Original Article

Effects of Streptozotocin - Induced Diabetes on the Tongue of Adult Male Albino Rat and the Possible Protective Role of Cod Liver Oil

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ABSTRACT

Introduction: Oral problems are among the common diabetes induced complications. Cod liver oil (CLO) is a rich source of polyunsaturated fatty acids that was found to provide a better control of glucose and lipid metabolism.

Aim of the Work: To study the histological changes of streptozotocin (STZ) induced diabetes on rat's tongue and evaluate the possible protective role of CLO.

Material and Methods: Thirty-six adult male albino rats, aging 4-6 months and weighting 180-200 gm, were used in this study. Animals were divided into three equal groups. Group I: was equally subdivided into three subgroups; subgroup IA: kept as negative control, subgroup IB: received single intraperitoneal injection of citrate buffer and Subgroup IC: received CLO by gastric tube daily for 12 weeks. Group II: received single intraperitoneal injection of STZ to induce diabetes. Group III: diabetes was induced as group II then rats were given CLO daily by gastric tube for 12 weeks. At the end of the experiment for each group, animals were anesthetized and tongues were dissected out and processed for light and scanning electron microscopic examination and immunohistochemical studies.

Results: The present study revealed that diabetes induced marked irregularities, flattening and hyperkeratosis of tongue papillae in addition to distortion of the muscle layer. Group III revealed evident improvement of these changes.

Conclusion: Obvious diabetes induced changes of tongue histoarchitecture were detected. However, CLO exerted a marked protective role against these changes. Thus, it could be a promising protector against diabetes induced tongue hazards.

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Key Words: Cod liver oil, diabetes, tongue muscles, tongue papillae.

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INTRODUCTION

Diabetes mellitus has become a worldwide health problem affecting nearly 8.5% of adult population. Unfortunately, the number of diabetic adults has risen from 108 million recorded in 1980 to 422 million in 2014. Moreover, owing to their poor eating habits and their increased prevalence of obesity, younger population became more liable to diabetes nowadays[1,2]. Diabetic patients are liable to a high incidence of oral problems such as dental caries, xerostomia, periodontal disease, sensory disorders, taste problems, salivary gland dysfunction and oral infections[3].

The tongue is the strongest muscular organ in the body and is responsible for critical functions including taste, speaking, chewing and swallowing[4]. Tongue complications as leukoplakia, taste impairment, changes in innervation and atrophic lesions up to tumors are added to the oral diabetic complications[5]. One of the frequently reported leading causes of these diabetic complications, is the free radical mediated oxidative stress[6].

Since ancient times, natural compounds have been used in various attempts aiming to manage multiple medicinal purposes including glycemic disorders[7]. Cod liver oil (CLO) is rich in polyunsaturated fatty acids, docosahexaenoic acid and vitamin A which are vital substances for the proper development and function of various tissues. It has been also reported that it lowers lipids, increases HDL, exerts an anti-inflammatory and anti-thrombotic effects in addition to

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providing protection against free-radical-induced damage and arteriosclerosis[8]. Previous studies recorded that, using CLO in treatment of diabetes in rats provided better control of their glucose level and lipid metabolism[9].

Reviewing the literature, it has been found that studies concerning the effect of diabetes on rat tongue were somehow few. On the other hand, the beneficial effects of CLO on multiple diabetic rat tissues, other than the tongue, were investigated. Thus, the objectives of this study were to examine the histological changes of STZ induced diabetes on rat tongue and to evaluate the possible role of CLO in ameliorating these changes.

MATERIAL AND METHODS

Drugs

1. Streptozotocin (STZ): from Sigma (St. Louis, Mo, USA), purchased in a powder form as 1 gm vial. Each rat was given a single intraperitoneal injection of 55 mg/kg body weight freshly dissolved in 1 ml citrate buffer (pH 4.5). Diabetes induced in rats by STZ is considered as a type1 diabetes mellitus model[9].
2. Cod liver oil: commercially known as Ultra Seas (Cod Liver Oil) 120 mL syrup, purchased from Egyptian Group for Pharmaceutical Industries (EGPI). It was given in a dose equivalent to 0.5mL/kg daily by gastric tube for 12 weeks[9].

Animals

Thirty-six adult male albino rats, aging from 4-6 months and weighting 180-200 gms, were used in this study. Animals were obtained and bred locally at the animal house of the Medical Research Center of Faculty of Medicine, Ain-Shams University. Rats were housed in medium sized metal cages in room temperature about 21 ±10˚C, humidity 45-50% with regular dark/light cycles and good ventilation. Free diet and water access were allowed and all rats were kept under the same circumstances throughout the experiment. All animal procedures followed the guidelines of Ain Shams University Research and Ethics Committee.

Experimental Protocol

Animals were divided into three equal groups as follows:

Group I (Control Group): It was composed of 12 adult male albino rats that were further subdivided into three equal subgroups:

- Subgroup IA (Negative Control): including 4 rats which were not given any treatment.
- Subgroup IB (Citrate Buffer Control): including 4 rats that received a single intraperitoneal injection of 1ml citrate buffer.
- Subgroup IC (CLO control): including 4 rats that were given 0.5mL/kg of CLO daily by gastric tube for 12 weeks.

Group II (Diabetic Group): It included 12 adult male rats in which diabetes was induced using a single intraperitoneal injection of 55 mg/kg body weight STZ, following overnight fasting. Blood samples were taken from the tail vein 48 hours after STZ injection and rats with blood glucose level of 250 mg/dl or more were considered to be diabetic[9].

Group III (CLO Treated Diabetic Group): It included 12 adult male rats in which diabetes was induced and confirmed as in group II, then 0.5mL/kg of CLO was administered daily by gastric tube beginning 48 h after STZ injection for 12 weeks[9].

By the end of the experiment for each group, rats were anesthetized, using diethyl ether inhalation, tongues were carefully removed and the anterior 2/3 were dissected and processed for examination by light and scanning electron microscopes and immunohistochemical studies. Image morphometric analysis has been also done.

Processing of Samples

For light microscopic studies, specimens were fixed in 10% buffered formalin, processed and embedded in paraffin blocks, sectioned at 5µm, cut and stained by Hematoxylin and Eosin (Hx.&E.)[10], and Masson trichrome special stain for collagen detection[11]. For immunohistochemical studies for the proliferating cell nuclear antigen (PCNA), 5µm sections were incubated in mouse monoclonal anti-PCNA antibody (Dako PC10) at a dilution of 1 in 100 in TRIS-buffered saline (TBS) (pH 7.6) and then washed in TBS. Visualization of PCNA was done using the standard alkaline phosphatase-anti-alkaline-phosphatase labelling method[12]. The middle third of the specimen was examined and photographed using light microscope (Olympus 268M microscope) equipped with an automatic photomicrographic camera system.

For scanning electron microscopic studies; specimens were immediately fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.4) for 24 hrs. The samples were treated with 8N hydrochloric acid at 60° for 30 minutes to remove mucus from the surface of the tongue. Samples were then dehydrated through a graded ethanol series and dried by the t-butyl alcohol freeze-drying method[13]. Specimens were then coated with gold sputter prepared for scanning electron microscope study[14]. The middle third of the specimen was examined and photographed with JEOL, JSM-5500 electron microscope, at the Regional Center of Mycology and Biotechnology, Al-Azhar University.

Morphometric analysis

It was carried out on routine Hx. & E. stained slides using image analyzer Leica Q win V.3 program installed on a computer in the Histology Department, Faculty of Medicine, Ain Shams University. The computer was connected to a Leica DM 2500 microscope (Wetzlar, Germany). Six randomly chosen fields in six sections obtained from six different animals from the same group were used for measuring the epithelial thickness of filiform papillae from the basal epithelial cells at the top of the papilla till the end of keratin layer in the three groups. Pixels were calibrated for actual measurements in micrometer using the objective lens of 40X.

Analysis of data was performed by MedCalc® Version 11.1.1.0 for Windows (MedCalc Software, Belgium) and Microsoft Office Excel 2010 (Microsoft, USA) where Analyses of Variance (ANOVA test), mean, standard deviation (SD) and T-test were done. T test result was considered to be highly significant when $P \leq 0.001$, significant when $P \leq 0.05$ and insignificant when $P > 0.05$.

RESULTS

Histological Results

Group I (Control Group): Light microscopic examination of sections of the three subgroups IA, IB and IC showed almost the same histoarchitecture of tongue. Hematoxylin and Eosin stained sections showed that the dorsal surface of tongue had numerous regularly arranged filiform papillae most of which appeared as sharp bristle like projections with few small short ones. They were lined by keratinized stratified squamous epithelium without taste buds and enclosed thin connective tissue cores (Figures 1,2). Fungiform papillae were fewer, wider and scattered in-between the filiform ones. These papillae had relatively thin covering keratinized epithelium with wide connective tissue core. A single onion like, pale staining multicellular taste bud was detected at the central top part of each fungiform papilla (Figure 3). The deeper muscle layer was composed of regular bundles of muscle fibers running in different directions with small blood vessels in between (Figure 4). Thin collagen fibers were detected around the muscle bundles by Masson trichrome stain (Figure 5).

Examination of immunohistochemically stained sections for PCNA for the three subgroups showed positive immunoreaction mainly in the basal epithelial cells of papillae (Figures 6,7).

Scanning electron microscopic examination of the three subgroups showed numerous sharp conical filiform papillae with uniform keratinized tips and smooth covering epithelium. They were arranged in parallel rows with regular inclination. Few rough keratinized cells were also seen at their bases. Moreover, scattered mushroom like fungiform papillae with broad apices were seen among the numerous filiform ones. These fungiform papillae showed multiple thin epithelial cell layers arranged around a central well-defined gustatory pore surrounded by shallow indentation (Figures 8-10).

Group II (Diabetic Group): Examination of Hx. & E. stained tongue sections of the diabetic rats revealed obvious histoarchitectural tongue changes with disfiguring of its papillae. Most of the filiform papillae appeared short with wide connective tissue core together with multiple areas of flattened papillae, thickening of epithelial covering and evident hyperkeratosis (Figures 11,12). Mitotic figures were also detected in their covering epithelium (Figure 13). Fungiform papillae appeared irregular with thick covering keratinized epithelium. Some taste buds were decentered and acquired a peripheral top position and others showed evident vacuolation (Figures 11,14). The muscle layer revealed wide areas of separation of its muscle bundles (Figure 15). Moreover, groups of signet ring shaped rounded to oval cells with empty cytoplasm and peripheral nucleus were observed interrupting the muscle bundles (Figure 16). Masson trichrome stain revealed excessive collagen fibers separating the muscle bundles (Figure 17).

Examination of immunohistochemically stained sections for PCNA showed positive immunoreaction in most epithelial cells (Figures 18,19).

Scanning electron microscopic examination showed filiform papillae with obvious disturbance in orientation and inclination and areas of flattening. Some showed blunted, notched ends or excess keratinized tips with rough covering epithelium. Distorted fungiform papillae appeared mostly either with very thin epithelial layers and absent gustatory pores or thick covering epithelial layers with ill-defined gustatory pores (Figures 20-23).

Group III (CLO Treated Diabetic Group): Examination of Hx. & E. stained tongue sections of CLO treated diabetic rats revealed regular arrangement of most of filiform papillae which appeared sharp bristle like with almost normal keratinized stratified squamous epithelial covering and thin connective tissue core (Figures 24,25). Most of fungiform papillae appeared regular with top central taste buds and thin covering keratinized epithelium (Figure 26). The muscle layer showed almost regular muscle bundles (Figure 27) and Masson trichrome stain revealed thin collagen fibers around them (Figure 28).

Immunohistochemical stained sections examination for PCNA showed positive immunoreaction mainly in the basal epithelial cells (Figures 29,30).

Scanning electron microscope examination revealed regular orientation and inclination of filiform papillae. However, few filiform papillae showed blunt or serrated tips. Fungiform papillae mostly showed regular smooth thin covering epithelial layers with well-defined gustatory pores (Figures 31,32).

Morphometric Results

Using morphometric studies, the mean thickness of the covering epithelium of filiform papillae in the three groups was measured and values are mentioned in (Table 1).

Statistical analysis revealed highly significant difference between group I and group II with $P<0.001$. Similarly, a highly significant difference between group II and group III with $P<0.001$ has been also found. On the other hand, the difference between group I and group III was statistically non-significant with $P>0.05$ (Table 1).

Comparisons among the morphometric results of the three groups were further illustrated in (Column Chart 1).

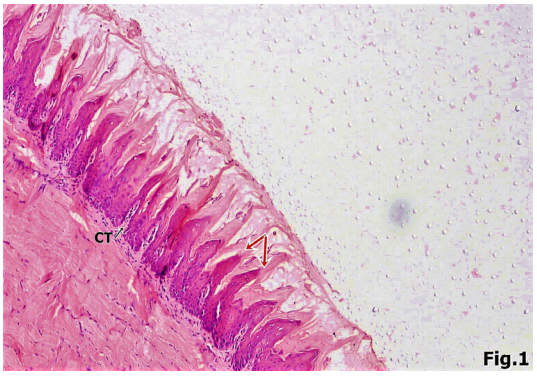


Fig. 1: A photomicrograph of a section of the tongue of group I albino rat showing regular arrangement of filiform papillae (red arrows) with thin connective tissue core (CT). (Hx.&E. X100)

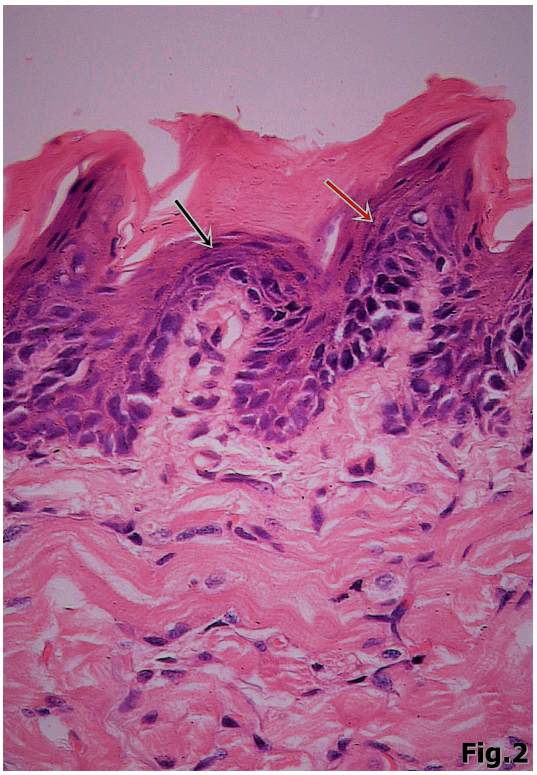


Fig. 2: A photomicrograph of a section of the tongue of group I albino rat showing sharp bristle like (red arrow) and small short (black arrow) filiform papillae covered with keratinized stratified squamous epithelium. (Hx.&E. X400)

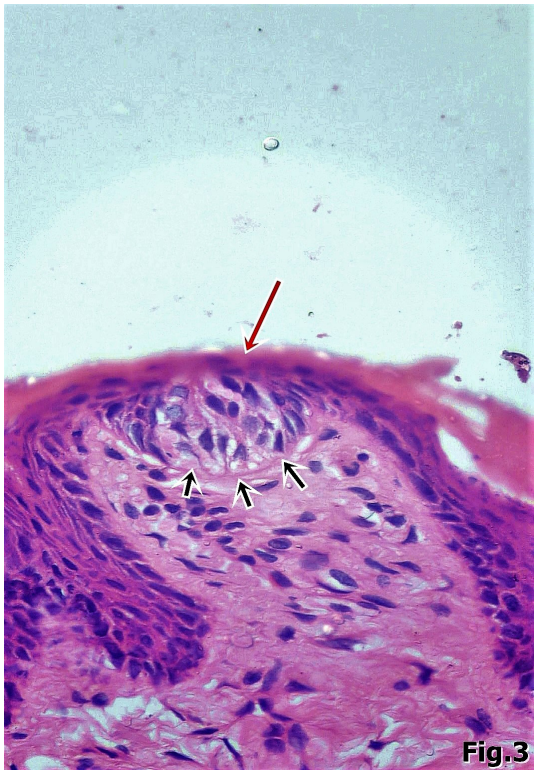


Fig. 3: A photomicrograph of a section of the tongue of group I albino rat showing fungiform papilla with onion like single taste bud (black arrows) at the top central part and thin covering keratinized epithelium (red arrow). (Hx.&E. X400)

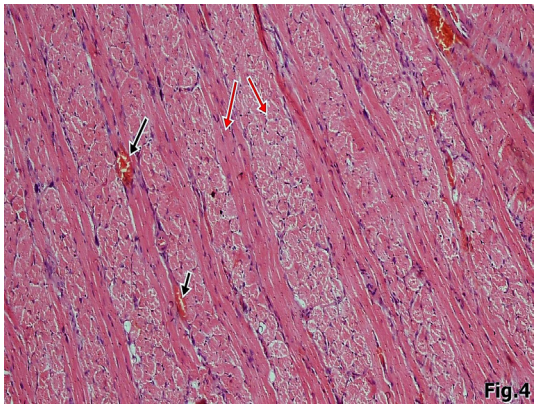


Fig. 4: A photomicrograph of a section of the tongue of group I albino rat showing bundles of muscle fibers (red arrows) with small blood vessels in-between (black arrows). (Hx.&E. X100)

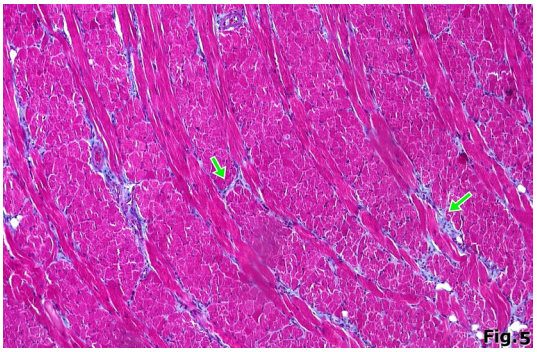


Fig. 5: A photomicrograph of a section of the tongue of group I albino rat showing thin collagen fibers (arrows) around the muscle bundles. (Masson trichrome stain X100)

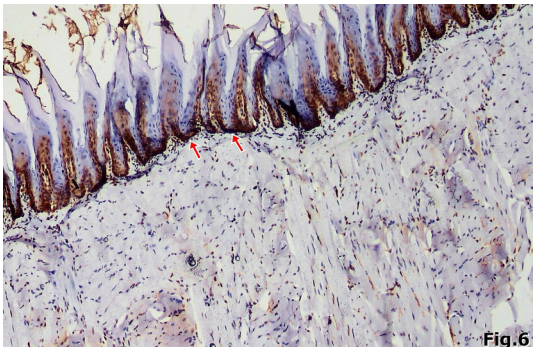


Fig. 6: A photomicrograph of a section of the tongue of group I albino rat showing positive immunoreaction to PCNA (brown color) mainly in basal epithelium cells (red arrow). (Immunostaining for PCNA X100)

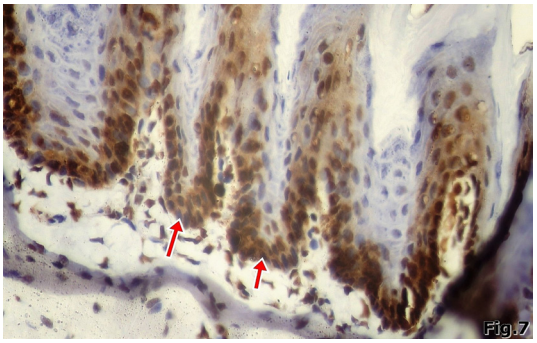


Fig. 7: A higher magnification of the previous photomicrograph of a section of the tongue of group I albino rat showing positive immunoreaction to PCNA (brown color) mainly in the basal epithelial cells (arrow). (Immunostaining for PCNA X400)

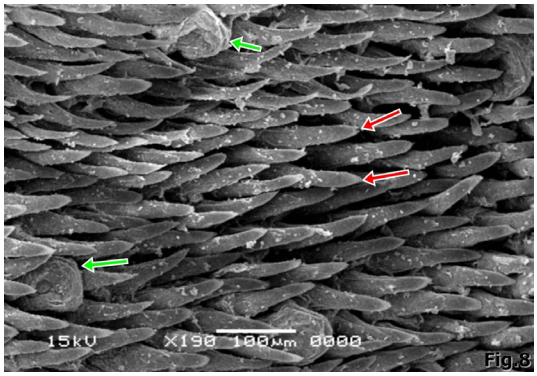


Fig. 8: A scanning electron micrograph of a section of the tongue of group I albino rat showing numerous sharp conical filiform papillae arranged in parallel rows with regular inclination (red arrows) with scattered fungiform papillae with broad apices (green arrow). (SEM X190)

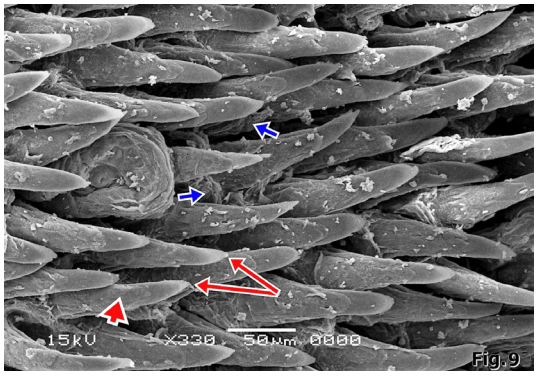


Fig. 9: A scanning electron micrograph of a section of the tongue of group I albino rat showing filiform papillae with uniform keratinized tips (red arrow) and smooth covering epithelium (red arrow head), Few rough keratinized cells were seen at their bases (blue arrows). (SEM X330)

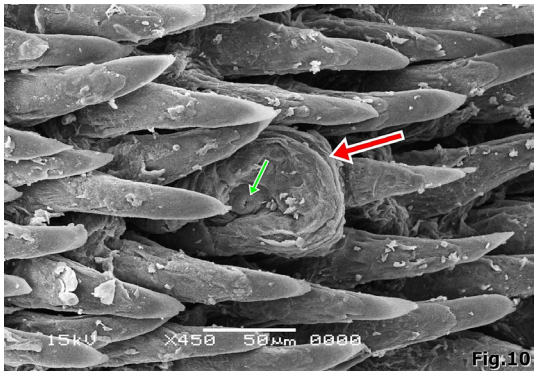


Fig. 10: A higher magnification of the previous scanning electron micrograph of a section of the tongue of group I albino rat showing Mushroom like fungiform papilla with thin multiple epithelial cell layers (red arrow) and well-defined gustatory pore surrounded by shallow indentation (green arrow). (SEM X450)

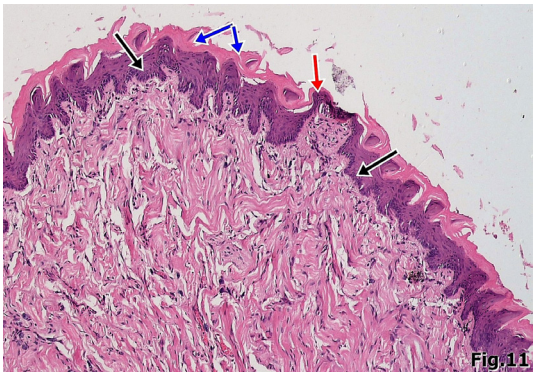


Fig. 11: A photomicrograph of a section of the tongue of group II albino rat showing flattened filiform papillae (black arrows) with hyperkeratosis (blue arrows). Notice the irregular fungiform papillae with decentered taste bud (red arrow). (Hx.&E. X100)

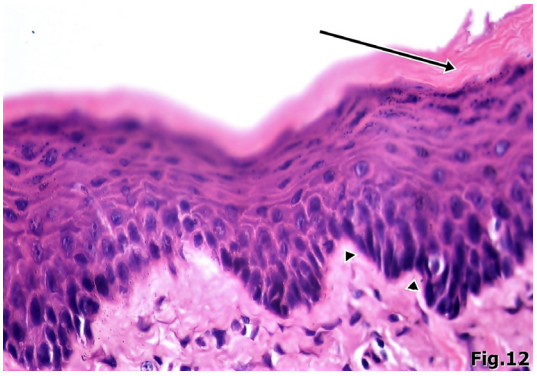


Fig. 12: A photomicrograph of a section of the tongue of group II albino rat showing shortened papillae with wide connective tissue core (black arrow heads) and thickened covering epithelium with hyperkeratosis (black arrow). (Hx.&E. X400)

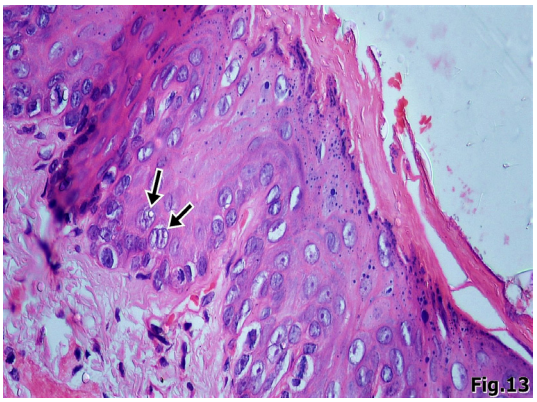


Fig. 13: A photomicrograph of a section of the tongue of group II albino rat showing mitotic figures (black arrows) in the covering epithelium of filiform papillae. (Hx.&E. X400)

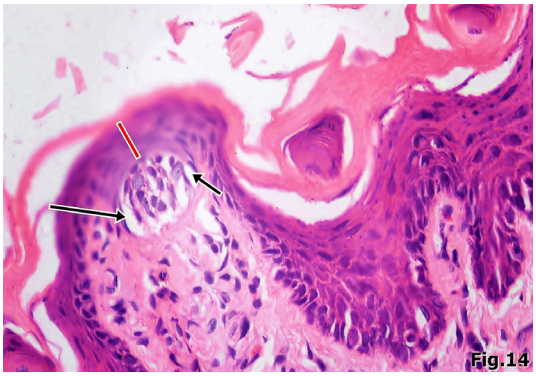


Fig. 14: A photomicrograph of a section of the tongue of group II albino rat showing fungiform papillae with thick covering epithelium (red line) and vacuolations in its taste bud (black arrows). (Hx.&E. X400)

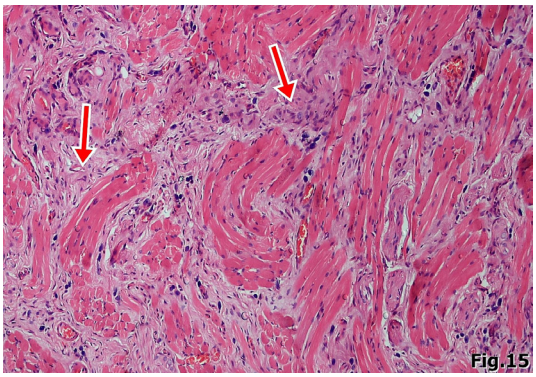


Fig. 15: A photomicrograph of a section of the tongue of group II albino rat showing wide areas of separation (red arrow) between muscle bundles. (Hx.&E. X100)

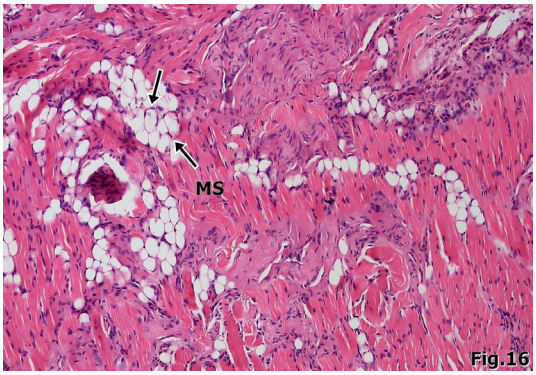


Fig. 16: A photomicrograph of a section of the tongue of group II albino rat showing groups of signet ring shaped cells with peripheral nuclei (arrows) interrupting the muscle bundles. (Hx.&E. X100)

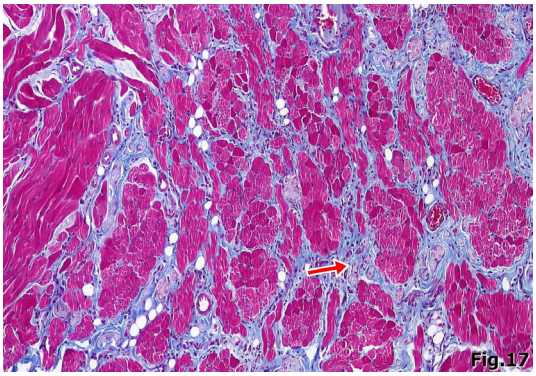


Fig. 17: A photomicrograph of a section of the tongue of group II albino rat showing excessive collagen fibers (red arrow) separating the muscle bundles. (Masson trichrome stain X100)

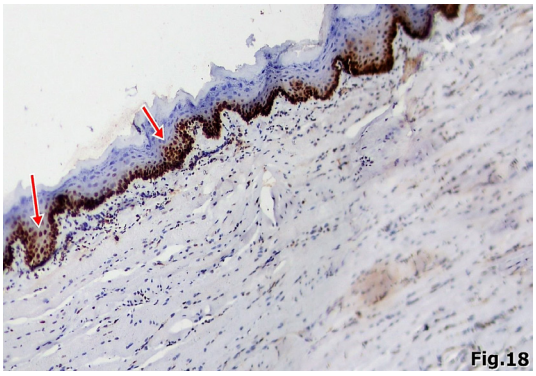


Fig. 18: A photomicrograph of a section of the tongue of group II albino rat showing increased positive immunoreaction to PCNA (brown color) in the epithelial cells (arrows). (Immunostaining for PCNA X100)

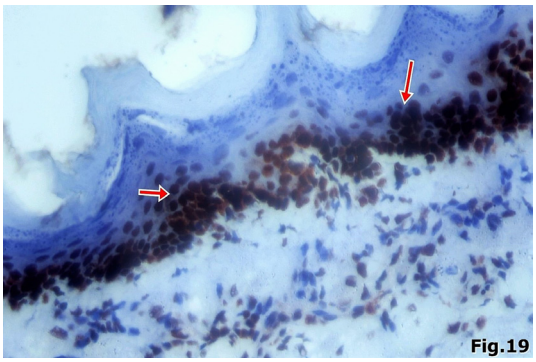


Fig. 19: A higher magnification of the previous photomicrograph a section of the tongue of group II albino rat showing strong positive immunoreaction to PCNA (brown color) of most epithelial cells (arrows). (Immunostaining for PCNA X400)

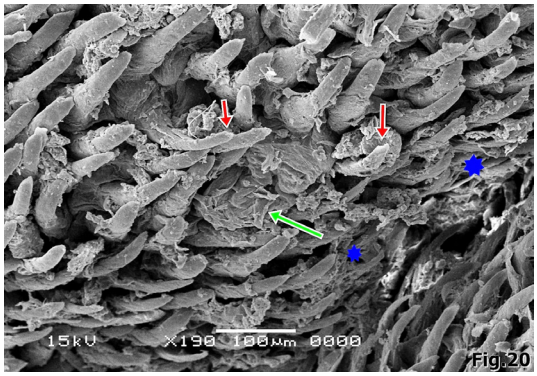


Fig. 20: A scanning electron micrograph a section of the tongue of group II albino rat showing filiform papillae with disturbed orientation and inclination (red arrows) with areas of flattening (*). Notice the distorted fungiform papilla (green arrow). (SEM X190)

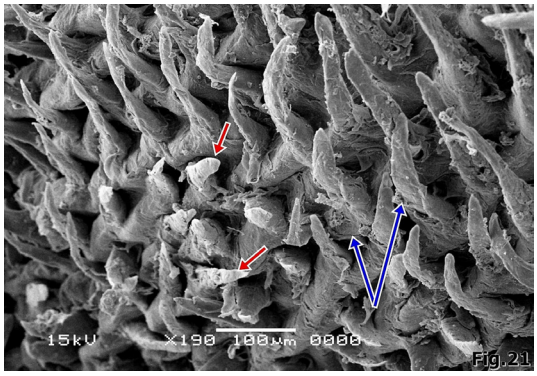


Fig. 21: A scanning electron micrograph of a section of the tongue of group II albino rat showing filiform papillae with disturbed orientation and inclination with excess keratinized tips (red arrows). And rough covering epithelium (blue arrows). (SEM X190)

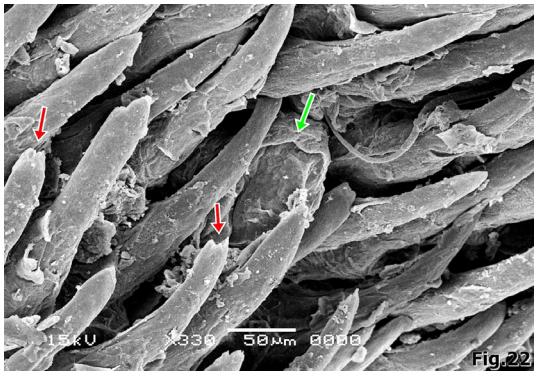


Fig. 22: A scanning electron micrograph a section of the tongue of group II albino rat showing filiform papillae with blunted and notched ends (red arrows). Notice the fungiform papilla with very thin epithelial layers and absent gustatory pores (green arrows). (SEM X330)

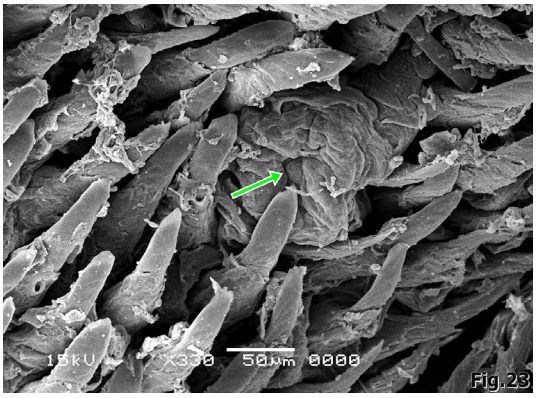


Fig. 23: A scanning electron micrograph of a section of the tongue of group II albino rat showing fungiform papilla with thick covering epithelial layers and ill-defined gustatory pores (green arrows). (SEM X330)

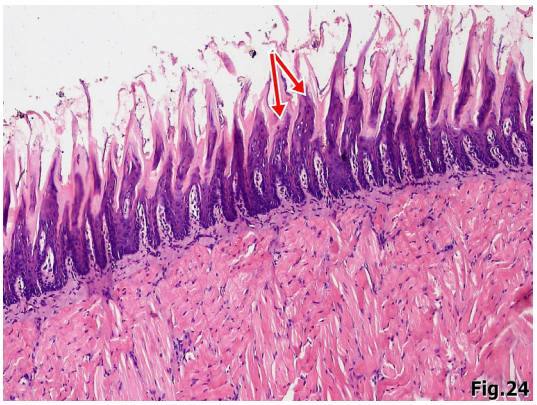


Fig. 24: A photomicrograph of a section of the tongue of group III albino rat showing regular arrangement of sharp bristle like filiform papillae (red arrows). (Hx.&E. X100)

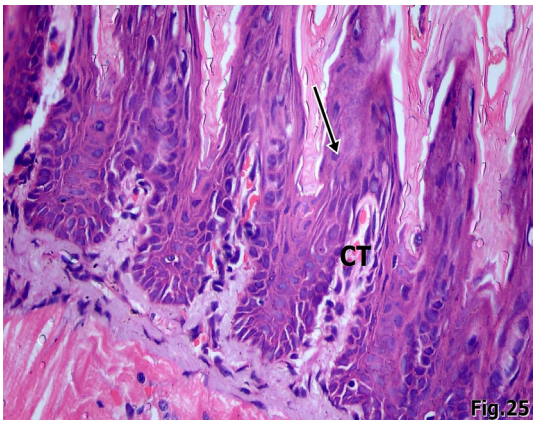


Fig. 25: A photomicrograph of a section of the tongue of group III albino rat showing filiform papillae with thin connective tissue core (CT) and covering keratinized stratified squamous epithelium (arrow). (Hx.&E. X400)

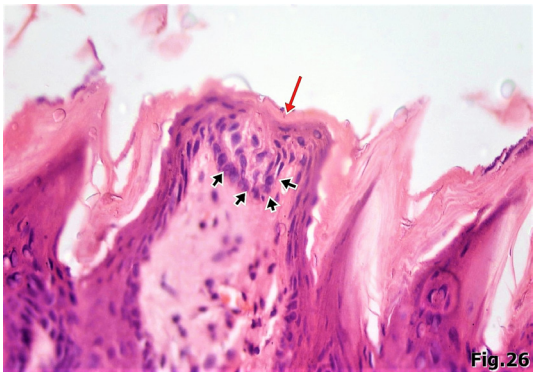


Fig. 26: A photomicrograph of a section of the tongue of group III albino rat showing fungiform papilla appeared regular with top central taste bud (black arrows) and thin covering keratinized epithelium (red arrow). (Hx.&E. X400)

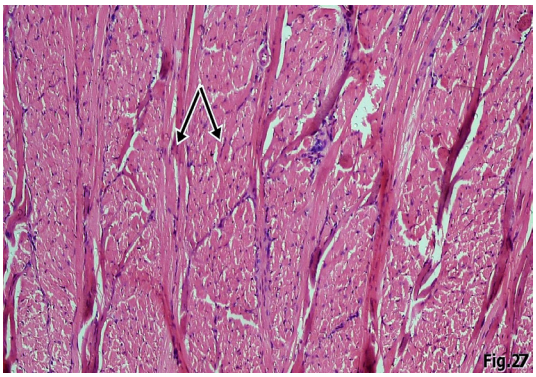


Fig. 27: A photomicrograph of a section of the tongue of group III albino rat showing mostly regular muscle bundles fibers (black arrows). (Hx.&E. X100)

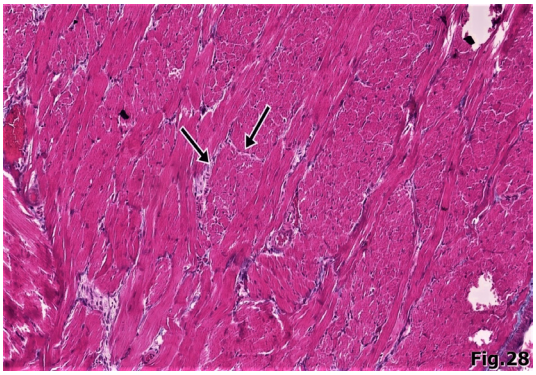


Fig. 28: A photomicrograph of a section of the tongue of group III albino rat showing thin collagen fibers (arrows) around the muscle bundles. (Masson trichrome stain X100)

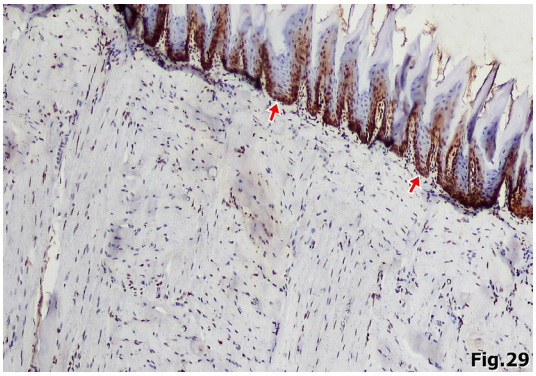


Fig. 29: A photomicrograph of a section of the tongue of group III albino rat showing positive immunoreaction to PCNA (brown color) mainly in the basal epithelial cells (red arrows). (Immunostaining for PCNA X100)

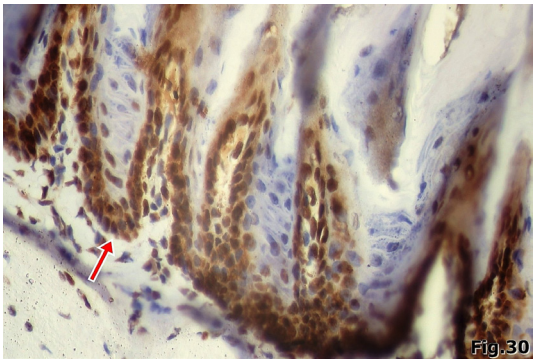


Fig. 30: A photomicrograph of a section of the tongue of group III albino rat showing positive immunoreaction to PCNA (brown color) mainly in the basal cells (arrow) of the covering epithelium. (Immunostaining for PCNA X400)

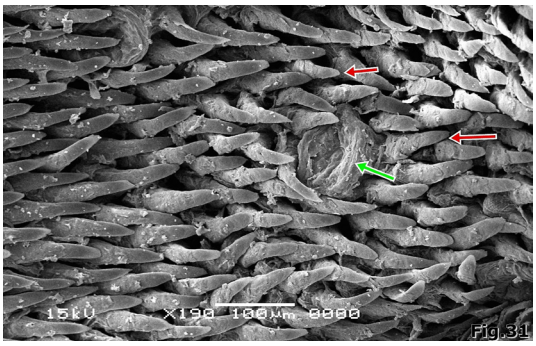


Fig. 31: A scanning electron micrograph of a section of the tongue of group III albino rat of group III albino rat showing regular orientation and inclination of filiform papillae (red arrows). Notice the regular fungiform papilla with covering epithelial layers (green arrow). (SEM X190)

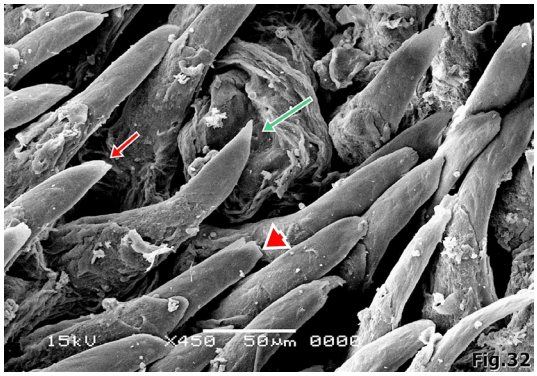
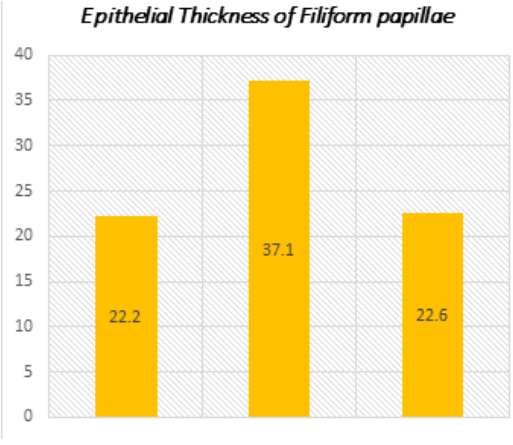


Fig. 32: A scanning electron micrograph of a section of the tongue of group III albino rat showing few filiform papillae with blunt (red arrow head) or serrated tips (red arrow). Notice the regular fungiform papilla with thin covering epithelial layers and well-defined gustatory pore (green arrow). (SEM X330)

Table 1: comparing the thickness of the covering epithelium of filiform papillae of the tongue in Hx.&E. stained sections among the three groups showing *P value* either; non-significant (ǂ) or highly significant (**)

		Group I (Control Group)	Group II (Diabetic Group)	Group III (CLO Treated Diabetic Group)
Epithelial thickness of filiform papillae Mean ±SD (in µm)		22.2±1.687	37.1±1.91	22.6±1.65
T test	Between Group I&II		*P*= 0.0001 *P* < 0.001**	
	Between Group II&III		*P* = 0.0001 *P* < 0.001**	
	Between Group I&III		*P*= 0.5 *P* > 0.05ǂ	



Column chart 1: demonstrating the morphometric comparison between the three groups as regards; thickness of the covering epithelium of filiform papillae

DISCUSSION

Diabetes mellitus causes many oral complications, the second most common site of which, after periodontal tissue, is the tongue[15]. Management of medical diseases as diabetes using natural compounds, has been a growing interest nowadays. However, most studies usually focus on the ability of these natural compounds to maintain blood glucose levels more than their role in ameliorating secondary complications of diabetes including oral lesions. Thus, the current study aimed at examining the histological changes of STZ induced diabetes on rat tongue and evaluating the possible role of CLO as an antioxidant in ameliorating these histological changes.

The results of the present study revealed marked diabetes induced histopathological tongue affection. Regarding papillae, Hx. and E. stained sections showed shortening of filiform papillae with widening of their connective tissue core together with flattening of some of the papillae. This was further revealed by scanning electron microscopic examination that showed marked disturbance in their orientation and inclination with areas of flattening in addition to blunting and sometimes notching of their ends. Filiform papillae are known to have a highly specialized mechanical function where their sharp projecting shape serves for the efficient taking of food into the oral cavity and its subsequent grinding with the teeth in coordination with the palate[16]. Therefore, alteration in their characteristic arrangement may subsequently alter their mechanical function.

Moreover, the epithelial covering of these filiform papillae appeared markedly thickened with hyperkeratosis in Hx. and E. stained sections which was also similar to their scanning electron microscopic picture where they appeared with excessively keratinized tips and rough covering epithelium. This thickening was further reinforced by the image morphometric studies that revealed a highly significant increase in their thickness when compared to those of group I.

Gurvits and Tan (2014) stated that, hyperkeratosis, which is a benign condition characterized by abnormal hypertrophy of filiform papillae, is usually associated with advanced age, poor general conditions as well as neurological disorders affecting tongue movement. They added that this condition is largely attributed to the decreased normal friction with subsequent decrease in the continuous desquamation that

occurs normally in the keratinized layers of filiform papillae thus leading to its accumulation[17].

Additionally, the current study detected the presence of mitotic figures in the epithelial lining of filiform papillae. It has been stated that, normal growth and maintenance of oral mucosa requires proper regulation of cell division in order to maintain proper coordination between epithelial differentiation and desquamation. Disruption of this normal growth control mechanisms or any dysregulation of cell cycle machinery leads to dysplastic transformation of epithelium and higher risks of cancer development[18]. Mitotic figures are said to be atypical if they show abnormal chromosomal distribution or increased number of mitotic spindles with multipolar morphologic appearance[19]. Moreover, Tandon et al., (2016) stated that, the more advanced the pathology is, the greater the number of mitotic figures and added that undue proliferation of cells due to mitosis is an important mark in precancer and cancer[20].

As for the fungiform papillae, Hx. & E. stained sections revealed that they were markedly irregular in shape with obvious thickening of their covering keratinized epithelium. Moreover, some of their taste buds acquired a peripheral top position and others showed vacuolations. Scanning electron microscopic examination further clarified their distorted appearance where some of them appeared with very thin covering epithelial layers and absent gustatory pores while others had thick covering epithelial layers with ill-defined gustatory pores. Negrato and Tareza (2010) reported that, diabetic rats had marked decrease in their salivary flow with altered composition which might lead to absence of gustin, responsible for the continuous maturation of taste buds[21]. Upon studying the innervation of taste buds using morphometric and quantitative immunohistochemical analysis, it has been reported that diabetic animals had significant reduction in their taste buds' innervation[22].

Regarding the muscle layer, wide spaces in between muscle bundles were noted in Hx. and E. stained sections that could be explained by the excessive collagen fibers detected around the muscle bundles separating them in the Masson trichrome stained sections. Sun et al., (2008) reported that diabetes was associated with atrophy, dysfunction, metabolic disturbance and structural changes of skeletal muscles and linked these changes to diabetes-associated metabolic changes, hemodynamic and vascular impairments[23]. Additionally, Muramatsu et al., (2017) recorded a notable reduction in the number of muscle spindles of gastrocnemius muscle of diabetic rats and attributed this to the diabetes induced loss of alpha and gamma motor neurons which induced atrophy of muscle spindles[24].

Another striking finding in the muscle layer of diabetic rats in the current study was the aggregation of groups of rounded to oval signet ring cells with peripheral compressed nuclei interrupting the muscle bundles. Computed tomographic scan revealed denervation atrophy of the right side of the tongue with fatty infiltration following damage of the hypoglossal nucleus or hypoglossal nerve[25]. Additionally, excessive fat tissue infiltration in leg skeletal muscle in individuals with diabetes mellitus, peripheral neuropathy and obesity associated with impaired performance and function has been previously recorded[26]. Thus, those detected groups of cells could represent a sort of fatty infiltration in the tongue following diabetes and diabetic neuropathy might be one of its leading causes.

Immunohistochemically stained sections of this group for PCNA showed positive immunoreaction of most epithelial cells that appeared increased as compared with the control group where, the positive reaction was mainly at the basal epithelial cells. It has been reported that, PCNA acts as an auxiliary protein to DNA delta polymerase and its synthesis begins to increase in the late G1 phase of the cell cycle and after reaching a peak in the middle S-phase, it returns to its initial condition in the late S phase[27]. Thus, it plays an important role in DNA synthesis, repair, cell cycle progression and cell proliferation[28]. Regarding the tongue tissue in specific, El Tokhy et al., (2012) reported that PCNA localization in rat tongue epithelium was at the basal cell layers as they were the only cell layers that contain proliferating cells[29]. Additionally, it has been declared that, increased PCNA expression in tongue epithelium indicates increased cellular turnover[28].

The above described histological findings of diabetic rat tongue could explain the occurrence of impaired taste, fissuring of the dorsum of the tongue and the appearance of painless median rhomboid glossitis which is a central de-papillated area on the middle third of the dorsum of the tongue with diabetes mellites as reported by Vijayabala et al. (2013)[30]. Moreover, Batbayar et al., (2004)

attributed the diabetes induced histological tongue changes to the chronic inflammatory state, changes in innervations and microvasculature frequently associated with diabetes[5].

On the other hand, examination of tongue of diabetic rats treated with CLO revealed marked improvement where the histological structure almost mimicked that of the control group. Hematoxylin and Eosin stained tongue sections showed regular arrangement of filiform papillae as most of them appeared sharp bristle like, with thin connective tissue core and thin covering keratinized stratified squamous epithelium which was almost back to normal. This was further confirmed by the morphometric studies where the thickness of their covering epithelium significantly decreased compared to that of group II and showed non-significant changes compared to group I. Moreover, scanning electron microscopic examination of these papillae showed almost regular orientation and inclination. However, few papillae showed blunt or serrated tips. As for the fungiform papillae, Hx. & E. stained sections revealed that they almost appeared regular with top central taste buds and thin covering keratinized epithelium and this was further clarified by scanning electron microscopic examination which showed regular thin covering epithelial layers with well-defined gustatory pore.

Regarding the muscle layer, Hx. & E. stained sections revealed almost regular bundles of muscle fibers and Masson trichrome stain reinforced this almost back to normal picture by reviling thin collagen fibers around the muscle bundles in contrast to the excessive collagen fibers seen separating the muscle bundles in group II.

Immunohistochemical stained sections examination for PCNA of group III showed positive immunoreaction mainly in the basal epithelial cells which was nearly similar to those of the control group.

It has been reported that diabetes mellitus is associated by abnormalities in the antioxidant pathways with subsequent increase in production of reactive oxygen species[31]. Hyperglycemic state has been also found to generate free radicals by many mechanisms such as metal-catalyzed oxidation of glucose, oxidative degeneration, and protein glycation[9]. The histological improvements observed in the group treated with CLO could be explained by the study of Hunkar et al., (2002) which reported that CLO supplementation decreases triacylglycerol, plasma cholesterol, lipid peroxidation products and gave a better glucose control in diabetic animals. Cod liver oil has been also found to improve the cardiovascular and the metabolic abnormalities in diabetic rats[8]. Moreover, it has been recorded that oral administration of CLO during pregnancy decreased the incidence of type 1 diabetes[32]. Further explanation has been recently offered by Das (2017) who stated that polyunsaturated fatty acid containing compounds, including fish oil, suppress the production of reactive oxygen species and enhance antioxidant defenses of pancreatic β cells that ultimately prevents development of type 1 diabetes[33].

CONCLUSION

Streptozotocin - induced diabetes led to marked histoarchitectural tongue changes. However, CLO greatly improved such changes. Thus, it could be considered as a highly promising protector for ameliorating diabetes induced tongue hazards with subsequent improvement of quality of life for diabetic patients. Further researches on the effect of CLO on other oral diabetic complications are strongly recommended.

CONFLICT OF INTERSTS

There are no conflicts of interest.

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تأثير داء السكري المستحث بالستربتوزوتوسين على لسان ذكر الجرذ الأبيض البالغ والدور الوقائي المحتمل لزيت كبد سمك القد

ايناس انور بخيت و مارى رفعت اسحق
قسم التشريح و علم الأجنة كلية الطب جامعة عين شمس

ملخص البحث

المقدمة: تعد المشاكل الفموية من بين المضاعفات الشائعة التي يسببها داء السكري. يعتبر زيت كبد سمك القد مصدراً غنياً للأحماض الدهنية المتعددة غير المشبعة ، وقد وجد أنه يوفر تحكما أفضل لعمليه استقلاب الجلوكوز والدهون.

هدف البحث: دراسة التغيرات النسيجية لداء السكري المستحث بالستربتوزوتوسين على لسان الجرذان وتقييم الدور المحتمل لزيت كبد سمك القد في تحسين هذه التغيرات.

المواد و الطرق المستخدمة : استخدم في هذه الدراسة ستة وثلاثون من ذكور الجرذان البيضاء البالغه ، تتراوح أعمارهم بين 4-6 أشهر و وزنهم من 180 إلى200- جم.

و قد تم تقسيم الحيوانات بشكل عشوائي إلى ثلاث مجموعات تحتوي كل منها على اثني عشر فأراً.

• المجموعة الأولى: و قد قسمت بالتساوي إلى ثلاث مجموعات فرعية ؛

o المجموعة الفرعية IA: تم الاحتفاظ بها كمجموعه ضابطه سلبية

o المجموعة الفرعية IB: تم الحقن مره واحدة بمعادل السيترات داخل الغشاء البريتونى

o المجموعة الفرعية IC: تلقّت زيت كبد سمك القد بواسطة أنبوب معدي يومياً لمدة 12 أسبوع.

• المجموعة الثانية: تم الحقن بجرعه واحده من الستربتوزوتوسين داخل الغشاء البريتونى لحث داء السكري.

• المجموعة الثالثة: بعد حث داء السكري كالمجموعة الثانيه، تم إعطاء الفئران زيت كبد سمك القد يومياً بواسطة أنبوب معدي لمدة 12 أسبوع.

في نهاية التجربة لكل مجموعة ، تم تخدير الحيوانات ثم تشريح الألسنة ومعالجتها من أجل الفحص بالمجهرين الضوئي و الماسح الإلكترونى بالإضافه إلى الدراسات المناعية.

النتائج: أظهرت الدراسه الحاليه أن داء السكرى قد أدى إلى عدم إنتظام ، مناطق تسطيح و فرط تقرن فى حليمات اللسان بالإضافه إلى تشوه فى طبقة العضلات. فيما كشفت المجموعة الثالثة عن تحسنا واضحا في هذه التغييرات وكانت الصورة مشابهة تقريبًا للمجموعة الأولى.

الخلاصه: أوضحت هذه الدراسه تغيرا ملحوظا فى التركيب النسيجى للسان ناجما عن داء السكرى. بينما أدى زيت كبد سمك القد دورا وقائيا ملحوظا ضد هذه التغييرات. وبالتالي، يمكن إعتبار زيت كبد سمك القد كواقى واعد ضد الآثار الضارة لداء السكري على اللسان.