

The synergistic effects of *Ixeris dentata* and *Lactobacillus gasseri* extracts on a diabetes-induced dry mouth model by enhancing antioxidation

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Abstracts

Dry mouth, hyposalivation, or xerostomia is a significant problem in diabetic patients; however, there was no way to relieve these symptoms. This study was aimed to evaluate the effects of *Ixeris dentata* (IXD) in combination with lactobacillus extract on the salivation rate in diabetes-induced dry mouth, and its mechanism was also investigated. In the streptozotocin-induced diabetes model, dry mouth condition was established as a model. Both control and diabetic rats were treated with a sublingual spray of either water or IXD and subsequently treated with or without a spray of lactobacillus extract. In diabetes condition, the salivary flow rate, amylase activity, and aquaporin-5 and Na⁺/H⁺ exchanger (NHE-1) expressions were markedly decreased, whereas they were more significantly recovered in the sequential treatment of IXD-lactobacillus extract than each single treatment. Furthermore, oxidative stress and its related ER stress response were especially regulated in the IXD/lactobacillus extract condition, where the following anti-oxidative enzymes; GSH:GSSG ratio, superoxide dismutase (SOD), and glutathione peroxidase (GPx) were involved. This study suggests that the combination of IXD

and lactobacillus would be a potential alternative medicine against diabetes-induced hyposalivation and xerostomia.

Keywords: *Ixeris dentata*; *lactobacillus*, salivation; xerostomia

1. Introduction

Diabetes mellitus is a metabolic disorder that has physical effects on patients. Xerostomia (dry mouth) is one of these effects [1], which causes difficulties in swallowing, chewing, and increased risk of oral problems. Several studies have examined the prevalence of oral lesions and xerostomia in diabetic patients. Previous studies reveal that 76.4% of diabetic patients suffer from xerostomia [1], which has affected the quality of life. Hence, dry mouth needs to be diagnosed and treated to improve oral health and better quality of life [2].

IXD is one of the herbal medicine routinely used in Korea, Japan, and China to reduce the treatment of diabetes, indigestion, and allergies [3,4]. Additionally, in these countries, IXD extract is widely popular as functional or healthy food [5]. Previously, we used a diabetic rat model to identify *Ixeris dentata* (IXD) as a regulator of salivary secretion [6]. Additionally, several studies have reported the nutritional value of IXD and its components [5]. Antioxidant effects of IXD have been confirmed similar to flavonoid-enriched natural extracts. However, its specific functions in diabetes-induced dry mouth model need to be investigated.

It has been reported that oxidative stress plays a role in reducing the saliva secretion [7], and oxidative stress is influenced by reactive oxygen species (ROS), which also affects the age-related diseases including diabetes, obesity, and hyperlipidemia [8]. Also, metabolic diseases increase the mitochondrial production of ROS, decreasing the antioxidative potential of the body [9]. Consequently, it becomes challenging for the body to maintain the redox balance, ultimately accumulating ROS. Furthermore, accumulated oxidative stress is deleterious to cell

membrane proteins and phospholipids and leads to cellular dysfunction [10]. Dysmetabolism-associated saliva dysfunction has been reported to be related with redox imbalance and ROS accumulation [11].

Moreover, the use of lactic acid bacteria is popular in fermented foods around the world and is well accepted by society. Also, few strains of lactic acid bacteria are routinely used in probiotics for their health benefits. Lately, several reports suggest the beneficial effects of lactic acid bacteria, such as immunoregulatory, antioxidative, and anti-inflammatory effects have been reported [12-14] representing the safe and valuable functional food ingredients. Considering the natural health benefits of IXD and lactic acid bacteria, synergistic effects of IXD and lactobacillus extract were investigated to improve the dry mouth condition in diabetes-associated dry mouth model. Utilization of IXD and lactobacillus extract may indicate, the potential activity of the combination against the hyposalivation and its related redox disturbance mechanisms.

2. Materials and Methods

2.1. Chemicals and Reagents

Pilocarpine hydrochloride, streptozotocin (STZ) and citric acid were procured from Sigma Chemical Company (St. Louis, MO, USA). The following proteins were used in this study : Antibodies against anti-amylase (#4017, Cell Signaling Technology, Danvers, MA, USA), anti-NHE-1 (sc-28758, santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), anti-AQP-5 (sc-514022, santa Cruz Biotechnology), anti-GRP78 (sc-376768, santa Cruz Biotechnology), anti-CHOP (#2895, Cell Signaling Technology), p-IRE1 α (ab48187, Abcam, Cambridge, MA, USA), IRE-1 α (#3294, Cell Signaling Technology), anti-p-eIF2 α (#9721, cell signaling), anti-eIF2 α (sc-133132, santa Cruz Biotechnology), and anti- β -actin (sc-130300, Santa Cruz

Biotechnology). Horseradish peroxidase-conjugated secondary antibodies were obtained from Enzo Life Sciences, Inc. (Farmingdale, NY, USA).

2.2. Plant Material Preparation

Ixeris dentata roots were harvested in 2014 in Dangin, Korea (ID 2014-01), and were identified and verified at the National Institute of Horticultural and Herbal Science (NIHHS), Rural Development Administration (RDA), Wanju, Korea, and deposited at the College of Pharmacy, Yonsei University, Incheon, Korea [6]. Roots were dried and powdered, about 40g of powdered root is extracted with 300 mL of water and ethanol in gradient manner (20%, 40%, 60%, 80%, and 100% ethanol) using ultrasonic apparatus for 3 h at 50 °C. These extracts were suspended in water to get the desired concentration before use.

2.3. Preparation of *Lactobacillus* Extracts

Lactobacillus gasseri MJM6064 was isolated from human saliva and stored in 20% glycerol at -80 °C. It was activated on DeMan-Rogosa-Sharpe (MRS) agar plate at 37 °C for 24 h. A single colony was inoculated into 3 mL MRS broth and incubated at 37 °C for 16 h. Then 0.5 mL of the seed culture was inoculated into 500 mL of MRS broth and cultured at 37 °C for 24 h. After fermentation, the supernatant was extracted with the same volume of ethyl acetate (EtOAc), and the extract was concentrated to dryness by a rotary evaporator under vacuum. The dryness was suspended in water for further use.

2.4. Induction of diabetes

Type 1 diabetes was induced as described previously [15]. Briefly, 65 mg/kg of STZ is dissolved in 0.1 M citrate buffer. Citrate buffer used as a vehicle, and after 3 days of injection,

basal blood glucose levels were measured in overnight-fasted rats. Glucometer (Accu-Chek, Roche, Germany) was used to assess systemic glucose concentration. Blood glucose concentrations of 300 mg/dL or higher are regarded as diabetic and these diabetic, animals were used for the study [16].

2.5. Experimental Design

Sprague-Dawley male rats (250-270 g) were procured from the Samtako (Daejeon, Republic of Korea) and maintained in specific pathogen-free housing condition at 22 ± 2 °C and $55 \pm 5\%$ humidity under 12 h light/dark cycle. Rats were cared for in accordance with the regulations of the Institutional Animal Care and Use Committee of Jeonbuk National University Laboratory Animal Center (cuh-IACUC-2018-2). All the animals used in the study were acclimatized to our laboratory conditions for one week before use in the experiments. Rats were randomly separated into 8 groups, consisting of 10 rats in each group. Briefly, two weeks after either water or streptozotocin injection, the rats were anesthetized prior to sublingual treatment (1 spray=50 μ L). Different groups of the study are as follows; normal control group was sprayed with water (control + water); second normal group sprayed with IXD extract (control+IXD, 10 mg/kg); third normal group sprayed with lactobacillus extract (control+*Lactobacillus gasseri*, 0.5mg/kg); fourth normal group sprayed with IXD and cotreated lactobacillus extract (control+IXD/*Lactobacillus gasseri*). Similarly, diabetic rats were grouped as control rats sprayed with water (STZ + water); rats sprayed with water (STZ + water); rats sprayed with IXD extract (STZ+IXD, 10 mg/kg); rats sprayed with lactobacillus extract (STZ+*Lactobacillus gasseri*, 0.5 mg/kg); rats sprayed with IXD and cotreated lactobacillus extract (STZ + IXD/*Lactobacillus gasseri*). Post saliva collection animals were sacrificed, the bilateral submandibular glands were carefully excised and weighed. One lobe

was immediately frozen, and the remaining section was kept in formalin (formaldehyde 3.7%; Dana Korea, Seoul, Republic of Korea) for histological examinations (Supplementary figure 1).

2.6. Collection of total saliva

Saliva was collected as described previously [17]. Briefly, two weeks after either vehicle or STZ injection, a single spray of water or IXD extract (10 mg/kg) was given sublingually to anesthetized rats and left for 5 min. Later, 0.6 mg/kg of pilocarpine is injected intraperitoneally, and saliva was collected using pre-weighed, cotton balls for up to 30 min [6].

2.7. Immunoblotting

Immunoblotting was performed as described previously [6] using submandibular gland tissue homogenates and whole saliva. The homogenate was prepared by homogenizing the submandibular gland tissue in radioimmunoprecipitation assay lysis buffer. Total protein was quantified using the Bio Rad Protein assay (Bio-Rad, CA, USA). Samples containing 30 µg of total protein extract were separated on a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE; BioRad), and transferred to a polyvinylidene fluoride membrane (PVDF). The membrane was blocked with 5% skim milk in tris-buffered saline (0.137 M NaCl, 0.025 M tris, pH 7.4) containing 0.1% Tween 20 (T-TBS). Blots were probed with relevant antibodies, washed and probed again with species-specific secondary antibodies. Protein signals were visualized with the help of enhanced chemiluminescence reagent. For Coomassie Brilliant Blue R-250 staining (CBB), samples were stained with CBB and de-stained using a 30% methanol/10% acetic acid solution.

2.8. Hematoxylin and eosin staining

Hematoxylin and eosin (H&E) staining was performed as described previously [6]. Briefly, processed paraffin-embedded submandibular gland tissue was sectioned, deparaffinized, and rehydrated in decreasing concentrations of alcohol. Later, washed with water and stained with Harris hematoxylin and 1% eosin. Then sections were dehydrated with increasing concentrations of alcohol and observed under the light microscope.

2.9. Immunohistochemistry

Immunohistochemistry was performed as described previously [6]. Formalin-fixed, paraffin-embedded salivary gland tissues were deparaffinized and rehydrated in xylene, followed by decreasing ethanol concentration. Antigen retrieval was accomplished using Target Retrieval Solution in a decloaking chamber (Biocare Medical, Concord, CA, USA). Further, sections were cooled, rinsed, and blocked with phosphate buffer containing hydrogen peroxide and detergent to block endogenous peroxidase activity. Sections were washed with Tris-Buffered Saline-Tween 20 (TBST) prior to incubation with the primary antibody at 4 °C. Then, the sections were rinsed in T-TBS buffer and treated with secondary antibodies at room temperature. Later, sections were washed and incubated with 3-amino-9-ethylcarbazole (AEC) substrate chromogen before counterstaining with Harris hematoxylin (Sigma-Aldrich Co., St Louis, MO, USA). All the sections were washed thoroughly and mounted in an aqueous medium.

2.10. Dihydroethidine (DHE) staining

DHE staining was done as described previously. Submandibular tissues were cut into sections,

treated with DHE and incubated in a humidified chamber at 37 °C for 30 min. Fluorescence was detected with fluorescent microscope (Olympus, Melville, NY).

2.11. Statistical analysis

All observations are expressed as mean \pm SEM. Mean and standard deviations of the analyzed samples were determined, and groups were compared using one-way ANOVA, followed by t-test. A *P* value of <0.05 was considered statistically significant.

3. Results

3.1. IXD and cotreated lactobacillus extract shows synergistic effect against dry mouth

Dry mouth is associated with decreased salivary secretion, decreases salivary secretion is reported to be closely associated with diabetic condition [18,19]. These close associations between salivary secretion, dry mouth and diabetic condition lead the current investigation where effects of type 1 diabetes mellitus on salivation and the beneficiary effects of IXD, lactobacillus extract, and its cotreated extracts on the diabetes-induced dry mouth were studied. A single spray of IXD or lactobacillus extracts or cotreatment was administered sublingually and observed a significant reduction in salivary secretion in STZ-induced diabetic rats whereas no significant changes in saliva secretion in water-, IXD-, or lactobacillus-treated control rats. However, cotreatment with IXD and lactobacillus extract significantly increased salivary secretion and salivary flow rate in diabetic rats than IXD or lactobacillus extract (Figure 1A, B). Further, to investigate the effects of IXD and lactobacillus extract on the morphology of the submandibular glands, histological examination with H&E was performed. The streptozotocin-induced diabetic group showed depleted acinar cells and irregular ductal cell morphology in the submandibular glands (Figure 1C).

Both the sublingual route and spray form of the IxD treatment may have enhanced salivation without improving the morphology of the salivary gland. We found no significant differences in submandibular glands weight in STZ-diabetic rats or IxD and cotreated lactobacillus extract (Figure 1D). The total protein concentration remained unchanged in all the groups (Figure 1E), suggesting that IxD/lactobacillus extract increase saliva secretion not due to the change of salivary gland weight.

3.2. IxD and cotreated lactobacillus extract increase salivary amylase expression in diabetic rats

In addition to saliva secretion, the expression of α -amylase in saliva and salivary gland lysates represents an oral functional state [20]. In immunoblot analysis, no significant differences were observed in control rats treated with either water, IxD, lactobacillus extract or IxD/lactobacillus extract (Figure 2A). However, significant reduction in amylase expression in both saliva and submandibular glands tissue were observed in vehicle-treated diabetic animals. Interestingly, the expression of amylase was more significantly increased in both saliva and tissue lysates under the cotreated diabetic condition (Figure 2A) when compared with each single treated condition. Amylase activity was also regulated in the cotreated condition (Figure 2B), suggesting that cotreatment with IxD/lactobacillus extract restore the decreased amylase folding and secretion seen in the salivary glands of diabetic condition.

3.3. IxD and cotreated lactobacillus extract increase salivary secretion through the activation of AQP5 and NHE-1

Multiple reports have shown the expression of aquaporin 5 (AQP5) and sodium-hydrogen exchanger (NHE-1) in various sites of the submandibular glands. AQP5, the main water

channel to control water secretion, is localized in the apical, basal, and lateral membranes of submandibular gland acinar cells in SD rats [21]. Localization of NHE-1 to acinar and duct cells was also reported, suggesting that the Na^+/H^+ antiporter isoform 1 contributed to saliva secretion [22]. Our study showed a uniform distribution of AQP5 expression in control rats (Figure 3A); however, the diabetic rats had faint AQP5 expression in both the ductal and acinar cells of the submandibular glands. Weak expression of NHE-1 in both the submandibular glands duct and acinar cells of diabetic rats (Figure 3B) was also observed. As expected, IXD and cotreated lactobacillus extract-treated rats showed higher expression of NHE-1 in both the ductal and acinar cells of submandibular glands compared with each treated rats. These results suggest that the cotreated IXD/lactobacillus extract increases the expression of AQP5, which controls water balance in saliva environment, i.e., submandibular acinar cells and that the extract enhances the expression of NHE-1, which contributes to fluid secretion from acinar cells and of NaCl by duct cells. In immunoblotting data, diabetic rats had significantly lower expressions of AQP5 and NHE-1, compared with control rats (Figure 3C). Cotreated spray of IXD and lactobacillus extract more significantly recovered the reduced expressions of AQP5 and NHE-1 in diabetic submandibular glands tissue homogenates compared with either IXD or lactobacillus extract.

3.4. IXD and cotreated with lactobacillus regulates diabetes-associated ER stress

Further, to evaluate the effect of IXD/lactobacillus on ER stress immunoblot assays were performed using submandibular gland tissue lysate to measure the expression of several ER stress markers. It was observed that the expressions of ER stress response proteins were upregulated in diabetic rats (Figure 4). Also, no differences in the ER stress response proteins in vehicle and IXD-treated control rats was noticed. In the diabetes condition, the cotreated

IXD/lactobacillus significantly inhibited the ER stress protein expression although, each single treated IXD or lactobacillus also controlled the expression, suggesting that the IXD cotreated with lactobacillus extract reduced ER stress in the diabetic submandibular gland.

3.5. IXD and cotreated with lactobacillus protects against streptozotocin-induced diabetes model

Various reports have suggested that oxidative stress significantly increases with diabetes [23,24]. We performed the dihydroethidium (DHE) fluorescent staining to detect ROS accumulation in the submandibular gland. As shown in figure 5A, we observed high DHE fluorescence in the submandibular glands of diabetic rats, and IXD and cotreated lactobacillus extract reduced the ROS fluorescence intensity. There were no differences in control rats treated with either water, IXD or lactobacillus extract. We analyzed protein oxidation, membrane lipid peroxidation, glutathione redox status, glutathione peroxidase (GPx), and superoxide dismutase (SOD) activity in the diabetes-induced dry mouth models. The increase in protein oxidation observed in the dry mouth models was significantly reduced by the IXD combined with lactobacillus extract (Figure 5B). Since protein oxidation may be linked to ROS [25], we assessed MDA assays, the GSH/GSSG ratio, GPx, and SOD activity. The MDA levels were reduced in the presence of IXD combined with lactobacillus extract (Figure 5C). The GSH:GSSG ratio, GPx, and SOD activities were also decreased in the diabetes models and were restored by the IXD combined with lactobacillus extract (Figure 5D-F).

4. Discussion

In this study, the potential synergistic efficacy of IXD and lactobacillus extract against diabetes-induced dry mouth was evaluated. Observations suggest that IXD combined with

lactobacillus extracts exhibited a synergistic effect on salivary secretion compared with a single treatment of either IXD or lactobacillus extract. The effect was also reflected by amylase, aquaporin 5, and NHE-1 activities and its anti-oxidative effect controlling reactive oxygen species, suggesting that the IXD/lactobacillus extract might contribute to dry mouth.

The cotreated IXD/lactobacillus showed a synergistic effect against diabetes-associated dry mouth. In our study, submandibular gland weight and morphology were not different in diabetic rats with or without the cotreated IXD/lactobacillus; however, showing a recovery in salivary secretion in diabetic rats with the cotreated IXD/lactobacillus (Figure 1). Specifically, in diabetic conditions, it was reported that reduction in salivary flow contributed to symptomatic drying of the oral tissues and loss of the protective effects of salivary buffers, proteins, and mucins [26]. Along with the salivary flow rate, salivary α -amylase is also one of the essential enzymes in saliva, which is an indirect saliva function marker [27,28]. In this study, the expression of α -amylase was also significantly recovered under the cotreated IXD/lactobacillus, suggesting the possibility of IXD and its compounds as a potential candidate against dry mouth [29]. Furthermore, probiotics including lactobacillus, have been reported to have a regulatory effect on the oral functional state, such as hygiene and anti-inflammation [30,31]. The single treatment of IXD and its compounds also enhanced saliva flow rate and amylase secretion in diabetic conditions (Figure 1, 2), [17]. The synergistic effect of the cotreated IXD/lactobacillus showed a potential therapeutic/preventive approach to control dry mouth.

ER stress, a key signal to explain the disturbed folding/secretion process, was also synergistically regulated in the cotreated condition. The ER secretory capacity is overwhelmed, causing alterations in ER folding and secretion along with ER redox uncoupling phenomenon leading to ROS accumulation [18]. The ER stress and its associated ER-ROS accumulation contribute to hyperglycemia-associated salivary gland dysfunction and irreversible salivary

gland cell damage under chronically high concentrations of glucose [19]. The ER stress and its related ROS accumulation have also been studied in IxD-treated diabetes rats [6]. In this study, the ER stress and ROS accumulation were controlled with the cotreated IxD/lactobacillus (Figure 4, 5), restoring the diminished saliva flow rate and amylase secretion. ROS has been suggested as a mechanism of salivary gland hypofunction in Sjogren's syndrome also [32]. A rapid decline in glutathione and an increase in intracellular ROS suppressed the amylase release that was induced by a beta-adrenergic agonist in rat parotid acinar cells, suggesting that oxidative stress in salivary gland tissue induces an alteration in the secretory function and reduces salivary proteins [33]. Treatment with IxD combined with lactobacillus extract more significantly activated antioxidant enzymes, like superoxide dismutase and reduced malondialdehyde in the dry mouth conditions compared with each single treatment (Figure 5 D-H), indicating that IxD shows synergistic antioxidant effects when combined with lactobacillus extract. Considering that polyphenol compounds are important plant constituents with their free radical scavenging ability by virtue of their hydroxyl groups [34,35], the rich phenolic content of the IxD extracts would support their antioxidant activity. More specifically, the high relative contents of luteolin 7-O-glucoside and luteolin 7-O-glucuronide combined with the lactobacillus' antioxidative effect [36] are suggested to contribute to the ROS scavenging effect in the IxD/lactobacillus-cotreated condition. The regulatory effect of ER stress and its related or unrelated ROS are explaining the IxD/lactobacillus-synergistic effect, a potential mechanism in this study.

Primarily, this study is designed to enhance saliva secretion without a systemic effect on the serum glucose level. The previous study has uncovered the systemic effect of an oral formula of IxD on blood glucose level, also showing the significant controlling effect against dry mouth [6]. In this study, an oral spray of IxD with lactobacillus was demonstrated to be effective

against the dry mouth under diabetic condition, suggesting that the local application of the extracts might be successful in general dry mouth condition.

In conclusion, the present study indicates that the IXD and lactobacillus extract treat or prevent diabetes-associated dry mouth, justifying their use as functional food-originated spray formulation. The cotreated extracts exhibited ER stress regulatory effect with the related or unrelated free radical scavenging properties. This effect was helpful in controlling dry mouth without systemic effect on blood glucose concentration in diabetic conditions.

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Author Contributions

Conceptualization, H.Y.L. and H.J.C.; methodology, M.G., J.C., and J.W.S.; formal analysis, H.Y.L.; writing-original draft preparation, H.Y.L. and H.J.C.; writing-review and editing, J.W.S. and H.J.C.

Conflicts of Interest

The authors declare no conflict of interest.

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Figure Legends

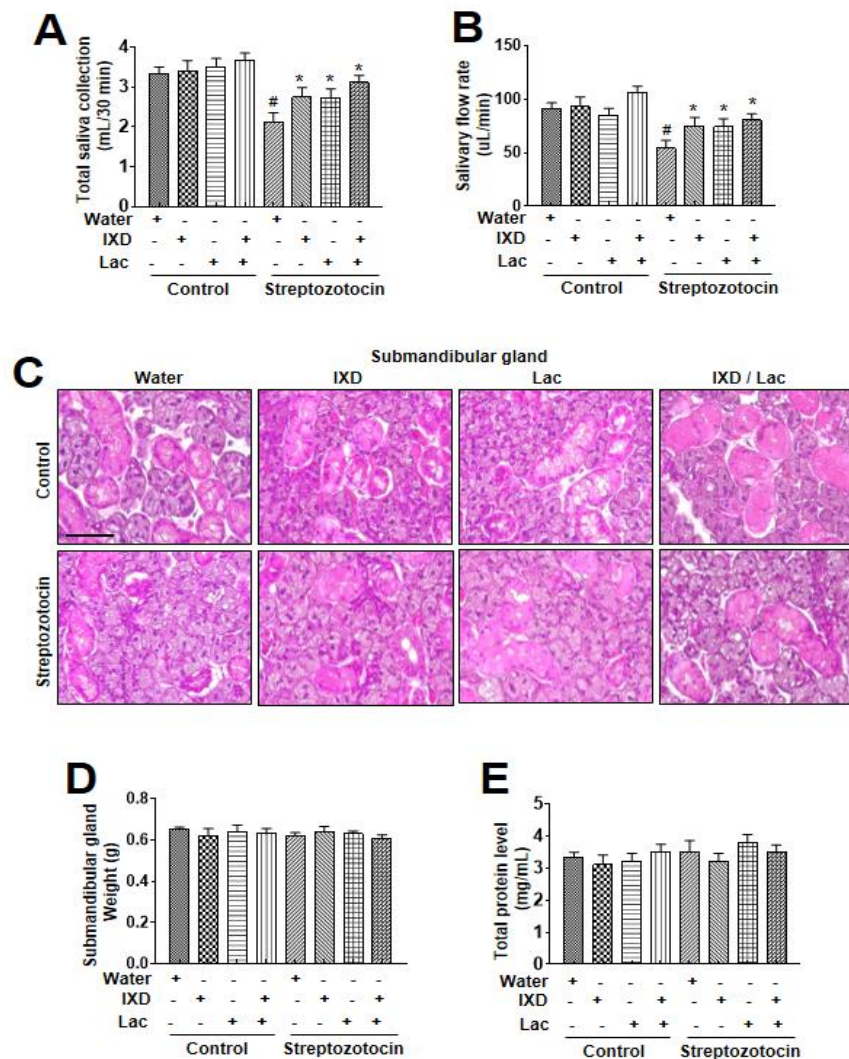


Figure 1. *Ixeris dentata* and lactobacillus extracts improve salivary function in diabetes model. Water, IXD, or lactobacillus extract was given as a spray or IXD, and subsequently, lactobacillus was given as a spray to streptozotocin-induced diabetes models. Saliva and the submandibular glands were collected after sacrificing the animals. Total saliva collected in 30 minutes (A) and salivary flow rate (B) were measured. (C) Hematoxylin and eosin staining was performed on paraffin-embedded submandibular gland tissues from normal and diabetic rats; either treated with or without water, IXD, lactobacillus extract, or cotreated IXD and

lactobacillus extracts. Magnification=20 \times , scale bar=100 μ m. Weight of submandibular gland (D) and total salivary protein concentration (E) were measured. #significant difference vs water-treated control rats; *significant difference vs streptozotocin-induced diabetic control rats ($p<0.05$). Values are represented as mean \pm SEM (n=10 rats per group). STZ; streptozotocin.

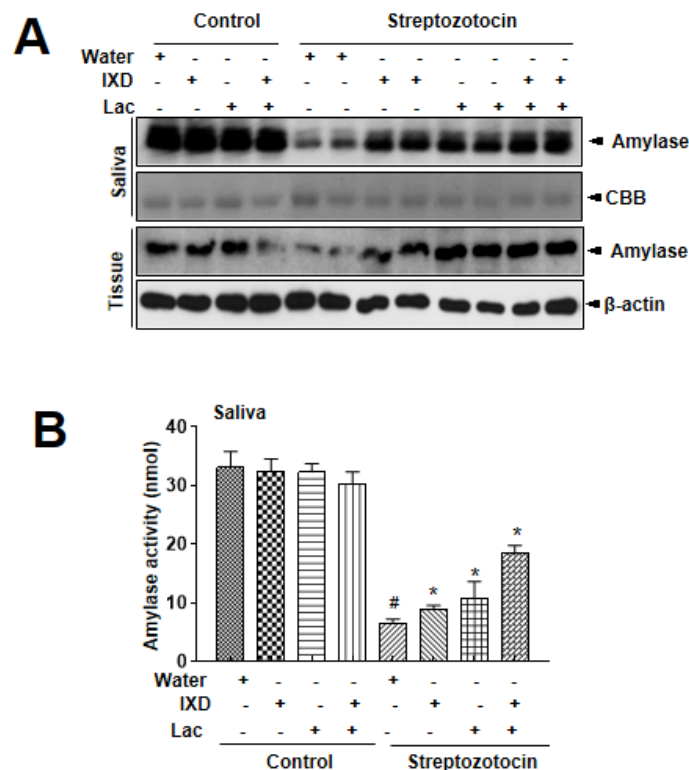


Figure 2. *Ixeris dentata* and lactobacillus extracts increase α -amylase expression in salivary glands from diabetic rats. Water, IXD, or lactobacillus extract were given as a spray or IXD, and subsequently, lactobacillus were given as a spray to streptozotocin-induced diabetes models. Western blotting was performed with anti-amylase antibody in submandibular gland tissue homogenates (A) and amylase activity was analyzed as described in Materials and Methods (B). β -actin was used as a loading control. #significant difference vs. water-treated

control rats; *significant difference vs. STZ-induced diabetic control rats ($p < 0.05$). Values are represented as mean \pm SEM ($n = 10$ rats per group). STZ; streptozotocin.

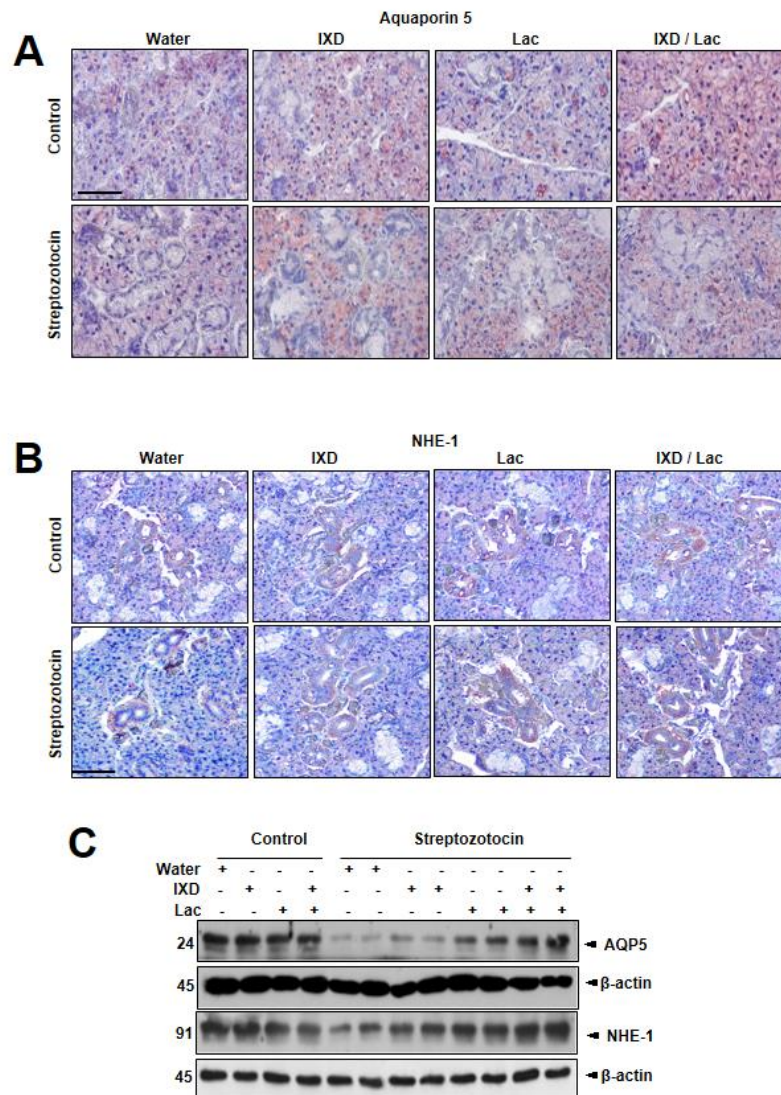


Figure 3. *Ixeris dentata* and lactobacillus extracts recover the disrupted aquaporin5 and sodium hydrogen exchanger1 expressions in salivary gland from diabetic rats. Water, IXD, or lactobacillus extract was given as a spray or IXD, and subsequently, lactobacillus were given as a spray to streptozotocin-induced diabetes models. Immunohistochemistry (A, B) and immunoblotting (C) were performed with anti-aquaporin-5 or NHE-1 antibody.

Magnification=20×, scale bar=100 μm. AQP5; aquaporin5, NHE-1; sodium hydrogen exchanger1.

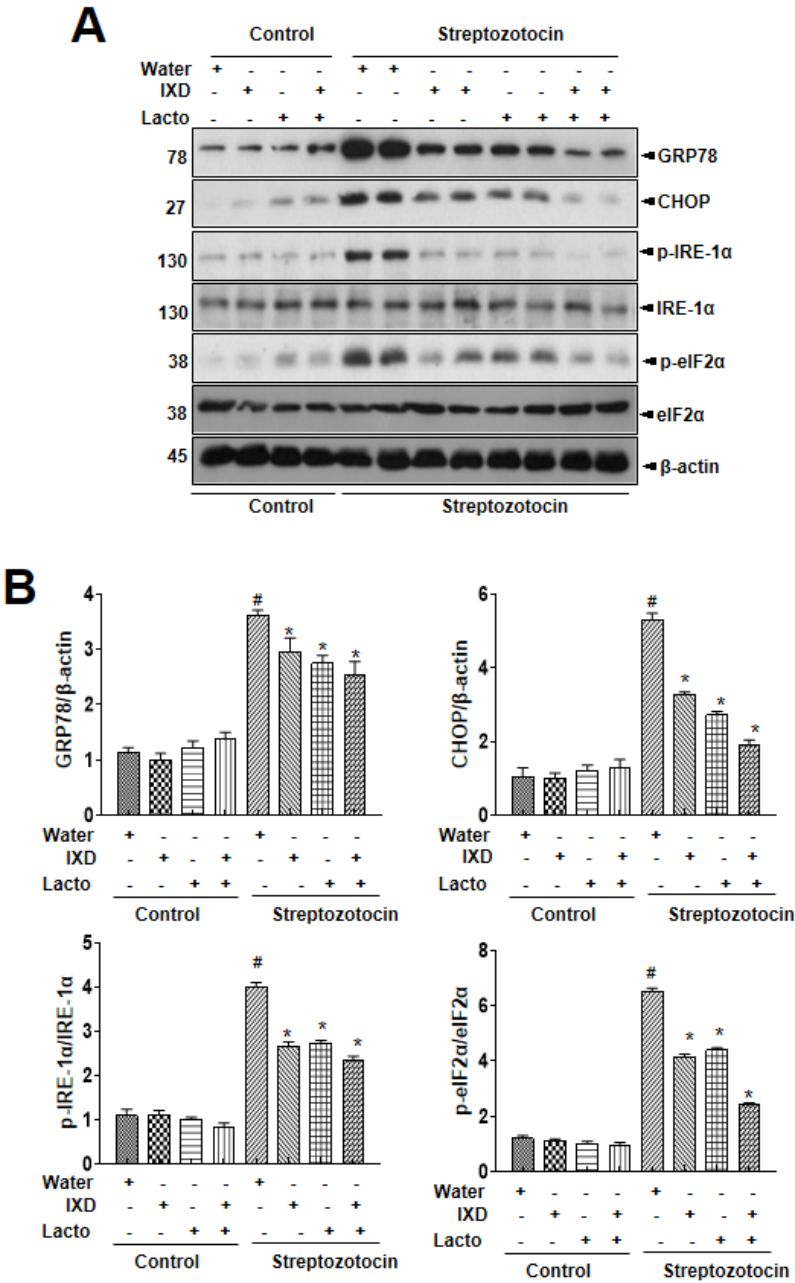


Figure 4. *Ixeris dentata* and lactobacillus extracts regulate ER stress response in salivary glands from diabetic rats. Water, IXD, or lactobacillus extract was given as a spray or IXD,

and subsequently, lactobacillus were given as a spray to streptozotocin-induced diabetes models. Immunoblotting was performed with anti-GRP78, CHOP, p-eIF2 α , total eIF2 α , p-IRE1 α , and total IRE1 α antibodies. β -actin was used as a loading control.

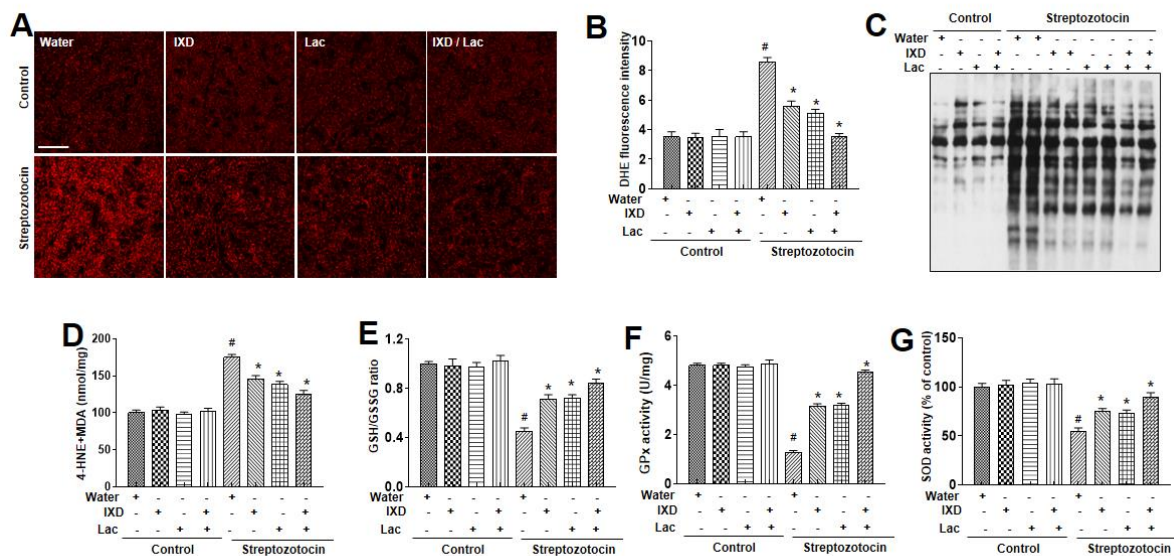


Figure 5. *Ixeris dentata* and lactobacillus extracts reduce lipid peroxidation and ROS production in salivary glands of diabetic rats. Water, IXD, or lactobacillus extract was given as a spray or IXD, and subsequently, lactobacillus were given as a spray to streptozotocin-induced diabetes models. In the submandibular gland tissues, dihydroethidium staining was performed and quantified as described in Materials and Methods (A, B). On lysates from submandibular gland tissues, OxyBlot (C), 4-HNE and malondialdehyde analysis (D) were performed. The GSH/GSSG ratios (E), glutathione peroxidase (F), or superoxide dismutase (G) were analyzed in the submandibular gland tissues. #significant difference vs. vehicle-treated control rats; *significant difference vs. streptozotocin-induced diabetic control rats ($p < 0.05$). Values are represented as mean \pm SEM ($n = 10$ rats per group). STZ; streptozotocin, GPx; glutathione peroxidase.