



Possible Ameliorative Impact of Curcumin on Regressive Changes Induced by Diabetes on Parotid Glands of Albino Rats

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ABSTRACT

Purpose: Diabetes mellitus (DM) has deleterious effect on various body organs especially salivary glands. Therefore, this study was conducted to evaluate the therapeutic effect of curcumin on parotid salivary glands in diabetic rats. **Material and methods:** Fifty-six adult male albino rats weighting 150-200 g were selected and divided into two equal groups n= 28 rats each. Diabetes was induced in both groups I (Diabetic) & II (Diabetic & curcumin) with a single intraperitoneal injection of streptozotocin (STZ) at a dose of 55 mg/kg. After 2 weeks of diabetes confirmation; rats of group II received 200 mg/kg of curcumin orally once daily. Both groups I and II were divided into 2 subgroups (A, B, C and D respectively) 14 rats each and were sacrificed after 15 and 20 days of curcumin administration, and the parotid glands sections were stained with Hematoxylin and Eosin, Anti-iNOS, and Anti-IL-2. Then were subjected to digital image analysis followed by two-way ANOVA statistical analysis. **Results:** Curcumin treated group revealed improvement in parotid glands in H&E sections especially at 20 days when compared to diabetic group with reduced histomorphological alterations. While, immunohistochemically, curcumin treated group showed decreased expression in the levels of both iNOS and IL-2 with significant statistical difference with diabetic group in all time periods. Post hoc LSD test showed a significant difference between time periods of diabetic group while showed no significance between curcumin group. **Conclusion:** Curcumin can be possible candidate for hindering the regressive changes that affect salivary glands caused by diabetes.

INTRODUCTION

Diabetes mellitus (DM) is a high prevalence chronic metabolic disease among population. It is characterized by a chronic persistent state of hyperglycemia mediated by defects in pancreatic insulin secretion and/or insulin resistance by tissue cells⁽¹⁾.

KEYWORDS

Parotid Glands,
Diabetes Mellitus,
Curcumin, Inos, IL-2.

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Chronic hyperglycemia results in elevation of advanced glycation end products (AGEs) that directly affect the cells leading to various cellular and organ disorders ⁽²⁾. AGEs accumulate in the endothelial and tissue cells causing endothelial dysfunction, inflammatory provocation and increased oxidative stress. Which subsequently, generates reactive oxygen species (ROS) resulting in increased protein fragmentation, DNA damage, generation of free fatty acid, and increased vascular disorders with resultant multi-organ damage ⁽³⁾.

DM has deteriorating effect on the oral hard and soft tissues, specially salivary glands as it causes oxidative damage and decreased salivary secretion ⁽⁴⁾, disrupted protein content, and elevated levels of myeloperoxidase, salivary IgA, and salivary amylase ⁽⁵⁾.

Curcumin is an active molecule in the rhizomes of *Curcuma longa* plant (turmeric) ⁽⁶⁾. It has been used as a natural therapy for several gastrointestinal and hepatic diseases ⁽⁷⁾. Curcumin has attracted interest of researchers due to several biological and pharmacological effects detected by both in vivo and in vitro studies. These studies reported that curcumin possess antioxidant, anti-inflammatory, immune-modulatory, anti-microbial, anti-neoplastic, cardio-protective, hepato-protective, nephro-protective, hypoglycemic, and anti-rheumatic properties ^(8,9).

Several therapeutic potentials of curcumin have been attributed to its anti-inflammatory capacity. Different studies related the anti-inflammatory effect of curcumin to several mechanisms including its potential in decreasing pro-inflammatory cytokines and lipid peroxidation products ^(10,11). Moreover, curcumin decreases mRNA production of pro-inflammatory mediators as inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 ⁽¹²⁾. Also, curcumin reduces neutrophil chemotaxis, chemokine release and suppresses T-helper cells ⁽¹³⁾.

Curcuminoids enhance insulin resistance, reduce glucose and insulin levels, increase adiponectin release, and decrease interleukin (IL)-6, IL-1 β , and tumor necrosis factor- α levels in diabetes mellitus patients ⁽¹⁴⁾. Owing to these pharmacologi-

cal properties, the therapeutic potential of curcumin has been targeted by several studies including cellular, animal studies, and clinical trials. It was found that curcumin has various therapeutic effects that differ according to the chemical constituents and the supplied dose ⁽¹⁵⁾.

So herein, we tried to evaluate the impact of curcumin on the inflammatory process induced by diabetes on parotid salivary glands via detecting the levels of iNOS and IL-2.

MATERIALS AND METHODS

Animals

Fifty-six adult male albino rats weighting 150-200 g were selected in the present study. Rats were kept in quarantine for seven days with free access to water and rodent food. Then, they were allocated randomly into two equal groups, 28 rats each. Rats of group I were considered as control (diabetic group), while rats of group II were considered as the test one (curcumin group). All procedures were approved by Ethics Committee of Faculty of Dentistry, Mansoura University, Egypt. The National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) was followed.

Diabetes induction

Diabetes was induced in both groups I & II with a single injection of streptozotocin (STZ) dissolved in 0.1 M cold sodium citrate buffer, pH 4.5, at a dose of 55 mg/kg intraperitoneally ⁽¹⁶⁾. To confirm diabetes was induced, blood glucose levels of overnight fasting animals were detected, 48 hours post STZ injection. Rats with blood glucose levels ranged from 300-500 mg/dL were used in the study (Fig.1).

Curcumin administration

After 2 weeks of diabetes confirmation; rats of groups II received a freshly prepared 0.5% carboxymethylcellulose (CMC) suspension of 200 mg/kg of curcumin (95%) (Nanjing Tianshu Biological Eng. Co. China) orally once daily ⁽¹⁷⁾, while rats of group

I received only freshly prepared 0.5% carboxymethylcellulose (CMC) ⁽¹⁸⁾ (Fig. 1). This day was considered day 0.

Sample collection

Animals of each group were randomly divided into 2 subgroups 14 rats each, rats were sacrificed by euthanization as follows: group I was divided into subgroup A after 15 days, and subgroup B after 20 days of curcumin administration. While group II was divided into subgroup C which was sacrificed after 15 days, and subgroup D which was sacrificed 20 days after curcumin administration.

Histological evaluation

Parotid glands were fixed for 24 hour in 10% neutral buffered formalin followed by tissue processing, and paraffin-embedding methods. Sections were cut at 4 μ m, deparaffinized, and then stained using H&E.

Immunohistochemical evaluation

The sectioned glands were immune-stained using avidin-biotin technique. The sections were stained with rabbit anti-rat iNOS, and IL-2.

Five non overlapped different fields from each slide were visualized using Olympus® digital camera installed on Olympus® microscope. The images were inspected on an Intel® Core I3® based computer using Video Test Morphology® software (Russia) with a specific built-in routine for quantification of immune stain. The targeted positive immuno-stained areas were automatically selected and separated from the original image depending on the positive stain hue range, the selected area was thresholded and defined as region of interest and then a 3D histogram was constructed, and integrated density of the area was calculated.

Statistical analysis

Data was analyzed by Statistical Package for Social Science (SPSS) version 26.0. Descriptive statistics were tested in Mean $\pm$ Standard deviation

(SD). The significance of difference was calculated using two-way ANOVA for comparison between the different groups followed by post hoc LSD test. Probability P values < 0.05 was accepted to be statistically significant.



Figure (1) Photograph showing streptozotocin (A), intraperitoneal injection of STZ in albino rat (B), curcumin (C) and its supplementation via oral gavage.

RESULTS

Hematoxylin and eosin

H&E sections of diabetic rat parotid glands revealed various alterations in gland morphology such as the appearance of lipid vacuoles in serous acini and degenerative changes of the acinar cells at 15 days, with increased damage intensity at 20 days. While these changes were reduced after curcumin administration, with acinar and ductal structures almost restored (Fig. 2).

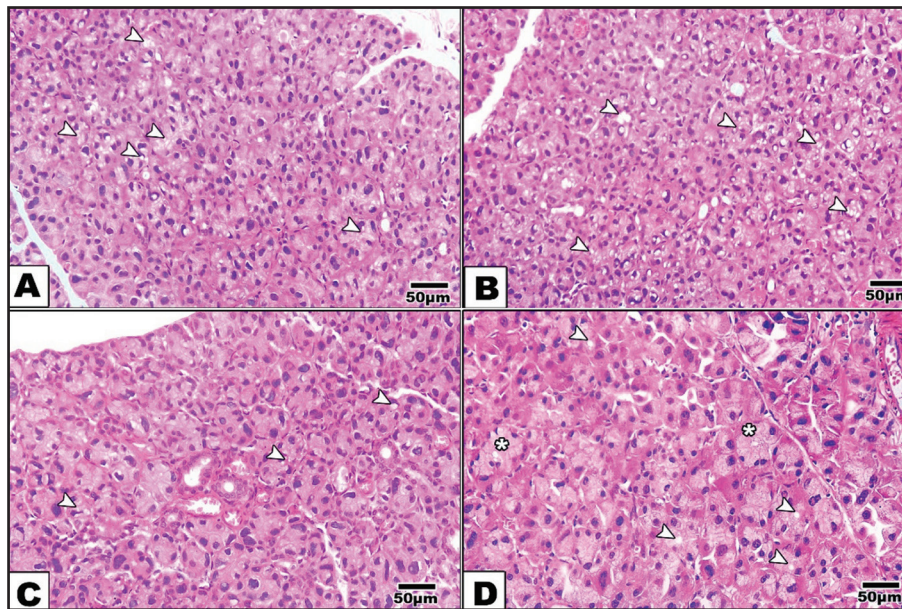


Figure (2): Photomicrograph of diabetic group showing vacuolation (arrowhead) and altered acinar structure at 15 days (subgroup A) and 20 days (subgroup B). Curcumin group with organization of acinar (*) and ductal structures at 15 days (C) and 20 days (D). (H&E x 200)

Immunohistochemical results

A. Inducible nitric oxide synthase (iNOS)

Positive reaction was observed as brown staining of cytoplasm of acini. Diabetic group showed significantly high expression of iNOS at day 15 (11.31 ± 0.34) with highest expression at day 20 (13.5 ± 0.44). While curcumin group revealed marked reduction in iNOS expression at 15 and 20

days (5.68 ± 0.45) & (3.3 ± 0.55) respectively (Fig. 3).

B. Interleukin-2 (IL-2)

Diabetic group showed high expression of IL-2 at day 15 (8.17 ± 0.56) with highest expression was observed at day 20 (10.72 ± 0.57). While curcumin group showed marked reduction in IL-2 expression at 15 days (5.25 ± 0.63) while least expression was observed at 20 days (2.88 ± 0.19) (Fig. 4).

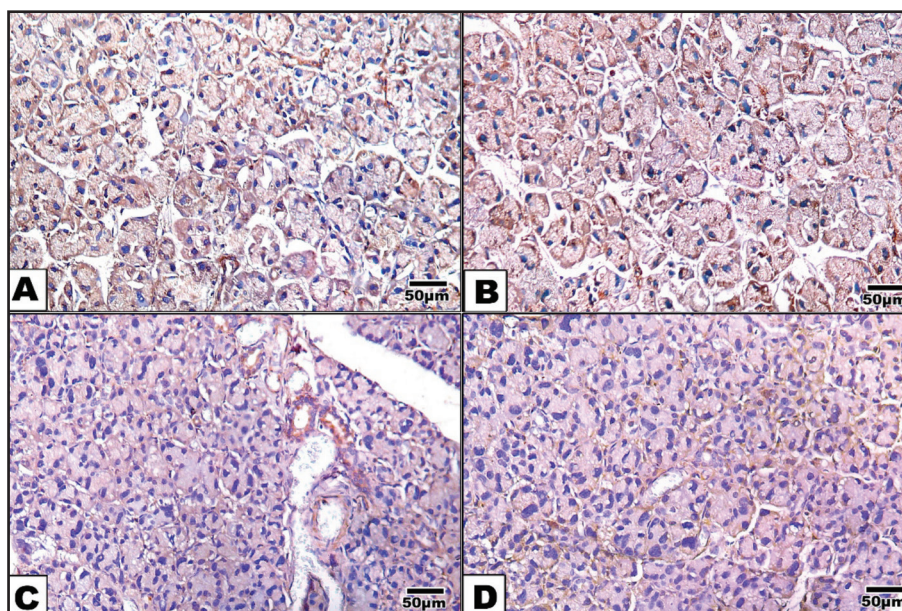


Figure (3) Photomicrograph of diabetic group showing increased iNOS expression at 15 days (subgroup A) and 20 days (subgroup B). Curcumin group with decreased acinar expression of iNOS at 15 days (subgroup C) and 20 days (subgroup D). (IHC x 200).

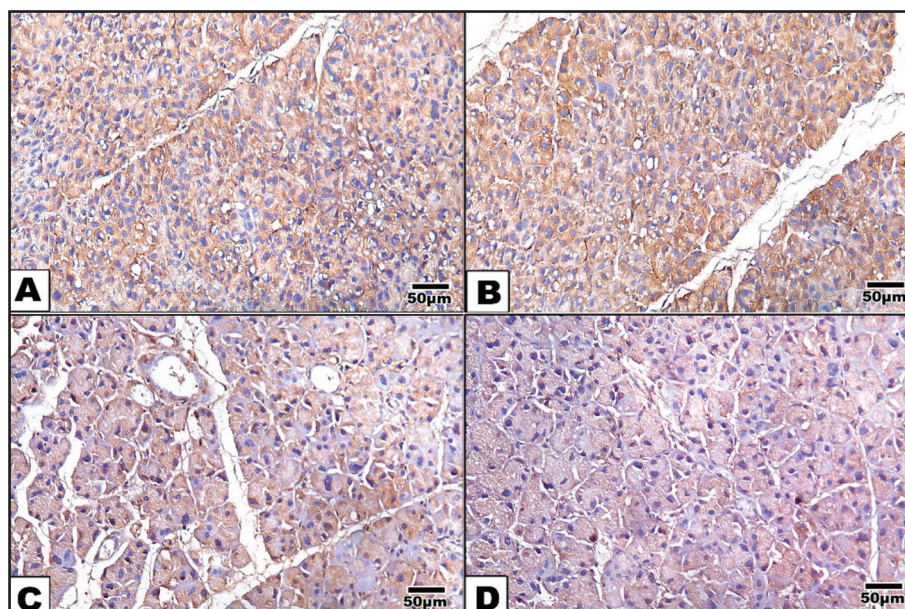


Figure (4) Photomicrograph of diabetic group showing high IL-2 expression at 15 days (subgroup A) and 20 days (subgroup B). Curcumin group with reduced expression of IL-2 at 15 days (subgroup C) and 20 days (subgroup D). (IHC x 200).

Statistical analysis

Two-way ANOVA showed a significant statistical difference between diabetic and curcumin group in all time periods for iNOS and IL-2. Post hoc LSD test showed a significant difference between time periods of diabetic group while showed no significance between curcumin group (Table 1).

Table (1) Showing mean $\pm$ SD of area percentage of iNOS & IL-2 stains, two-way ANOVA test and post hoc test

		Diabetic Gr I		Curcumin Gr II		P
		A	B	C	D	
iNOS	Mean	11.31	13.5	5.68	3.3	
	$\pm$ SD	0.34	0.44	0.45	0.55	<0.001*
	Post hoc		P1=<0.001*		P2=<0.07	
IL-2	Mean	8.17	10.72	5.25	2.88	
	$\pm$ SD	0.56	0.75	0.63	0.19	<0.001*
	Post hoc		P1=<0.001*		P2=<0.09	

P1: significance relative to A (diabetic Group)

P2: significance relative to C (Curcumin Group)

DISCUSSION

Streptozotocin was used to induce experimental diabetes which mimics diabetes mellitus with non-ketosis hyperglycemia, with changes in the morphology and secretory function of parotid glands (19,20).

In this study, histologic sections of diabetic rats' parotid glands revealed various morphological alterations such as lipid vacuolation and degeneration in the serous acini and acinar cells. Similarly, previous study reported that diabetic groups showed smaller acinar cells and presence of lipid intracytoplasmic droplets (21).

After 20 days the glandular damage increased, this may be attributed to persistent hyperglycemia produced by DM, leading to presence of chronic oxidative stress state, generating excessive reactive oxygen species (ROS), thus progressing the complications accompanying DM (22).

In curcumin treated group, the glandular and ductal structures showed improvement when compared to the diabetic group, this may be to curcumin's antioxidant mechanism as classical phenolic chain breaking antioxidant, donating H-atoms from the

phenolic groups reducing free radical production, so ameliorating the damage caused by ROS produced by diabetes ⁽²³⁾.

Additionally, curcumin caused significant reduction on MDA levels and increased total activity of antioxidant enzymes due to enhanced access to glutathione reductase, and promoted production of Superoxidase dismutase, catalase, and glutathione reductase ⁽²⁴⁾.

In this study the immunohistochemical results for iNOS showed increased expression in acinar cells of parotid glands, this is in accordance with another study which stated that diabetes promote inflammation by stimulating glucose use, with changes in oxidative phosphorylation. The metabolic dysregulation promotes a pro-inflammatory state in macrophages, causing up-regulation of the inflammatory response including iNOS, caspase 1, COX-2, VEGF and NF- κ B ⁽²⁵⁾. STZ induction was found to increase expression of iNOS in rat submandibular salivary glands. iNOS produces NO and O₂, resulting in peroxynitrite-mediated cell injury, so enhancing inflammation and oxidative stresses ⁽²⁶⁾.

Curcumin treated group showed marked decrease of iNOS expression specially after 20 days, concomitantly curcumin aided in the degradation of iNOS expressed in murine macrophage-like RAW 264.7 cells stimulated by LPS. Curcumin suppresses iNOS tyrosine phosphorylation via inhibiting ERK 1/2 activation, thus reducing the activity of iNOS enzyme ⁽²⁷⁾.

Moreover, curcumin was found to be a potent anti-inflammatory agent by reducing NO formation as well as iNOS expression at protein and mRNA levels, also curcumin might decrease iNOS gene via suppression of c-Jun/ AP-1 activation ⁽²⁸⁾.

The current study showed marked expression of IL-2 in diabetic group with most sever expression at 20 days. These results are similar to previous research where elevated messenger RNA production of various inflammatory cytokines, including IL-1 β , IL-2, IL-10, tumor necrosis factor- α , and interferon- γ was found in the submandibular glands

of both NOD and BALB/c mice ⁽²⁹⁾. Also, IL-2 expression associated with inflammatory reaction and insulin resistance in adipose tissue ⁽³⁰⁾

However, another study reported that IL-2 showed suppressed levels in newly diagnosed insulin dependent diabetes and increases with progression of inflammation and late stage of diabetes. Also, IL-2 levels were found to be elevated, depressed, or unchanged in patients with insulin dependent DM. These different reactions can be the cause of different metabolic status or/and a different stage of the autoimmune process ⁽³¹⁾.

In curcumin group the expression of IL-2 was markedly reduced in comparison with diabetic group, similarly it has been stated that curcumin suppressed the IL-2- induced phosphorylation of STAT5A and JAK3 in CTLL-2 cells ⁽³²⁾. Moreover, Curcumin showed potent immunosuppressive potential by reducing IL-2 formation and IL-2 induced activation of human lymphocytes, mediated by NF kappa B inhibition ⁽³³⁾.

CONCLUSION

With limitations of this study, curcumin is a possible candidate for reducing the damage caused by diabetes on salivary glands via its antioxidant and anti-inflammatory potential through its capacity to decrease expression of iNOS. Also, via immunomodulatory effect by reducing IL-2 expression.

RECOMMENDATIONS

Further studies are recommended with different doses of curcumin. Also, study may be conducted for longer time periods to monitor the changes.

Conflict of interest:

All authors ensure that there is no conflict of interest in this work.

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