Effect of Aloe vera gel on the parotid glands of streptozotocin-induced diabetic albino rats: histological and immunohistochemical study

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Background and Objectives: Diabetes mellitus is a disease of international health threat. Among several plants, Aloe vera extract has antidiabetic action thereby validating the rationale of its use in medicine. This study aimed to evaluate the effect of Aloe vera gel on parotid gland of streptozotocin-induced diabetic male albino rats, and also effect of diabetes on the parotid glands

Methods: Diabetes mellitus was induced in rats by intraperitoneal injection of 60 mg/kg Streptozotocin dissolved in saline solution. A total of 40 adult albino rats were divided equally into 4 groups. The groups consist of normal untreated group, normal treated with Aloe vera gel group (300mg/kg/day administered orally once daily for 21 consecutive days), untreated diabetes mellitus group and diabetes mellitus treated with Aloe vera gel (300mg/kg/day administered orally once daily for 21consecutive days). The parotid salivary glands of all animals were dissected after 2 months and prepared for histological and immunohistochemical examinations. The change in body weight also was measured.

Results: Histopathological examination of the parotid salivary glands in the diabetic group revealed a loss of the standard glandular architecture, including the acini, duct, and connective tissue stroma. Most of these alterations and degenerative changes disappeared or were markedly decreased in the group treated with Aloe vera gel.

Conclusion: Aloe vera gel had a noticeable antidiabetic effect on Streptozotocin-induced diabetic alterations in the parotid salivary glands of rats. Hence, Aloe vera gel may be beneficial as a dietary supplement for reducing diabetes complications.

keywords: Aloe vera, Streptozotocin induced diabetic, Parotid salivary gland, Ki-67, Caspase-3.

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Introduction

Diabetes mellitus is a metabolic disorder of multiple etiologies. Diabetes mellitus is caused by either absolute insulin lack in type 1 diabetes or insulin resistance in type 2 diabetes.[1] Chronic hyperglycemia leads to glucotoxicity to body tissues with the formation of advanced glycation end products.[2] These mechanisms are responsible for the chronic complications of diabetes. The clinical diagnosis of diabetes is often indicated by the presence of symptoms such as polyuria, polydipsia, and unexplained weight loss, and is confirmed by measurement of abnormal hyperglycemia.[3] Experimentally, diabetes mellitus can be induced by selective destruction of the insulin-producing Beta-cells of the pancreas with a single rapid injection of streptozotocin (STZ), a glucose moiety with a very reactive nitrous urea group from the mold Streptomyces griseus. STZ has been used as diabetogenic factor in experimental animals.[4,5]

Aloe vera is a plant that has been used for thousands of years for its medicinal properties to cure different human diseases and disorders.[6] Several researches have reported different bioactive compounds such as amino acids, anthraquinones, sugars, enzymes, polyphenols, minerals, and vitamins (A, B, C, and E) in Aloe vera leaves and gel.[7]

These bioactive compounds provide properties, such as antioxidant, anti-hypercholesterolemic, anti-diabetic, anti-

ulcer, antibacterial activity, antiviral activity, antifungal activity, anti-acne, nutraceutical, humectant, skin protection against UV-A and UV-B, wound healing properties, the prevention of diabetes and cancer, cardiovascular diseases, and the generation of antibodies.[8-10] The major salivary glands consist of paired submandibular, sublingual, and parotid glands that work simultaneously with other minor salivary glands scattered all over the oral cavity. Each one of these major salivary glands consists of a specific combination of both mucous and serous acinar cells, which are responsible for synthesizing protein components of saliva and transporting water and electrolytes.[11] Regarding the morphology of salivary gland, experiments conducted on chemically induced diabetic rats and autoimmune diabetic mice have demonstrated a reduction in acinar volume, growth retardation, a weight reduction of the parotid and submandibular glands, a decline in the number of granular ducts and in the density of secretory granules, as well as accumulation of lipid droplets in acinar cells and intercalated ducts.[12,13]

The Ki-67 index is a well-known proliferation marker. This nuclear antigen is expressed in the G1, S, G2 and M cell cycle phases. During these phases the level of expression varies: levels are low in G1 and early S phase and increase to a maximum at the time of mitosis. A rapid decrease in Ki-67 expression occurs in anaphase and telophase of cell cycle.[14] Current study was aimed to investigate the effect of diabetes on the parotid glands, and also effect of Aloe vera gel on parotid gland of streptozotocin induced diabetic male albino rats. As variable to evaluate the grade of damage or protection, we used histopathological and immunohistochemical investigations to clarify its effect on cell proliferation and apoptosis.

Methods

The current work was conducted in accordance with the guidelines for the care and use of laboratory animals of the Hawler Medical University. The protocol of the present study was approved by the Local Committee on the Ethical Use of Animals of the Medical University in Kurdistan region/Iraq.

Study animals: The study was carried out on forty male albino rats, weighing about (250-280) gm; The animals were kept in a metallic cage at the animal house in the college of medicine Hawler Medical University.

The animals were allocated into 4 groups (10 animals each group)

Group I: (Saline/ Distilled water treated group): Intraperitoneally received saline solution (single dose of 60 mg/kg body weight). Distilled water was administered orally once daily by intragastric gavage needle for 21 consecutive days (300 mg/kg/day).

Group II: (Saline treated group /Aloe vera gel): Intraperitoneally received saline solution (single dose of 60 mg/kg body weight), and the extract of Aloe vera gel (300 mg/kg/day) was administered orally once daily by intragastric gavage needle (days 1-21) for 21 consecutive days.[15]

Group III: (STZ/Distilled water treated group): Intraperitoneal injection of Streptozotocin (STZ) at a single dose of 60 mg/kg body weight dissolved in saline solution[16], the third group were consider as diabetic. Distilled water was administered orally once daily by intragastric gavage needle for 21 consecutive days (300 mg/kg/day).

Group IV (STZ/Aloe vera gel treated group): Intraperitoneal injection of Streptozotocin (STZ) at a single dose of 60 mg/kg body weight dissolved in saline solution The extract of Aloe vera gel (300 mg/kg/day) was administered orally once daily by intragastric gavage needle (days 1-21) for 21 consecutive days.

Preparation of Aloe Vera Gel Extract:

Fresh succulent leaves of aloe vera were collected, the inner gel component removed and the leafy exudate homogenized in an electric blender. Subsequently stored at 4° C.[17]

Induction of diabetes

Experimental Diabetes mellitus was induced in overnight fasted rats by intraperitoneal injection of Streptozotocin (STZ) (Sigma- Aldrich Corp, St. Louis, MO, USA) at a single dose of 60 mg/kg body weight dissolved in saline solution. After injection, the rats had free access to food

and water. Diabetes mellitus was allowed to develop and stabilize in these STZ treated rats over a period of four days. Blood glucose levels were measured in overnight-fasted rats, blood was collected from the tail tip. A glucometer (Accu-Chek, Roche, Germany) was used to measure the level of blood glucose. Concentrations of 300 mg/dL or higher of blood glucose are regarded as diabetic[18.] Rats body weights were evaluating at the start of the experiment and during the variable time intervals of two months using electronic digital weighing balance (Fujian, China). Body weight was measured at the same time at morning.

Histopathological and immunohistochemical analysis

At the end of the 2 months, all animals were anesthetized by ketamine over dose (Hameln Pharmaceuticals GmbH, Germany). Careful removal of the skin of the neck and the face reveal the presence of the salivary glands. Rat parotid gland is located behind and below the ear, caudally bordering the submandibular gland[19]. Specimens were taken quickly from the right and left parotid gland tissues, then specimens were preserved in 10% neutral buffered formalin for 24 hours, then were processed by standard paraffin embedded methods. Sections were cut at 4-5µm, deparaffinized, and stained with Hematoxylin and eosin (H&E) for the examining of cells of each compartment in the parotid gland (acini, intercalated duct, striated duct, granular convoluted tubules, excretory duct and the connective tissue).

Immunohistochemical analysis:

Cell proliferation was assessed by Ki-67 immunohistochemistry, while the apoptosis was assessed by cleaved caspase-3 immunostaining. Positive cells expressing Ki-67 were identified by brown nuclei, while cleaved caspase-3 was demonstrated brown cytoplasmic staining. Five sections were randomly chosen for each animal. Approximately 1000 cells from 5 high spots areas at a magnification of 400 X of each section were taken, and the number of Ki-67 and caspase-3 immunopositive cells were calculated by two observers and the mean values for each group were determined. Mean group values were statistically compared. All microscopic analyses were performed using a light microscope (Olympus, Tokyo, Japan). The level of Ki-67 and cleaved caspase-3 expression was evaluated according to the scoring system of Seleit et al.[20]

Statistical Analysis: Data was expressed as mean ± standard deviation. Comparative analyses between and amongst variables were done using analysis of variance (ANOVA). A Kruskal-Wallis test was performed to further ascertain significant differences between means. Statistical significance was set at $P < 0.01$.

Results

Body weight: During the experimental study, the animals from control group remained alive. The animals in group I and group II appeared healthy, active and gained body weight. Three rats in the group III died in an average of 5 days following STZ injection and one rats of the group IV died in an average of 2 days.

Analysis of variance (ANOVA) indicated lack of significant differences in initial values of body weight between four groups of rats ($P > 0.01$). However, significance differences in final values of body weight (end of second month) observe between Group I / Group III, Group I / Group IV, Group II/ Group III, and Group II/ Group IV respectively ($P < 0.01$).

A- Microscopical feature

Group I and Group II (Saline/ Distilled water treated group and Saline treated group /Aloe vera gel)

Examination of control group sections of parotid salivary glands in Albino rat revealed that the glands were composed of pure serous acini. These serous acini appeared round and had a very narrow lumen. The acini were lined by pyramidal cells with apical acidophilic cytoplasm. Their nuclei were basally situated and prominent, deeply stained, spherical in shape. The duct system represented by the granular convoluted tubules (GCTs) were the most prominent structure in the salivary glands and were lined with tall columnar cells with rounded or oval basally situated nuclei and abundant eosinophilic granules.

The striated ducts had a single layer of co-

lumnar cells with rounded, centrally located, and darkly stained nuclei and eosinophilic cytoplasm with apparent acidophilic basal striations. The excretory ducts were seen between the lobes of the gland surrounded by thin fibrous connective tissue stroma. These ducts were lined with a pseudostratified columnar epithelium with rounded or oval deeply stained nuclei appearing at different levels. Normal blood vessels with numerous RBCs were also seen (Figure 1).

Group III (STZ/distilled water treated group)

Microscopically, the salivary gland of diabetic rats showed severe pathological changes; signs of acini degeneration represented by disfigured lobular structure and loss of normal architecture of the secretory portions were seen.

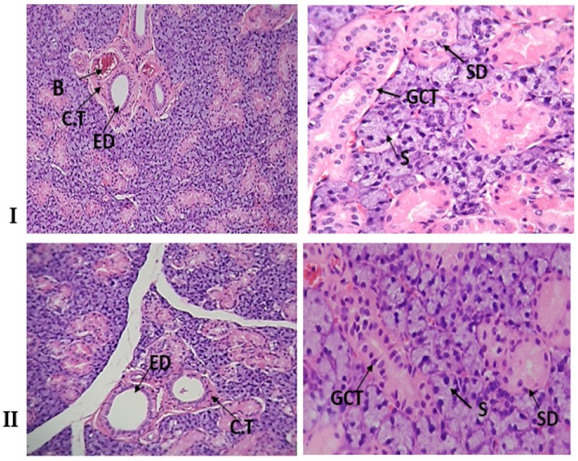

Figure 1. Microphotograph of rat parotid salivary gland in group I and group II (H&E)
Group (I) showing: normal architecture of the gland with pure serous acini (S), normal architecture of the excretory duct (ED), striated ducts (SD), excretory ducts (ED), thin fibrous connective tissue surround ED and blood vessels (B) (x200, x400)
Group II: showing normal architecture of the glands, regular structured serous acini (S). The CT distributed in the stroma between the serous acini (S), granular convoluted tubules (GCTs), and striated ducts (SD) (x200, x400).

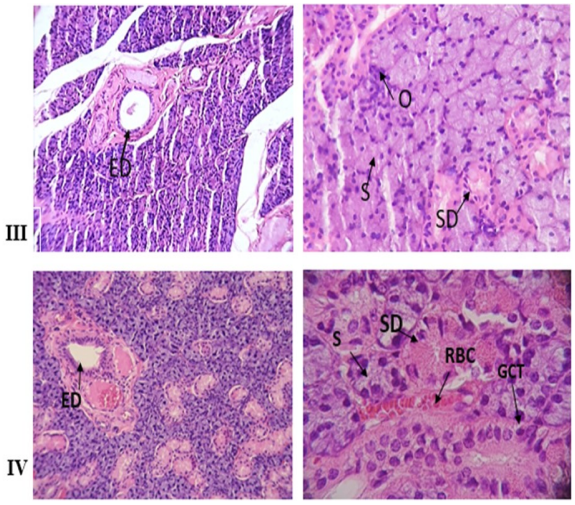

Figure 2. Microphotograph of rat parotid salivary glands (H&E) Group (III) showing: loss of normal architecture of the secretory portions were seen, serous acini with apparent reduction in size. acinar cells with ill-defined outline (S). Acinar cells undergo degeneration(O). striated duct cells with ill-defined boundaries, apparent decrease in the columnar appearance, intracytoplasmic vacuolizations, hyperchromatic nuclei, decreased eosinophilic granules of the striated ducts, degeneration and indistinct basal striations of the striated ducts (SD) (x200, x400).
Group (IV) showing: marked improvement in cells of serous acini, and relatively preserved their shape (S). Nearly normal architecture of the striated ducts, the numbers of vacuoles decreased (SD), Few striated ducts lumina are filled by stagnated secretion. The granular convoluted tubules were lined by simple columnar epithelium with eosinophilic cytoplasm and basal rounded nuclei (GCT). Somewhat dilated BVs and engorged with RBCs (x200, x400).

There was hydropic degeneration of the cytoplasm and ill-distinct cell boundaries and vacuolization in the acini. Nuclei of the acinar cells revealed different sizes and shape, the intercalated, striated and interlobular ducts were dilated and their cytoplasm showed signs of degeneration. The connective tissue stroma, both intra and interlobular revealed increase in collagen fiber thickness and showed hyalinization.

All blood vessels were dilated and engorged with blood (Figure 2).

Group IV (STZ/ Aloe vera gel treated group)

Histological examination of the parotid gland in this group showed that the ducts become dilated with discontinuity of their epithelial lining in some areas, also its revealed marked improvement in cells of acini as well as cells of ducts lining, and the acini relatively preserved their shape. The numbers of vacuoles decreased and well- formed striated ducts were also detected. They restored their basal striations and had intact epithelial lining. The intercalated ducts were noticed in between the acini. Blood vessels were seen around these ducts; no congestion or areas of hemorrhage were seen (Figure 2).

Immunohistochemical evaluation

Immunohistochemical analysis using Ki-67 immunostaining to study the cellular proliferation in Albino rat parotid glands showed that a significant difference between group I and group III, also there was a significant difference between group II/group III ($p<0.05$), also significant difference was seen between group II and group IV. Most of the positive cells was seen in association with ductal cell (Table 1 and Figure 3).

Regarding to caspase-3, significant increase in mean expression of caspase-3 between group I/ group III, group I / group IV, Group II/ Group III and Group II/ Group IV respectively ($p<0.01$), as shown in Table 2 and Figure 4.

Table 1. Means and standard deviations of Ki-67 immune-expression of the parotid gland after water or Aloe vera gel treatment in the experimental animals after saline or streptozotocin induced diabetic in all groups in the A in the end of second months

Groups	Ki-67	
	(Mean± SD)	*p*-value
Group I / Group II	1.280±0.487 1.428±0.534	0.5909
Group I / Group III	1.280±0.487 0.142±0.377	0.002214 *
Group I / Group IV	1.280±0.487 0.571±0.786	0.06076
Group II/ Group III	1.428±0.534 0.142±0.377	0.002058 *
Group II/ Group IV	1.428±0.534 0.571±0.786	0.04094
Group III/ Group IV	0.571±0.786	0.2268

Table 2. Means and standard deviations of caspase-3 immune-expression of the parotid gland after water or Aloe vera gel treatment in the experimental animals after saline or streptozotocin induced diabetic in all groups in the end of second month.

Groups	caspase-3	
	(Mean± SD)	*p*-value
Group I / Group II	0.571±0.786 0.714±1.112	0.9428
Group I / Group III	0.571±0.786 4.142±2.115	0.00186 2*
Group I / Group IV	0.571±0.786 3.428±1.511	0.00416 9*
Group II/ Group III	0.714±1.112 4.142±2.115	0.00286 6*
Group II/ Group IV	0.714±1.112 3.428±1.511	0.00624 1*
Group III/ Group IV	4.142±2.115 3.428±1.511	0.2165

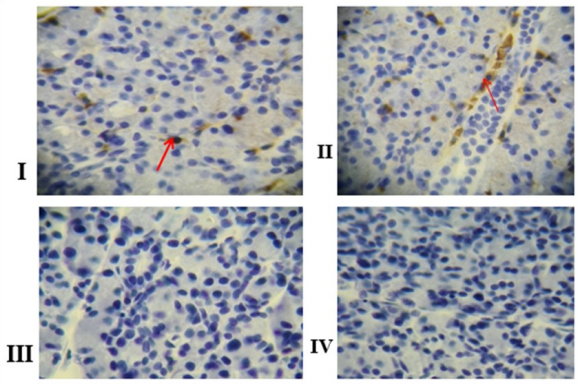

Figure 3. Microphotograph of Ki-67 immune expression in parotid salivary glands of rat x400
Group I: mild nuclear Ki 67 immunoreactivity in the acini and duct (red arrow). Group II: mild nuclear Ki 67 immunoreactivity in the acini and duct (red arrow). Group III: negative Ki-67 immuno reactivity in nuclei of cells of ducts and Group III: negative Ki-67 immunoreactivity in nuclei of cells of ducts and acini. Group IV: negative Ki-67 immunoreactivity in nuclei of cells of ducts and acini

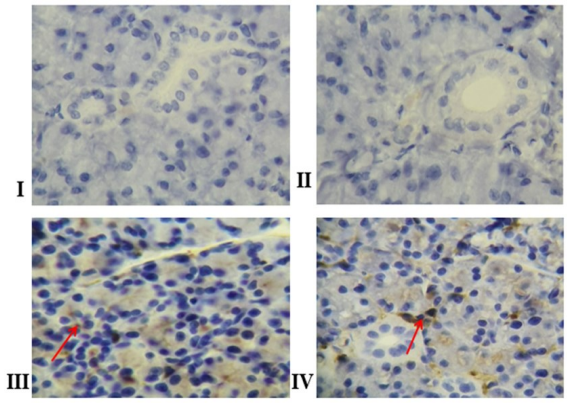

Figure 4. Microphotograph of caspase-3 immune expression in parotid salivary glands of rat x400
Group I: negative cytoplasmic reaction to caspase-3. Group II: negative cytoplasmic reaction to caspase-3.
Group III: mild cytoplasmic reaction to caspase-3 (red arrow). Group IV: mild cytoplasmic reaction to caspase-3 (red arrow).

Discussion

The present experiment showed that the diabetic animals had significant decrease in their body weight (group III), whereas in animals submitted to prolonged glycemic control, recovery of the body weight was observed (group IV). The diabetes mellitus provokes metabolic disorders in various organ systems, including a reduction in body weight and destruction of different tissues.[21-23] However, slight increase in body weight has been observed in diabetic rats, these findings indicate that diabetes compromises general body metabolism, leading to weight loss, and that glycemic control is an effective treatment for the recovery of body weight in diabetic animals, this comes in agreement with the result of Anderson.[24]

In group I and group II, the parotid gland has an acinus (spherical) shape which consist of pure serous cells with a small central lumen. The pyramidal epithelial cells have a truncated apical vertex and a nucleus surrounded by condensed chromatin, shifted basally, characteristics present in human, mouse and rabbit, an observation similar to what was reported by Amano et al.[25] In group III the parotid gland showed multiple histopathological changes, including vacuolization and atrophy of some acinar cells, and reduced secretory granules. A previous studies had reported the same structural changes of parotid glands.[26,27] The presence of these vacuoles could be attributed to the intracytoplasmic lipid accumulation within the cells. However, a large proportion of the lipid seems to dissolve and be removed during tissue fixation and processing.[28] Furthermore, clinical and experimental studies have demonstrated a reduction in secretory and salivary components, as well as a severe inflammatory reaction accompanied by the presence of mononuclear cells first located around blood vessels and then around acini and ducts.[29-31]

Immunohistochemical results confirm our histological findings observed in Group III of the present study, in which caspase-3 antibodies revealed intense positive reaction to activated caspase-3 in the cytoplasm of the acinar and ductal cells, connective tissue cells as well as the endothelial cells. Our findings could be attributed to the fact that the toxicity of STZ is related to the inhibition of the enzyme O-GlcNAcase (N-acetylD-glucosaminidase) which removes protein linked GlcNAc.[32,33]

There are several mechanisms that could be responsible for the higher rate of apoptosis

noted in Group III of the present study. One mechanism may be through the cytokine activation of receptors with 'death domains', such as TNF receptor-1 (TNFR-1) or fas.[34] Diabetes is associated with both enhanced TNF and fas/fasligand expression.[35] Another mechanism is through the increased oxidative stress which is one of the common pathogenic factors of diabetes complications as it leads to the formation of excess ROS which lead to severe oxidative damage of the cell's components like lipids, proteins and DNA by inhibiting many of the enzymes involved in DNA synthesis. These pathways lead to one result which is apoptosis through activation of caspases.[36]

In the present study, histopathological examination of the rat parotid gland of treated group (Group IV) revealed that the entire glandular structure was markedly improved compared to that of the diabetic group, though the glandular architecture was not entirely similar for that of the control group, these results are consistent with those of several studies confirming the anti-diabetic, antioxidant effects of Aloe vera.[37,38] Aloe vera treated group had less vacuolation in the cytoplasm of acini cells and less degeneration in salivary structures that appeared after 4 weeks. In agreement with these results, Nejaim et al, found that A. vera has a protective action on salivary gland in rats exposed to ionizing radiation which cause free radical formation and revealed this radioprotective action to the antioxidant effect of A. vera, so, the beneficial effect of Aloe vera on parotid gland may be related to antioxidant effects that can protect cells against inflammatory processes and oxidative damage caused by diabetes.[39,40]

Various reasons can be given for the mechanism of action of Aloe vera on lowering blood sugar. Clinical evaluations have shown that the pharmacologically active substances are concentrated in the gel of Aloe vera leaves. An anthropoid called barbaloin is isolated from the Aloe vera plant, which protects the beta cells of the islets of Langerhans from damage caused by free radicals.[41]

A recent in vitro study showed that the action mechanism of Aloe vera polysaccharides antidiabetic effect is related to its ability to inhibit apoptosis and endoplasmic reticulum stress signaling.[42]

Conclusion

The beneficial effect of Aloe vera gel at a concentration of 300 mg/kg/day on the parotid salivary glands in streptozotocin induced diabetes in rats detected with improvement in cellular architecture and decrease apoptosis on parotid glands; therefore, it can be used as a protective natural product to the salivary glands in individuals suffering from diabetes mellitus.

Conflict of interest

The author reported no conflict of interests.

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