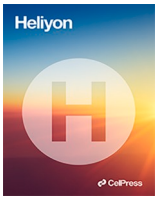
Heliyon 10 (2024) e29362

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Improvement effect of gemigliptin on salivary gland dysfunction in exogenous methylglyoxal-injected rats

Woo Kwon Jung, Su-Bin Park, Hwa Young Yu, Junghyun Kim *

Department of Oral Pathology, School of Dentistry, Jeonbuk National University, Jeonju, 54896, South Korea

ARTICLE INFO

Keywords:
Gemigliptin
Methylglyoxal
Hyposalivation
Salivary hypofunction
Anti-glycation
Diabetes

ABSTRACT

The symptom of hyposalivation associated with hypofunction of the salivary glands is a common feature of diabetes. Inadequate saliva production can cause tissue damage in the mouth, making it susceptible to infections and leading to oral health diseases. Previous studies have highlighted the harmful effects of methylglyoxal (MGO) and MGO-derived advanced glycation end products (AGEs) in diabetes. In this study, we investigated the protective effects of gemigliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor, against MGO-induced salivary gland dysfunction. MGO treatment of immortalized human salivary gland acinar cells induced apoptosis via reactive oxygen species (ROS)-mediated pathways, but this effect was mitigated by gemigliptin. In vivo experiments involved the simultaneous administration of MGO (17.25 mg/kg) with aminoguanidine (100 mg/kg) and gemigliptin (10 and 100 mg/kg) daily to rats for two weeks. Gemigliptin increased the saliva volume and amylase levels in MGO-injected rats. Gemigliptin reduced the DPP-4 activity in both the salivary glands and serum of MGO-injected rats. Furthermore, gemigliptin exerted anti-glycation effects by reducing the accumulation of AGEs in the saliva, salivary glands, and serum and suppressing the expression of the receptor for AGEs. These actions protected the salivary gland cells from ROS-mediated apoptosis. Overall, gemigliptin protected the salivary gland cells from ROS-mediated cell death, reduced the accumulation of amylase and mucins in the salivary glands, and enhanced the salivary function by upregulating aquaporin 5 expression, and it exerted protective effects against MGO-induced salivary gland dysfunction by enhancing the anti-glycation, antioxidant, and salivary secretion activities. Our findings suggest gemigliptin as a potential therapeutic for patients with salivary gland dysfunction caused by the complications of diabetes.

1. Introduction

Saliva contributes significantly to the maintenance of oral health and homeostasis, and it acts as an antimicrobial and assists digestion, taste, and cleansing [1,2]. The symptom of hyposalivation associated with hypofunction of the salivary glands is a common feature of diabetes [3]. When saliva production is insufficient, the oral cavity is vulnerable to infection due to tissue damage [4]. Salivary secretion disorders are currently treated temporarily, but underlying causes remain unaddressed [5]. Therefore, mechanisms

Abbreviations: AGEs, advanced glycation end products; AQP5, aquaporin 5; DPP-4, dipeptidyl peptidase-4; GLP-1, glucagon-like peptide 1; MGO, methylglyoxal; RAGE, receptor for advanced glycation end products; ROS, reactive oxygen species.

* Corresponding author.
E-mail address: dvmhyun@jbnu.ac.kr (J. Kim).

https://doi.org/10.1016/j.heliyon.2024.e29362
Received 20 November 2023; Received in revised form 1 April 2024; Accepted 7 April 2024
Available online 9 April 2024

of salivary gland dysfunction caused by underlying conditions should be further explored to improve the salivary gland functions.

Persistently high blood glucose levels increase the accumulation of advanced glycation end products (AGEs), which alter the protein function [6]. Under hyperglycemic conditions, methylglyoxal (MGO) is the precursor of AGEs that affects aging and diabetes via dietary intake [7]. MGO stress, characterized by the abnormal accumulation of MGO, increases the protein and DNA modifications. This triggers the formation of AGEs, impairs the cell and tissue functions, and contributes to the development of various diseases. MGO induces the formation of AGEs in both intracellular and extracellular proteins, lipids, and DNA [8,9], acts as a regulator of insulin resistance [10], and causes structural and functional abnormalities in insulin, contributing to the development of insulin resistance [11]. AGEs are significantly associated with dental and periodontal pathologies. Furthermore, AGE levels in the saliva correspond to their levels in other bodily fluids and are associated with both general and oral pathologies [12].

In metabolic disorders, such as diabetes, elevated levels of MGO increase the oxidative stress, thereby affecting the cell and tissue functions [13]. Hyperglycemia induces excess superoxide production, leading to oxidative damage in patients with hyperglycemia and diabetes [14]. ROS contribute substantially to the complications associated with diabetes because diabetics produce more reactive oxygen species (ROS) and have reduced antioxidant defense mechanisms [15]. Oxidative stress triggers lipid peroxidation, DNA oxidation, protein oxidation, and disturbances in nitric oxide synthesis in cells [16]. Polydatin improves the diabetes-related salivary gland dysfunction via its antioxidant and anti-glycation effects [17,18]. Increased ROS levels can lead to salivary gland dysfunction. A person with diabetes may suffer from hyposalivation and hypofunctioning salivary glands as a result of poor blood circulation in the salivary glands, dehydration, and impaired blood sugar control [5]. Accumulation of MGO contributes to salivary gland dysfunction by inducing oxidative stress, circulatory disturbances, and internal functional impairment in patients with diabetes and associated complications.

For the treatment of type 2 diabetes, dipeptidyl peptidase-4 (DPP-4) inhibitors are used because of their low side effects and excellent safety profile. In 2012, the Korean Food and Drug Administration approved gemigliptin, a DPP-4 inhibitor developed by LG Life Sciences (Seoul, Korea), as a therapeutic for type 2 diabetes. Through inhibition of glucagon-like peptide 1 (GLP-1), DPP-4 inhibitors inhibit incretin hormone inactivation and influence their physiological effects. This incretin hormone stimulates glucose-dependent insulin secretion and suppresses glucagon secretion in the L cells to regulate blood glucose levels [19,20]. There is evidence that DPP-4 inhibitors protect against diabetic complications in various ways [21]. Gemigliptin exerts anti-glycation effects. In vitro and in vivo studies have shown that gemigliptin inhibits AGE cross-linking and exerts anti-glycation effects [22]. Moreover, gemigliptin promotes salivary gland function through antioxidant and antiapoptotic mechanisms in diabetes [23]. However, the anti-glycation and salivary gland function-improving effects of gemigliptin remain ambiguous, warranting further studies on its action mechanisms and therapeutic effects. Therefore, we examined whether gemigliptin may protect against MGO-induced salivary gland dysfunction regardless of its ability to lower blood glucose, one of these DPP4 inhibitors' intrinsic functions.

2. Materials and methods

2.1. Cell cultures

Immortalized human salivary gland acinar cells (SGACs) were kindly provided by Professor Sang-Gun Ahn (Department of Oral Pathology, School of Dentistry, Chosun University, South Korea) and cultured on a keratinocyte serum-free medium (Gibco BRL, Grand Island, NY, USA) supplemented with 100 U/mL penicillin and 100 μg/mL streptomycin. Cultures were maintained in a humidified atmosphere of 95 % air and 5 % CO_2 at 37 °C.

2.2. Cell viability assay

Cells were seeded in a 96-well plate at a density of 1×10^4 cells. After reaching 70 % confluency, the cells were treated with various concentrations of gemigliptin (0, 2.5, 5, 10, 25, 50, and 100 μM; LG Life Sciences, Seoul, Korea), aminoguanidine (AG; 0, 2.5, 5, 10, 25, 50, and 100 μM; Sigma Aldrich, Louis, MO, USA), and MGO (0, 50, 100, 200, 300, 400, 500, and 600 μM; Sigma Aldrich) for 24 h, following the group conditions. Cell survival rates were assessed using the 3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide (MTT) assay kit (Sigma Aldrich). Cell viability in each well was determined using the Spark Multimode Microplate Reader (Tecan, Männedorf, Switzerland).

2.3. Measurement of ROS levels

Cells were seeded in a 96-well plate at a density of 1×10^4 cells. After 24 h of treatment with MGO (500 μM) and gemigliptin (0, 0.25, 0.5, 1, 2.5, 5, and 10 μM), cells were treated with a final concentration of 10 μM 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA; Sigma Aldrich) for 30 min and washed with Hanks' Balanced Salt solution (Welgene, Gyeongsan, Korea). DCFH-DA-positive signal intensity was measured using the Spark Multimode Microplate Reader (Tecan). Green fluorescence intensity of DCFH-DA-positive cells was detected using a fluorescence microscope (BX51; Olympus, Tokyo, Japan).

2.4. Western blotting analysis

Protein analysis was performed using cell and tissue lysates. Western blotting was performed as described previously [24]. The membranes were probed with Bax rabbit monoclonal (1:1000; Cat. # ab32503, Abcam, Waltham, MA, USA), Bcl2 mouse monoclonal

(1:1000; Cat. # ADI-AAM-072-E, Enzo, Beverly, MA, USA), caspase-3 rabbit monoclonal (1:1000; Cat. # 14220, Cell Signaling Technology, Beverly, MA, USA), caspase-9 mouse monoclonal (1:1000; Cat. # 9508, Cell Signaling Technology), cleaved caspase-9 rabbit monoclonal (1:1000; Cat. # 9507, Cell Signaling Technology), PARP rabbit monoclonal (1:1000; Cat. # 9532, Cell Signaling Technology), β-actin mouse monoclonal (1:2000; Cat. # sc-8432, Santa Cruz Biotechnology, CA, USA), catalase rabbit monoclonal (1:2000; Cat. # 14097, Cell Signaling Technology), SOD1 rabbit monoclonal (1:2000; Cat. # 37385, Cell Signaling Technology), AGE mouse monoclonal (1:1000; Cat. # KH001, TransGenic, Inc., FUK, JP), and aquaporin 5 (AQP5) rabbit polyclonal (1:1000; Cat. # BS3477, Bioworld Technology, Louis Park, MN, USA) antibodies followed by incubation with appropriate secondary antibodies.

2.5. Animals

Sprague-Dawley (SD) rats aged six weeks were obtained from Damul Science in Daejeon, Korea. MGO was administered for two weeks at a dosage of 17.25 mg/kg to induce salivary gland dysfunction. A week after acclimatization, the rats were randomly split into five groups as shown in Table 1. The administration was repeated once daily for two weeks. All animal procedures were approved by the Institutional Animal Care and Use Committee of Jeonbuk National University Laboratory Animal Center (IACUC approval no.: JBNU 2020-0148).

2.6. Saliva collection

Savliva collection was performed as described previously [24]. To collect saliva, cotton balls were placed in the rats' mouths for 15 min, centrifuged, and the saliva was collected.

2.7. Histopathological examination via hematoxylin and eosin (H&E) staining

Salivary gland tissues were fixed with 10 % formalin and rinsed with running tap water to remove all residual fixatives. After dehydration with ethanol, the tissues were embedded in paraffin and sectioned into 4-μm slices. Finally, H&E staining was performed for histological evaluation. The microscopic evaluation was analyzed by three oral pathologists in a blinded fashion.

2.8. Apoptosis analysis via TdT-mediated dUTP nick-end labeling (TUNEL) staining

We analyzed apoptosis in the salivary gland tissues of rats. The samples were fixed with formaldehyde, dehydrated, embedded in paraffin blocks, and sectioned. Next, the tissue sections were deparaffinized twice in xylene (5 min each) and rehydrated with ethanol. TUNEL assay was performed as described previously [24]. ImageJ 1.53 software (NIH, Bethesda, MD, USA) was used to determine the number of TUNEL-positive cells. Salivary gland sections were analyzed in five different fields from each (n = 5) section.

2.9. Histochemical examination via periodic acid-Schiff (PAS) and alcian blue staining

We stained the sections with PAS and alcian blue to assess neutral mucin accumulation and acidic mucin accumulation, respectively. Staining with PAS and alcian blue was performed as described previously [24].

2.10. Immunohistochemistry (IHC) assay

Immnunohistochemical staining was performed as described previously [24]. The following primary antibodies were used: 8-hydroxy-2′-deoxyguanosine (8-OHdG; 1:1000; Abcam), high mobility group box 1 (HMGB1; 1:1000; Cell Signaling Technology), AGEs (1:500; TransGenic, Inc.), receptor for AGEs (RAGE; 1:500; Santa Cruz Biotechnology), and AQP5 (1:500; Bioworld Technology). In order to quantify the optical density, the average optical density was measured at 200 × magnification using the ImageJ software (NIH). Salivary gland sections were analyzed in five different fields from each (n = 5) section.

2.11. Measurement of DPP-4 activity and DPP-4 and GLP-1 levels

An lysis buffer containing protease inhibitors was prepared for homogenization of frozen salivary gland tissues, which contained 150 mM NaCl, 1 % Triton X-100, and 10 mM Tris, pH 7.4. The volume of lysis buffer was determined in relation to the amount of

Table 1
Animal experimental groups.

Name of groups	Numbers of animals	Drug intervention
Normal group	7	PBS only
MGO group	7	rats treated with MGO via an intraperitoneal (i.p.) injection
AG group	7	rats treated with MGO via an i.p. injection and orally administered 100 mg/kg of AG
GG10 group	7	rats treated with MGO via an i.p. injection and orally administered 10 mg/kg of gemigliptin
GG100 group	7	rats treated with MGO via an i.p. injection and orally administered 100 mg/kg of gemigliptin

salivary gland tissue present. lysis was performed by determining the volume of lysis buffer based on the salivary gland weight ratio. DPP-4 activity and GLP-1 and DPP-4 levels were measured using the DPP-4 activity assay kit (Enzo), rat GLP-1 ELISA Kit (MyBioSource, CA, USA), and rat DPP-4 ELISA kit (MyBioSource), respectively, following the manufacturers' instructions. The results were measured using the Spark Multimode Microplate Reader (Tecan).

2.12. Measurement of amylase alpha 1 and AGE levels

Salivary amylase levels were determined with a Rat Amylase Alpha 1 ELISA Kit (MyBioSource, CA, USA), following the manufacturer's instructions. The levels of AGEs in the salivary gland tissues, saliva, and serum were mesured using rat AGEs ELISA kits (MyBioSource) according to the manufacturer' protocol.

2.13. Statistical analyses

Following Levene's test, a one-way ANOVA was performed, and significant differences between groups were detected. According to the homogeneity of dispersion, a post-hoc test was conducted (Tukey's multiple comparison test for homogeneous dispersion or Dunnett's T3 test for heterogeneous dispersion) using a Graphpad Prism 8.0 software (GraphPad, San Diego, CA, USA). We expressed all data as means ± standard errors. A p-value less than 0.05 was considered significant.

3. Results

3.1. Gemigliptin inhibits MGO-induced apoptosis and increases GLP-1 levels in SGACs

Cytotoxic effects of MGO on SGACs were assessed by culturing the cells with various concentrations of MGO for 24 h. MGO

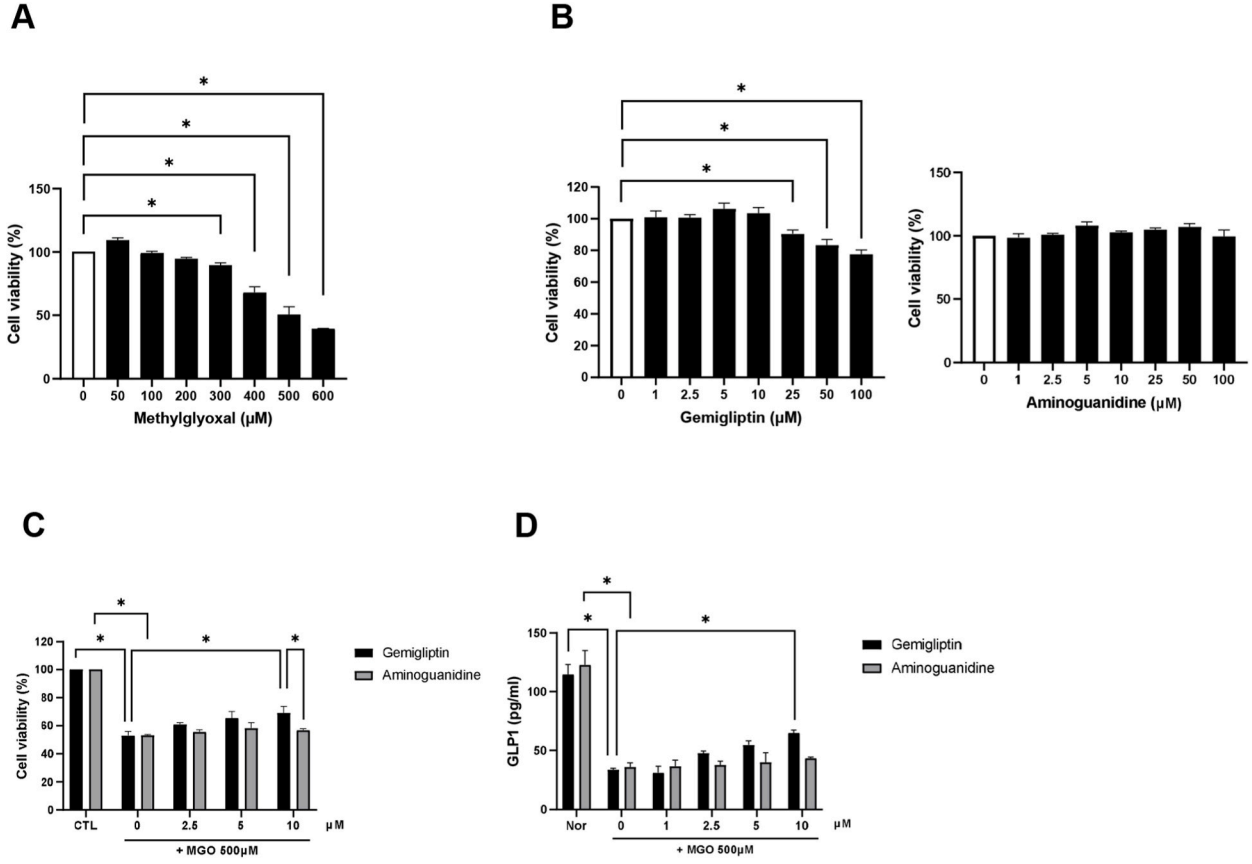


Fig. 1. Effects of gemigliptin (GG) on methylglyoxal (MGO)-treated immortalized human salivary gland acinar cells (SGACs). (A) Effects of 24-h treatment with methylglyoxal (MGO) on the viability of SGACs. (B) Cell viability of SGACs treated with GG and aminoguanidine (AG) for 24 h. (C) Effects of gemigliptin and AG on the viability of immortalized human SGACs treated with MGO for 24 h. (D) Glucagon-like peptide 1 (GLP-1) levels in SGACs treated with different concentrations of gemigliptin and AG. All data are presented as the mean ± standard error of the mean (n = 5). *p < 0.05.

suppressed the cell viability in a dose-dependent manner. Cell viability was maintained at approximately 50 % when the concentration of MGO was set to 500 μM (Fig. 1A). Based on these results, we chose a concentration of 500 μM MGO for 24 h as the optimal modeling condition for further studies. In the cytotoxicity assay of gemigliptin and AG, gemigliptin exhibited cytotoxicity starting from a concentration of 25 μM, while AG did not show toxicity up to a concentration of 100 μM (Fig. 1B). Experiments were conducted using non-toxic concentrations of gemigliptin, which were 10 μM or lower. Gemigliptin (0, 2.5, 5, and 10 μM) dose-dependently inhibited the decrease in cell viability caused by MGO. Also, when compared to the control compound 10 μM AG, cells treated with 10 μM gemigliptin exhibited higher cell viability (Fig. 1C). To investigate the effect of gemigliptin on GLP-1 expression in the MGO-treated SGACs, GLP-1 levels were examined. The results confirmed that GLP-1, which was significantly decreased in the MGO group compared to the control group, exhibited a dose-dependent increase with gemigliptin treatment (Fig. 1D). Therefore, it has been confirmed that gemigliptin inhibits the cytotoxicity of MGO in SGACs and increases GLP-1 levels.

3.2. Gemigliptin protects against MGO-induced oxidative stress and apoptosis in SGACs

To understand the inhibition of MGO-induced apoptosis by gemigliptin, the levels of relevant apoptotic proteins and ROS were examined. After culturing SGACs in a medium with 500 μM MGO for 24 h, a significant increase in fluorescence intensity was observed. However, co-treatment with MGO and gemigliptin attenuated the increase in ROS production in a dose-dependent manner. While the treatment with AG also reduced the ROS induced by MGO in a dose-dependent manner, it is noteworthy that at a concentration of 10 μM gemigliptin exhibited a stronger inhibition of ROS production compared to AG (Fig. 2A and B). To investigate the effect of gemigliptin on MGO-induced apoptosis in SGACs, western blotting analysis was performed to examine the expression of Bax, caspase-3, caspase-9, Bcl2, and PARP. MGO treatment significantly increased the levels of pro-apoptotic proteins (Bax, cleaved caspase-3, cleaved caspase-9) and cleaved PARP, while decreasing the levels of the anti-apoptotic protein (Bcl2). Compared to the MGO group, significant dose-dependent downregulation of Bax, cleaved caspase-3, and cleaved caspase-9 levels and upregulation of Bcl2 levels were observed in the gemigliptin-treated group (Fig. 2C and D). These results confirmed that gemigliptin dose-dependently reduced the ROS levels in SGACs increased by MGO and inhibited apoptosis.

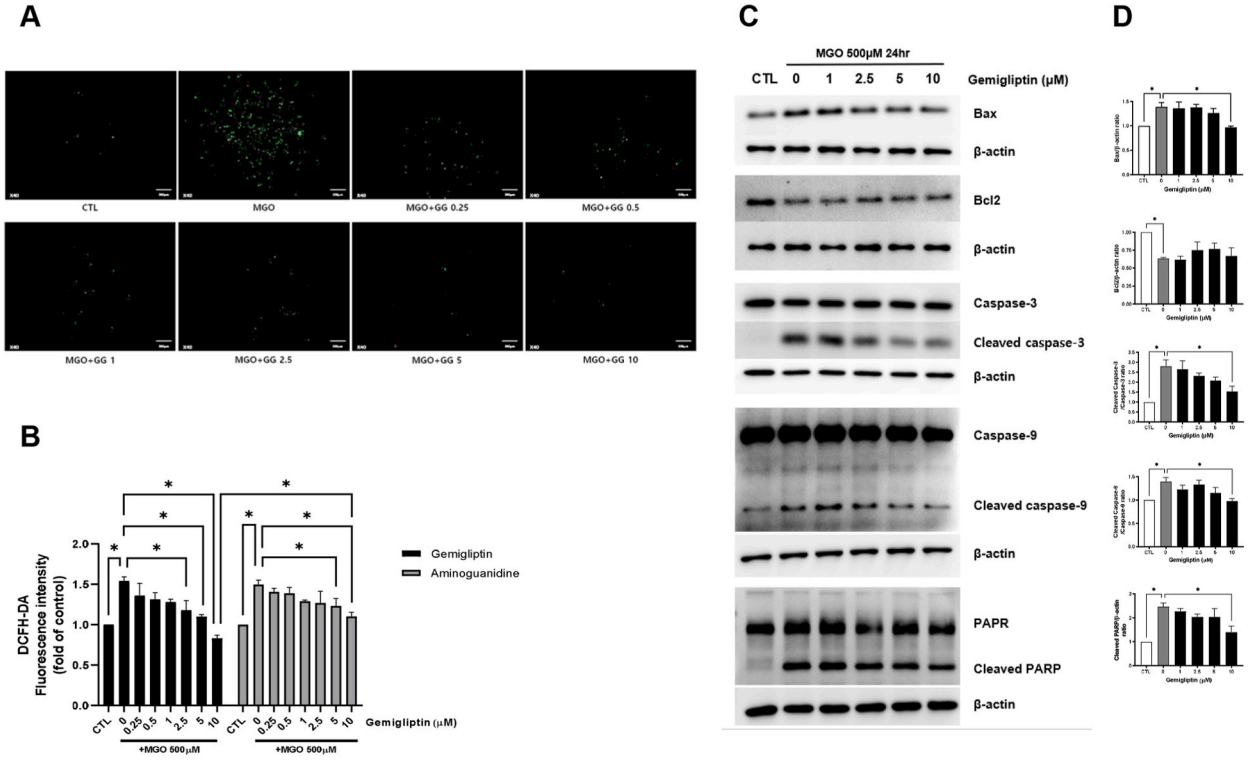
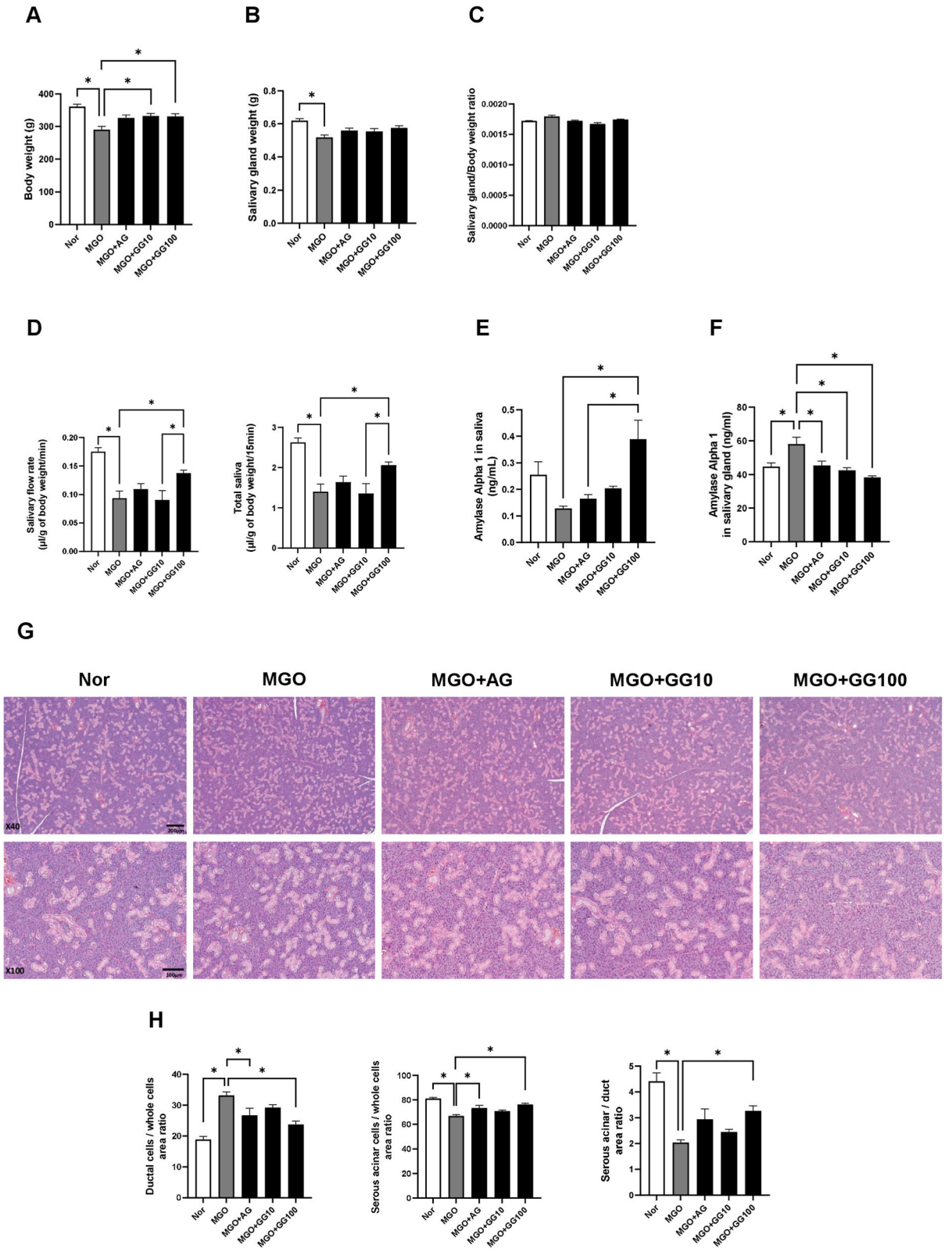


Fig. 2. GG reduces MGO-induced reactive oxygen species (ROS) levels and apoptosis in immortalized human SGACs. (A) ROS levels were determined via fluorescence microscopy with 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) as a fluorescent probe. (B) Quantitative analysis of DCFH-DA staining intensity. (C) Western blotting analysis of apoptosis-related proteins in SGACs. SGACs were treated with 0, 1, 2.5, 5, and 10 μM of GG and 500 μM of MGO for 24 h. (D) Optical density values of the protein bands from the Western blot were statistically quantified. β-actin was used as an internal control. All data are presented as the mean ± standard error of the mean (n = 5). *$p < 0.05$.



(caption on next page)

Fig. 3. GG ameliorates salivary gland dysfunction and pathological alterations in MGO-injected rats. (A) Body weight in each group. (B) Salivary gland weight in each group. (C) Bilateral salivary gland weight divided by body weight. (D) Total salivary secretion and flow in each group. (E) Measurement of amylase concentration in the collected saliva. (F) Measurement of amylase concentration in the salivary gland lysate. (G) Representative salivary glands stained with hematoxylin and eosin (H&E) (scale bar = 100 μm). (H) Quantitative evaluation of acinar cell and duct regions in salivary glands. All data are presented as the mean ± standard error of the mean (n = 5). *p < 0.05.

3.3. Gemigliptin prevents MGO-induced dysfunction and pathological alterations of the salivary gland

We conducted in vivo experiments to investigate the effect of gemigliptin on the impairment of salivary gland function in MGO-injected rats. The rats were simultaneously administered MGO (17.25 mg/kg), AG (100 mg/kg), and gemigliptin (10 mg/kg and 100 mg/kg) daily for two weeks. Body weight and salivary gland weight decreased in the MGO group, but increased with AG and gemigliptin treatment (Fig. 3A and B). The ratio of salivary gland weight to body weight showed a slight increase in the MGO group, but no significant difference was observed compared with the other groups (Fig. 3C). The salivary flow was significantly decreased in the MGO group than in the normal group. However, treatment with gemigliptin (100 mg/kg) significantly increased the salivary flow (Fig. 3D). Amylase levels in saliva decreased in the MGO group and increased in a dose-dependent manner in the gemigliptin-treated groups. In addition, gemigliptin-treated rats (100 mg/kg) had significantly higher concentrations of amylase than AG-treated rats (100 mg/kg) (Fig. 3E). However, there was an increase in salivary amylase concentration in the MGO group and a decrease in the gemigliptin group. (Fig. 3F). In order to observe changes in morphology in salivary glands, H&E staining was employed. The ratio of the ductal area to the serous acinar area was examined in the salivary glands, and it was observed that the area of ductal cells increased, whereas the area of serous acinar cells decreased in the MGO group. Conversely, in the gemigliptin-treated group, the area of the ductal cells decreased, whereas that of the serous acinar cells increased (Fig. 3G and H). Therefore, we confirmed that the MGO-induced reduction in salivary flow rate, changes in amylase levels in saliva and salivary glands, and morphological changes contributed to

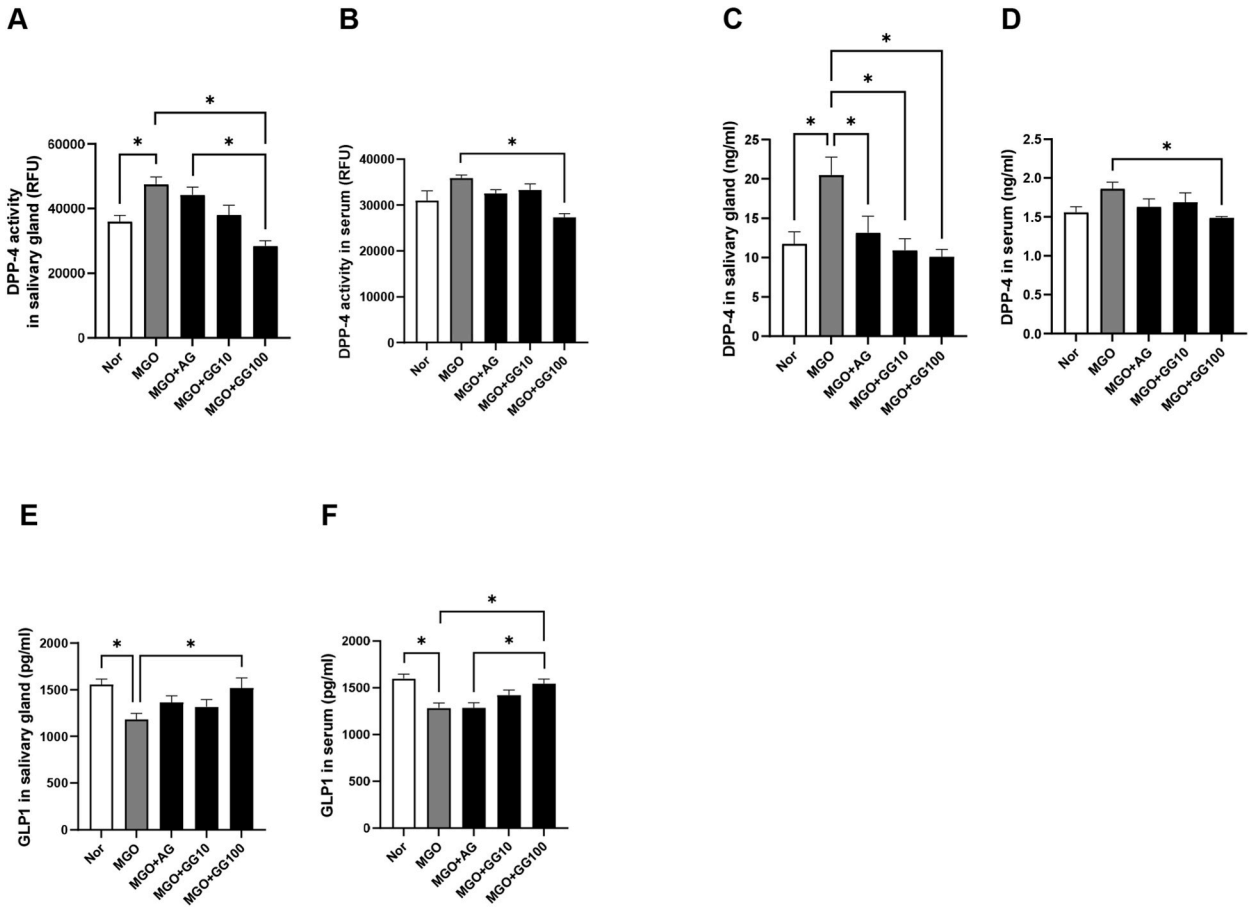
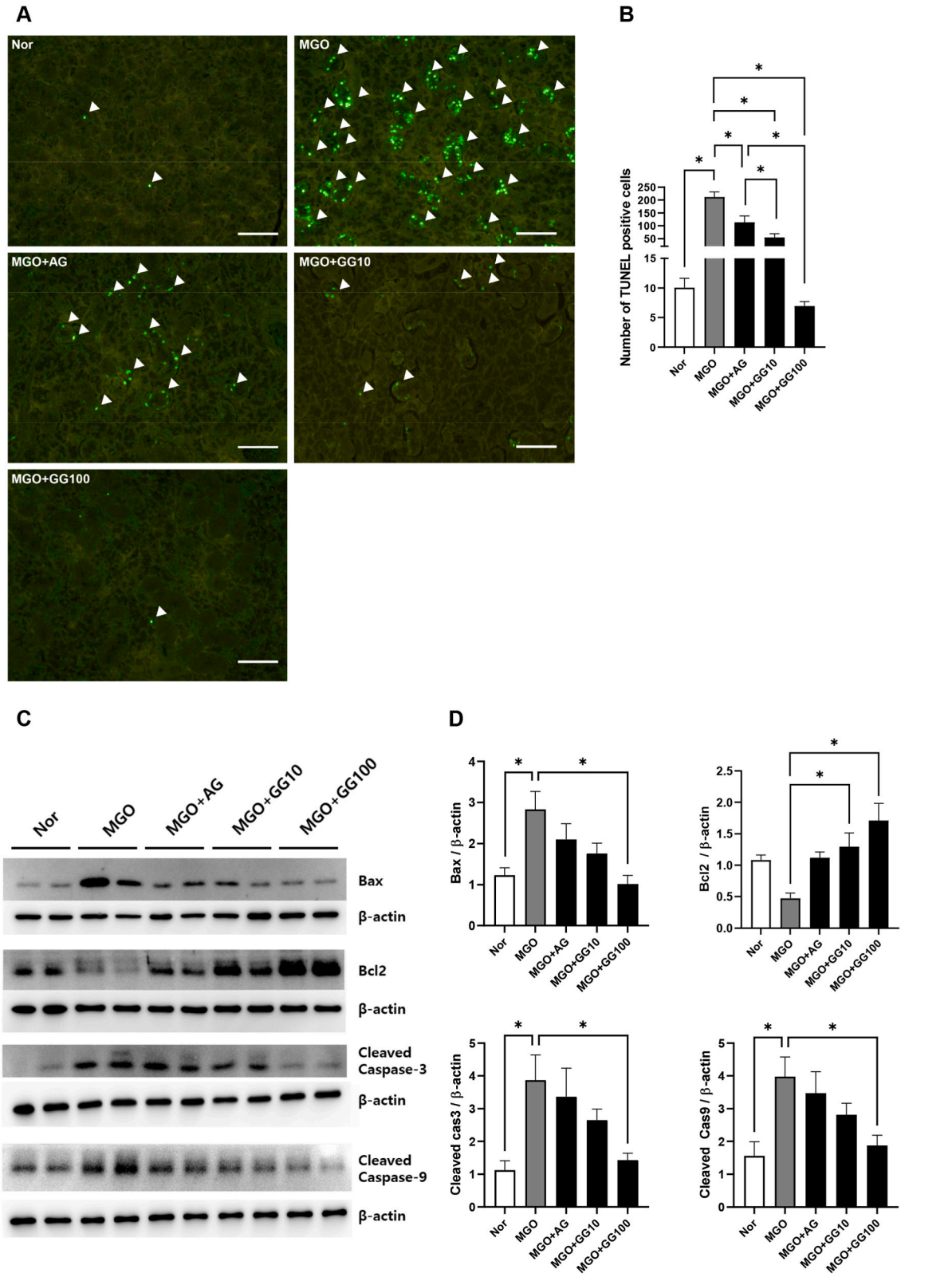


Fig. 4. Effects of GG on dipeptidyl peptidase-4 (DPP-4) activity and DPP-4 and GLP-1 levels in the salivary glands and serum of MGO-injected rats. (A) DPP-4 activity in the salivary glands. (B) DPP-4 activity in the serum. (C) DPP-4 levels in the salivary glands. (D) DPP-4 levels in the serum. (E) GLP-1 levels in the salivary glands. (F) GLP-1 levels in the serum. All data are presented as the mean ± standard error of the mean (n = 5). *p < 0.05.



(caption on next page)

Fig. 5. TdT-mediated dUTP nick-end labeling (TUNEL) assay and western blotting of apoptosis-related proteins in salivary gland tissues. (A) TUNEL staining of apoptotic cells in the salivary gland sections (scale bar = 50 μm). Arrow heads indicate TUNEL-positive cells. (B) Quantitative analysis of TUNEL-positive cells in the salivary glands. (C) Effects of GG on the expression levels of Bax, Bcl2, caspase-3, and caspase-9 proteins. (D) Optical density values of the protein bands from the Western blot were statistically quantified. β-actin was used as an internal control. All data are presented as the mean ± standard error of the mean (n = 5). *p < 0.05.

salivary gland dysfunction, which was improved by gemigliptin treatment.

3.4. Effects of gemigliptin on DPP-4 activity and DPP-4 and GLP-1 levels in the salivary gland and serum

This study aimed to investigate the effects of gemigliptin on DPP-4 activity and level in the salivary glands and serum, along with its effect on GLP-1 levels. There was a significant reduction in DPP-4 activity in both the salivary gland and serum in the gemigliptin-treated group compared to the MGO-treated group. (Fig. 4A and B). Similarly, The gemigliptin group had significantly lower

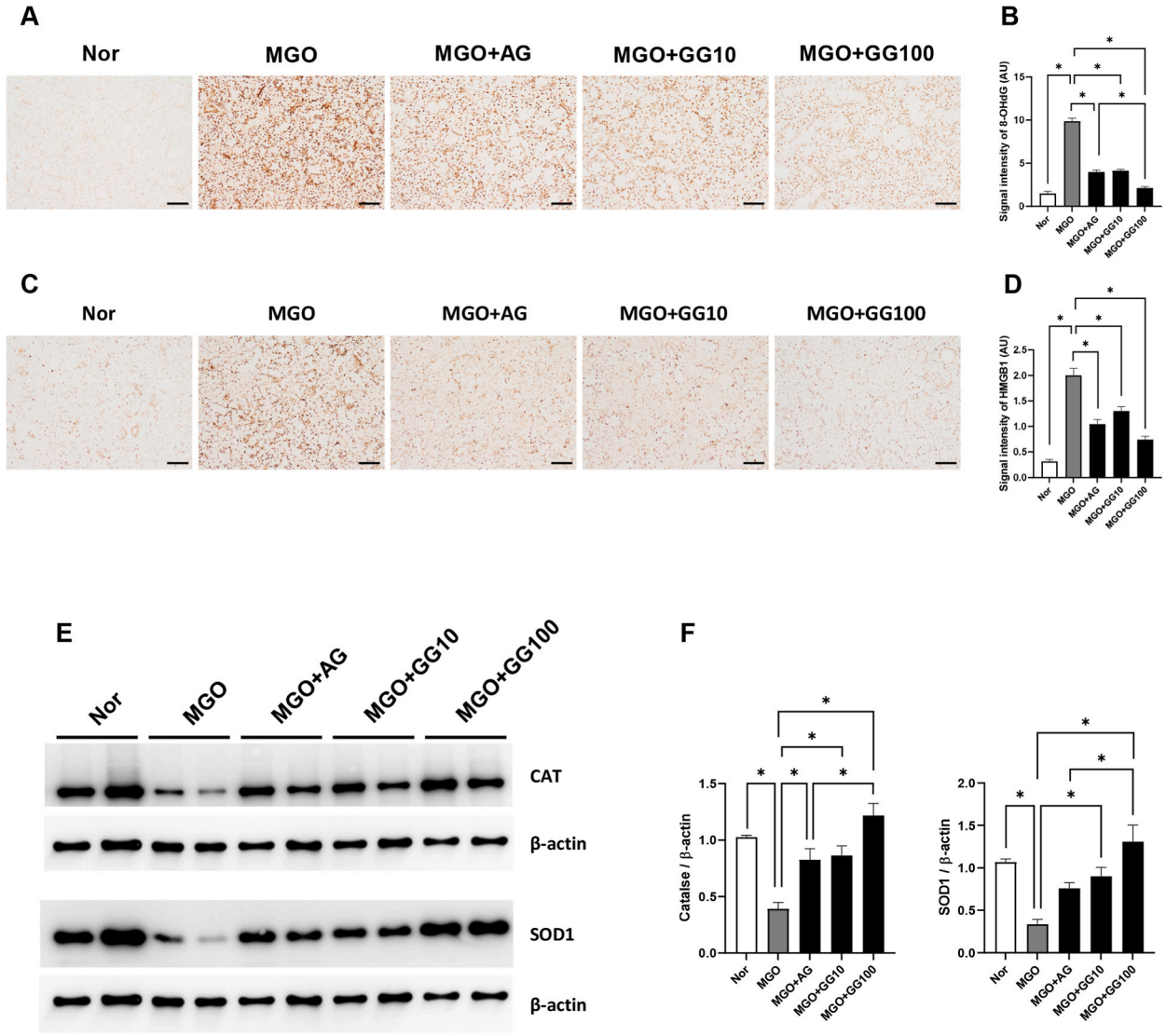
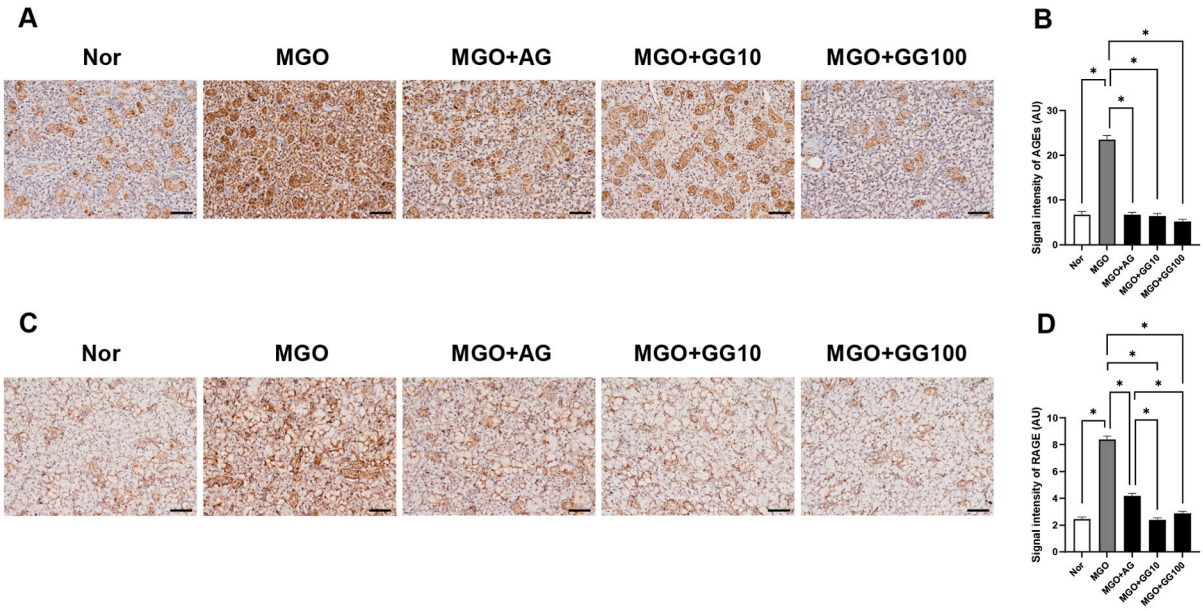
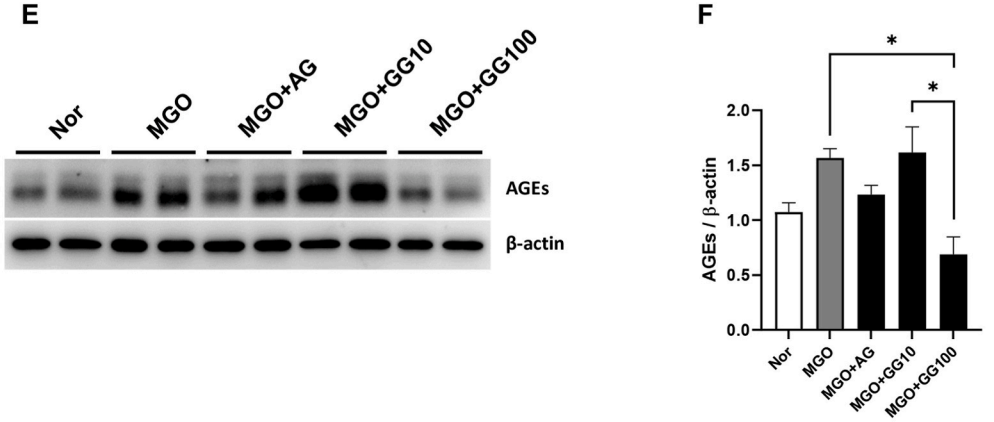
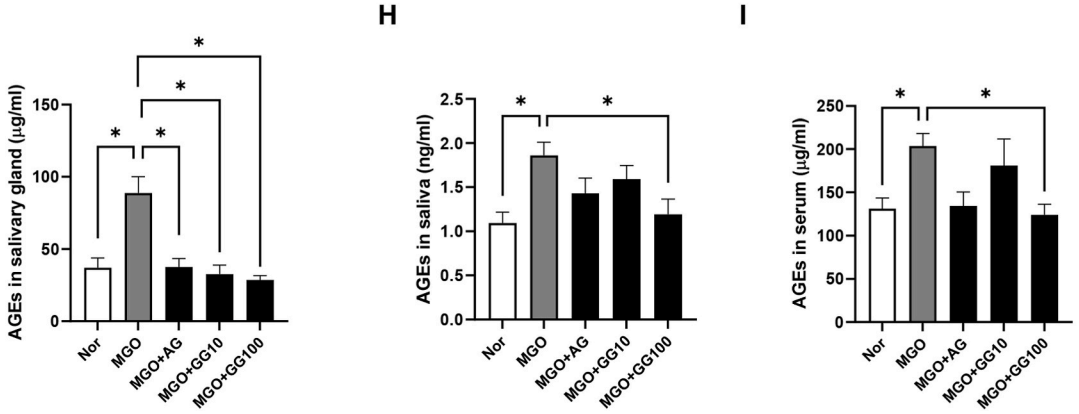


Fig. 6. Effect of GG on ROS production in the salivary glands of MGO-injected rats. (A) Immunohistochemical analysis of 8-hydroxy-2′-deoxyguanosine (8-OHdG) expression in the salivary glands (scale bar = 50 μm). (B) Quantification of 8-OHdG signal intensity. (C) Immunohistochemical analysis of high mobility group box 1 (HMGB1) expression in the salivary glands (scale bar = 50 μm). (D) Quantification of HMGB1 signal intensity. (E) Effects of GG on the expression levels of catalase (CAT) and superoxide dismutase 1 (SOD1) proteins were determined via western blotting. (F) Optical density values of the protein bands from the Western blot were statistically quantified. β-actin was used as an internal control. All data are presented as the mean ± standard error of the mean (n = 5). *p < 0.05.







(caption on next page)

Fig. 7. Effect of GG on the accumulation of advanced glycation end products (AGEs) in the salivary glands of MGO-injected rats. (A) Immunohistochemical analysis of AGE expression in the salivary glands (scale bar = 50 μm). (B) Quantification of AGE signal intensity. (C) Immunohistochemical analysis RAGE expression in the salivary glands (scale bar = 50 μm). (D) Quantification of RAGE signal intensity. (E) Effects of GG on the expression levels of AGEs were determined via western blotting. (F) Optical density values of the protein bands from the Western blot were statistically quantified. β-actin was used as an internal control. (G) Measurement of AGE concentration in the salivary glands. (H) Measurement of AGE concentration in the saliva. (I) Measurement of AGE concentration in the serum. All data are presented as the mean ± standard error of the mean (n = 5). *$p < 0.05$.

salivary gland and serum DPP-4 levels than the MGO group (Fig. 4C and D). In contrast, There was a significant increase in GLP-1 levels in the salivary glands and serum in the gemigliptin-treated group compared to the MGO-treated group, suggesting an enhanced GLP-1-mediated effect (Fig. 4E and F). Based on these findings, gemigliptin appears to be effective in decreasing DPP-4 activity in salivary glands and serum, thereby increasing GLP-1 levels. This could have implications for the treatment of salivary gland dysfunction.

3.5. Gemigliptin inhibits apoptosis in the salivary glands of MGO-injected rats

To confirm that gemigliptin alleviates apoptosis in rats injected with MGO, TUNEL assays were performed as well as Western blot analysis. In the salivary gland TUNEL assay, the number of TUNEL-positive apoptotic cells, which was significantly increased in the

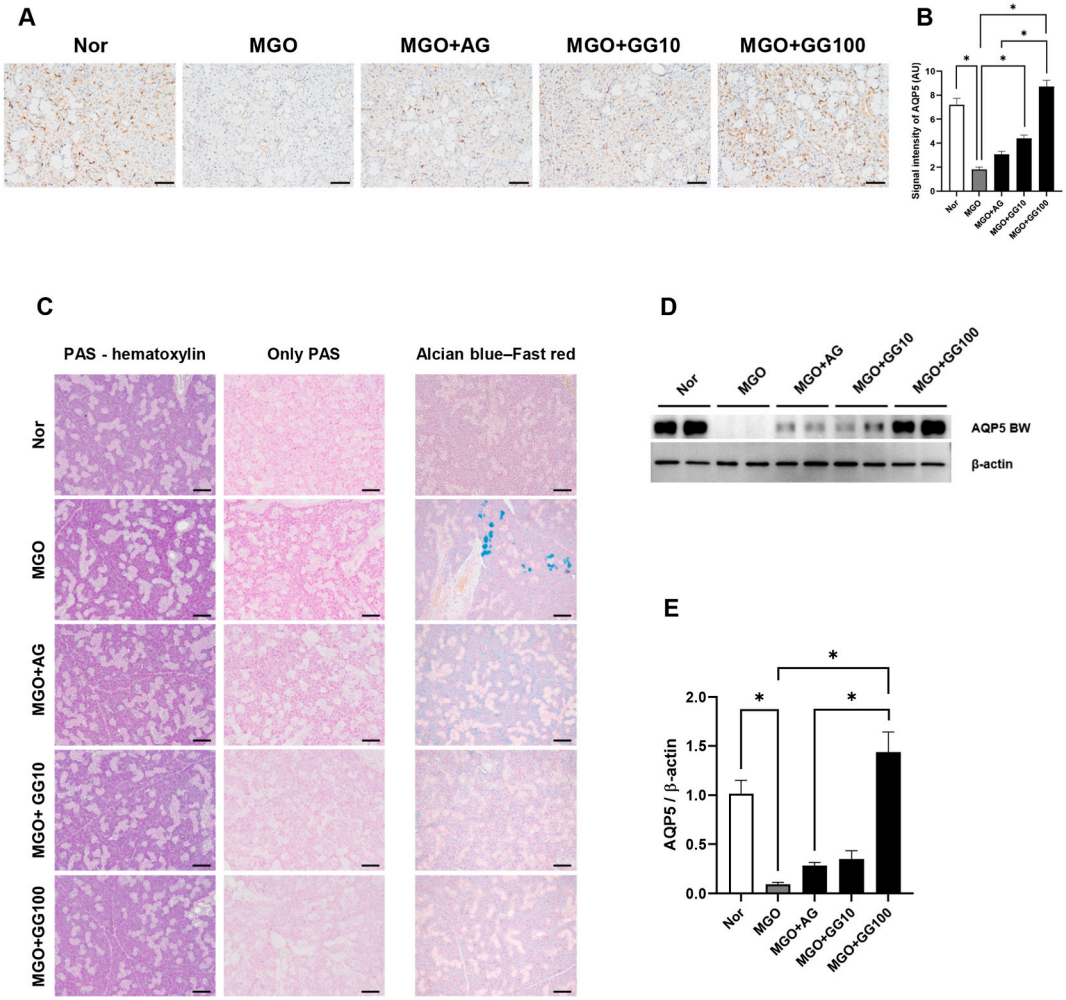


Fig. 8. Effects of GG on mucin accumulation and aquaporin 5 (AQP5) expression in the salivary glands of MGO-treated rats. (A) Immunohistochemical analysis of AQP5 expression in the salivary glands (scale bar = 50 μm). (B) Quantification of AQP5 signal intensity. (C) Periodic acid-Schiff (PAS) and Alcian blue (AB) staining of the salivary glands (scale bar = 100 μm). (D) Effect of GG on the expression of AQP5 was determined via western blotting. (E) Optical density values of the protein bands from the Western blot were statistically quantified. β-actin was used as an internal control. All data are presented as the mean ± standard error of the mean (n = 5). *$p < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

MGO group, exhibited a concentration-dependent decrease in the gemigliptin-treated group. Moreover, in comparison with the control group treated with AG at 100 mg/kg, the gemigliptin 100 mg/kg group demonstrated a notable reduction in TUNEL-positive cells (Fig. 5A and C). As compared to MGO-exposed glands, gemigliptin-treated samples showed altered expression profiles of apoptosis-associated proteins. Apoptosis-promoting proteins Bax, cleaved caspase-3, and cleaved caspase-9 increased in the MGO group. Further, Bcl2 was found to be less expressed, which would be an indication of an increase in apoptosis. However, apoptosis-promoting proteins, Bax, cleaved caspase-3, and cleaved caspase-9, decreased in a dose-dependent manner after gemigliptin administration, while anti-apoptotic proteins, Bcl2, increased (Fig. 5C and D). It seems that gemigliptin inhibits MGO-induced salivary gland apoptosis.

3.6. Gemigliptin inhibits oxidative stress in the salivary glands of MGO-injected rats

IHC was performed to assess whether oxidative stress-induced cellular damage was evident in 8-OHdG and HMGB1 accumulation. MGO-treated animals accumulated more 8-OHdG, whereas AG and gemigliptin-treated animals accumulated less. Further, gemigliptin 100 mg/kg showed a greater reduction in 8-OHdG accumulation than AG and MGO (Fig. 6A and B). A similar decrease in HMGB1 accumulation was observed in the MGO group after AG and gemigliptin treatments. There was a greater reduction in the gemigliptin 100 mg/kg group compared to the AG group (Fig. 6C and D). Further analysis of oxidative stress markers was done by western blotting. MGO-injected rats' salivary glands showed decreased expression of the antioxidant markers CAT and SOD1, which increased with AG and gemigliptin. These markers were significantly more expressed in the group treated with gemigliptin at 100 mg/kg than in the group treated with AG (Fig. 6E and F). In conclusion, Ageing rats treated with MGO showed a significant reduction in oxidative stress in their salivary glands when gemigliptin was administered.

3.7. Gemigliptin reduces AGE accumulation and RAGE expression in the salivary glands of MGO-injected rats

Experiments were conducted to observe the anti-glycation effects of gemigliptin in rats injected with MGO. The accumulation of AGEs and RAGE expression in the salivary glands was examined. A reduction in AGE accumulation occurred in the MGO group after treatment with the control drug AG as well as gemigliptin (Fig. 7A and B). Similarly, the expression of RAGE, as shown by the IHC results, decreased with gemigliptin treatment, following an increase observed in the MGO group (Fig. 7C and D). AGE expression was also measured via Western blotting. AG and gemigliptin treatment reduced AGE levels in the MGO group, in accordance with IHC results. There was the greatest reduction observed in the group treated with 100 mg/kg of gemigliptin (Fig. 7E and F). Saliva, salivary gland lysate, and serum were also tested for AGE levels. Saliva and salivary gland levels of AGEs increased in the MGO group, but decreased with AG and gemigliptin treatment. Serum levels of AGE were increased in the MGO group, but they decreased with AG and gemigliptin treatment. Consequently, both AG and gemigliptin reduced AGE accumulation and RAGE expression levels in the salivary glands, saliva, and serum of MGO-injected rats. Furthermore, the group administered 100 mg/kg gemigliptin showed a superior anti-glycation effect than the AG group.

3.8. Gemigliptin increases AQP5 expression and mucin secretion in the salivary glands of MGO-injected rats

Salivary glands were tested for AQP5 expression using IHC. Following gemigliptin administration, the decreased expression of AQP5 in the salivary glands of the MGO group increased dose-dependently (Fig. 8A and B). To determine whether SGACs accumulate neutral or acidic mucus, PAS and AB staining were conducted. When salivary gland acini were stained with AB and PAS reagents, moderate staining was observed in the normal group. There was, however, an increase in staining intensity in the MGO group, indicating that both acidic and neutral mucus were accumulating in the acini (Fig. 8C and D). Acini of salivary glands stained strongly with PAS in the MGO group, but weakened in the gemigliptin group (Fig. 8C). Gemigliptin-treated individuals had a less pronounced neutral mucine blockade than MGO-treated individuals. The intensity of AB staining was relatively high in the MGO group and relatively low in the gemigliptin 100 mg/kg group compared to the normal group. It is interesting to note that AB staining was strongly positive in some salivary gland acini in the MGO group (Fig. 8D). Furthermore, analyses of salivary gland lysates using western blotting confirmed the presence of AQP5 protein. In accordance with IHC data, these results support AQP5 findings. AQP5 expression was significantly reduced in the MGO group and increased concentration-dependently with gemigliptin administration (Fig. 8E and F). The results showed that gemigliptin improved AQP5 expression in the salivary glands of MGO-treated rats and reduced mucin accumulation.

4. Discussion

Saliva plays an important role in the maintenance of homeostasis in oral health [2]. Deficiency of saliva can lead to damage and infection in the oral tissues, which can be a cause of oral disease [4]. Salivary gland dysfunction is a common symptom in patients with diabetes. In metabolic disorders, such as diabetes, elevated levels of MGO can increase oxidative stress, affecting cell and tissue function [3]. However, the mechanism underlying MGO-related salivary gland function, which is closely associated with diabetes, remains unclear [3]. In this study, gemigliptin, a DPP-4 inhibitor, was found to increase GLP-1 levels and exhibit an anti-glycation effect in the salivary glands of mice injected with MGO. These effects alleviated salivary gland dysfunction and increased salivation.

The inhibition of DPP-4 enhances pancreatic insulin secretion through GLP-1 and suppresses hepatic glucose production by inhibiting its activity [25]. Gemigliptin, the drug used in the experiment, is a DPP-4 inhibitor that is used to treat type 2 diabetes [19]. Previous in vitro and in vivo experiments have shown that gemigliptin exhibits several additional activities, including anti-glycation

and anti-inflammatory effects [22,26]. We confirmed that gemigliptin has a cytoprotective effect against MGO-induced cytotoxicity in SGACs. Additionally, a relatively high cell survival rate was observed in the gemigliptin group compared to that in the control AG group. These results are consistent with previous findings, indicating that MGO-induced cytotoxicity could be inhibited by the increased GLP-1 levels following the DPP-4 inhibitory effect of gemigliptin [27]. Moreover, gemigliptin reduced DPP-4 activity in MGO-injected rats' salivary glands and serum and increased GLP-1 levels in a dose-dependent manner. These results demonstrated that gemigliptin functions as a DPP-4 inhibitor in the salivary glands.

For patients with diabetes, gemigliptin is recommended at a dose of 50 mg daily. Gemigliptin, however, reduced plasma AGE levels in db/db mice when given orally 100 mg/kg/day [22]. A mouse model of ureteral obstruction was also shown to be prevented from developing renal interstitial fibrosis by oral gemigliptin consumption at 150 mg/kg/day [28]. Based on previous studies, we found that gemigliptin has a similar effect on the salivary gland in rodents. A previous report measured gemigliptin plasma concentrations to be 62.7 ng/ml after a single 50 mg oral dose, with an area under the plasma concentration time curve (AUC) of 743.1 ng/ml h-1. It has a half-life of 17.1 h to eliminate gemigliptin from the body. DPP-4 activity was inhibited for 24 h after administration [29]. However, it was found that mice consuming 100 mg/kg of gemigliptin, had a peak plasma concentration of 2614 ng/ml, but had a 1.5-h elimination half-life. In mice, gemigliptin had an AUC of 4510 ng/ml h-1 [30]. Humans have a 6-fold lower AUC value than mice after oral administration, however, the human half-life of gemigliptin is 11-fold longer. Further, gemigliptin's inhibitory activity against DPP-4 was similar for 24 h after oral administration of 100 mg/kg gemigliptin in mice because of its short half-life.

We examined ROS-induced apoptosis caused by ROS in the SGACs and MGO-injected rats. Various studies have shown that MGO increases the intracellular ROS [31,32]. ROS are important contributors to hyperglycemia-induced cell death, since antioxidants can block MGO-induced oxidative stress [33]. In the salivary glands of MGO-injected rats, the expression of pro-apoptotic proteins decreased with gemigliptin administration in a dose-dependent manner, whereas the levels of anti-apoptotic proteins increased. Additionally, the number of TUNEL-positive cells, which increased in the MGO group, decreased after gemigliptin administration.

An increase in DPP-4 and a decrease in GLP-1 levels may be responsible for MGO-induced ROS production. As a result of its antioxidant properties, GLP-1 suppresses ROS production and inflammation during hyperglycemia and in diabetic animals [34,35]. The production of ROS by endothelial cells is increased by DPP-4, while the production of ROS by cardiomyocytes is inhibited by DPP-4 deficiency [36,37]. Using animal models of diabetes and its complications as models, DPP-4 inhibitors lower ROS production [38,39]. It has also been reported that gemigliptin can alleviate mitochondrial dysfunction and reduce the production of reactive oxygen species in liver disease and diabetes [40,41]. Previous studies and our findings indicate that gemigliptin's cytoprotective properties are due to its ability to reduce ROS levels by inhibiting DPP-4 activity.

MGO has already been shown to cause cytotoxicity in rat Schwann cells, human vascular endothelial cells, and bovine retinal pericytes [42]. The present study showed that exposure to MGO led to a reduction in body weight and absolute salivary gland weight. However, relative salivary gland to body weight slightly increased in the MGO group. No significant difference was observed compared with the other groups. As previously reported, drinking water containing MGO resulted in about 12 % weight loss for mice when given in drinking water. The relative organ weight was slightly increased, but no significant change was observed in MGO compared with the control group [43].

Anti-glycation effects of gemigliptin were confirmed in this study. Controlling MGO-induced AGE formation by increasing GLP-1 levels in DPP-4-deficient mice improves diabetes and its complications [44]. Anti-glycation effects of several DPP-4 inhibitors, including gemigliptin, have been verified in numerous studies [45,46]. Anti-glycation effect of gemigliptin was verified by capturing MGO and inhibiting the cross-linking reaction of AGEs and proteins [22]. In diabetic rats, gemigliptin's antioxidant effect prevented salivary gland function decline [23]. We also confirmed the anti-glycation effect of AG, a well-known AGE inhibitor. These results suggest that glycation is the major cause of aging in this animal model [47]. Salivary glands were damaged by MGO induced apoptosis through ROS-induced apoptosis. A protective effect of gemigliptin was observed by inhibiting DPP-4, increasing GLP-1 levels, and decreasing AGE accumulation.

In previous studies, gemigliptin has shown various pharmaceutical beneficial effects independent of glycemic control. There is widespread expression of DPP-4 throughout the body [48]. Increased DPP-4 activity promoted the development of oxidative stress and inflammation [49]. The deficiency of DPP-4 protects against oxidative stress caused by H2O2 [37]. Further, DPP-4 is known to increase RAGE gene expression, and DPP-4 inhibitor, linagliptin, inhibits mRNA levels of RAGE in endothelial cells [50]. When AGEs interact with RAGE, oxidative stress occurs, which causes cellular dysfunction [51]. The expression of RAGE protein, salivary AGEs, and salivary DPP-4 activity were clearly decreased with gemigliptin treatment. The present study did not show direct inhibition of ROS production by gemigliptin or ROS generation by DPP-4, but these findings suggest that gemigliptin inhibition of DPP-4 could reduce oxidative stress-related hypofunction of salivary glands.

In our study, we found that in rats injected with MGO, salivary secretion increased with gemigliptin administration. As observed morphologically, the MGO group had a decreased number of acinar cells and increased numbers of ductal cells. The gemigliptin-treated group showed an increase in the ratio of acinar to ductal cells. Similar results have been reported in several established models of salivary gland dysfunction models [52,53]. Although we did not observe any distinct morphological abnormalities in the salivary glands, there were differences in the composition ratio of salivary gland cells associated with salivary gland function.

MGO-induced ROS-mediated apoptosis causes salivary gland dysfunction. Among the most important enzymes present in saliva is α-amylase, which is responsible for the initial digestion of starch. Thus, it is a reliable indicator of salivary gland function. Decreased salivary volume is associated with salivary glands [54,55]. Our study found that both salivary volume and amylase levels decreased in the MGO group, whereas gemigliptin administration increased both variables concentration-dependently. Conversely, amylase levels increased in the salivary glands of the MGO group but decreased in the gemigliptin group. Low salivary amylase levels are linked to insulin resistance, diabetes, and obesity [56,57]. Diabetes-related dry mouth has also been associated with changes in salivary flow

rate and decreased salivary amylase activity [58,59]. Thus, we anticipated difficulties with the secretion of amylase from the salivary gland. When ROS accumulate in salivary glands, they cause oxidative stress that can alter saliva secretion, salivary amylase levels, and secretory functions like calcium regulation [60]. As a result of the findings, it appears that salivary oxidative stress can result in salivary secretion disorders and that gemigliptin can alleviate salivary dysfunction by inhibiting salivary oxidative stress.

Mucins, composed of various compounds, play a crucial role in mucosal lubrication, which is a primary function of saliva [61]. Here, strong AB and PAS staining demonstrated increased mucin accumulation in the acini of salivary glands of the MGO group. However, mucin accumulation decreased after gemigliptin administration, suggesting that gemigliptin reduced mucin accumulation in the acini by improving salivary gland dysfunction. Diabetes-related salivary gland accumulation of acidic and neutral mucins is associated with strong AB and PAS staining [17,62]. When salivary glands become dysfunctional, mucin composition can change [63]. MGO-induced salivary gland dysfunction may be more related to impaired secretion than to disorders affecting saliva production. Consequently, under diabetic conditions mucous accumulates in the salivary glands, indicating that the salivary glands have abnormal secretory function. In saliva formation, the primary fluid is generated and secreted by acini, which are the cells of the oral cavity [64]. Acinar cells synthesize these secretions and store them in cytoplasmic granules before releasing them [65]. In this study, MGO's salivary glands accumulated more mucins and less amylase than the control group. Our findings suggest a potential issue in the primary saliva secretion process. However, additional experiments are required to verify this.

In order to assess the expression levels of AQP5 in salivary glands, we assessed the importance of AQP5 in salivary secretion. In the salivary glands of the MGO group, we found a decrease in the expression of AQP5. A crucial function of AQP5 is to control salivary gland secretion [66]. The salivary flow stimulated by pilocarpine is significantly reduced in mice lacking AQP5, indicating the importance of AQP5 in regulating the water permeability in acinar cells [67]. Additionally, rats with type 2 diabetes exhibit reduced salivary flow and decreased expression of AQP5 [68]. In this study, gemigliptin increased salivary gland expression of AQP5 in a concentration-dependent manner. This suggests that gemigliptin improves MGO-induced salivary gland dysfunction by enhancing AQP5 expression levels in the salivary glands.

In conclusion, gemigliptin decreased ROS-induced apoptosis in salivary glands of rats injected with MGO, which was caused by DPP-4 activity and AGE accumulation. Consequently, gemigliptin protected salivary gland cells from damage, increasing salivary flow rate, increasing salivary amylase levels, reducing mucin accumulation, and increasing AQP5 expression (Fig. 9). There is no concrete evidence of how gemigliptin directly increases salivary secretion. Pilocarpine is the FDA approved oral drug for dry mouth. As a cholinergic agonist, this drug binds to muscarinic receptors in salivary glands and stimulates secretory nerves to secrete saliva. However, gemigliptin improved salivary gland hypofunction rather than direct salivation simulation, thereby improving salivary secretion. Overall, gemigliptin improved the salivary gland function, suggesting its potential as a therapeutic agent for patients with diabetes-associated salivary gland dysfunction.

Funding

This research was carried out with the support of the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (No. NRF-2023R1A2C1005120).

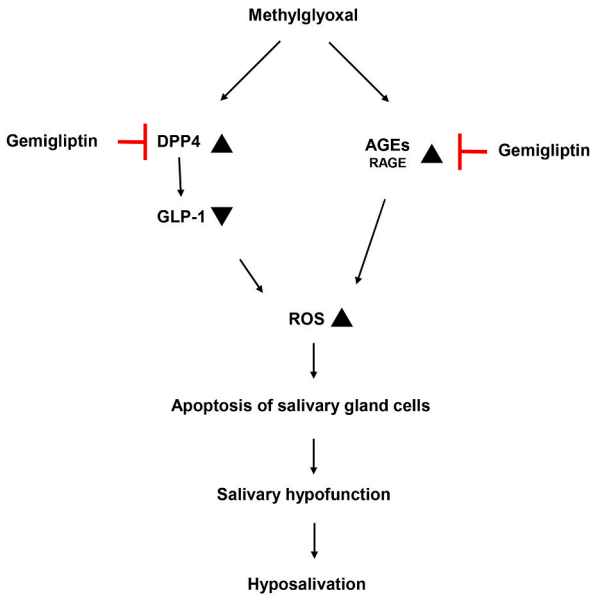


Fig. 9. Potential action mechanism of GG in MGO-induced hypofunction of the salivary glands.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Woo Kwon Jung: Writing – original draft, Methodology, Investigation, Data curation. **Su-Bin Park:** Investigation. **Hwa Young Yu:** Investigation. **Junghyun Kim:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29362.

References

[1] M.W. Dodds, et al., Health benefits of saliva: a review, J. Dent. 33 (2005) 223–233.
[2] N. Farsi, Signs of oral dryness in relation to salivary flow rate, pH, buffering capacity and dry mouth complaints, BMC Oral Health 7 (2007) 1–6.
[3] C. Carda, et al., Structural and functional salivary disorders in type 2 diabetic patients, Med. Oral, Patol. Oral Cirugía Bucal 11 (2006) 209.
[4] F. D'Aiuto, et al., Evidence summary: the relationship between oral diseases and diabetes, Br. Dent. J. 222 (2017) 944–948.
[5] J. Saleh, et al., Salivary hypofunction: an update on aetiology, diagnosis and therapeutics, Arch. Oral Biol. 60 (2015) 242–255.
[6] V.P. Singh, et al., Advanced glycation end products and diabetic complications, KOREAN J. PHYSIOL. PHARMACOL.: official journal of the Korean Physiological Society and the Korean Society of Pharmacology 18 (1) (2014).
[7] M. Shinohara, et al., Overexpression of glyoxalase-I in bovine endothelial cells inhibits intracellular advanced glycation endproduct formation and prevents hyperglycemia-induced increases in macromolecular endocytosis, The Journal of clinical investigation 101 (1998) 1142–1147.
[8] Y. Li, et al., The structural modification of DNA nucleosides by nonenzymatic glycation: an in vitro study based on the reactions of glyoxal and methylglyoxal with 2′-deoxyguanosine, Anal. Bioanal. Chem. 390 (2008) 679–688.
[9] N. Shoji, et al., LC-MS/MS analysis of carboxymethylated and carboxyethylated phosphatidylethanolamines in human erythrocytes and blood plasma [S], Journal of lipid research 51 (2010) 2445–2453.
[10] J.T. Mey, J.M. Haus, Dicarbonyl stress and glyoxalase-1 in skeletal muscle: implications for insulin resistance and type 2 diabetes, Frontiers in cardiovascular medicine 5 (2018) 117.
[11] C. Schalkwijk, et al., Modulation of insulin action by advanced glycation endproducts: a new player in the field, Horm. Metab. Res. 40 (2008) 614–619.
[12] A. Ilea, et al., Advanced glycation end products (AGEs) in oral pathology, Arch. Oral Biol. 93 (2018) 22–30.
[13] L. de Bari, et al., Interplay among oxidative stress, methylglyoxal pathway and S-glutathionylation, Antioxidants 10 (2020) 19.
[14] M. Brownlee, Biochemistry and molecular cell biology of diabetic complications, Nature 414 (2001) 813–820.
[15] I.C. West, Radicals and oxidative stress in diabetes, Diabet. Med. 17 (2000) 171–180.
[16] L. Gate, et al., Oxidative stress induced in pathologies: the role of antioxidants, Biomed. Pharmacother. 53 (1999) 169–180.
[17] H.R. Kim, et al., Polydatin alleviates diabetes-induced hyposalivation through anti-glycation activity in db/db mouse, Pharmaceutics 14 (2021) 51.
[18] H.R. Kim, J. Kim, Preventive effect of polydatin on diabetes-related hypofunction of salivary gland in streptozotocin-induced diabetic rats, Journal of Biomedical and Translational Research 22 (2021) 159–167.
[19] S.-H. Kim, et al., Gemigliptin, a novel dipeptidyl peptidase 4 inhibitor: first new anti-diabetic drug in the history of Korean pharmaceutical industry, Arch Pharm. Res. (Seoul) 36 (2013) 1185–1188.
[20] S.-H. Kim, et al., Pharmacological profiles of gemigliptin (LC15-0444), a novel dipeptidyl peptidase-4 inhibitor, in vitro and in vivo, Eur. J. Pharmacol. 788 (2016) 54–64.
[21] E.J. Bae, DPP-4 inhibitors in diabetic complications: role of DPP-4 beyond glucose control, Arch Pharm. Res. (Seoul) 39 (2016) 1114–1128.
[22] E. Jung, et al., Gemigliptin, a novel dipeptidyl peptidase-4 inhibitor, exhibits potent anti-glycation properties in vitro and in vivo, Eur. J. Pharmacol. 744 (2014) 98–102.
[23] W.S. Kang, et al., Gemigliptin suppresses salivary dysfunction in streptozotocin-induced diabetic rats, Biomed. Pharmacother. 137 (2021) 111297.
[24] W.K. Jung, et al., Gemigliptin improves salivary gland dysfunction in D-Galactose-injected aging rats, Pharmaceutics 16 (2023) 35.
[25] D.J. Drucker, M.A. Nauck, The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes, Lancet 368 (2006) 1696–1705.
[26] N. Lee, et al., Anti-inflammatory effects of empagliflozin and gemigliptin on LPS-stimulated macrophage via the IKK/NF-κB, MKK7/JNK, and JAK2/STAT1 signalling pathways, Journal of Immunology Research 2021 (2021).
[27] M.S. Mohiuddin, et al., Glucagon prevents cytotoxicity induced by methylglyoxal in a rat neuronal cell line model, Biomolecules 11 (2021) 287.
[28] H.S. Min, et al., Dipeptidyl peptidase IV inhibitor protects against renal interstitial fibrosis in a mouse model of ureteral obstruction, Lab. Invest. 94 (2014) 598–607.
[29] K.S. Lim, et al., Pharmacokinetics, pharmacodynamics, and tolerability of the dipeptidyl peptidase IV inhibitor LC15-0444 in healthy Korean men: a dose-block-randomized, double-blind, placebo-controlled, ascending single-dose, Phase I study, Clin Ther 30 (2008) 1817–1830.
[30] E. Jung, et al., Gemigliptin improves renal function and attenuates podocyte injury in mice with diabetic nephropathy, Eur. J. Pharmacol. 761 (2015) 116–124.
[31] K. Seo, et al., Methylglyoxal induces mitochondrial dysfunction and cell death in liver, Toxicol. Res. 30 (2014) 193–198.
[32] C.M. Chan, et al., Methylglyoxal induces cell death through endoplasmic reticulum stress-associated ROS production and mitochondrial dysfunction, J. Cell Mol. Med. 20 (2016) 1749–1760.
[33] W.H. Chan, H.J. Wu, Methylglyoxal and high glucose co-treatment induces apoptosis or necrosis in human umbilical vein endothelial cells, J. Cell. Biochem. 103 (2008) 1144–1157.

[34] Y. Ishibashi, et al., Glucagon-like peptide-1 (GLP-1) inhibits advanced glycation end product (AGE)-induced up-regulation of VCAM-1 mRNA levels in endothelial cells by suppressing AGE receptor (RAGE) expression, Biochemical and biophysical research communications 391 (2010) 1405–1408.
[35] H. Oeseburg, et al., Glucagon-like peptide 1 prevents reactive oxygen species–induced endothelial cell senescence through the activation of protein kinase A, Arterioscler. Thromb. Vasc. Biol. 30 (2010) 1407–1414.
[36] Y. Ishibashi, et al., Advanced glycation end products evoke endothelial cell damage by stimulating soluble dipeptidyl peptidase-4 production and its interaction with mannose 6-phosphate/insulin-like growth factor II receptor, Cardiovasc. Diabetol. 12 (2013) 1–9.
[37] H.-C. Ku, et al., DPP4 deficiency exerts protective effect against H2O2 induced oxidative stress in isolated cardiomyocytes, PLoS One 8 (2013) e54518.
[38] K. Chinda, et al., Cardioprotective effect of dipeptidyl peptidase-4 inhibitor during ischemia–reperfusion injury, Int. J. Cardiol. 167 (2013) 451–457.
[39] M. Barbieri, et al., Decreased carotid atherosclerotic process by control of daily acute glucose fluctuations in diabetic patients treated by DPP-IV inhibitors, Atherosclerosis 227 (2013) 349–354.
[40] E. Jung, et al., Gemigliptin improves renal function and attenuates podocyte injury in mice with diabetic nephropathy, Eur. J. Pharmacol. 761 (2015) 116–124.
[41] G. Nguyen, et al., Gemigliptin alleviates succinate-induced hepatic stellate cell activation by ameliorating mitochondrial dysfunction, Endocrinology and Metabolism 37 (2022) 918–928.
[42] J. Kim, et al., Methylglyoxal induces hyperpermeability of the blood-retinal barrier via the loss of tight junction proteins and the activation of matrix metalloproteinases, Graefes Arch. Clin. Exp. Ophthalmol. 250 (2012) 691–697.
[43] M.G. de Oliveira, et al., Methylglyoxal, a reactive glucose metabolite, induces bladder overactivity in addition to inflammation in mice, Front. Physiol. 11 (2020) 290.
[44] M.K. Sarker, et al., Attenuation of diabetic kidney injury in DPP4-deficient rats; role of GLP-1 on the suppression of AGE formation by inducing glyoxalase 1, Aging (Albany NY) 12 (2020) 593.
[45] N. Dietrich, et al., The DPP4 inhibitor linagliptin protects from experimental diabetic retinopathy, PLoS One 11 (2016) e0167853.
[46] K. Wongchai, et al., Protective effects of liraglutide and linagliptin in C. elegans as a new model for glucose-induced neurodegeneration, Horm. Metab. Res. 48 (2016) 70–75.
[47] P.J. Thornalley, Use of aminoguanidine (Pimagedine) to prevent the formation of advanced glycation endproducts, Arch. Biochem. Biophys. 419 (2003) 31–40.
[48] A. Barnett, DPP-4 inhibitors and their potential role in the management of type 2 diabetes, Int. J. Clin. Pract. 60 (2006) 1454–1470.
[49] T. Zheng, et al., Increased plasma DPP4 activity predicts new-onset hypertension in Chinese over a 4-year period: possible associations with inflammation and oxidative stress, J. Hum. Hypertens. 29 (2015) 424–429.
[50] Y. Ishibashi, et al., Advanced glycation end products evoke endothelial cell damage by stimulating soluble dipeptidyl peptidase-4 production and its interaction with mannose 6-phosphate/insulin-like growth factor II receptor, Cardiovasc. Diabetol. 12 (2013) 125.
[51] K. Prasad, M. Mishra, AGE-RAGE stress, stressors, and antistressors in health and disease, Int. J. Angiol. 27 (2018) 1–12.
[52] G.d.O. Lopes, et al., Effects of lead exposure on salivary glands of rats: insights into the oxidative biochemistry and glandular morphology, Environ. Sci. Pollut. Control Ser. 28 (2021) 10918–10930.
[53] K. Satoh, et al., E2f1-deficient NOD/SCID mice have dry mouth due to a change of acinar/duct structure and the down-regulation of AQP5 in the salivary gland, Pflügers Archiv-European Journal of Physiology 465 (2013) 271–281.
[54] P.D.V. de Almeida, et al., Saliva composition and functions: a comprehensive review, J. Contemp. Dent. Pract. 9 (2008) 72–80.
[55] J. Santos, et al., Copy number polymorphism of the salivary amylase gene: implications in human nutrition research, J. Nutrigenetics Nutrigenomics 5 (2012) 117–131.
[56] A.L. Mandel, P.A. Breslin, High endogenous salivary amylase activity is associated with improved glycemic homeostasis following starch ingestion in adults, The Journal of nutrition 142 (2012) 853–858.
[57] K. Nakajima, Low serum amylase and obesity, diabetes and metabolic syndrome: a novel interpretation, World J. Diabetes 7 (2016) 112.
[58] E.M. Chavez, et al., Salivary function and glycemic control in older persons with diabetes. *Oral Surgery, Oral Medicine, Oral Pathology*, Oral Radiology, and Endodontology 89 (2000) 305–311.
[59] C.-C. Lin, et al., Impaired salivary function in patients with noninsulin-dependent diabetes mellitus with xerostomia, J. Diabetes Complicat. 16 (2002) 176–179.
[60] K. Okabayashi, et al., Effect of oxidative stress on secretory function in salivary gland cells, Oxidative Stress-Environmental Induction and Dietary Antioxidants (2012) 189–200.
[61] E.S. Frenkel, K. Ribbeck, Salivary mucins in host defense and disease prevention, J. Oral Microbiol. 7 (2015) 29759.
[62] S. Zhang, et al., Artesunate combined with metformin ameliorate on diabetes-induced xerostomia by mitigating superior salivatory nucleus and salivary glands injury in type 2 diabetic rats via the PI3K/AKT pathway, Front. Pharmacol. 12 (2021) 774674.
[63] A.S. High, et al., A morphometric study of submandibular salivary gland changes in streptozotocin-induced diabetic rats, Arch. Oral Biol. 30 (1985) 667–671.
[64] C. Delporte, et al., Aquaporins in salivary glands: from basic research to clinical applications, Int. J. Mol. Sci. 17 (2016) 166.
[65] C. Porcheri, T.A. Mitsiadis, Physiology, pathology and regeneration of salivary glands, Cells 8 (2019) 976.
[66] J.D. Kawedia, et al., Interaction between transcellular and paracellular water transport pathways through Aquaporin 5 and the tight junction complex, Proc. Natl. Acad. Sci. USA 104 (2007) 3621–3626.
[67] T. Ma, et al., Defective secretion of saliva in transgenic mice lacking aquaporin-5 water channels, J. Biol. Chem. 274 (1999) 20071–20074.
[68] S.Y. Chen, et al., Decreased basal and stimulated salivary parameters by histopathological lesions and secretory dysfunction of parotid and submandibular glands in rats with type 2 diabetes, Exp. Ther. Med. 19 (2020) 2707–2719.