Comparative Study between the Effectiveness of Bone Marrow Derived Stem Cells and Insulin on Circumvallate and Foliate Papillae of Diabetic Rats: Histological and Morphological Study

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Abstract

Objectives. To explore the effectiveness of BM-MSCs versus insulin on circumvallate and foliate papillae of diabetic rats.

Materials and methods. The study was carried out on 56 male albino rats that were divided into 4 groups: Control group (Gp 1): rats received no drug. Diabetic group (Gp 2): rats received a single intra-peritoneal injection of Streptozotocin (STZ) (40mg/kg). BM-MSCs treated diabetic group (Gp 3): After diabetes confirmation; rats were given a single intravenous injection of PKH26 labeled BM-MSCs (million units) through tail vein. Insulin treated diabetic group (Gp 4): After diabetes confirmation; rats received subcutaneous injection of insulin (5IU/kg/day). After 4 weeks, half of the tongue specimens were processed and stained by Hematoxyline & Eosin, then examined by light microscope. While the other half were examined by scanning electron microscope.

Results. Circumvallate and foliate papillae of Gp 2 showed significant histological and morphological alterations. While in Gp 3 and 4, the papillae showed noticeable improvements, being more obvious in insulin treated one. Moreover, in Gp 3 and 4, there was a significant increase in body weight in comparison to Gp 2.

Conclusions. BM-MSCs administration could help in reducing the damaging effects of DM on circumvallate and foliate papillae. Insulin therapy caused more efficient improvements in diabetic rats than BM-MSCs.

Keywords
Diabetes, BM-MSCs, Insulin, circumvallate, foliate, SEM

Introduction

Diabetes mellitus (DM) is a devasting chronic metabolic disease that characterized by hyperglycemia with carbohydrates, fat and protein metabolism disturbances. It results from insulin production deficiency (type I) or produced insulin ineffectiveness (type II) [1].

The consequences of long term uncontrolled DM include: Micro-angiopathy that results in nephropathy, retinopathy and neuropathy, and Macro-angiopathy that results in heart and cerebrovascular diseases [2]. Many oral manifestations are usually associated with DM as increased risk of gingivitis and periodontitis with alveolar bone loss, reduction in salivary flow with changes in its composition, increased incidence of buccal infections, candidiasis, dental caries, alteration in taste perceptions and oral burning sensation, finally, delayed wound healing [3].

The World Health Organization [4] reported that the increasing number of diabetics is related to population growth, aging and urbanization that associated by diet alteration, obesity, decreased physical activity and increased stress. The worldwide increase in DM prevalence has highlighted the need for increased researches into treatment modalities for both the disease and its complications.

Insulin is a polypeptide hormone that synthesized and secreted by the beta cells of the pancreas. Insulin secretion is triggered by high blood glucose that taken up by glucose receptors into the beta cells [5]. The strategies for type I DM treatment are based on administration of different regimes of insulin injections with blood glucose levels careful monitoring; however, this cannot mimic the precise pancreatic beta cell regulation of glucose homeostasis and frequently associated with hypoglycaemic episodes [6].

The main goal of future treatment of DM is to promote beta cell regeneration and differentiation with the use of stem cell [7]. BM-MSCs are self-renewing and multi-potent cell populations, having the capacity to differentiate into mesenchymal tissues lineage and efficient trans-differentiate into endodermal and ectodermal linages [8,9,10]. The therapeutic potentials of BM-MSCs in DM are their ability to differentiate into insulin producing cells with their immunomodulation activity and subsequent protective effects on damaged tissues [11].

The current study aim was to investigate the comparative effect of BM-MSCs and insulin on circumvallate and foliate papillae of streptozotocin (STZ) induced diabetic albino rats.

Materials and methods

In this study, 56 male albino rats with weight range from 200-250g were used. All the animals were housed in a sterile, controlled environment (temperature 23±5°C and 12 h dark/light cycles) that fed a standard pellet diet and tap water ad libitum. The experimental procedures were conducted in the animal house of "The Medical Research Center" Ain-Shams University, in accordance with the recommendations and approval of the "Research Ethics Committee" Faculty of Dentistry-Ain Shams University. (Approval No: FDASU-RECD071701).

After a week of acclimatization, the rats were equally divided into 4 groups, 14 rats in each:

- Control group (Gp 1): Included normal healthy rats without receiving any drug.
- Diabetic group (Gp 2): A single intra-peritoneal injection of STZ (40mg/kg) was given to the rats which dissolved before administration in 1ml citrate buffer [12]. DM was defined as fasting blood glucose (FBG) reading of greater than (250mg/dl) after 3 days of STZ injection.
- BM-MSCs treated diabetic group (Gp 3): After diabetes confirmation; a single intravenous injection of (1×10^6 cells) of PKH26 labeled BM-MSCs (obtained from "The Tissue Culture Unit", Biochemistry Department, Faculty of Medicine, Cairo University) suspended in (1ml) phosphate buffer saline was given to the rats through the tail vein [13].
- Insulin treated diabetic group (Gp 4): After diabetes confirmation; a single subcutaneous injection of insulin (5IU/kg/day) was given to the rats for 4 weeks [14].

After 4 weeks of diabetes confirmation, the rats were separately sacrificed by over dose of general anesthesia then their tongues were immediately dissected. Half of the collected specimens were processed and examined by light and fluorescent microscopes, while the other half examined by scanning electron microscope.

a- Light microscopic (LM) examination

The specimens were fixed immediately in 10% neutral formalin for 2 days, then dehydrated in ascending grades of alcohol and embedded in paraffin wax. Histological sections (4μm) were prepared and subjected to Hematoxylin and Eosin (H&E) stain.

b- Fluorescent microscopic (FM) examination

Unstained sections after deparaffined by xylene, examined by phase contrast FM for detecting cells labeled with PKH26 dye. The examination was done at "The Tissue Culture Unit", Biochemistry Department, Faculty of Medicine, Cairo University.

c- Scanning electron microscopic (SEM) examination

The other half of tongue specimens were fixed by 2.5% glutaraldehyde for 1.5-2h, then placed in post-fixative for 2h. Dehydration of the samples was done by ethanol that was replaced with carbon dioxide. Finally, the samples were coated with gold and examined by the SEM at "the Regional Center of Mycology and Biotechnology", Al Azhar University.

d- Statistical Analysis

Data were checked for normality by using Kolmogorov-Smirnov and Shapiro-Wilk tests. To compare between the four groups, ANOVA test was performed. When ANOVA test was significant, Bonferroni's post-hoc test was done for pair-wise comparisons. The significance level was set at $P \leq 0.05$. Statistical analysis was performed using SPSS for Windows Version 21.

Results

1. Light microscopic results for H&E stained sections

A. Circumvallate papilla

- Gp 1: An inverted cone-shaped papilla was detected that surrounded by a well-developed deep trough. The papilla was covered by orthokeratinized stratified squamous epithelium with numerous well-defined taste buds in the epithelial wall of the trough. A central core of dense intact fibrous connective tissue (c.t) was observed (Fig.1a & 2a).
- Gp 2: The papilla was markedly shrunken with wide trough. It covered by torn and separated keratin. In addition, the taste buds appeared shrunken and distorted. The c.t revealed degenerated areas with dilated blood vessels (b.vs) and inflammatory cells infiltrate (Fig.1b & 2b).
- GP 3: The papilla showed normal shape with narrow trough. Most of taste buds were well-defined, while others were distorted. Few areas of c.t destruction, inflammatory cells infiltrate and dilated b.vs were seen (Fig.1c & 2c).
- GP 4: The trough surrounding the papilla was narrow and taste buds in its wall were well-defined. The c.t showed few inflammatory cells infiltrate (Fig.1d & 2d).

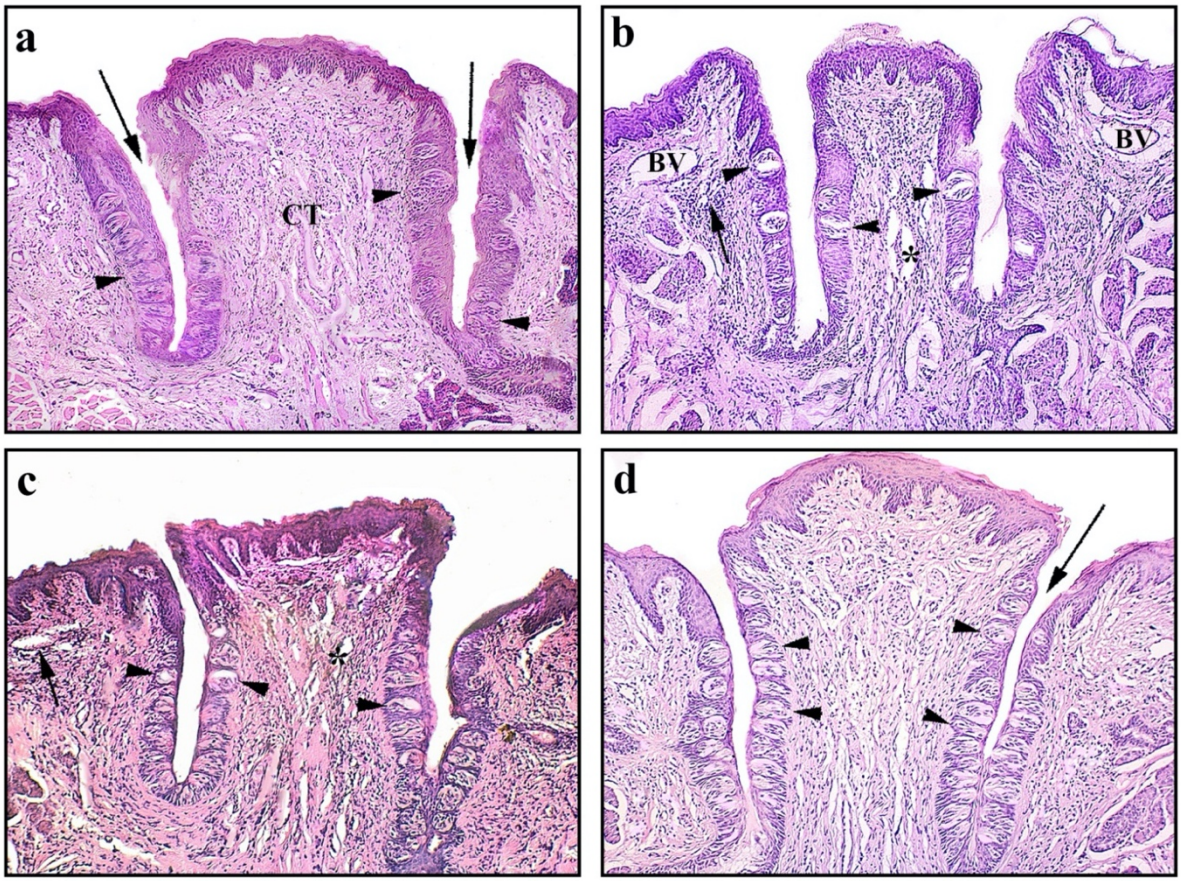

Fig.1. Photomicrographs of circumvallate papillae. **a)** Gp 1 showing; a well-developed deep and narrow trough (arrows) with many taste buds (arrow heads) and dense fibrous c.t (CT). **b)** Gp 2 showing; markedly shrunken papilla, surrounded by wide trough with distorted taste buds (arrow heads), degenerated areas in the c.t (*) with

dilated b.vs (BV) and inflammatory cells infiltrate (arrow). **c)** Gp 3 showing; most of taste buds were well-defined, while others were distorted (arrow heads), some areas of c.t destruction (*) with dilated b.v (arrow). **d)** Gp 4 showing; inverted cone shaped papilla that surrounded by narrow trough (arrow) with numerous taste buds (arrow heads) (H&E, original mag. x 100).

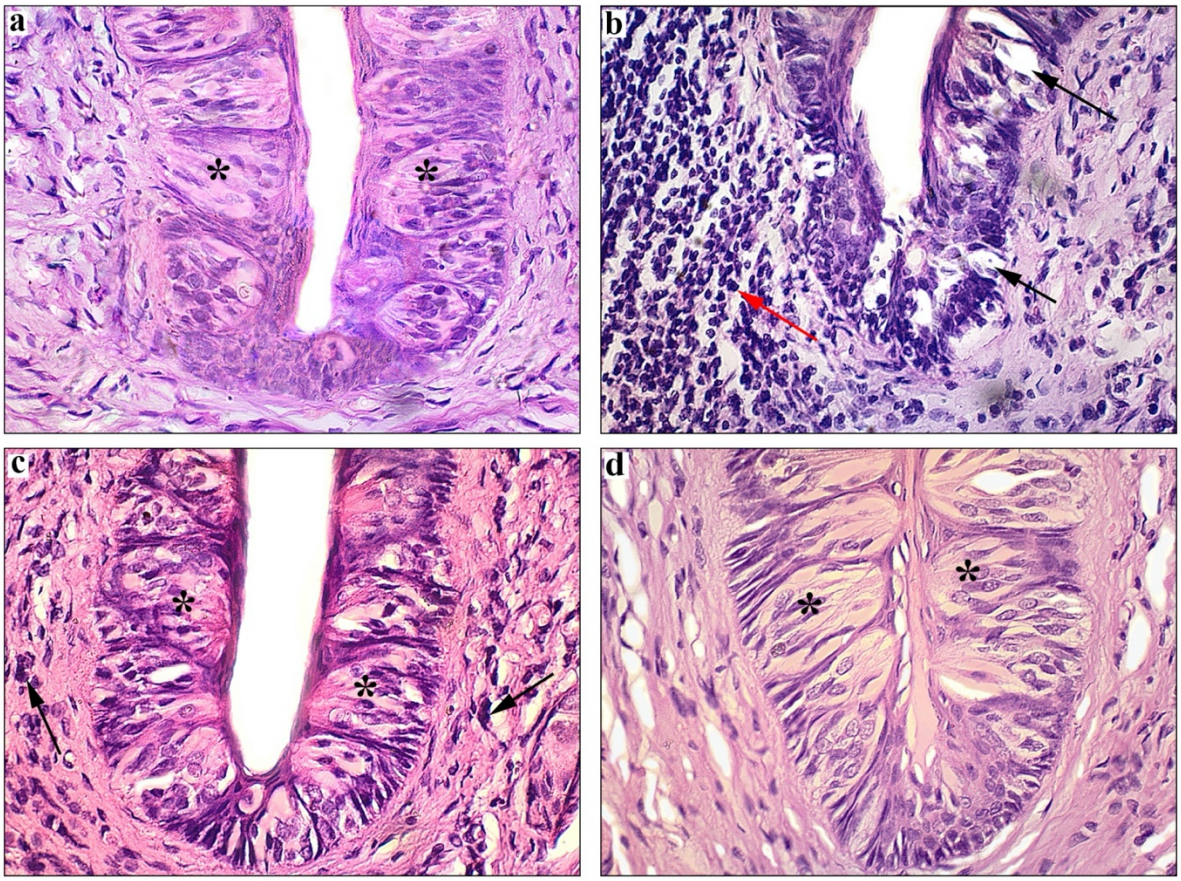

Fig.2. Photomicrographs of lower part of circumvallate papillae troughs. **a)** Gp 1 showing; a well-formed taste buds with normally arranged cells (*). **b)** Gp 2 showing; distorted taste buds (black arrows) and inflammatory cells infiltrate (red arrow). **c)** Gp 3 showing; well-defined taste buds (*) with few inflammatory cells (arrows). **d)** Gp 4 showing; numerous normal taste buds (*) (H&E, original mag. x 400).

B. Foliate papillae

- Gp 1: The papillae appeared as series of parallel ridges, covered by orthokeratinized stratified squamous epithelium. The troughs between papillae appeared narrow and uniform with several taste buds along their wall. Intact fibrous c.t cores were observed with many secondary c.t papillae (Fig.3a & 4a).
- Gp 2: The papillae appeared shrunken with apparently wide troughs with areas of torn and separated keratin layer. The taste buds were distorted and the c.t was degenerated with many congested and dilated b.vs (Fig.3b,c & 4b).
- GP 3: The foliate papillae were surrounded by wide troughs. The taste buds appeared normal. In some areas within c.t, inflammatory cells infiltration and congested b.vs were detected (Fig.3d & 4c).

- GP 4: The papillae appeared as parallel ridges, separated by narrow troughs and the taste buds were well-defined. Fibrous and intact c.t cores were seen (Fig.3e & 4d).

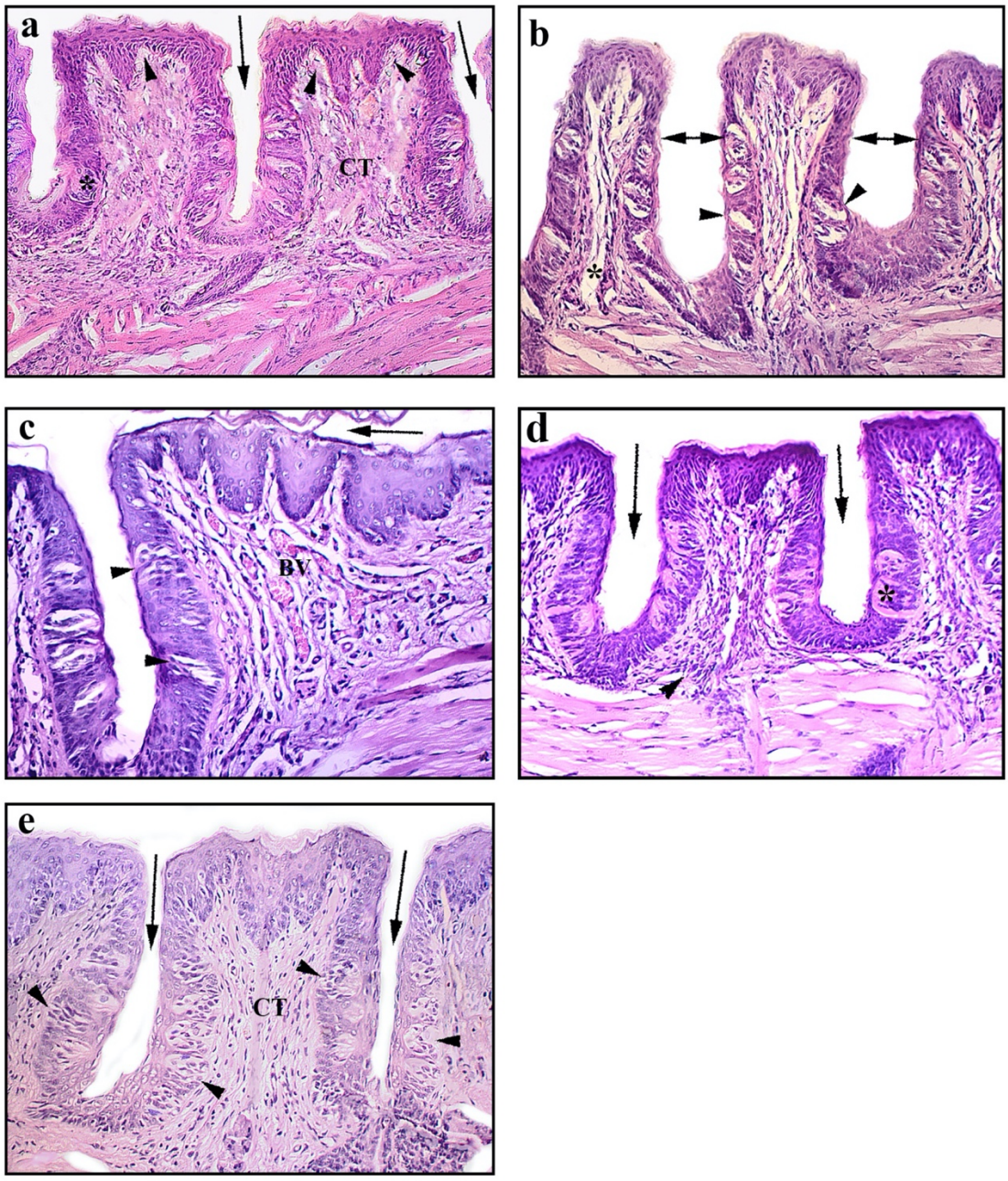

Fig.3. Photomicrographs of foliate papillae. a) Gp 1 showing; deep and narrow troughs (arrows) with well-defined taste bud (*), intact c.t cores (CT) and many secondary papillae (arrow heads). b) Gp 2 showing; shrunken papillae with wide troughs (double arrow) and distorted taste buds (arrow heads) with degenerated c.t (*). c) Gp 2 showing; separated keratin layer (arrow) and ill-defined taste buds (arrow heads) with dilated and congested b.vs (BV). d) Gp 3 showing; wide troughs between papillae (arrows) and normal taste bud (*) with inflammatory cells infiltration (arrow head). e) Gp 4 showing; parallel ridges, separated by narrow troughs (arrows), well-defined taste buds (arrow heads) and intact fibrous c.t cores (CT) (H&E, original mag. x 200).

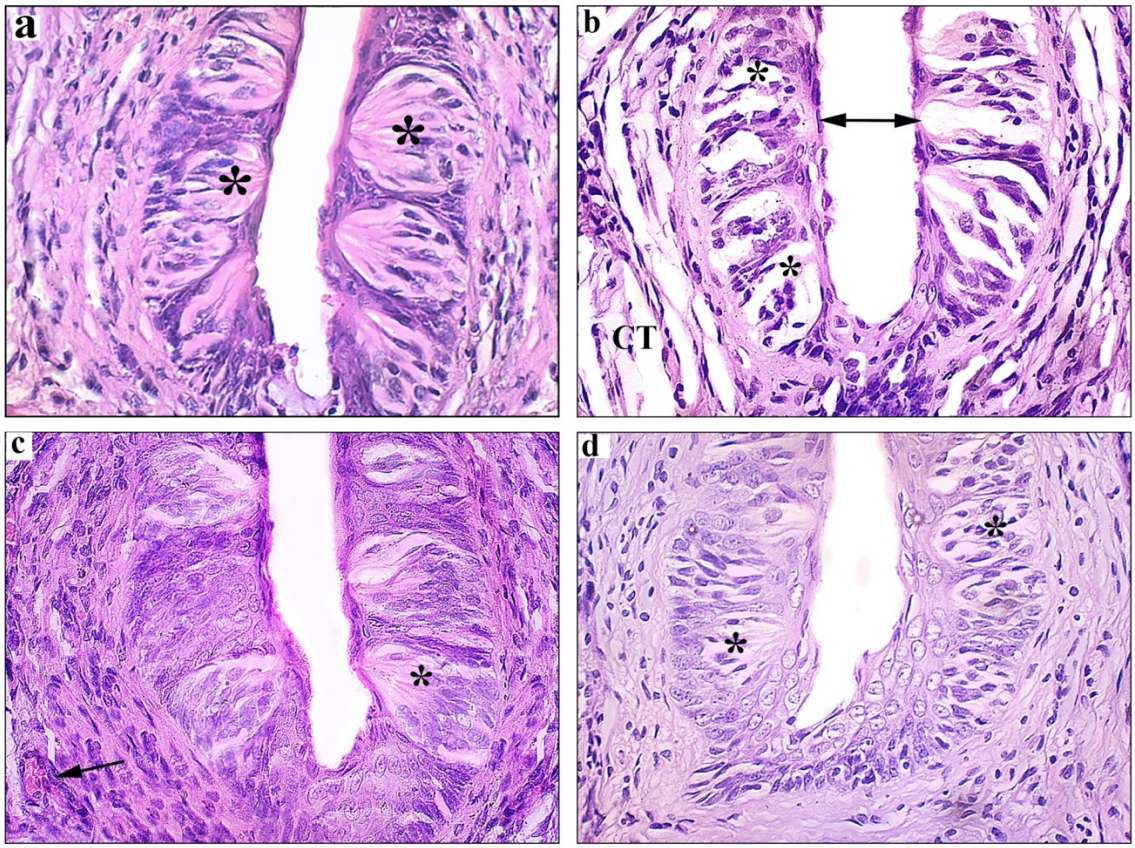

Fig.4. Photomicrographs of lower part of foliate papillae troughs. a) Gp 1 showing; well-defined taste bud (*). b) Gp 2 showing; distorted taste buds (*) with degenerated c.t (CT). c) Gp 2 showing; separated keratin layer (arrow) and ill-defined taste buds (arrow heads) with dilated and congested b.vs (BV). d) Gp 3 showing; normal taste bud (*) with congested b.v (arrow). e) Gp 4 showing; well-defined taste buds (*) (H&E, original mag. x 400).

2. Fluorescent microscopic results

The phase contrast fluorescent microscopic examination of Gp 3 showed red fluorescent PKH26 labeled cells in the epithelium, c.t and taste buds of circumvallate and foliate papillae (Fig.5a,b).

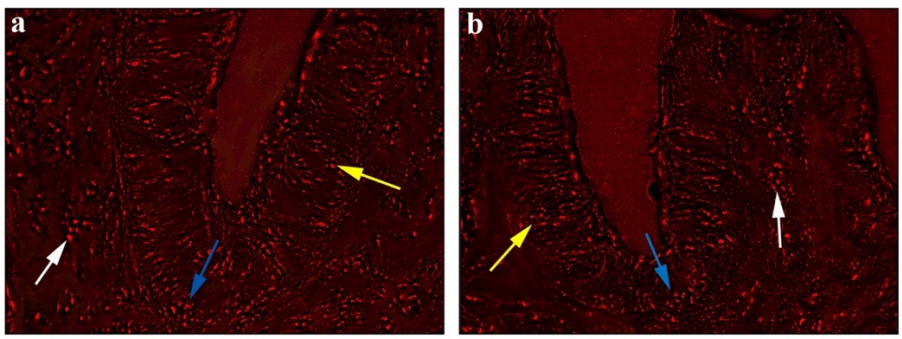

Fig. 5. Fluorescent photomicrographs of Gp 3. a) Circumvallate papillae, b) foliate papilla showing; red fluorescent PKH26 labeled cells in the epithelium (blue arrow), c.t (white arrow) and taste buds (yellow arrow). (PKH26, original mag. x 200).

3. Scanning electron microscopic results

A. Circumvallate papilla

- Gp 1: The central papillary structure of the papilla had tapered end and micro ridges on its surface with horse shoe shaped elevated flanking papillary structure and narrow trough surrounding it (Fig.6a).
- Gp 2: The central part of the papilla appeared noticeably distorted with less detected micro ridges on its surface. The surrounding trough was markedly widened (Fig.6b).
- GP 3: The papilla appeared normal and surrounded by trough which was wide in some areas and narrow in others (Fig.6c).
- GP 4: The central papillary part appeared to be somewhat bulging. In some areas, the flanking papillary structure was well-defined, while in others, it was ill-defined. The surrounding trough was narrow (Fig.6d).

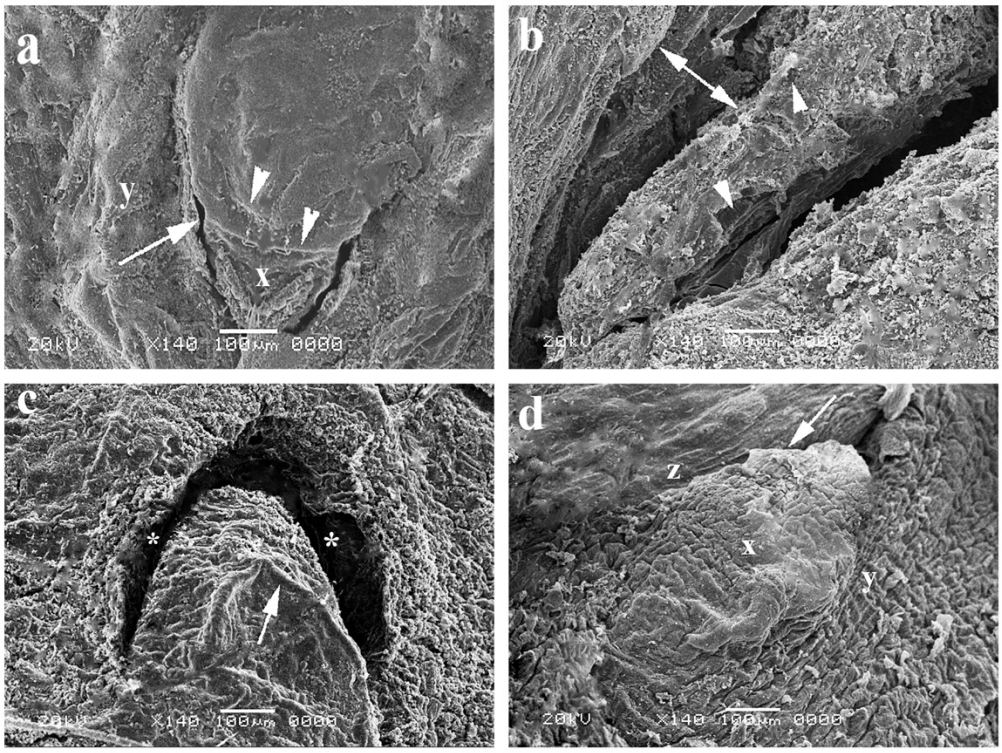

Fig.6. Scanning electron micrographs of circumvallate papillae. a) Gp 1 showing central papillary structure (x) and micro ridges on its surface (arrow heads) that surrounded by elevated flanking papillary structure (y) and narrow trough (arrow). b) Gp 2 showing; torn keratin (arrow heads) and ill-defined flanking papillary structure with widened trough (double arrow). c) Gp 3 showing; micro ridges on central papillary structure surface (arrow) surrounded by uneven thickness of the trough (*). d) Gp 4 showing; bulging central papillary structure (x) surrounded by well-defined flanking papillary structure in some areas (y) and ill-defined in others (z) and narrow trough (arrow) (x 140).

B. Foliate papillae

- Gp 1: The papillae appeared as 3-4 prominent parallel ridges that separated by narrow grooves (Fig.7a).
- Gp 2: The papillae showed noticeable distortion in their shape which appeared shrunken with wide grooves between them (Fig.7b).

- GP 3: The papillae revealed series of prominent ridges, separated by slightly wide grooves (Fig.7c).
- GP 4: The papillae demonstrated four parallel ridges which were separated by narrow grooves (Fig.7d).

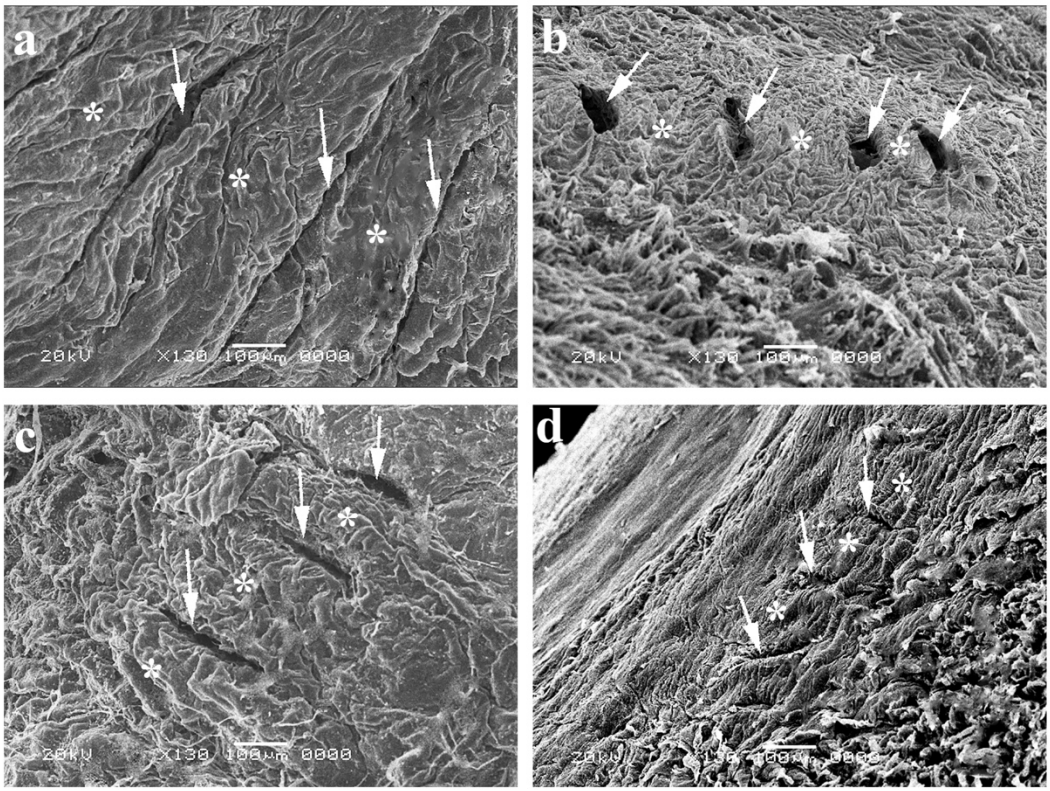

Fig.7. Scanning electron micrographs of foliate papillae. a) Gp 1 showing; 3-4 prominent parallel ridges (*) which were separated by narrow grooves (arrows). b) Gp 2 showing; distorted shrunken papillae (*) with wide grooves (arrows). c) Gp 3 showing; series of prominent ridges (*) that were separated by slightly wide grooves (arrows). d) Gp 4 showing; parallel ridges (*) and narrow grooves (arrows) (x 130).

4. Statistical results

Comparison of BW after 4 weeks of diabetes confirmation between the different groups:

- Gp 1 showed the significantly highest mean BW. Gp 4 showed significantly lower value followed by Gp 3. Gp 2 showed the significantly lowest mean BW (Table 1& Fig.8).

Table 1. The mean, standard deviation (SD) values and results of repeated measures ANOVA test for comparison of BW between the different groups

	Gp 1 (n = 14)		Gp 2 (n = 14)		Gp 3 (n = 14)		Gp 4 (n = 14)		*P*-value	Effect size (Partial Eta Squared)
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
BW	255 ^{A}	3.1	171 ^{D}	4	200 ^{C}	3.4	230 ^{B}	4.4	<0.001 *	0.987

- *: Significant at $P \leq 0.05$, Different superscripts in the same row are statistically significantly different.

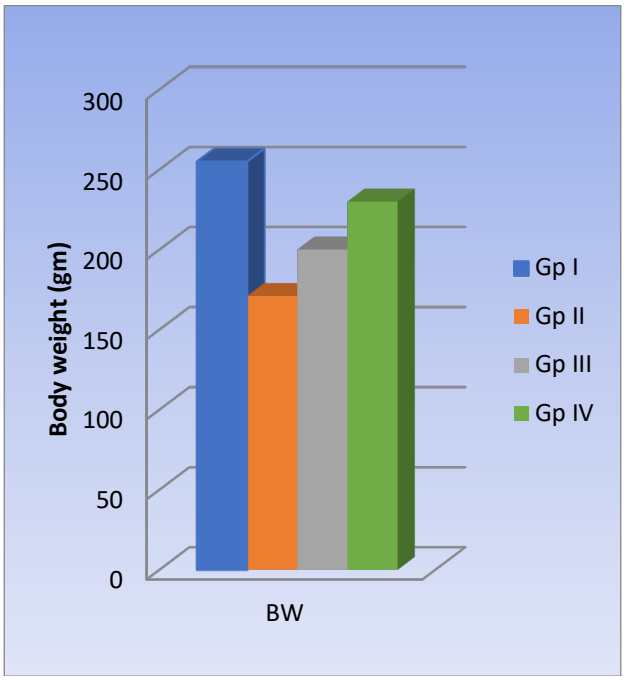

Fig.8. Bar chart representing comparison of mean BW between the four groups.

Discussion

The present study was conducted to evaluate the possible therapeutic effect of BM-MSCs in comparison to insulin therapy on circumvallate and foliate papillae of diabetic rats.

In this research, STZ was the drug of choice for diabetic induction as single intra-peritoneal injection of (40mg/kg) STZ caused beta cells irreversible destruction and induced experimental DM in 2-3 days, similar to previous study [12].

Intravenous injection of ($1x10^6$) BM-MSCs per rat through the tail vein was used in this study which could influence regeneration of pancreatic islet in STZ-induced diabetes with significant reduction of FBG level as revealed by Afifi [13].

In this work, circumvallate and foliate papillae of diabetic group showed detectable disturbance in their shape that was demonstrated in both LM and SEM examinations. The papillae were shrunken with markedly widened surrounding troughs. These atrophic lesions of the tongue were contributed to changes in innervation and microvasculature (that leads to tissue hypoxia), in addition to chronic inflammation associated with DM [15].

It is worth mentioning here that patients with DM were reported to suffer from lower rates of saliva with marked dysfunction of the secretory capacity of the salivary glands [16,17]. It was also proved that in diabetic rats there was a decrease in the concentration of epidermal growth factor in saliva which might greatly affect the rate of cell division [18]. So, it could be suggested that the dramatic effect of DM on salivary function might indirectly cause atrophy

to the circumvallate and foliate papillae seen in the present study as documented by American Dental Association [19].

In consistence with taste buds observations in diabetic group of this study, prior researchers [20] examined the innervations of the circumvallate papillae and taste buds in diabetic and control rats by a morphometric and quantitative immunohistochemical analysis. They concluded that in diabetic group, the innervation and number of taste cells was significantly reduced. These findings suggest that taste buds are highly dependent on innervations for maintenance and function, so any change in the taste buds could be related to neuropathological changes of diabetes. Additionally, it might be possible that the lower blood supply as a result of DM associated micro-angiopathy to gustatory lingual papillae led to decrease in the total number of taste buds and taste cells [21].

In BM-MSC treated diabetic group, the examinations revealed significant improvements in the histological and morphological picture of circumvallate and foliate papillae compared to the diabetic group. This result came in accordance with Aboushady et al. [22] and ElSaadany et al. [23] who displayed in their studies few smaller areas of c.t degeneration and fewer dilated blood vessels with increased epithelial thickness of the irradiated rat's tongue after intravenous administration of mesenchymal stem cells (MSCs). They stated that "MSCs are capable to restore the integrity of epithelium by maintaining cellular and tissue homeostasis, through the specific regulation of epithelial cells and reduce radiation induced-apoptosis in tongue epithelium". As well, it was previously suggested that the significant promoted recovery of dextran sulfate sodium induced-colitis by MSC transplantation was due to enhancing cell cycle and inhibiting apoptosis in the epithelium [24].

A major finding in the current study was that maintenance of a normoglycemic state in diabetic animals was vital to preserve the normal structure of circumvallate and foliate papillae. Thus, in comparison to the deteriorative effects experienced by rats with uncontrolled hyperglycemia recorded here, the papillae in animals that are diabetic but received insulin succeeded to maintain their histological and morphological features nearly comparable to that seen in control group. Sun et al. [25] reported that insulin, as glucose-lowering hormone in the body, not only alleviates hyperglycemia detrimental effects through its metabolic regulation, but also directly modulates inflammatory mediators and acts upon immune cells. In addition; prior work [26] revealed that diabetic rats treated with insulin showed increase in ventral prostate epithelial thickness with reduced apoptotic index.

In statistical results, Gp 2 in this study showed a significant decreased BW. Prior research claimed that BW loss detected in diabetic rats could be associated with their inability to metabolize carbohydrates [27]. According to Gp 3, the rats revealed a significant increase in their BW that were significantly higher than that of the diabetic rats. This result could be related to the effect of BM-MSCs in improving the plasma insulin level with decreasing hyperglycemia and correction of the BW [28]. The rats in Gp 4 of the current study also showed increased values that were significantly higher than both Gp 2 and Gp 3. This might be related to the retained levels of glucose and insulin [29].

Conclusions

BM-MSCs and insulin therapies could help in decreasing the harmful effects of diabetes on circumvallate and foliate papillae, while being more obvious in insulin one.

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