



The Anticipated Effect of Both Zamzam Water and Epidermal Growth Factor (EGF) Separately on Tongue Tissues and Lingual Salivary Glands of Diabetic Albino Rats.

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ABSTRACT

Purpose: The aim of this study was to evaluate the anticipated effect of both Zamzam water and Epidermal growth factor (EGF) separately on tongue tissues and associated lingual salivary glands of diabetic albino rats, (Histological and Ultrastructure evaluation). **Materials and Methods:** Eighteen male albino rats were selected and divided into four groups. Group I (control group) received no drugs, while rats of group II (diabetic group) received a single dose of alloxan intraperitoneal injection. Moreover, rats of group III diabetic and received Zamzam water for 3 months and group IV diabetic and received EGF. All rats were sacrificed; tongue was dissected and processed for hematoxylin & eosin and transmission electron microscopy evaluation. Results the diabetic group showed histological & ultrastructural changes in the tongue epithelium and underlying lamina propria, a condition which almost restored to normal in zamzam treated group and relatively restored in EGF group. **Conclusion:** Zamzam water provides safer alternative nutritional strategy in restoring the integrity of the damaged epithelium of the tongue tissues after degenerative effect of DM. EGF causes relatively improvement of the damaged epithelium especially in basal cell layer, keratin layer and blood vessels in lamina propria of tongue tissue.

INTRODUCTION

Diabetes mellitus is represented a chronic heterogeneous group of disorders affecting carbohydrate, fat and protein metabolism, causing hyperglycaemia, which is due to impaired carbohydrate (glucose) utilization resulting from a defective or deficient insulin secretory response. It was estimated that the disorder affects more than 100 million people worldwide and it is predicted to reach 366 million by 2030 ⁽¹⁾.

KEYWORDS

Tongue Tissue,
Diabetes Mellitus,
Zamzam Water, (EGF),
Albino Rats.

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Egypt is one of the top ten countries in the world in relation to diabetes incidence and it is on rise. More than 11% of Egyptians suffer from diagnosed DM. It is associated with long-term complications damage, dysfunction and/or failure of several organs, (heart, liver, kidney, eye, bone, joints, skin and mucous membrane, blood and nerve system) and autonomic dysfunction, including sexual dysfunction, stroke, diabetic neuropathy, retinopathy, amputations, renal failure and blindness⁽²⁾. Several oral changes have been reported to be associated with DM. These complications include periodontal diseases, xerostomia, multiple abscesses, dental caries, candida infection, burning mouth syndrome, impaired healing, and increasing incidence for secondary infection in oral mucous membrane⁽³⁾. Tongue one of the most oral tissues affected by diabetes.

Zamzam is natural water consumed by millions of Muslims worldwide owing to their religious belief. The well is found in Makkah within the holy mosque (Haram)⁽⁴⁾. The alkaline nature of zamzam water and presence of minerals (inorganic elements) such as (Na), (Ca), (Mg), (K), (HCO), (Cl), (Fl), (SO), and totally dissolved salts (TDS) and high calcium content making its potential antioxidant, agent antitumor, anxiolytic and antidepressant agent^(5,6). EGF is a family of growth factors of low molecular and small 53-amino acid, single chain polypeptide, is found in many mammalian tissues including kidney, small intestine, liver, pancreas, to lesser extent, in the lactating mammary glands, and occurs in nano-gram quantities in plasma⁽⁷⁾. It regulates cell proliferation, migration and differentiation through binding to receptor tyrosine kinases on target cells. Also help in promotion of epidermal development, wound healing, eruption of the incisors, activation of various transport systems and changes in cellular metabolism⁽⁸⁾. The aim of this study to evaluate the anticipated effect of both Zamzam water and Epidermal growth factor (EGF) separately on tongue tissues and associated lingual salivary glands of diabetic albino rats, (Ultrastructure and Histological)

MATERIALS AND METHODS

Materials

- Alloxan (25g. ALX monohydrate, Sigma Aldrich pharmaceutical company)
- Zamzam water was obtained from inside the Holy Mosque in a well in Makkah Al-Mukarramah.
- Epidermal Growth Factor (EGF) lyophilized from 1 mg/ml solution after extensive dialysis against 20mM phosphate buffer, PH 7.4 and 130mM NaCl obtained from (Sigma Aldrich)

Methods:

80 adult male Wistar albino rats weighing between (200±20 g) was used in this experimental study. The rats were housed in stainless steel cages (5 rats / cages) under standard conditions of a relative humidity and temperature. Diabetes will induce in normal rats by intra peritoneal injection of alloxan monohydrate. Each rat was injected with a single dose of 1 ml from the prepared alloxan solution (200mg/kg bodyweight) as a single dose in order to induce DM. Since alloxan is capable of producing fatal hypoglycemia due to massive pancreatic insulin release, rats were treated with 20% glucose solution orally after 6 h for 3 days. The animals were randomly divided into different groups as follows (each group containing 20 rats):

- (Group I);The Control group: received saline
 - The experimental groups (After induction of diabetes, the diabetic rats)
1. (Group II); (-ve control). Diabetic rats without any treatment
 2. (Group III); Oral treatment of diabetic rats with zamzam water as their sole source of drinking water.(100 mL/24 h/cage).
 3. (Group IV); Oral treatment of diabetic rats with EGF at the dose of 1.25 µg of EGF (sigma)\kg. (Using an insulin injector, a 1.25 microgram/kg dose of EGF containing the saline solution was administered to the rats in the group IV).

After 3 months, at the end of the study, the dissected tongue tissue from rats used for dorsal surface and associated minor lingual salivary gland examination by light and transmission electron microscope examination.

RESULTS

Histological results:

The tongue papillae in diabetic group revealed that basal cell showed degeneration of the cells and fragmentation of the basement membrane in some areas. In the all cell layers there were marked degeneration and loss of architecture, presence of vacuolation, widening of the intercellular spaces between adjacent cells. Area of mitosis are also observed. Zamzam group showed by light microscopic examination that all types of filiform papillae, fungiform and circumvallate papillae appeared in normal shape and articheture with relatively arrangement of cell layering as control group. The muscle fibers revealed its normal arrangement in bundles in the underlying lamina propria. Restored taste buds in both fungiform and circumvallate papillae were also observed. In EGF group by light

microscopic examination that all types of tongue papillae appeared in relatively normal articheture and histological appearance with mitotic activity of cells in different epithelial cell layering. The muscle fibers revealed arrangement in bundles in the underlying lamina propria in different directions. Slight restoring of taste buds in both fungiform and circumvallate papillae was also observed. Histological section of diabetic SG showed cytoplasmic vacuolation, pyknotic nucleus, focal loss of salivary architecture. Widening in interacinar spaces were obviously observed. Congested dilated blood vessels blood vessels engorged with red blood cells (RBCs) was also noted. Microscopic examination of lingual salivary gland of zamzam group showed the acini with esinophilic stain, relatively normal histologic features and normal acinar arrangement. The muscle fiber septa appeared with restored architecture separating the acini. Lingual salivary gland of EGF group showed some serous acini were still lacking acinar articheture, the acini appeared basophilic in stainability and some scattered acini were also observed . Atrophied muscle fibers , little area of vacuolation were also observed. There were widening in intercellular and interlobular spaces. **Fig (1)**

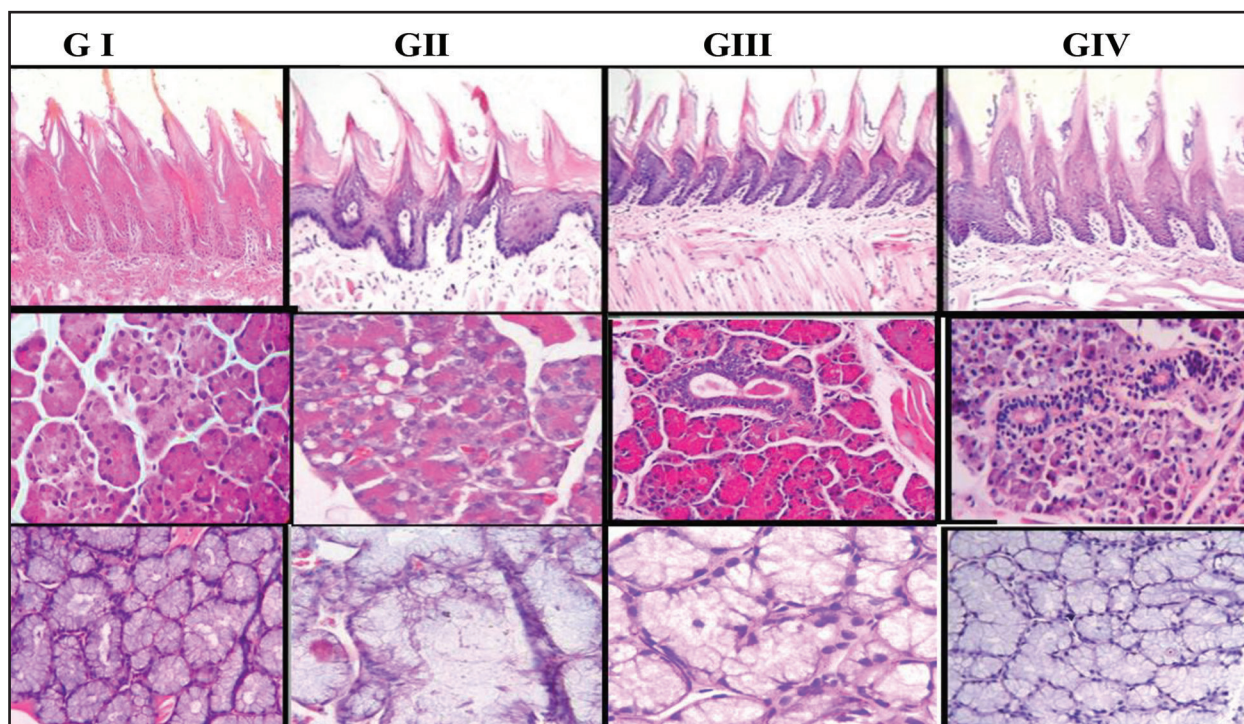


Figure (1) A photomicrograph showing tongue papillae and lingual salivary gland of tongue tissue. Control group(GI), diabetic group(GII) , Zamzam group(GIII), EGF group(GIV) (H&E, Orig. Mag x100)

Transmission electron microscope result

In electron microscopic examination of diabetic group, there was wide basal lamina, detached in some areas, loss of appearance of hemidesmosome. Most of cell layers appeared with less orientation, with pleomorphism (different size and shape). Desmosomal junction between cells were detached in some areas with increase of inter cellular space in some areas. Detached keratin layer were noticed. The cells appeared with the cytoplasm showing vacuolated mitochondria, ill developed of cell organelles, loss of normal chromatin distribution. Apoptosis of some cells and cell division appear in some areas. In the underlying connective tissue, Apoptotic fibroblast. Areas of collagen fiber separation, less density and thickness. less oriented muscle fibers and loss of continuous Z-line. There were some of inflammatory cells, dilated blood vessels with thick walls and proliferated swollen endothelial cells. While in zamzam treated group, there were restoring of hemidesmosome between basal cell layer and underlying lamina propria and desmosomal attachment between adjacent cells. The

cytoplasm contained mitochondria with relatively defined cristae, large abundant tonofilament and free ribosomes. In underlying lamina propria, active fibroblast with clea cross banding of collagen fibers were appeared. Normal orientation of Muscle fiber with continuity of Z line were appeared. Moreover, in EGF treated group the cytoplasm of the basal cells containing free ribosomes, abundant tonofibrils, some restored mitochondria and its cisternae and their nuclei showing intact continuous nuclear membrane. Hemidesmosome appeared to be restored at certain areas. Spinous cells appeared with restored desmosomal junction between the cells. The stratum corneum appeared with thick keratin layer with the interdigitation between the keratin squamous and obviously increasing in keratohyline granules in granular cell layer. In lamina propria, Spindle shaped fibroblast appeared with euchromatin nucleus surrounded by L.S&T.S abundant collagen fibers. The muscle fibers appeared with slight arrangement and relatively restored shape than diabetic group, slightly with normal fiber appearance. There was much number of mitochondria. **Fig (2)**

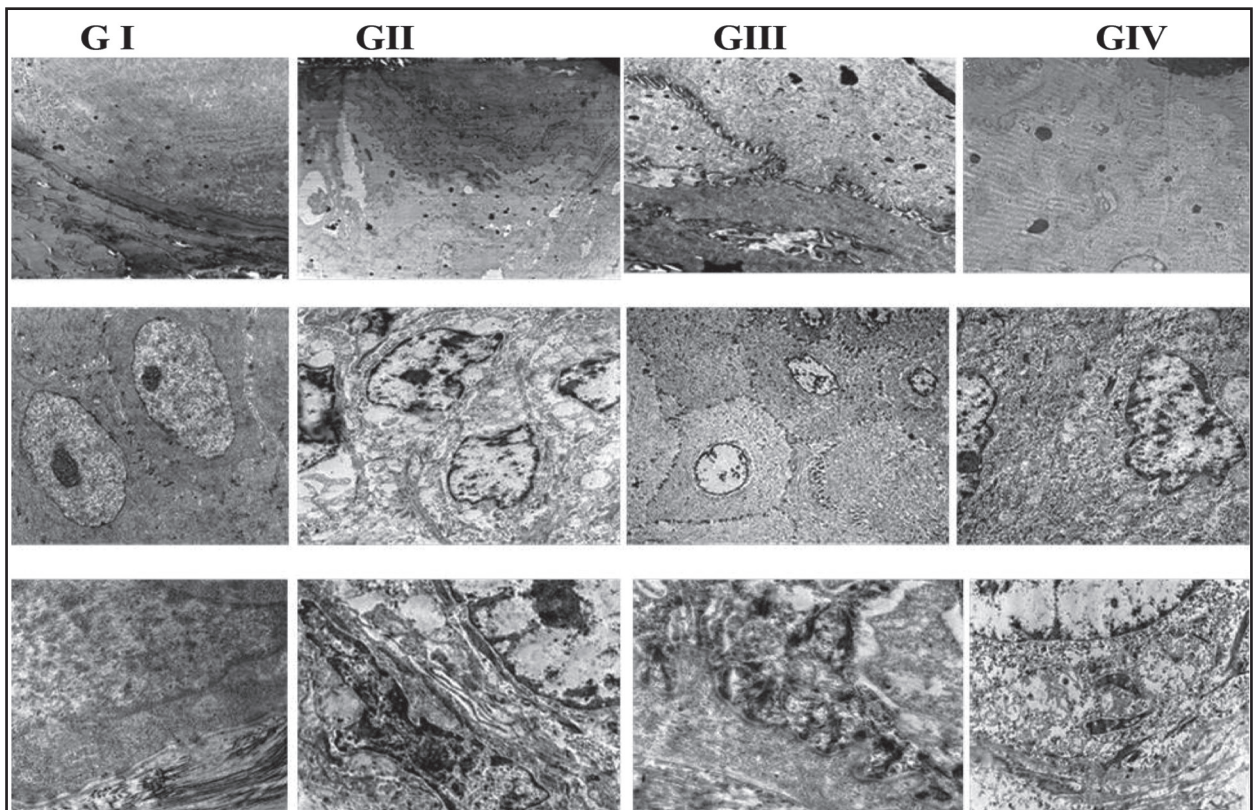


Figure (1) A photomicrograph showing electron microscope examination (TEM) of tongue papillae. Control group(GI), diabetic group(GII) , Zamzam group(GIII), EGF group(GIV)

DISCUSSION

Diabetes mellitus was selected because this disease accounts for approximately 5th most common chronic condition and the sixth most frequent cause of death among the elderly. There were an association between diabetes mellitus and alterations in the oral mucosa in experimental studies and clinical practice ⁽⁹⁾. The choice of alloxan in this study due its low mortality rate, high tolerance, easily given by different routes and its diabetogenic action where was rapid and permanent as it destructed the beta cells of islets of Langerhans ⁽¹⁰⁾. Rats were chosen for conduction of the present study for many reasons; first of all they are the mammalian whose physiological body functions are nearly similar to those of human being. Besides, they were easily obtained, housed, bred, handled, controlled and tested ⁽¹¹⁾. The choice of the tongue tissue was occur as the tongue was seen as a mirror that reflects the general health status. The tongue papillae are of a relatively high metabolic activity, so any enzymatic disturbance, or drug toxicity may result in alternation of tongue tissue ⁽¹²⁾.

Examination of diabetic group by light and electron microscope showed that, there was disruption of the basement membrane, decreased in numbers of hemidesmosomes, hyperkeratosis, decrease epithelial layering, degenerative cells throughout layers of covering epithelium and wide intercellular spaces mitochondrial cytolysis and cytoplasmic vacuolations. This finding may be due to alteration in the basement membrane components and integrins as a result of increase in their degradation by proteases elevated in diabetic corneas ⁽¹³⁾. DM generated an excess of superoxide and AGEs which liberate due to glycosylation of protein led to increase in the cytosolic (ROS) which facilitated the production of mitochondrial superoxide which might mediate tissue injury in the DM ⁽¹⁴⁾. While in zamzam groups, both light and microscopic results were concomitant where there was intact and continuous basement membrane as well as multiple restored hemidesmosomal and desmosomal junctions,

restoring of all epithelial cell layering and cell organelles were observed. Zamzam water was founded to be up-regulated gap junctional intercellular communication and connexin 43 antibodies due to the high calcium and magnesium content of Zamzam water ⁽¹⁵⁾. The antioxidant action of ZW lead to its protective effect and scavenging action to free radicals and ameliorated the toxic effects on DNA ⁽¹⁶⁾. In EGF treated groups, there were partly disrupted basement membrane was observed. This may be due to alterations of some constituents in the basement membrane, such as a loss of heparan-sulfate proteoglycan ⁽¹⁷⁾. Relative orientation of the epithelial cell layers. Intact cell membrane as well as cytoplasmic cell organelles (increase number of free ribosomes, tonofilament, some mitochondria and restored mitochondria cristae) bases that EGF produces a cascade of cellular events that are part of the mitogenic responses including initiation of DNA synthesis of extracellular macromolecules and stimulate cell proliferation, differentiation and maturation ⁽¹⁸⁾. The light microscope examination of lingual salivary gland revealed that there were structural changes in diabetic rats showing hypotrophic changes in the cells of the serous and mucous acini as cytoplasmic vacuolation, pyknotic nucleus and focal loss of salivary architecture in the diabetic rats. These findings may be due to oxidative stress and generation of free radicals that increased in diabetes ⁽¹⁹⁾. Zamzam water treated group had no vacuolation in the cytoplasm of acini cells and less degeneration in the salivary structures with regeneration of the normal salivary architecture. These revealed the role of zamzam water in regulating vital cellular functions, including cell proliferation and differentiation and its free radical scavenging capability potent and antioxidant activity ⁽²⁰⁾.

In EGF group, lingual salivary gland appeared with partially recovery of the glandular architecture of both serous and mucous acini, EGF promoted the differentiation of progenitor cells to acinar cells in an in vitro study and promotes cell proliferation and differentiation ⁽²¹⁾.

CONCLUSION

Zamzam water provides safer alternative nutritional strategy in restoring the integrity of the damaged epithelium of the tongue tissues, configuration of all layers of epithelium, and associated salivary glands after degenerative effect of DM

EGF cause relatively improvement of the damaged epithelium especially in basal cell layer, keratin layer and blood vessels in lamina propria of tongue tissue. Also, there was slight repair in associated salivary glands. This study confirms the degenerative effect of DM on tongue tissues and associated salivary glands.

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