole gland architecture was greatly restored, as evidenced by numerous mitotic figures and well defined acinar outlines. The acinar portion also displayed granular deeply basophilic cytoplasm (Figures 2d,3d). The GCTs rarely showed degeneration, with preserved intercellular boundaries and normal eosinophilic granular content. Both the acinar and GCT cells displayed fewer intracellular vacuolizations. The striated ducts showed normal cell lining with rare vacuolizations (Figure 2d). Some excretory ducts were dilated, with normal lining and dilated blood vessels congested with RBCs could be seen nearby the excretory duct (Figure 3d).

Immunohistochemical Results

A very weak to mild immunoreactivity for iNOS occured among the whole glandular elements which was slightly enhanced in the duct system in the control group (Figure 4a). While in the untreated diabetic group (STZ group), in the glandular parenchyma, there was a significantly increase in cytoplasmic iNOS immunoexpression (Figure 4b). And in the BV treated diabetic Group (BV+STZ Group), comparing to the STZ group, there was a decrease in iNOS immunoreactivity (Figure 4c). Finally, examining the immunostained sections of the BMSCs treated diabetic group, compared to the STZ group, showed a marked reduction in iNOS immunoreactivity throughout the whole glandular parenchyma (Figure 4d).

Statistical Results

a) iNOS Immunoexpression area %

The STZ group had the highest mean area percent, whereas the control group had the lowest, with a statistically significant difference between groups ($p<0.05$). In a pairwise comparison, the STZ group had a statistically significant larger area percent than other groups ($p<0.05$). The STZ+BV group had a statistically significant higher area % than the STZ+MSCs ($p=0.001$) and control ($p<0.05$) groups. Furthermore, in comparison to the control group, the STZ+MSCs groups had a statistically significant larger area percent ($p<0.05$). (Figure 5).

b) qRT-PCR assay

The STZ+MSCs group had the highest mean HO-1 gene expression, whereas the control group had the lowest, with a statistically significant difference between groups ($p<0.05$).

The pairwise comparison revealed a statistically significant higher expression of the gene in the STZ+MSCs group comparing with STZ+BV ($p=0.012$), STZ and control group ($p<0.05$). Additionally, a statistically significant higher gene expression was noticed in the STZ+BV group as compared to STZ and control group ($p<0.05$). The expression of the gene is statistically significant increased in the STZ group in comparison to the control group($p<0.05$) as well (Figure 6).

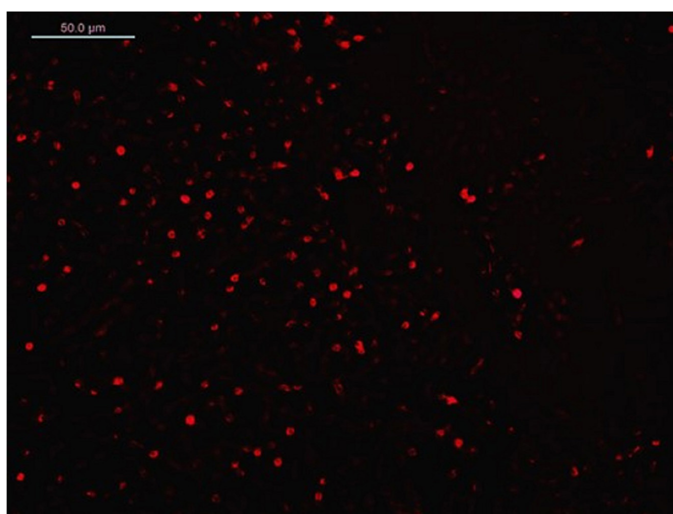

Fig. 1: PKH26-labelled injected BMSCs showing their engraftment in the rat's submandibular salivary gland tissue

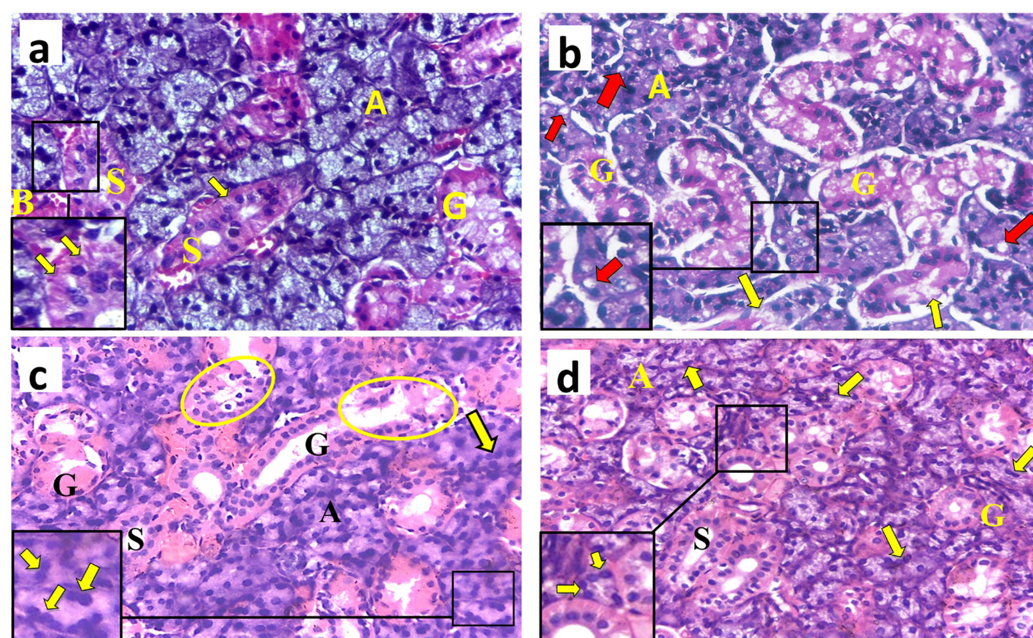

Fig. 2: Photomicrographs of H&E stained sections:
(a) Control group showing normal histological structure; acinar cells (A), GCTs (G), striated ducts (S) and nearby blood vessels (B); a higher magnification inset inset: showing the basal striations of the striated duct (yellow arrows) (b) STZ group with a large number of intracytoblasmic vacuoles (red arrows) in the acinar portions (A) and GCTs (G); a higher magnification inset: showing the cytoplasmic vacuolations, as well as areas of degeneration (yellow arrows). (c) BV + STZ group showing: well-defined boundaries of the acinar portions (A); a higher magnification inset: show some mitotic figures (yellow arrows), GCTs (G) showed in some areas degeneration (yellow circles) and the striated duct (S) rarely displayed degeneration. (d) BMSCs+ STZ group showing: few intracytoplasmic vacuolizations in the acini (A); a higher magnification inset: show some mitotic figures (yellow arrows), GCTs (G) and striated ducts (S) showed normal lining (H&E, Orig. Mag. X400).

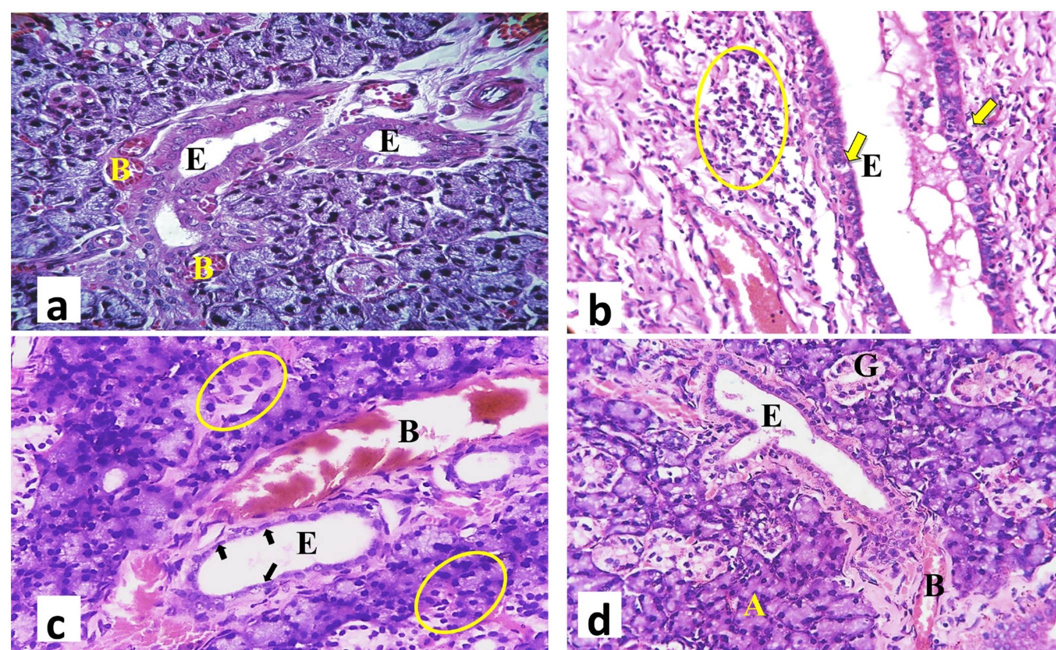

Fig. 3: Photomicrographs of H&E stained sections:
(a) Control group showing: excretory ducts (E), lined with pseudostratified columnar epithelium and blood vessels (B). (b) STZ group showing: dilated excretory duct (E) with loss of pseudostratification, areas of degeneration (yellow arrows) and retained secretion as well as numerous chronic inflammatory cells (yellow oval) fibrous C.T. septa (C). (c) BV + STZ group showing: small areas of loss of glandular architecture (yellow circles), dilated excretory ducts (E) with flattened lining nuclei (black arrows) and dilated blood vessels (B). (d) BMSCs+ STZ group showing: acini (A) & GCTs (G) with few vacuolizations, excretory duct (E) having an almost normal lining and congested blood vessels (B) could also be seen in the C.T. septa (C) which rarely displayed chronic inflammatory cell infiltration (H&E, Orig. Mag. X400).

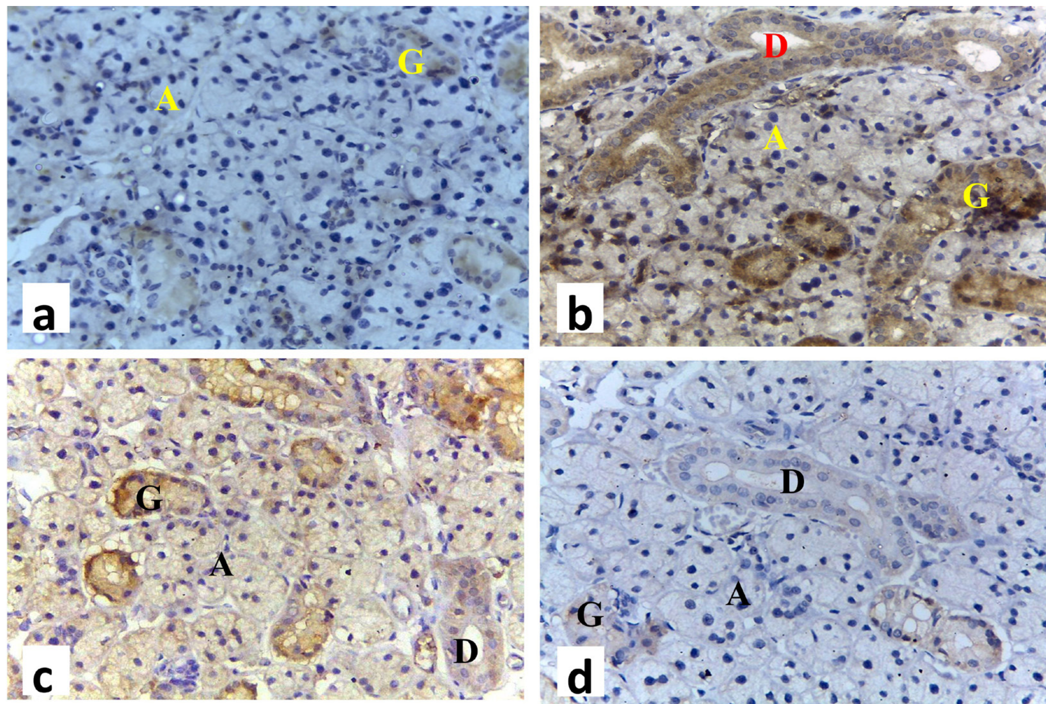

Fig. 4: Photomicrographs of iNOS immunostained sections:
(a) Control group showing: very weak immunoreactivity for iNOS in the acini (A) and mild iNOS immunoreaction the GCT (G). (b) STZ group showing: mild to moderate cytoplasmic iNOS immunoreactivity among the acini (A), while the duct system (G&D) reacted strongly to iNOS. (c) BV + STZ group showing: mild cytoplasmic iNOS immunoreactivity among the acini (A), moderate reaction occurred in the GCT (G), while mild to moderate immunoreactivity was detected in other ducts (D). (d) BMSCs+ STZ group showing: weak immunoreactivity for iNOS in the acinar cells (A) ,while mild to moderate iNOS immunoexpression occurred in the GCT (G), however, the remaining ducts (D) reacted mildy to iNOS (DAB, Orig. Mag. X400).

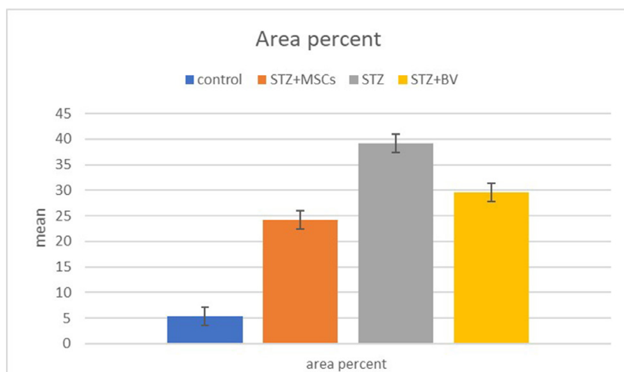

Fig. 5: Bar chart showing mean value of area percent with 95% CI error bars

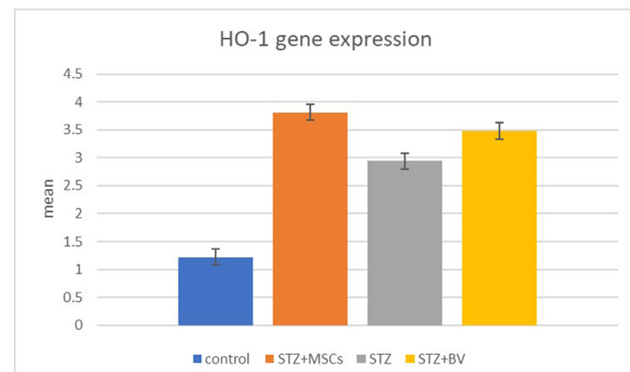

Fig. 6: Bar chart showing mean value of HO-1 gene expression relative to β-actin with 95% CI error bars

DISCUSSION

Hyperglycemia caused by abnormalities in insulin production, insulin effect, or both is a symptom of diabetes mellitus(DM)[1]. The prevalence of DM has increased in growth in recent years which suggests an urgent need to develop additional, innovative ways to improve diabetes management and prevention for global populations[20]. Initial treatment of DM generally includes antidiabetic drugs, however; these drugs have unwanted adverse effects necessitating the development for new treatment modalities[21].

BMSCs have attracted significant attention in DM treatment as they are known to promote the renewal of pancreatic islet β-cells, protect them from apoptosis and ameliorate insulin resistance of peripheral tissues. In addition, venomous products of some animals have been related to the therapies development for a diversity of diseases, as diabetes[22]. Due to its therapeutic effects on disorders such as neurological, cardiovascular, haematological, musculoskeletal, and dermatological diseases, BV is an agent that has been utilised as a medication[23]. Thus, the present study aimed to assess BV therapy versus BMSCs on STZ-induced salivary gland alterations in an attempt to further explore their possible therapeutic applications.

In the ongoing study, diabetes induced degenerative alterations in the rat's submandibular salivary glands including vacuolizations and ductal dilation. Similar

findings were previously reported by Nurdiana et al.[24] who demonstrated that diabetic rats'pancreas displayed destruction in the pancreatic islets with swollen acinar cells filled by small vacuoles. These vacuoles may be attributed to the lipid droplets accumulation, resulting from fatty acids utilization due to reduced cellular activity[25].

In addition, the excretory ducts dilatation and retention of their secretion detected in the current work is greatly in agreement with the results of Rabea[26] who studied parotid salivary glands in STZ-induced diabetic rats and found the histological changes in ductal system. Those alterations could be related to the glandular injury and dysfunction caused by diabetes[27].

In the ongoing investigation, the histological structures of the diabetic submandibular .salivary gland were apparently improved following BV administration. This could be supported by several studies confirming the effects of BV as a well-known antioxidant and anti-inflammatory agent[15]. Regarding the dose and route of administeration which was choosen in the current study was in accordance with Mousavi et al.[15] who found that this dose is the most effective dose to lower blood glucose and lipids in diabetic rats. Additionally, Elkotby et al.[28] reported that it is capable to regenerate the β-cells of islets of langerhans diabetic pancreatic tissues. This was in agreement with Raafat and Hamam[29] who concluded that administering BV improved the histological and ultrahistological structural changes induced by STZ.

In this study, an obviously improved glandular architecture following BMSCs treatment was noticed. These findings could be supported by the most recent findings of Denewar and Amin[30] who noticed a more regular glandular parotid gland architecture following BMSCs injection in diabetic rats. The therapeutic effects of BMSCs on diabetic salivary glands could be attributed to the potent antioxidant effects of BMSCs which provided protection against hyperglycemia induced oxidative stress[31,32]. Besides, Alfaifi et al.[33] suggested that various trophic factors secreted by BMSCs play key therapeutic roles through reducing apoptosis, inflammation, fibrosis in addition to stimulating angiogenesis and tissue regeneration in damaged liver.

Regarding iNOS immunoexpression findings in the current research, they greatly supported the obtained histological findings, where the immunostained sections of the control.group presented very weak to mild immunoreaction for iNOS throughout the whole gland. These findings are similar to those obtained by Zhang et al.[34], who reported that iNOS was shown to be slightly dispersed in some salivary ducts of normal cases, however it was unnoticed in the parenchyma. And this could be related to the fact that iNOS is not constantly present in cells and is only expressed when the cells are induced or stimulated[35]

The STZ group, on the contrary, showed moderate to strong iNOS cytoplasmic immunoreactivity in the acini and ducts of the gland. These findings are confirmed by statistical results as they they revealed a statistically significant higher iNOS area percent as compared to the control group. These results could be supported by the results of Ceriello et al.[36] who demonstrated that hyperglycemia-induced oxidative stress enhanced iNOS expression.

Following BV treatment, a reduction in the cytoplasmic immunoreactivity for iNOS was detected as comparing to the STZ group. Statistically, these results are significantly decreased compared to STZ group. This could be supported by the results of Lee et al.[37], who reported that BV suppresses LPS-induced iNOS activation through regulation of protein kinase C, an intracellular mediator of LPS-induced iNOS and eNOS expression[38,39].

On the other hand, in the BMSCs+STZ group, the cytoplasmic immunoreactivity for iNOS was decreased than both the STZ and BV+STZ groups. these findings are emphasized statistically as the area percent of iNOS immunoexpression significantly decreased than both the STZ and BV+STZ groups. These results are similar to those of Ibrahim et al.[40] who demonstrated that MSCs homed the injured sites, resulting in renal tissue regeneration and decrease in iNOS expression.

Hence, the improved histological glandular architecture and the reduced iNOS immunoexpression level in the BMSCs+STZ group could be related to the potential regenerative properties of MSCs demonstrated by various studies. Among these, a study by Cho et al.[41] concluded that DM progression is excacerbated by diabetic hyperglycemia induced oxidative stress injury. So, the antioxidative and antiapoptotic capability of MSCs may further promote pancreatic islet cell survival.

HO-1 which was used in this study is considered the main protein in diseases arising as a result of oxidative and inflammatory insults[42]. This could be explained according to Yuan et al.[43] who reported that HO-1 is a cytoprotective enzyme that plays a critical role in defending the body against oxidant-induced injury during inflammatory processes, by regulating intracellular levels of pro-oxidant heme, or by other benefits of its byproducts, such as carbon monoxide and biliverdin[44].

In the ongoing study, statistical qRT-PCR results of HO-1 gene expression were greatly coincident with the obtained histological and immunohistochemical iNOS gene expression results. In the diabetic (STZ) group, HO-1 gene expression was significantly increased. It was further significantly increased in both treated groups, being significantly higher in the BMSCs+STZ group than the BV +STZ one. This is in coincidence with Kim et al.[45] who demonstrated that HO-1 mRNA and protein levels of HO-1 were largely increased after LPS injection and were further increased by apamin, a component of BV. The authors suggested that apamin alleviates LPS-induced oxidative stress, at least partially, by modulating HO-1 expression. These results were further confirmed by Kim et al.[46], who

showed that administering MEL significantly reduced acute renal failure and structural damage caused by LPS injection, a main component of BV. The authors attributed this to the significantly enhanced mRNA and protein expression of HO-1 following MEL adminsteration.

On the other hand, in the BMSCs+STZ group, HO-1 gene expression was significantly upregulated more than that in both the STZ and BV+STZ groups. Similar results were observed by Zhang et al.[47] who investigated the preventive and therapeutic potential of IV administered BMSCs in acute liver failure. They concluded that BMSCs ameliorated acute liver failure through significantly increasing HO-1 expression which, consequently, reduced polymorphonuclear leukocyte infiltration and function, therefore, playing important anti-inflammatory and anti-apoptotic roles.

Conclusively, BMSCs treatment exerted an obvious enhancement in a diabetic rat's submandibular salivary gland histological picture, reduced the elevated iNOS immunoexpression and upregulated HO-1 gene expression more than in the BV treated diabetic (BV+STZ) group. These findings are in agreement to a considerable extent with those of Abdelkader[48] who compared the therapeutic effect of BV and MSCs transplantation in treating experimental rabbit model of temporomandibular joint osteoarthritis. They concluded that better histological recovery occurred following MSCs treatment in comparison to BV one.

CONCLUSIONS

Based on the present findings; it could be concluded that STZ-induced DM causes degenerative effects in the submandibular salivary glands, reflecting possible influence on their secretory activity. Following BV or BMSCs treatment, an obvious improvement was noticed in the histological features of the diabetic rat salivary glands. Furthermore, it is noteworthy that BMSCs injection still exerted a more noticeable therapeutic effect as evidenced by histological, immunohistochemical, qRT-PCR and statistical results.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

التأثير العلاجى لسم عسل النحل مقابل الخلايا الجذعية الوسيطة على الغدد اللعابية تحت الفك السفلى فى الفئران المصابة بداء السكرى المستحث بالستربتوزوتوسين

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المقدمة : مرض السكري هو مرض خطير يثير القلق في جميع أنحاء العالم. استخدم علاج سم النحل و الخلايا الجذعية الوسيطة لنخاع العظام على نطاق واسع في علاج الأمراض المختلفة.

هدف العمل: هدفت هذه الدراسة إلى مقارنة تأثير سم النحل (BV) والخلايا الجذعية الوسيطة لنخاع العظام (BMSCs) على الغدد اللعابية تحت الفك السفلي التي يسببها الستربتوزوتوسين (STZ) الفئران المصابة بداء السكري.

المواد وأساليب العلاج: تم استخدام ٤٠ من ذكور الجرذان البيضاء في الدراسة الحالية. تم استخدام خمسة فئران لعزل وزراعة ال BMSCs ، خدمت ٥ جرذان كمجموعة ضابطة، بينما تم توزيع الفئران المتبقية إلى ثلاث مجموعات: مجموعة مرضى السكرى غير المعالجة: تم تلقي جرعة IP واحدة من STZ ، مجموعة مرضى السكري المعالجة بـ BV: تلقت الجرذان المصابة بداء السكري جرعة يومية من IP قدرها ٠,٥ مجم / كجم من BV لمدة ٤ أسابيع، ومجموعة مرضى السكري المعالجة ب BMSCs: تلقت الفئران المصابة بداء السكري حقنة وريدية واحدة من BMSCs. بعد شهر واحد ، تم التضحية بجميع الحيوانات ، تم تشريح الغدد اللعابية تحت الفك السفلي وتجهيزها من أجل فحوصات نسيجية و كيميائية مناعية وتفاعل البوليميراز المتسلسل الكمي في الوقت الحقيقي (qRT PCR). أخيرًا، تم تحليل جميع البيانات التي تم الحصول عليها إحصائيًا.

النتائج: كشفت المجموعة الضابطة عن بنية طبيعية للغدد اللعابية ، بينما كشفت المجموعة المصابة بداء السكري التغيرات الغددية التنكسية. أظهرت المجموعتان اللتان عولجتا من مرض السكري صورًا نسيجية محسّنة ، ومع ذلك؛ كان التحسن أكثر وضوحًا في المجموعة المعالجة بـ BMSCs مقارنةً بالمجموعة المعالجة بـ BV. فيما يتعلق بمجموعة مرضى السكري ، لوحظ زيادة بشكل ملحوظ التعبير المناعي لسينثيز أكسيد النيتريك المحرض (iNOS) والتعبيرلجين هيم أوكسجينيز ـ١ (HO-١). ومع ذلك ، بعد علاجات BV و BMSCs ، لوحظ انخفاض بشكل ملحوظ نشاط المناعة iNOS وزيادة التعبير الجيني HO-١.

الإستنتاج: علاجات BMSCs و BV خففت من الآثار التنكسية لمرض السكري ، حتى الآن ؛ BMSCs أظهر تأثير علاجي ملحوظ أكثر من BV.