EGYPTIAN DENTAL JOURNAL
www.eda-egypt.org
VOL. 71, 241:256, JANUARY, 2025
PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360
ORAL MEDICINE, X-RAY, ORAL BIOLOGY AND ORAL PATHOLOGY
Submit Date : 01-09-2024 • Accept Date : 27-09-2024 • Available online: 10-01-2025 • DOI : 10.21608/edj.2024.316760.3187

EFFECT OF GINGER VERSUS L-CARNITINE ON SUBMANDIBULAR SALIVARY GLANDS OF MALE INDUCED DIABETIC ALBINO RATS AGAINST CONVENTIONAL THERAPY (HISTOLOGICAL, ULTRASTRUCTURAL, AND BIOCHEMICAL STUDY)

Dina Ali Nagui*, Ashraf K Awaad** *and* Nesma Mohamed Khalil*

ABSTRACT

Background: Diabetes mellitus DM is a metabolic disorder associated with salivary dysfunction leading to xerostomia. Ginger is a medicinal plant known to have a positive effect on blood glucose level. L-Carnitine is a vitamin-like molecule with a noticeable hypoglycemic and antioxidant activity.

Aim: This study aimed to compare the antidiabetic effect of Ginger and L-Carnitine on submandibular salivary gland of streptozotocin-induced diabetic rats.

Materials and methods: Fifty male Wister albino rats were divided into five groups (10 rats each): Control group remained untreated. After induction of diabetes, the remaining forty rats were divided into: diabetic group, metformin group, ginger group, and l-carnitine group. After six weeks, rats were euthanized, and blood samples were collected for biochemical analysis. Submandibular glands on both sides were dissected out and prepared for light microscopic and transmission electron microscopic examination and histomorphometric analysis of acinar cell vacuolization, optical density of Periodic Acid Schiff and Alcian Blue staining and the data were statistically analyzed.

Results: The treated groups showed better histological results in restoring the normal structure of submandibular glands cells which appeared well defined with reduced vacuolation. On ultrastructural level, treated groups revealed euchromatic nuclei, slightly dilated cisternae of rER and numerous secretory granules. Ginger group showed better results compared to L-carnitine group.

Conclusions: Ginger and L-carnitine can be used as adjuvant treatment in management of DM as they are safe, inexpensive supplements. However, Ginger can be prescribed as a prophylactic antioxidant in healthy individuals as it resulted in better amelioration in the glandular structure.

KEY WORDS: Ginger, L-carnitine, Metformin, submandibular gland, Diabetes Mellitus

* Department of Oral Biology, Faculty of Dentistry, Alexandria University, Egypt.
** Biochemistry and Molecular Biology, Center of Excellence in Regenerative Medicine and Applications (CERRMA) Faculty of Medicine, Alexandria University, Egypt.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder manifested as hyperglycemia due to defect in insulin secretion, function, or both.[1] Despite hundreds of research had been performed, complications of diabetes are considered the most prevailing chronic diseases around the world.[2] When first diagnosed with DM, the patient suffers from metabolic dysregulations such as retinopathy, neuropathy, microvascular and macrovascular diseases that necessitate continuous and expensive care. Beside these well-known complications, oral complications of DM are also expected, including xerostomia, crown and root caries, gingival and periodontal disease, altered taste sensation, oral lichen planus, recurrent aphthous stomatitis and defective wound healing.[3]

Reduced salivary flow can result from salivary dysfunction. Among diabetic patients, the estimated global prevalence of xerostomia varies from 34% to 51%. Xerostomia is manifested as burning mouth, caries, difficulty speaking and swallowing, and an increased risk of infection. It might negatively impact the quality of life for patients.[4]

Oral pharmaceutical medications used to treat diabetes are linked to unfavourable side effects. Consequently, herbal drugs are nowadays used with other medications in the treatment of different illnesses because they are cheap, have many therapeutic effects and acceptance by patients. [5, 6]

Ginger (Zingiber officinale) is used as a spice in many countries with many medicinal properties include anti-inflammatory, hypolipidemic, analgesic, anti-thrombotic, anti-arthritic, anti-migraine, anti-nausea, anti-vomiting properties.[7, 8] Indeed, it has a positive effect on blood glucose level. Therefore, it may increase sensitivity of insulin and prevent the complications associated with diabetes. This promising antidiabetic effect of ginger may be due to its bioactive components, such as gingerols, flavonoids, zingerone, paradols and shogaols.[9]

L-Carnitine is a water-soluble, vitamin-like molecule that is formed from lysine and methionine amino acids in the liver and kidney. Its dietary sources are poultry, meat, dairy products, and fish. Since it is involved in the metabolism of lipids, it was found to be used in the management of depression and improvement of physical fitness.[10]. Animal model studies demonstrated that L-carnitine has noticeable anti-diabetic and hypoglycemic effects and is related to its antioxidant activity.[11] Furthermore, daily injections of L-carnitine led to enhancement in blood sugar level, and increased utilization of glucose avoiding the hyperglycemia seen in the untreated diabetic animal.[12]. For that reason, new therapeutic approaches based on adjuvant treatment of medicinal plants with antidiabetic effect are being assessed to reduce the complications among diabetic patients.[13]

In view of the aforementioned advantages of ginger and L-Carnitine, this study was performed to compare their antidiabetic effect on the structure of submandibular salivary gland of streptozotocin-induced diabetic rats. The null hypothesis of this study is that there will be no differences between ginger and l-carnitine on the structure of submandibular salivary glands of diabetic rats.

MATERIALS AND METHODS

The experimental protocol was approved by the ethical committee of Faculty of Dentistry, Alexandria University (IRB No. 00010556 – IORG 0008839). The work was carried out following the ARRIVE guidelines for animal research [14], the institutional guidelines and the National Research Council's Guide for the Care and Use of Laboratory Animals. [15]

Rats were obtained from the animal house of Medical Research Institute, Alexandria University and maintained under the same environmental conditions at 24± 1°C, 50%±10% humidity and 12-hour light/12-hour dark cycle. The rats were

housed four per one metal cage. A standard diet was provided to all rats with water ad libitum.

The minimal sample size was calculated based on a previous study aimed to describe the changes in the submandibular glands of the offspring of diabetic mothers, by detecting alterations in serous secretion using Periodic Acid Schiff (PAS) stain, concluding that maternal diabetes produces negative effects on submandibular salivary glands of the offspring. The sample size was calculated Adopting a power of 90% (β=0.20) to detect a standardized effect size in area percent of PAS of 0.518, and level of significance 5% (α error accepted =0.05), the minimum required sample size was found to be10 rats per group (number of groups=5) (Total sample size=50 rats) [16, 17]. Any specimen loss from the study sample due to any reason were replaced to maintain the sample size [18].The sample size was calculated using GPower version 3.1.9.2 [19]

After 15 days of acclimatization, fifty male Wister albino rats about 4 months old (200-220 grams) were divided randomly into 5 experimental groups, 10 rats each: group 1: control group, group 2: diabetic group, group 3: metformin treated group, group 4: ginger treated group, and group 5: l-carnitine treated group.

In groups 2, 3, 4 & 5 diabetes was induced by a single intraperitoneal injection of 45 mg/kg of streptozotocin (STZ) [20] (Sigma, St Louis, MO, USA) diluted in 0.1 M citrate buffer (PH 4.5).[21] Rats were then given glucose solution (5%) for the first 48 hours to prevent hypoglycemic mortality which may occur due to extensive insulin release from the pancreas after STZ injection. After 7 days, blood sugar level was measured by collecting a drop of blood from each rat using bionime GS100. Only rats with fasting blood sugar level ≥ 200gm/dl were considered diabetic and were included in the study.[20] In control group 1, rats were given intraperitoneal citrate buffer of the same volume as the other groups.

After confirmation of diabetes, diabetic rats were randomly divided into 4 experimental groups. Group 2 (diabetic group): rats remained untreated till the time of sacrifice. Group 3: (metformin treated group): rats received 100mg/kg metformin [22]. Group 4 (ginger treated group); rats received 500mg/kg of ginger [23]. Group 5 (l-carnitine treated group): rats received 600 mg/kg of L-carnitine [11]. All treatments were given daily by oral gavage syringe till the end of the experiment. Blood sugar level was measured every 7 days throughout the experimental period.

Six weeks after confirmation of diabetes, all rats were fasted overnight and euthanized by intraperitoneal injection of 100 mg/kg sodium pentobarbital. [24]. After sacrifice, blood samples were collected from the abdominal aorta for biochemical analysis. Submandibular glands were dissected out, the right gland was prepared for light microscopic examination, while the left gland was prepared for transmission electron microscopic examination.

Light microscopic examination

Submandibular gland specimens were fixed in 10% neutral buffered formalin, washed, dehydrated in alcohol, and cleared in xylene. Finally, they were embedded in paraffin blocks, and sections of five μm thickness were stained with Hematoxylin and Eosin stain (H&E) for general examination. In addition, sections were stained with Periodic Acid Schiff (PAS), and Alcian Blue and examined by light microscope (Optika, Ponteranica, Italy) for detection of serous and mucous secretions respectively. [6, 25]

Histomorphometric analysis

Fiji image j[26] was used to perform the histomorphometric analysis. In each group, all 10 specimens were included. In each specimen, 4 serial sections were used for the analysis and the mean was calculated. The following parameters were measured in x400 micrographs:

1. Percentage of acinar cells vacuolization:

In each field, the vacuoles in acinar cells were traced, measured and then divided by the total surface area of the field to obtain the percentage of vacuolization.

2. Optical density of PAS and Alcian Blue staining:

The mean intensity of each stain was obtained using Fiji image j. Then optical density was calculated using the following formula: OD = log (maximum intensity/mean intensity), maximum intensity = 255. [27]

Transmission electron microscopic examination

Submandibular glands were cut into small fragments and fixed overnight in buffered solution of formaldehyde-glutaraldehyde at 3C. Then specimens were washed in phosphate buffer and post fixed in 1% osmium tetra-oxide for 1.5 hour. Specimens were rinsed in phosphate buffer, dehydrated in ascending concentrations of ethanol, and cleared in propylene oxide. Afterwards, tissue samples were embedded in epoxy resin. Semi thin sections were cut and stained with toluidine blue and examined by light microscope to determine the area of interest. Ultra-thin sections were then cut, mounted on copper grids and stained with uranyl acetate and lead citrate.[28] The grids were examined using Jeol 100 CX electron microscope (Joel, Japan).

Biochemical analysis

Oxidative Stress and Antioxidant enzymes:

Lipid Peroxidation (Malondialdehyde) Determination: [29]

In this method, trichloroacetic acid was used to precipitate lipoproteins from the serum. This precipitate was treated with 0.05 M sulphuric acid and 0.67% thiobarbituric acid (TBA) in 2 M sodium sulphate. Upon heating in boiling water for 30 min., the lipid peroxide was coupled with TBA resulting in a colored chromogen. The produced chromogen was extracted in n-butanol and colorimetrically assessed at 530 nm.

Total antioxidant capacity assessment: [30]

The aantioxidant capacity was measured by Ferric ion reducing antioxidant power (FRAP) assay. Briefly, at low pH, the ferric ion was reduced to ferrous form in the presence of antioxidants. The maximum absorbance of the blue colored ferrous-tripyridyl-S-triazine (Fe (**III**)-TPTZ) complex was determined spectrophotometrically at 593nm.

Statistical analysis

Data were analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Continuous data were tested for normality by **Shapiro-Wilk test**, and quantitative data were presented as mean and standard deviation. **One way ANOVA test** was used to compare the different groups and followed by **Post Hoc test (Tukey)** for pairwise comparison. Significance of the obtained results was judged at the 5% level.

RESULTS

Light microscopic results

Light microscopic examination of the control specimens revealed the normal structure of the submandibular gland which constitutes mainly of serous acini with pyramidal cells and basal rounded nuclei. Granular convoluted tubules were seen with eosinophilic cytoplasm. Striated duct showed its characteristic basal striations (Fig.1A). In diabetic group, marked alterations were noted. Most of the acinar cells were ill defined, some pyknotic nuclei were observed and the cytoplasm exhibited many vacuolations. Disturbances were also seen in granular convoluted tubules and vacuolization of the striated duct (Fig.1B). On the other hand, metformin, ginger and l-carnitine treated groups showed well-defined acinar cells with basal rounded

nuclei compared to diabetic group. In metformin and ginger treated groups, some alterations were noted which include disorganization of some of granular convoluted tubules and few vacuolations in striated duct. The alterations were more pronounced in l-carnitine treated group in form of more cytoplasmic vacuolations and alterations in granular convoluted tubules and striated ducts. (Fig.1 C, D&E).

PAS staining results: Serous acini of the control group exhibited strong positive PAS reaction of the polysaccharide content of the cells (Fig.2A), while the diabetic group showed minimal reaction (Fig.2B). Metformin, Ginger, and l-carnitine groups revealed a stronger reaction in comparison to diabetic group. (Fig.2C, D &E respectively)

Alcian Blue staining results: Mucous acini in control group, showed a strong reaction (Fig.3A), while in the diabetic group, the mucous acini showed minimal reaction (Fig.3B). On the other hand, metformin group (Fig.3C) revealed more positive reaction followed by ginger (Fig.3D), and l-carnitine groups (Fig.3E) in comparison to diabetic group.

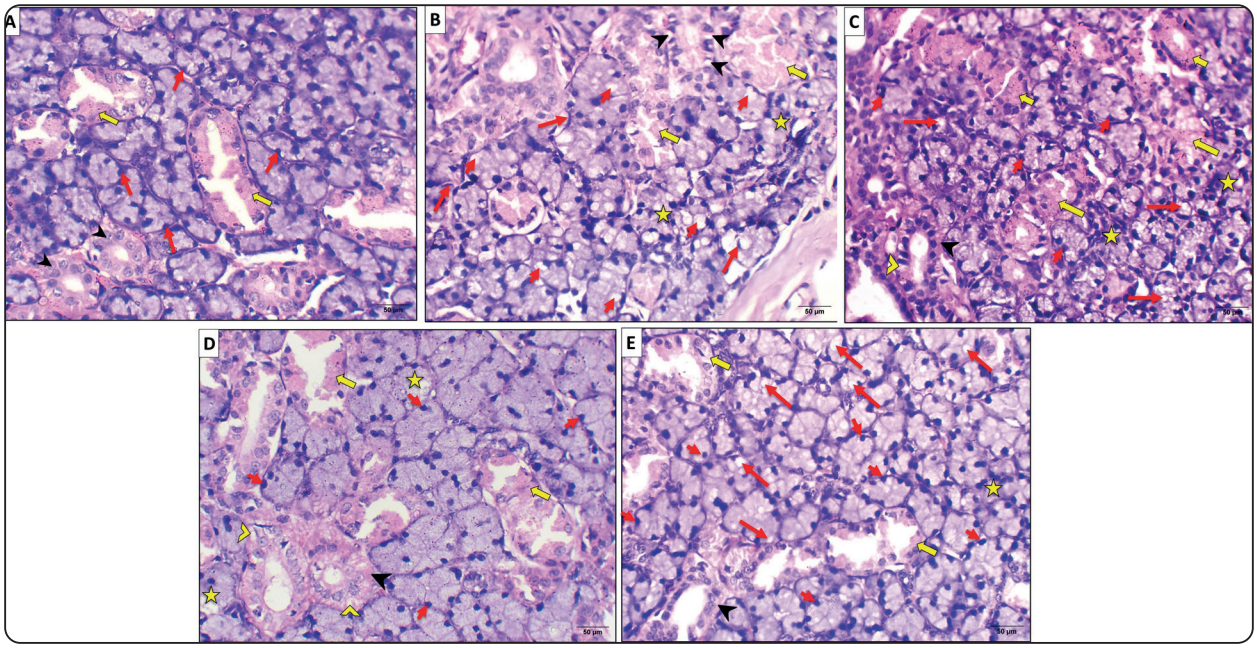

Fig. (1) Light micrograph (LM) of submandibular gland in different groups. **A (control group):** showing well defined serous acini with basal rounded nuclei (arrows), granular convoluted tubules with eosinophilic cytoplasm (yellow arrows) and secretory striated ducts with their characteristic basal striations (arrowheads). **B (diabetic group):** showing marked vacuolation of the acinar cells (short red arrows) with pyknotic nuclei (long red arrows). Some acini appear ill-defined (stars). Disturbances in the structural organization of granular convoluted tubules (yellow arrow) and vacuolization of the striated duct (arrowheads). **C (Metformin group):** most of the acini are well defined with basal rounded nuclei (short red arrows) while some appeared ill-defined (stars), some acinar vacuolization is noted (long red arrows), granular convoluted tubules appear well organized (short yellow arrows), while others appear disorganized (long yellow arrows). Secretory striated duct (black arrowhead) with few cytoplasmic vacuolations (yellow arrowhead). **D (Ginger group):** showing restoration of structural organization in most of the acini with basal rounded nuclei (red arrows). Few cytoplasmic vacuolations (stars) are seen. Granular convoluted tubules show structural disorganization (yellow arrows) and striated duct (black arrowhead) with some vacuolation (yellow arrowhead) & **E (L-carnitine group)**: showing well defined acini with rounded basal nuclei (short red arrows), few cytoplasmic vacuolations (long red arrows), an area with ill-defined acini (star) and some alterations in the granular convoluted tubules (yellow arrows). Striated duct is also noted (arrowhead). (H&E x400)

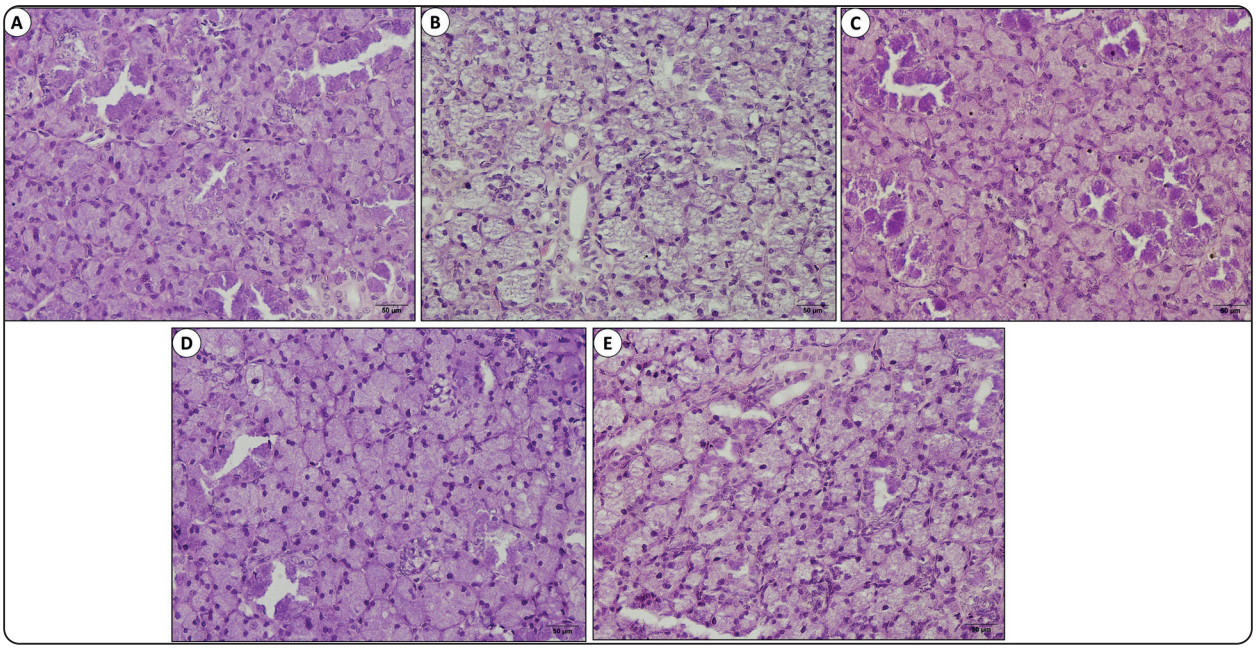

Fig. (2) LM of PAS reaction. **A:** control group serous acini showing strong positive reaction to PAS. **B:** diabetic group shows minimal reaction. **C & D & E:** (metformin & ginger, L-carnitine treated groups respectively) shows moderate reaction to PAS. (PAS, 400x)

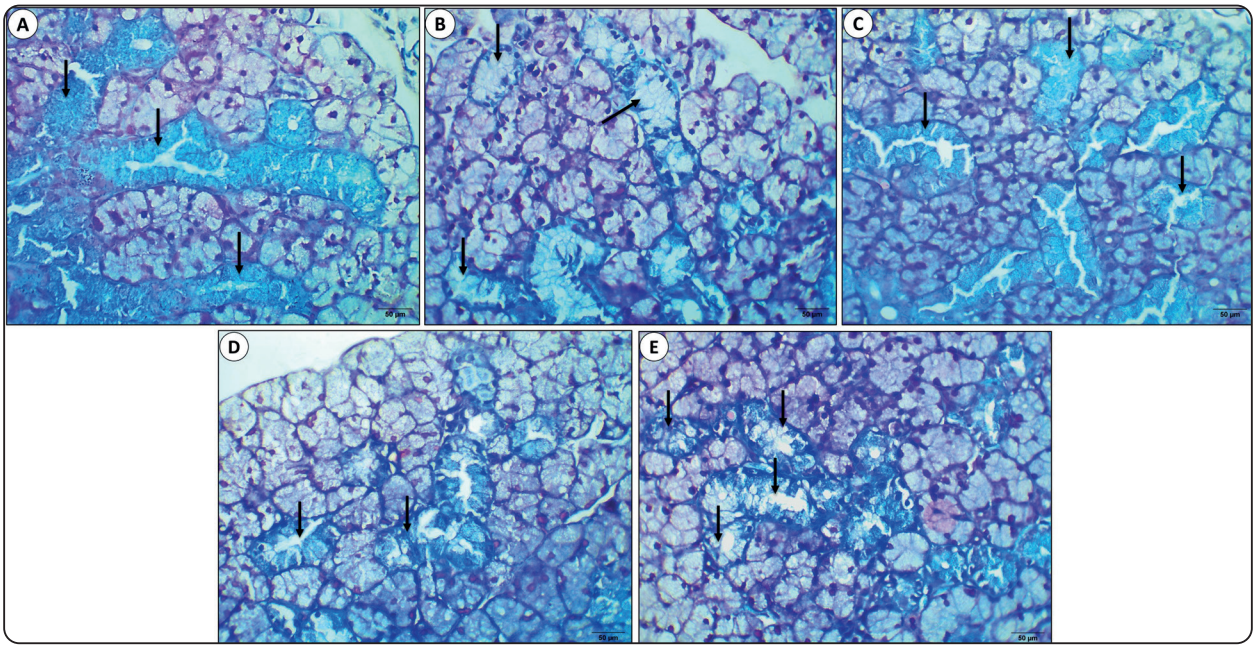

Fig. (3) LM of Alcian Blue staining in different groups. **A:** control group shows strong staining of mucous acini. **B:** diabetic group shows minimal staining. **C:** metformin treated group; mucous acini show strong staining. **D:** ginger treated group shows moderate staining. **E:** (L-carnitine treated group) shows mild staining of the mucous acini. (Alcian Blue, 400x)

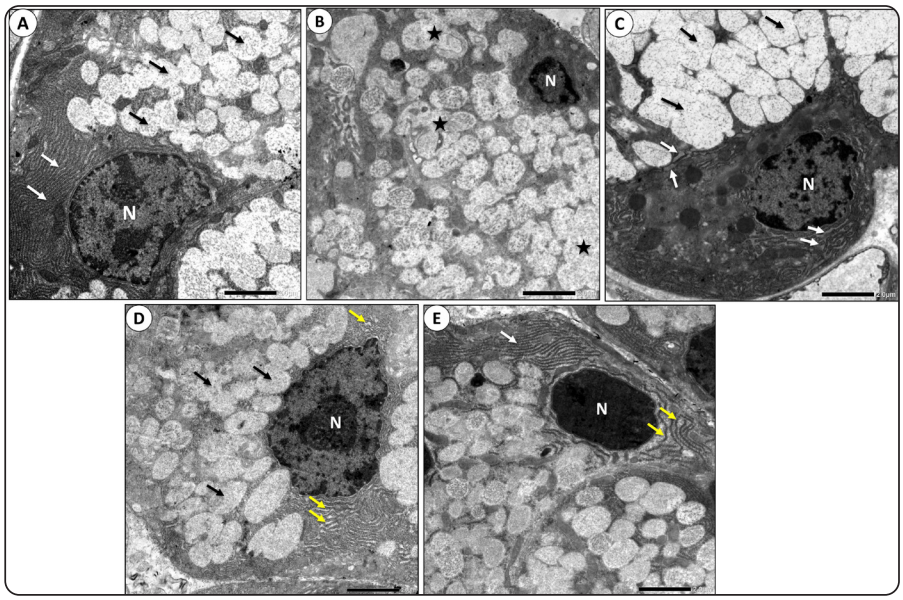

Fig. (4) Transmission electron micrograph (TEM) of secretory acini of submandibular gland in different groups. **A (control group):** shows basal euchromatic nucleus (N) surrounded by well-organized cisternae of rER (white arrows) and electron lucent secretory granules (black arrows). **B (diabetic group)**: shows shrunken nucleus (N) and coalescence of secretory granules (stars). **C (Metformin treated group):** shows basal slightly irregular euchromatic nucleus (N), slightly dilated cisternae of rER (white arrows) and numerous secretory granules (black arrows). **D (ginger treated group):** shows slightly irregular basal euchromatic nucleus (N), slightly dilated cisternae of rER (yellow arrows) and secretory granules (black arrows). **E (L-carnitine treated group):** shows basal heterochromatic nucleus (N), well organized rER (white arrow), while other part shows disorganized arrangement of rER (yellow arrows). x3000

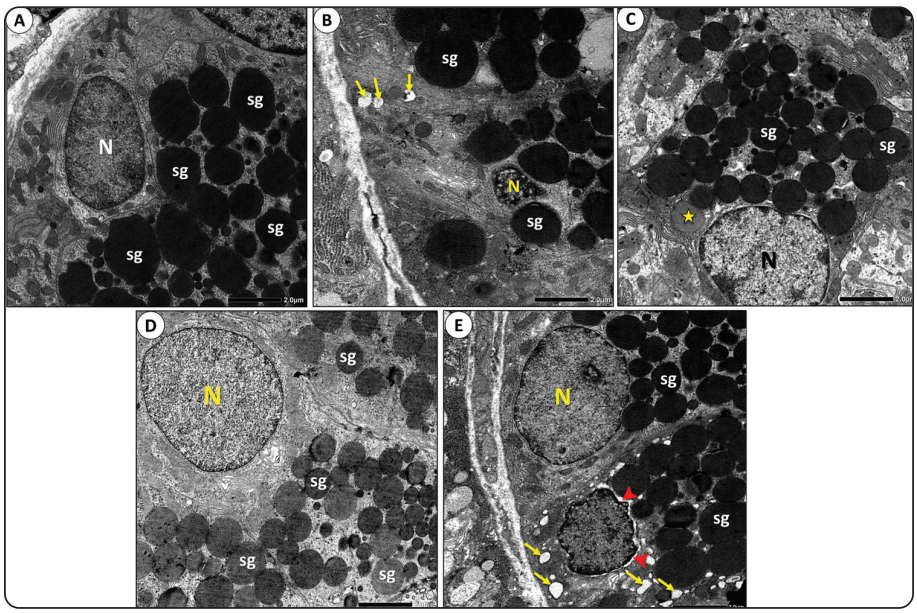

Fig. (5) TEM of granular convoluted duct in different groups. **A (control group)** shows basal euchromatic nucleus(N) and cytoplasm filled with electron dense secretory granules (sg). **B (diabetic group)** shows a shrunken nucleus (N), numerous secretory granules (sg) and some cytoplasmic vacuolations (arrows). **C (Metformin treated group):** basal euchromatic nucleus (N) is seen, numerous electron dense secretory granules (sg) and electron lucent secretory granule (star). **D (ginger treated group):** shows basal euchromatic nucleus (N) and numerous secretory granules with different electron density (sg). **E (L-carnitine treated group):** shows a duct cell with basal euchromatic nucleus (N) and numerous secretory granules (sg) and other cell which shows some changes including irregular nuclear outline, widening of the nuclear membrane (arrowheads) and dilatation of rER cisternae (arrows). X 3000

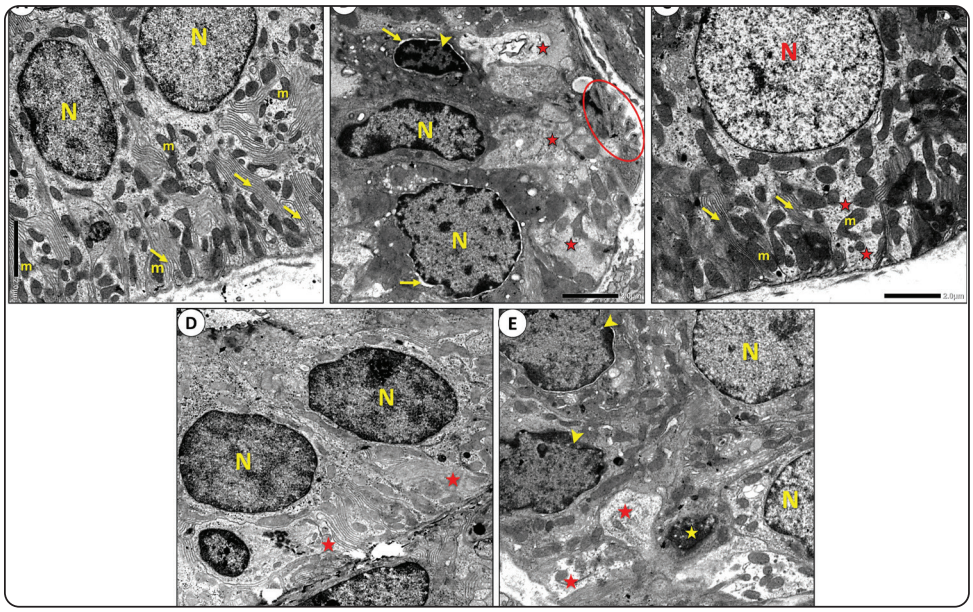

Fig. (6) TEM of striated duct in different groups. **(A): control grou**p showing central large euchromatic nuclei (N) and its characteristic basal membrane infoldings (arrows) and numerous mitochondria (m). **(B): diabetic group** showing loss of the basal membrane infoldings and mitochondria (stars) and variation in size and shape of nuclei (N). A shrunken nucleus (arrowhead) and widening of the nuclear membrane is seen (arrows). The basal lamina shows irregularity (encircled area). **(C): metformin treated group** showing euchromatic nucleus (N), the characteristic basal membrane infoldings (arrows) and radially arranged mitochondria (m) and a small area devoid of membrane infoldings (stars). **D (Ginger treated group)** showing euchromatic nuclei (N). and areas of loss of basal membrane infoldings (stars). **E (l-carnitine treated group)** showing variation in shape of the nuclei, some nuclei appear regular (N) and other show irregular outline (arrowheads), a shrunken nucleus (star) and areas of loss of the basal membrane infoldings and mitochondria (stars). x3000

Histomorphometric analysis

Table (1) shows the percentage of acinar cell vacuolization in different groups. A statistically significant increase in percentage of cell vacuolization was noted in diabetic group compared to control group ($P < 0.05$), where the values were 4.60 ± 1.25 and 0.34 ± 0.25 respectively. No significant differences were noted between each of metformin treated group & ginger treated compared to control group, where the values were 1.16, 0.76 & 0.34 respectively. In metformin, ginger and L-carnitine treated groups, a statistically significant decrease in percentage of vacuolization was detected compared to diabetic group ($p<0.05$), where the values were 1.16 ± 0.61, 0.76 ± 0.26 and 2.13 ± 0.59 respectively. However, significant difference was noted between metformin and L-carnitine group ($p<0.05$) and between ginger and L-carnitine groups ($p<0.05$).

Percentage of acinar cells vacuolization

TABLE (1) Percentage of acinar cells vacuolization

	Control (n = 10)	Diabetes (n = 10)	Metformin (n = 10)	Ginger (n = 10)	L-carnitine (n = 10)
			Percentage of vacuolization		
Mean ± SD.	0.34 ± 0.25	4.60[a] ± 1.25	1.16[b] ± 0.61	0.76[b] ± 0.26	2.13[abcd]±0.59

All data are represented as mean ± SD of *n = 10 rats per group.*
a ***P < 0.05 versus Control group;*** **b** ***P < 0.05 versus Diabetes group;*** **c** ***P < 0.05 versus Metformin group;*** **d** ***P < 0.05 versus Ginger group. The comparisons were made by one-way ANOVA using*** **Post Hoc Test (Tukey).**

Optical density of PAS staining:

Table (2) show the optical density of PAS staining in different groups. A statistically significant decrease in optical density of PAS between each of diabetic group and L-carnitine treated group compared to control group ($p<0.05$) was noted, where the values were 0.2, 0.24 & 0.32 respectively. Also, a statistically significant decrease in optical density of PAS between metformin & control group ($p<0.05$), where the values were 0.27 and 0.32 respectively. A statistically significant increase in optical density of PAS in metformin and ginger treated groups compared to diabetic group ($p<0.05$),

Optical density of Alcian Blue staining

Table (3) show the optical density of alcian blue staining in different groups. A statistically significant decrease in alcian blue optical density between each of diabetic, ginger and L-carnitine treated groups and control group ($p<0.05$) was noted, where the values were 0.21, 0.29, 0.24 and 0.36 respectively. The difference between each of metformin and ginger treated groups and diabetic group was significant ($p<0.05$). A statistically significant difference was noted between metformin treated group and ginger treated group ($p<0.05$). In addition, the difference between metformin and L-carnitine treated group was significant ($p<0.05$). A statistically significant difference was noted between ginger and L-carnitine treated group ($p<0.05$).

TABLE (2) Comparison between the different studied groups according to optical density of PAS

	Control (n = 10)	**Diabetes (n = 10)**	**Metformin (n = 10)**	**Ginger (n = 10)**	**L-carnitine (n = 10)**
			Optical density of PAS		
Mean ± SD.	0.32 ± 0.04	0.20^{a} ± 0.02	0.27^{ab} ± 0.04	0.28^{b} ± 0.04	0.24^{a} ± 0.03

All data are represented as the mean ± SD of n = 10 rats per group.

***a P < 0.05 versus Control group;* b *P < 0.05 versus Diabetes group;* c *P < 0.05 versus Metformin group;* d *P < 0.05 versus Ginger group. The comparisons were made by one-way ANOVA using* Post Hoc Test (Tukey).**

TABLE (3) Comparison between the different studied groups according to optical density of Alcian Blue

	Control (n = 10)	**Diabetes (n = 10)**	**Metformin (n = 10)**	**Ginger (n = 10)**	**L-carnitine (n = 10)**
			Optical density of Alcian Blue		
Mean ± SD.	0.36 ± 0.03	0.21^{a} ± 0.03	0.34^{b} ± 0.02	0.29^{abc} ± 0.05	0.24^{acd} ±0.03

All data are represented as the mean ± SD of n = 10 rats per group.

a *P < 0.05 versus Control group;* **b** *P < 0.05 versus Diabetes group;* **c** *P < 0.05 versus Metformin group;* **d** *P < 0.05 versus Ginger group. The comparisons were made by one-way ANOVA using* **Post Hoc Test (Tukey).**

Electron microscopic results:

TEM examination of control group specimens revealed secretory acini with basal euchromatic nucleus and well-organized cisternae of rER and numerous electron lucent secretory granules (Fig. 4A). In diabetic group, the nucleus appeared shrunken and heterochromatic, the cytoplasm revealed coalescing electron lucent secretory granules and ill-defined rER (Fig. 4B). On the other hand, in both metformin treated group (Fig. 4C) and ginger treated group (Fig. 4D), the acini showed basal slightly irregular euchromatic nuclei, slightly dilated cisternae of rER and numerous secretory granules. In L-carnitine treated group, the nucleus was heterochromatic and disorganization of the normal arrangement of rER was observes in some areas (Fig.4E).

Granular convoluted duct cells of control group showed basal euchromatic nuclei and numerous electron dense secretory granules (Fig.5A). In diabetic group, some nuclei appeared shrunken and cytoplasmic vacuolization were detected. Secretory granules showed variation in size and electron density (Fig. 5B) In metformin treated group, almost the normal structure was restored where euchromatic nuclei were seen with numerous electron dense secretory granules (Fig.5C). In ginger treated group, the nucleus appeared large and euchromatic, while the secretory granules exhibited different electron densities (Fig.5D). In L-carnitine treated group, some cells revealed the normal characteristic features, while others exhibited changes in form of irregular nuclear outline, slight widening of the nuclear membrane and dilatation of the rER cisternae (Fig.5E).

Striated duct in control group revealed central euchromatic nuclei and the characteristic basal membrane infoldings with numerous radially arranged mitochondria (Fig.6A). In diabetic group, numerous changes were observed including loss of the basal membrane infoldings and mitochondria, variation in shape of the nuclei, widening of the nuclear membrane and irregular basal lamina (Fig.6B). In both metformin treated group (Fig.6C) and ginger treated group (Fig.6C), relative restoration of the normal characteristic features was noted. Also, some changes were observed like localized small areas of loss of basal membrane infoldings. In L-carnitine treated group, more changes were observed including irregularity in the outline of some nuclei, other nuclei appeared shrunken and partial loss of the characteristic basal membrane infoldings and mitochondrial arrangement (Fig.6E).

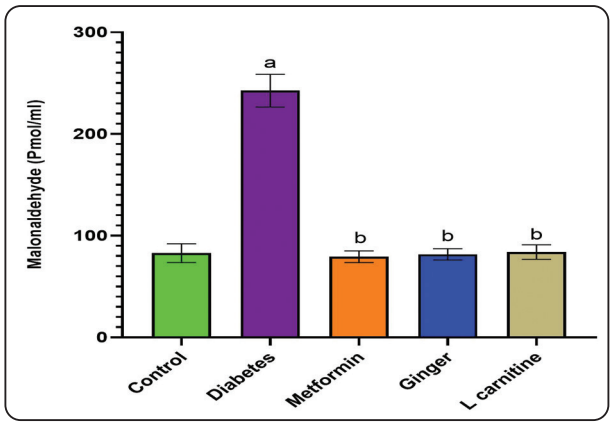

Fig. (7) Comparison between the different groups according to serum MDA level

Biochemical analysis

Effect of L-carnitine and ginger on lipid peroxidation

Serum MDA level was statistically ($P<0.05$) increased in diabetic rats by 212% when compared to the control group. However, treatment with metformin, ginger and L-carnitine were significantly decreased MDA levels by 67%, 66% and 65%, respectively ($P<0.05$) as compared with diabetic rats. No significant changes were detected with metformin, ginger and L-carnitine treatment between each other and in comparison, to control rats (Figure 7).

Effect of L-carnitine and ginger on total antioxidant capacity

Total antioxidant capacity showed a significant decrease (P<0.05) by 39% in diabetic rats when compared with the control group. Treatment with metformin, ginger and L-carnitine was accompanied with a statistical increase in total antioxidant capacity by 120%, 217%, and 140%, respectively when compared with diabetic rats. Ginger-treated group showed an increase in total antioxidant capacity by 44% as compared with metformin-treated rats. Total antioxidant capacity was also increased after L-carnitine treatment by 9% as compared with metformin-treated rats that are not significant (Figure 8).

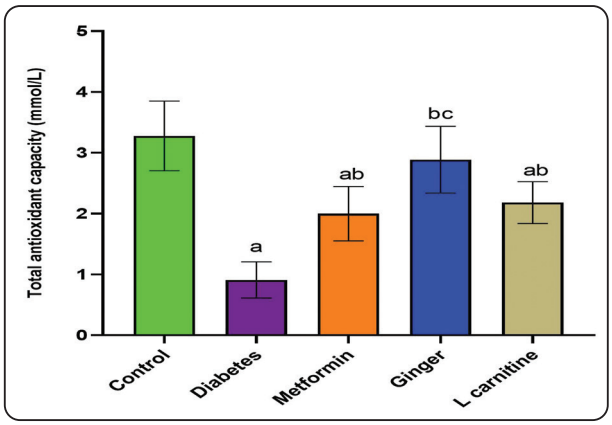

Fig. (8) Comparison between the different groups according to Total antioxidant capacity

DISCUSSION

Diabetes mellitus is a global metabolic disease affecting millions of people around the world. Its high prevalence and its microvascular and macrovascular complications can lead to severe morbidity and mortality. [31, 32]

Although Metformin is one of the oldest oral antidiabetic drugs, it is still the medication of choice with growing indications. Several studies showed that it has variable therapeutic effects in the management of different conditions such as prediabetes and type1and 2 diabetes mellitus. [33]. However, the continuous use of some synthetic antidiabetic drugs results in severe adverse effects, and thus the need for safe, inexpensive medications is still needed. Herbal and medicinal plants were proved to have a role in curing chronic diseases, e.g., cancer, fatty liver, and cardiovascular diseases. Furthermore, The World Health Organization (WHO) stated that more than 400 plants are available for the treatment of diabetes. [34, 35]

Khandouzi et al stated that ginger and its gingerol active ingredient have antihyperglycemic and antihyperlipidemic effects by improving glucose uptake and enhancing the phosphorylation of the insulin receptors.[36] Indeed, clinical reports claimed that the anti-inflammatory and antioxidant effects of L-Carnitine decreases the severity of diabetes, thus decreasing the release of reactive oxygen species (ROS). [37]

The present study was performed to compare the effect of ginger and L-carnitine versus Metformin on the submandibular salivary glands of diabetic rats.

In the current work, diabetes mellitus was induced in male Wister albino rats by a single intraperitoneal (i.p) injection of streptozotocin (STZ) 45 mg/kg. [38] STZ model depends on the fact that this drug causes damage to beta (β) cells of the pancreas and is applied as a clinically relevant animal model, used frequently to investigate human type I diabetes

Changes in respiratory enzyme activity and aerobic O_2 consumption are important physiological processes that lead to the generation of ROS. In many pathological conditions where the respiratory chain is compromised, free radicals are substantially elevated. The mitochondrial electron transport chain overproduces ROS during respiration when blood glucose levels are high. There is growing data that suggests oxidative stress, hyperglycemia, and diabetic complications are closely related. [39]

The decreased activity of antioxidant capacity, protein glycation, lipid peroxidation, and autoxidation of glucose are the causes of increased oxidative

stress observed in diabetic rats.[40] In accordance with this finding, the current investigation demonstrated that the STZ-induced diabetic rats had elevated serum MDA, and lowered activity of antioxidant enzymes.

Nonetheless, the current study's investigations demonstrated that the administration of metformin, L-carnitine, and ginger was linked to a significant rise in total antioxidant capacity, a remarkable decrease in MDA. Ahmed Mobasher M. et al. demonstrated that STZ-induced diabetic rats exhibited increased MDA levels and decreased antioxidants level. On the other hand, metformin treatment improved oxidative stress biomarkers, decreased hepatocyte histological alterations. [41]

Furthermore, our findings support the findings of Cao Y., Li X., et al., who concluded that L-carnitine stimulated endogenous antioxidant defense mechanisms while inhibiting the peroxidation of lipids and production of free radicals induced by high glucose levels. [42]

Moreover, our findings are in line with those of K.R. Shanmugam, et al., who showed that upon ginger supplementation to diabetic rats for 30 days, remarkable dose-dependent hypoglycemia and improvement in antioxidant enzymes parameters in the liver and kidney tissues except MDA were monitored. [43]

In our study, histological examination by light microscope revealed degenerative changes affecting the acini in the diabetic group. This was manifested in the form of cytoplasmic vacuolations and ill-defined acinar cell borders. These findings were confirmed by ultrastructural findings which also revealed coalescence of secretory granules, pyknosis of some nuclei in acinar cells and vacuolations in convoluted tubules.

These results were in accordance with the findings of Yasser and Shon who stated that diabetes mellitus adversely affects the acini of rat submandibular glands and attributed these changes to the oxidative stress and its underlying mechanisms causing detrimental changes to the cellular proteins and lipids. [44]

These results may be due to increased blood sugar level leading to the imbalance between the released free radicals and the natural antioxidants mechanisms occurring in the cell. Subsequent oxidative stress associated with diabetes is thought to occur not only by the generation of ROS, non-enzymatic glycosylation of protein, glucose auto-oxidation, defective metabolism of antioxidant enzymes, and lipid peroxidation. [45, 46]

In addition, in the current study, the most prominent change in the shape of the nuclei in diabetic group was decrease in size of the nuclei and irregularity in its outline. Irregularity in the nuclear outline in diabetes was also reported by Martinovic et al [47] & Oz et al [48].

This can be explained by imbalance between antioxidant enzymes and ROS generation caused by diabetes. This leads to oxidative stress which leads to DNA damage. [49, 50]

Histomorphometric analysis revealed a statistically significant increase in acinar cell vacuolization in diabetic group compared to control group ($p1<0.001$). This can be attributed to increase in free radicals resulting in liberation of lysosomal enzymes which interact with other proteins inside the cell leading to fragmentation of cellular structures and aggregation in vacuoles to maintain cellular metabolism as a cell defense mechanism. [51]

In our study, dilatation of rER cisternae was noted in diabetic group. This is in agreement with Schönthal et al who stated that oxidative stress can affect the rER resulting in improper protein production and gradual gathering of misfolded and unfolded proteins leading to distention of its cisternae. [52]

On the other hand, when rats were treated with metformin and ginger, most of the normal structure of the gland was restored. Marked improvement in the acini was observed except for few vacuolations.

Ultra-structurally, secretory acini showed normal organelles structure except for minor dilatation in rER cisternae.

Our results are in coordination with those of Saad et al who stated that ginger administration led to restoration of most of the detrimental effect of diabetes on soft palate mucosa in diabetic rats. [53]

Indeed, ginger extract and gingerol, have potent antihyperglycemic action via enhancing glucose uptake, increasing phosphorylation of insulin receptors and inhibiting lipid peroxidation. [54] Moreover, studies revealed that ginger has free radical scavenging effect, thus protecting the β-cells from diabetes-induced oxidative stress. [55] These findings are in agreement with Lee et.al and li et.al who confirmed through their studies that gingerols helps in maintaining glucose uptake and homeostasis by increasing the release of Glucose transporter type 4 and activating Adenosine Monophosphate-Activated Protein Kinase (AMPK) pathway [56, 57]

In the current study, PAS was used to detect neutral mucopolysaccharides and Alcian Blue stain for acidic mucopolysaccharides. In diabetic group, a statistically significant decrease in immunoreaction of both PAS and Alcian Blue stains compared to control group ($p<0.001$). However, in ginger treated group, a statistically significant increase in immunoreaction of both PAS and Alcian Blue compared to diabetic group ($p<0.001$).

The current results are in accordance with those of Bashandy et al who mentioned that ginger administration increased PAS/alcain blue staining intensity of gastric mucosa after 5-fluorocil administration. [58] Moreover, Abd El Hady Mousa et al reported almost restoration of PAS positive material in the group treated with ginger. [59]

In the present study, when diabetic rats were supplemented with L-Carnitine, although remarkable recovery in the gland structure was noticed, still more cytoplasmic vacuolations compared to the previous groups were detected by light microscope and by histomorphometric analysis. A statistically significant increase in percentage of acinar cell vacuolization was detected in L-carnitine group compared to metformin and ginger treated groups ($p<0.05$). Also, on the ultrastructural level, slight dilatation in rER cisternae, irregularity in the nuclear membrane or shrunken nucleus.

Our results are in line with those of Al Badawi et al who stated that L-carnitine showed a protective role against ionizing radiation in submandibular salivary gland of rats. L-carnitine administration caused a decrease in cytoplasmic vacuolization and increase in number of cytoplasmic organelles.[60] This improvement in the gland condition may be due to the ability of L-Carnitine to reduce the free radical generation and increase their scavenging via the cellular antioxidant content. In addition, it forms phospholipids and protein kinase c through its interaction with arachidonic acid, thus it reduces lipid peroxidation as well as oxidative stress. Indeed, Virmani A, Diedenhofen A mentioned that L-Carnitine itself acts as an antioxidant and protects the main antioxidant enzymes L-Carnitine caused a significant reduction in MDA level. [61]

In our study, PAS staining of L-carnitine group showed a non-significant increase in optical density compared to diabetic group. These results are in agreement with those of ElGhamrawy et al who found that L-carnitine administration with liquid diet resulted in moderate staining of acinar cells with PAS compared to liquid diet group only which exhibited weak staining of PAS. [62]

In conclusion, the current study results suggest that both ginger and L-carnitine therapy have beneficial effect in the management of the side effects of diabetes mellitus on the structure of submandibular salivary glands of rats. They protect against diabetes therapeutically by improving liver and kidney function, reducing oxidative stress, and boosting antioxidant enzymatic activity. However, when compared to metformin, ginger has more potent overall anti-diabetic benefits than L-carnitine.

Therefore, we recommend that ginger and L-carnitine to be used as safe, and inexpensive adjuvant therapy in case of diabetes beside conventional synthetic medications. Also, the use of ginger as a prophylactic antioxidant in healthy persons should be considered.

REFERENCES

1. Verhulst MJ, Loos BG, Gerdes VE, Teeuw WJ. Evaluating all potential oral complications of diabetes mellitus. Frontiers in endocrinology. 2019;10:56.
2. Deng L, Du C, Song P, Chen T, Rui S, Armstrong DG, et al. The role of oxidative stress and antioxidants in diabetic wound healing. Oxidative medicine and cellular longevity. 2021;2021(1):8852759.
3. Rohani B. Oral manifestations in patients with diabetes mellitus. World journal of diabetes. 2019;10(9):485.
4. Ahmad R, Haque M. Oral health messiers: Diabetes mellitus relevance. Diabetes, metabolic syndrome and obesity: targets and therapy. 2021;14:3001.
5. Nunes Carvalho GC, Garcia Lira Neto JC, Cunha Nunes LC, Parente Garcia Alencar AM, Lino Marques RL, Coelho Damasceno MM. Effectiveness of Ginger in the Treatment of Type 2 Diabetes Mellitus: A Pilot Study of the Randomized Clinical Trial Type. Aquichan. 2021;21(1).
6. Barkat MA, Goyal A, Barkat HA, Salauddin M, Pottoo FH, Anwer ET. Herbal medicine: clinical perspective and regulatory status. Combinatorial chemistry & high throughput screening. 2021;24(10):1573-82.
7. Hajimoosayi F, Sadatmahalleh SJ, Kazemnejad A, Pirjani R. Effect of ginger on the blood glucose level of women with gestational diabetes mellitus (GDM) with impaired glucose tolerance test (GTT): a randomized double-blind placebo-controlled trial. BMC complementary medicine and therapies. 2020;20(1):1-7.
8. Al-Rawaf HA, Gabr SA, Alghadir AH. Molecular changes in diabetic wound healing following administration of vitamin D and ginger supplements: biochemical and molecular experimental study. Evidence-Based Complementary and Alternative Medicine. 2019;2019.
9. Pang G-M, Li F-X, Yan Y, Zhang Y, Kong L-L, Zhu P, et al. Herbal medicine in the treatment of patients with type 2 diabetes mellitus. Chinese medical journal. 2019;132(1):78.
10. Karalis DT, Karalis T, Karalis S, Kleisiari AS. L-Carnitine as a Diet Supplement in Patients With Type II Diabetes. Cureus. 2020;12(5).
11. Shahouzehi B, Barkhordari K, Aminizadeh S, Masoumi-Ardakani Y. Effect of L-carnitine administration on serum insulin and adiponectin levels, and AMPK, APPL1 and PPARγ gene expression in STZ-induced diabetic rat liver. The Ukrainian Biochemical Journal. 2017(89,№ 6):48-55.
12. Savic D, Ball V, Curtis MK, Sousa Fialho MdL, Timm KN, Hauton D, et al. L-Carnitine Stimulates In Vivo Carbohydrate Metabolism in the Type 1 Diabetic Heart as Demonstrated by Hyperpolarized MRI. Metabolites. 2021;11(3):191.
13. Venkateswaran M, Jayabal S, Hemaiswarya S, Murugesan S, Enkateswara S, Doble M, et al. Polyphenol-rich Indian ginger cultivars ameliorate GLUT4 activity in C2C12 cells, inhibit diabetes-related enzymes and LPS-induced inflammation: An in vitro study. Journal of Food Biochemistry. 2021;45(2):e13600.
14. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. Journal of Cerebral Blood Flow & Metabolism. 2020;40(9):1769-77.
15. Albus U. Guide for the care and use of laboratory animals (8th edn). SAGE Publications Sage UK: London, England; 2012.
16. Charan J, Biswas T. How to calculate sample size for different study designs in medical research? Indian journal of psychological medicine. 2013;35(2):121-6.
17. El Sadik A, Mohamed E, El Zainy A. Postnatal changes in the development of rat submandibular glands in offspring of diabetic mothers: Biochemical, histological and ultrastructural study. PLoS One. 2018;13(10):e0205372.
18. Pannucci CJ, Wilkins EG. Identifying and avoiding bias in research. Plastic and reconstructive surgery. 2010; 126(2):619.
19. Faul F, Erdfelder E, Lang A-G, Buchner A. G* Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior research methods. 2007;39(2):175-91.
20. Abdelaleem RMA, Abdel Hameed H, Askar M, Hassan SHM, El-Batal AI. Modulatory role of selenium nanoparticles and grape seed extract mixture on oxidative stress biomarkers in diabetic irradiated rats. Indian Journal of Pharmaceutical Education and Research. 2016; 50(1):170-8.

21. Motyl K, McCabe LR. Streptozotocin, type I diabetes severity and bone. Biological procedures online. 2009; 11(1):296-315.

22. Abdulmalek SA, Balbaa M. Synergistic effect of nano-selenium and metformin on type 2 diabetic rat model: Diabetic complications alleviation through insulin sensitivity, oxidative mediators and inflammatory markers. PloS one. 2019;14(8):e0220779.

23. Rong X, Peng G, Suzuki T, Yang Q, Yamahara J, Li Y. A 35-day gavage safety assessment of ginger in rats. Regulatory Toxicology and Pharmacology. 2009;54(2):118-23.

24. Zatroch KK, Knight CG, Reimer JN, Pang DS. Refinement of intraperitoneal injection of sodium pentobarbital for euthanasia in laboratory rats (Rattus norvegicus). BMC veterinary research. 2016;13:1-7.

25. Feldman AT, Wolfe D. Tissue processing and hematoxylin and eosin staining. Histopathology: Springer; 2014. p. 31-43.

26. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, et al. Fiji: an open-source platform for biological-image analysis. Nature methods. 2012;9(7):676-82.

27. Mustafa HN, El Awdan SA, Hegazy GA, Jaleel GAA. Prophylactic role of coenzyme Q10 and Cynara scolymus L on doxorubicin-induced toxicity in rats: Biochemical and immunohistochemical study. Indian journal of pharmacology. 2015;47(6):649.

28. Press W, DCHayat M. Principles and techniques of electron microscopy: biological applications. Wiley Online Library; 2000.

29. Kei S. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clinica chimica acta. 1978;90(1):37-43.

30. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical biochemistry. 1996;239(1):70-6.

31. Shah NA, Levy CJ. Emerging technologies for the management of type 2 diabetes mellitus. Journal of Diabetes. 2021;13(9):713-24.

32. Cirano FR, Molez AM, Ribeiro FV, Tenenbaum HC, Casati MZ, Corrêa MG, et al. Resveratrol and insulin association reduced alveolar bone loss and produced an antioxidant effect in diabetic rats. Journal of Periodontology. 2021;92(5):748-59.

33. Drzewoski J, Hanefeld M. The current and potential therapeutic use of metformin—the good old drug. Pharmaceuticals. 2021;14(2):122.

34. Kumar S, Mittal A, Babu D, Mittal A. Herbal medicines for diabetes management and its secondary complications. Current diabetes reviews. 2021;17(4):437-56.

35. Mahdavi A, Bagherniya M, Mirenayat MS, Atkin SL, Sahebkar A. Medicinal plants and phytochemicals regulating insulin resistance and glucose homeostasis in type 2 diabetic patients: a clinical review. Pharmacological Properties of Plant-Derived Natural Products and Implications for Human Health. 2021:161-83.

36. Khandouzi N, Shidfar F, Rajab A, Rahideh T, Hosseini P, Taheri MM. The effects of ginger on fasting blood sugar, hemoglobin A1c, apolipoprotein B, apolipoprotein AI and malondialdehyde in type 2 diabetic patients. Iranian journal of pharmaceutical research: IJPR. 2015;14(1):131.

37. Fathizadeh H, Milajerdi A, Reiner Ž, Amirani E, Asemi Z, Mansournia MA, et al. The effects of L-carnitine supplementation on indicators of inflammation and oxidative stress: a systematic review and meta-analysis of randomized controlled trials. Journal of Diabetes & Metabolic Disorders. 2020;19:1879-94.

38. Goyal SN, Reddy NM, Patil KR, Nakhate KT, Ojha S, Patil CR, et al. Challenges and issues with streptozotocin-induced diabetes–a clinically relevant animal model to understand the diabetes pathogenesis and evaluate therapeutics. Chemico-biological interactions. 2016;244:49-63.

39. Simmons RA. Developmental origins of diabetes: the role of oxidative stress. Free Radical Biology and Medicine. 2006;40(6):917-22.

40. Sheweita S, Mashaly S, Newairy A, Abdou H, Eweda S. Changes in oxidative stress and antioxidant enzyme activities in streptozotocin-induced diabetes mellitus in rats: role of Alhagi maurorum extracts. Oxidative medicine and cellular longevity. 2016;2016.

41. Mobasher MA, El-Tantawi HG, El-Said KS. Metformin ameliorates oxidative stress induced by diabetes mellitus and hepatocellular carcinoma in rats. Reports of Biochemistry & Molecular Biology. 2020;9(1):115.

42. Cao Y, Li X, Shi P, Wang L-x, Sui Z-g. Effects of L-carnitine on high glucose-induced oxidative stress in retinal ganglion cells. Pharmacology. 2014;94(3-4):123-30.

43. Shanmugam KR, Mallikarjuna K, Nishanth K, Kuo CH, Reddy KS. Protective effect of dietary ginger on antioxidant enzymes and oxidative damage in experimental diabetic rat tissues. Food Chemistry. 2011;124(4):1436-42.

44. Yasser S, Shon A. Histomorphometric and immunohistochemical study comparing the effect of diabetes mellitus on the acini of the sublingual and submandibular salivary glands of albino rats. Open Access Macedonian Journal of Medical Sciences. 2020;8(A):49-54.

45. Rezaei N, Mardanshahi T, Shafaroudi MM, Abedian S, Mohammadi H, Zare Z. Effects of L-carnitine on the follicle-stimulating hormone, luteinizing hormone, testosterone, and testicular tissue oxidative stress levels in streptozotocin-induced diabetic rats. Journal of evidence-based Integrative Medicine. 2018;23:2515690X18796053.

46. Lucchesi AN, Freitas NTd, Cassettari LL, Marques SFG, Spadella CT. Diabetes mellitus triggers oxidative stress in the liver of alloxan-treated rats: a mechanism for diabetic chronic liver disease. Acta Cirurgica Brasileira. 2013;28:502-8.

47. Martinovic T, Ciric D, Pantic I, Lalic K, Rasulic I, Despotovic S, et al. Unusual shape and structure of lymphocyte nuclei is linked to hyperglycemia in type 2 diabetes patients. Tissue and Cell. 2018;52:92-100.

48. Oz ZS, Bektas S, Battal F, Atmaca H, Ermis B. Nuclear morphometric and morphological analysis of exfoliated buccal and tongue dorsum cells in type-1 diabetic patients. Journal of Cytology/Indian Academy of Cytologists. 2014;31(3):139.

49. Buttke TM, Sandstrom PA. Oxidative stress as a mediator of apoptosis. Immunology today. 1994;15(1):7-10.

50. Kujoth GC, Hiona A, Pugh TD, Someya S, Panzer K, Wohlgemuth SE, et al. Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. Science. 2005;309(5733):481-4.

51. Shubin AV, Demidyuk IV, Komissarov AA, Rafieva LM, Kostrov SV. Cytoplasmic vacuolization in cell death and survival. Oncotarget. 2016;7(34):55863.

52. Schönthal AH. Endoplasmic reticulum stress: its role in disease and novel prospects for therapy. Scientifica. 2012;2012(1):857516.

53. Saad FA. The influence of Zingiber officinale Roscoe on the histological changes of soft palatal mucosa in streptozotocin-induced type 3 diabetes rat model. Egyptian Journal of Histology. 2020;43(2):645-62.

54. El-Mehi AE-s, Faried MA. Effect of high-fructose diet-induced metabolic syndrome on the pituitary-gonadal axis from adolescence through adulthood in male albino rats and the possible protective role of ginger extract. A biochemical, histological and immunohistochemical study. Folia morphologica. 2020;79(4):690-708.

55. Araujo A, Jesus-Lima J, Otoch J, Pessoa A. Effect of Ginger (Zingiber officinale) Supplementation on Diabetes: An Update. Am J Phytomed Clin Ther. 2018;6(3):13.

56. Lee JO, Kim N, Lee HJ, Moon JW, Lee SK, Kim SJ, et al. [6]-Gingerol Affects Glucose Metabolism by Dual Regulation via the AMPKα2-Mediated AS160–Rab5 Pathway and AMPK-Mediated Insulin Sensitizing Effects. Journal of cellular biochemistry. 2015;116(7):1401-10.

57. Li Y, Tran VH, Koolaji N, Duke C, Roufogalis BD. (S)-[6]-Gingerol enhances glucose uptake in L6 myotubes by activation of AMPK in response to [Ca 2+] i. Journal of Pharmacy & Pharmaceutical Sciences. 2013;16(2):304-12.

58. Bashandy MA. Light and electron microscopic studies on the possible protective effect of ginger on the gastric fundic mucosa of adult male Albino rats treated with 5-fluorouracil. Egyptian Journal of Histology. 2020;43(1):36-50.

59. Abd El Hady Mousa M, Mansour H, Eid F, Mashaal A. Anti-inflammatory activity of ginger modulates macrophage activation against the inflammatory pathway of monosodium glutamate. Journal of Food Biochemistry. 2021;45(8):e13819.

60. Al Badawi MH, Salem NA, Mahmoud OM. Protective effect of l-carnitine on ionizing radiation-induced damage in rat submandibular gland: histological study. Egyptian Journal of Histology. 2015;38(2):346-55.

61. Virmani A, Diedenhofen A. The possible mechanisms involved in the protection strategies against radiation-induced cellular damage by carnitines. International Journal of Clinical Medicine. 2015;6(02):71-80.

62. ElGhamrawy T. The effect of liquid diet on the parotid gland and the protective role of L-carnitine: immunohistochemical and ultrastructural study. Folia morphologica. 2015;74(1):42-9.