

## EFFECT OF OLIVE OIL ON THE ATROPHIED TONGUE PAPILLA OF DIABETIC ALBINO RATS

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### ABSTRACT

**Aim:** This study aimed to investigate the effect of olive oil on the atrophied tongue filiform papillae of diabetic albino rats.

**Materials and methods:** Eighteen albino rats were used in this study and were divided into 3 groups. Group I: Control group which included 6 rats. Group II: Diabetic group which included 6 rats and Group III: Diabetic group treated with olive oil which included 6 rats. The experiment was terminated after fifteen and thirty days of diabetes induction. The tongue filiform papillae specimens were dissected and prepared for collection at days 15, 30, and they were analyzed by scan electron microscopy, histologically, histomorphometrically and statistically.

**Results:** SEM examination of group I showed that the dorsal surface was covered by elongated and conical shaped-filiform papillae. Filiform papillae in group II appeared destructed and irregular compared to control group I. Filiform papillae in group III began to regain their normal appearance. Histological analysis of group I showed that the filiform papillae appeared normal, characterized by keratinized structures with uniform thickness of the lingual epithelium, lamina propria. In group II, there was a complete loss of the conical shape of the filiform papillae with significant infiltration of inflammatory cells into the lamina propria. In group III, the filiform papillae began to regain their normal appearance.

**Conclusion:** Diabetes with chronic hyperglycemia has damaging effects on the lingual filiform papillae. On the other hand olive oil has a reparative and protective role towards these effects.

**KEY WORDS:** Olive oil, Diabetic, Tongue papilla.

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## INTRODUCTION

East Asian countries have investigated and approved the use of herbal substances for a significant portion of medical therapy in developing nations. As a result, it has grown in popularity in western nations and gained greater significance in the healthcare of people. Olive oil is one of these healthy natural agents.<sup>(1)</sup>

One good monounsaturated fat is olive oil. Olive oil works well as a cosmetic oil to cleanse and moisturize skin. Rich in protein,  $\beta$ -carotene, and vitamin C, olive oil is also a great source of natural antioxidants. Olive oil protects against the oxidative damage linked to diabetes. Both muscle and keratinocyte damage are brought on by this oxidative stress. Its antioxidant content, which includes flavonoids and transcription factor activation, is responsible for this. The antioxidants in olive oil improve circulation, blood supply to the tissues and the salivary flow rate.<sup>(2)</sup>

Olive oil is a major source of dietary fatty acids in Mediterranean regions. Olive oil lowers blood sugar because it contains terpenoids, which help to stimulate the liver's B-cells. Diabetic albino rats' blood glucose levels are hypoglycemic when olive oil is used. Some of hyperglycemia's negative consequences can be reversed by using olive oil.<sup>(3,4)</sup>

Diabetes mellitus is a chronic illness that causes elevated blood glucose levels due to compromised insulin secretion or action. In these patients, hyperglycemia must be controlled to lower the risk of micro and macrovascular complications. Because of their adverse effects, hyperglycemic medications are no longer as effective as they once were as antidiabetic medications. So ongoing effort was made to explore another therapeutic agent which can overcome the deleterious effect of diabetes.<sup>(5,6)</sup>

The prevalence of diabetes is rising quickly. With diabetes, the tongue is the oral location most frequently impacted, second only to periodontal

tissues. The tongue mucosa is protected by saliva under normal physiological settings; however diabetics have a decrease in salivary flow rate. Diabetes patients' hyperglycemia changes a number of signaling pathways, which leads to oxidative stress and the release of advanced glycation end products (AGEs) and reactive oxygen species (ROS). In vitro and in vivo, AGEs affect the function of skin keratinocytes.<sup>(7,8)</sup>

Inducing vasoconstriction and decreasing tissue vasodilatation, reactive oxygen species (ROS) lessen the flow of nutrients and waste products out of the tongue tissues. As a result, there are some degenerative alterations and an increased vulnerability to infection. One of the reasons why taste perception is diminished is because of reduced blood flow, which is particularly noticeable in nerve tissue. Among the anomalies linked to diabetes patients include aberrant matrix metalloproteinase expression, persistent inflammation, and poor angiogenesis.<sup>(9,10)</sup>

Hyperglycemia causes an increase in the activity of the proteasome, lysosome, and proteolytic enzymes, which affects the skeletal muscles of the tongue. Muscular atrophy is the outcome of these enzymes' increased rate of muscle protein breakdown. Diabetes disrupts this pathway, which results in muscle degeneration. Rapamycin is the main regulator of protein synthesis in skeletal muscle and is triggered by Akt via insulin.<sup>(11)</sup>

In order to facilitate mastication, clean teeth, aid in swallowing, and support vocalization in animals, the tongue's primary job is to manipulate food. It is therefore the primary organ of taste in the gustatory system and plays a crucial role in the digestive system. Its foliate, circumvallate, and fungiform papillae all have taste buds covering their dorsal surfaces. It receives a lot of blood vessel supply and is heavily innervated. This study will examine the impact of olive oil on the papilla of the diabetic albino rats' tongues.<sup>(12)</sup>

## MATERIAL AND METHOD

- Olive Oil: dietary supplementation with 15% virgin olive oil daily for thirty days through an oral gavage (tube).<sup>(13)</sup>
- Alloxan: Diabetes induction drug (Sigma-Aldrich). (This drug was given as 150 mg/kg 1<sup>st</sup> dose and 100mg/kg 2<sup>nd</sup> dose)<sup>(14)</sup>

### Animals

In this investigation, 18 albino rats weighing 200–250 grams were employed. The experiment was carried out in the pharmacy faculty's animal house at Al-Azhar University in Cairo.

As advised by the faculty's research ethics committee, the conditions in the animal house were in compliance with the rules of the animal house. Rats were housed under conventional laboratory conditions, with each group having its own cage at a temperature of 20 to 25 degrees Celsius. They were fed a standard meal and had unrestricted access to water.

The rats were randomly assigned to one of three groups which consisted of six rats each: Group I (control group), Group II (diabetic group) and Group III was the diabetic group that received dietary supplementation with 15% virgin olive oil for thirty days daily through an oral gavage. After thirty days of inducing diabetes, the experiment was stopped.

In order to induce diabetes, two intraperitoneal injections of alloxan (Sigma-Aldrich) were dissolved in ice-cold, ultra-pure water with a pH of 6.8 (Millipore system). For rats to stay diabetic during the trial, a first dosage of 150mg/kg was administered, followed by a second dose of

100mg/kg two days later. When blood glucose levels were greater than 250 mg/dL, diabetes was believed to have occurred.<sup>(14)</sup>

Each group's filiform papillae specimens were split into two subgroups at 15 and 30 days. Each had three rats in it. Animals were seen every day. At 15 days and 30 days of the experiment, the animals from all groups were euthanized by anesthetic overdose of thiopental. The tongue filiform papillae specimens were dissected and prepared for collection at days 15, 30, and they were analyzed by scan electron microscopy, histologically, histomorphometrically and statistically.<sup>(15)</sup>

### Sample size calculation:

Sample size calculation was performed using G\*Power version 3.1.9.2, **Faul et al., (2007 & 2013)** University Kiel, Germany. Copyright (c) 1992-2014.<sup>(16)</sup>

$$f = \frac{\sigma_{\mu}}{\sigma}$$

$$\sigma_{\mu}^2 = \frac{\sum_{i=1}^k n_i (\mu_i - \mu)^2}{N}$$

Where:

$F$ : is the effect size;  $\alpha = 0.05$ ;  $\beta = 0.05$ ; Power =  $1 - \beta = 0.80$  (80%)

The effect size  $f$  was **0.82** using alpha ( $\alpha$ ) level of 0.05 and Beta ( $\beta$ ) level of 0.05, i.e., power = 80%; the estimated sample size ( $n$ ) should be at least **18** samples (Rats). The sample size in this study agrees with **Eltokhey et al., 2013** who have published on this point.<sup>(17)</sup>

### Animal grouping:

Animal groups:		
Group I (control )	Group II	Group III
Consist of six rats and didn't receive any treatment	Diabetes induction was done in six rats and received two dose of alloxan. The 1st dose was 150 mg/kg and the 2nd dose was 100 mg/kg given 2 days after the 1 <sup>st</sup> dose	Diabetic induction was done in six rats then treated with olive oil given by an oral gavage

## Testing procedure

### Statistical analysis

Using the appropriate statistical tests, all gathered data was computed, tabulated, and statistically examined as follows:

- Shapiro-Wilk performed a normality test to verify that the data had a normal distribution.
- Mean  $\pm$  Standard deviation (SD) were used to compute descriptive statistics.
- One-way ANOVA (analysis of variance) was utilized to compare the groups under investigation, depending on the sorts of data. To assess the statistical significance between the groups, the Bonferroni post-hoc test or other post-hoc tests were used.
- Paired sample T test was used to comparing the time interval in each group.
- SPSS software for Windows version 26.0 (Statistical Package for Social Science, Armonk, NY: IBM Corp.) was used to conduct statistical analysis at significant levels  $< 0.05$  (P- Value)

### Scanning Electron Microscopic Results: (At 15 days)

Results of the Scanning Electron Microscopy (SEM) Analysis of the control group (group 1) at 15

days revealed that the dorsal surface was covered with many, elongated, conical-shaped filiform papillae with intact, slightly curved, tapering tips pointing in a single direction (Fig. 1). SEM of group 2 revealed that, in contrast to the control group, filiform papillae seemed damaged, short, and uneven, with keratin loss at their ends (Figs 2) when compared to the control group. The lingual filiform papillae in group 3 seemed almost normal. Filiform papillae were characterized by its long, conical shape, orderly arrangement, unbroken tapering tips, and essentially unidirectional orientation (Fig. 3).

### (At 30 days)

The dorsal surface of the control group (group 1) was covered by many elongated, conical-shaped filiform papillae with intact, slightly curved, tapering tips pointing in a single direction, (Fig. 4). When group 2 was compared to the control group, the filiform papillae in group 2 seemed damaged, short, and uneven, with keratin loss at the ends (Figs 5). When compared to the control group, the lingual filiform papillae in group 3 seemed almost normal. Filiform papillae were a long, conical shape with unbroken tapering tips that was regularly placed and pointed in a single direction. (Fig.6)

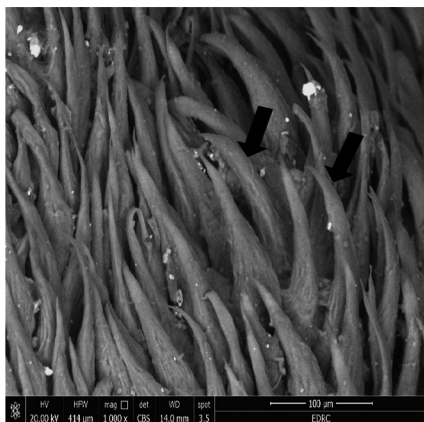


Fig. (1) A scanning electron micrograph of a tongue of group I showing regular orientation in one direction of short conical filiform papillae with tapering keratinized end (black arrows). X1000



Fig. (2) A scanning electron micrograph of a tongue of group II showing filiform papillae appeared destructed, short and irregular (white arrows). X500

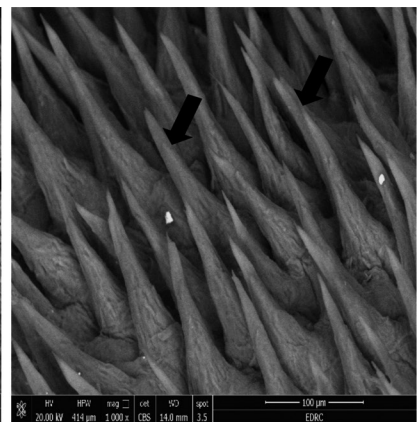


Fig. (3) A scanning electron micrograph of a tongue of group III showing regular orientation in one direction of short conical filiform papillae with tapering keratinized ends (black arrows). X500



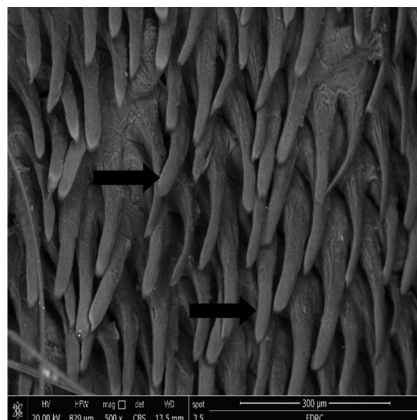


Fig. (4) A scanning electron micrograph of a tongue of group I showing regular orientation in one direction of long conical filiform papillae with tapering keratinized end (black arrows). X500

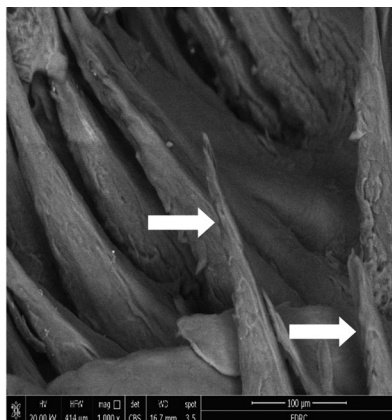


Fig. (5) A scanning electron micrograph of a tongue of group II showing filiform papillae appeared more destructed, short, atrophied and irregular (white arrows). X1000

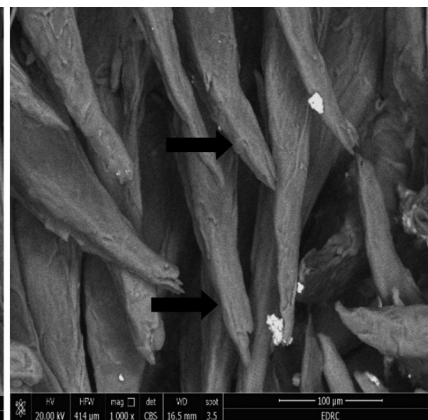


Fig. (6) A scanning electron micrograph of a tongue of group III showing regular orientation in one direction of long conical filiform papillae with tapering keratinized ends (black arrows). X1000

### Histological Results: (At 15 days)

The dorsal surface of the control group was covered by many, elongated, keratinized, conical-shaped filiform papillae with intact, slightly curved, tapering tips pointing in a single direction, according to histological analysis (Fig. 1). Group II revealed filiform papillae that were shorter, irregular and destructed with keratin loss at the ends in contrast to the control group. The epithelial cells looked shedded and destroyed. Inflammatory cell infiltration was clearly visible in the muscle layer and lamina propria (Fig. 2). Comparing group III to the control group, the lingual filiform papillae appeared almost normal, long, conical, consistently distributed, directed almost in a single direction and primarily with undamaged tapering points. (Fig. 3).

### (At 30 days)

The dorsal surface of the control group was covered by many elongated, keratinized, conical-shaped filiform papillae with intact, slightly curved, tapering tips pointing in a single direction (Fig. 4). Group II revealed filiform papillae that were shorter, irregular, and destructed, with keratin loss at the ends in contrast to the control group. The epithelial cells looked shedded and destroyed. Inflammatory cell infiltration was clearly visible in the muscle

layer and lamina propria. (Fig.5) Comparing group III to the control group, the lingual filiform papillae appeared almost normal. Filiform was characterized as long, conical, regularly arranged, directed almost in a single direction and primarily with undamaged tapering points. (Fig.6)

### Statistical analysis

Table 1, shows the comparison of the inter- and intra-group differences for L (Length) and W (Width) based on the provided data (Fig.7)

### Length

The analysis of the L measurement shows significant differences between groups over the two time periods (15 days and 30 days). comparing inter-group differences, the control group displayed the highest mean distance at both intervals, followed by the Olive group, while the Diabetes group had the lowest values. Intra-group comparisons reveal significant results for all groups, particularly highlighting that both the Control and Olive groups showed an increase in mean distance from 15 to 30 days, while the Diabetes group exhibited decrease. ANOVA results show a significant P value ( $<0.001$ ), indicating substantial variation among the groups over the 30-day period.

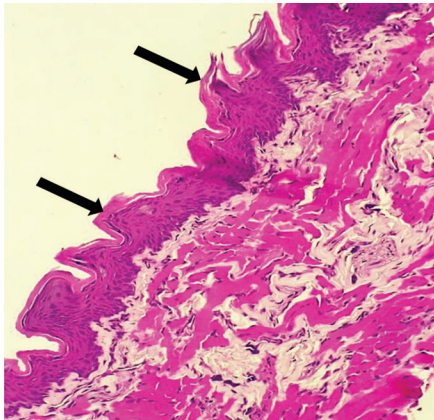


Fig. (1) A photomicrograph of a tongue of group I showing regular orientation in one direction of short conical filiform papillae with tapering keratinized end (black arrows). (H&EX100)

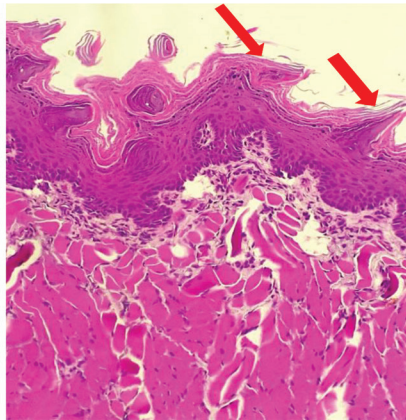


Fig. (2) A photomicrograph of a tongue of group II showing filiform papillae appeared destructed, short and irregular (red arrows). (H&EX100)

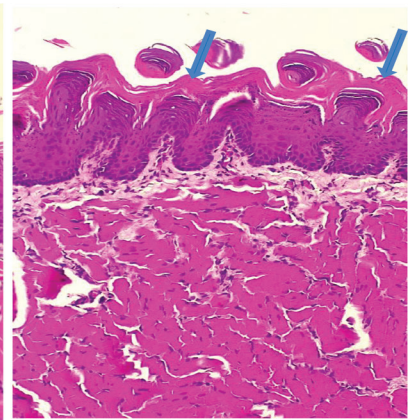


Fig. (3) A photomicrograph of a tongue of group III showing regular orientation in one direction of short conical filiform papillae with tapering keratinized ends (blue arrows). (H&EX100)

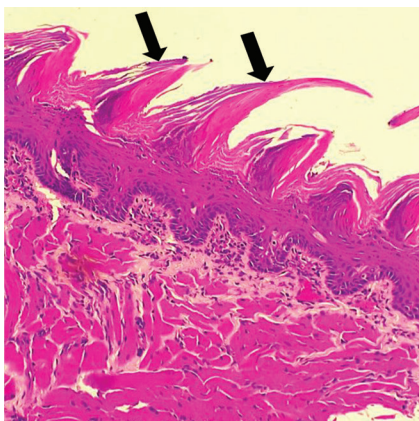


Fig. (4) A photomicrograph of a tongue of group I showing regular orientation in one direction of long conical filiform papillae with tapering keratinized end (black arrows). (H&EX100)

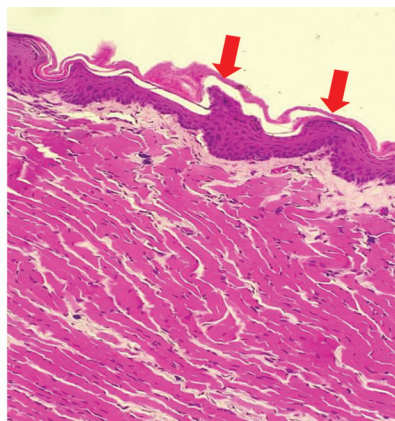


Fig. (5) A photomicrograph of a tongue of group II showing filiform papillae appeared more destructed, short, atrophied and irregular (red arrows). (H&EX100)

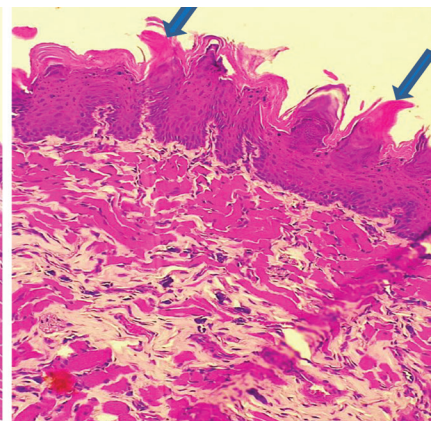


Fig. (6) A photomicrograph of a tongue of group III showing regular orientation in one direction of long conical filiform papillae with tapering keratinized ends (Blue arrow). (H&EX100)

## Width

For the W measurement, the results indicate no significant differences among the groups after 15days, as evidenced by a non-significant P value (0.35). However, after 30 days there are statistically significant between them, statistical analysis showed no significant difference in the intra-group comparisons, with P values ( $P > 0.05$ ). Although the Control and Olive groups showed an increase

in mean distance from 15to 30 days, while the Diabetes group's values decreased significantly, the control group displayed the highest mean distance at both intervals, followed by the Olive group, while the Diabetes group had the lowest values.

Table 2, shows the comparison of the inter- and intra-group differences for width in L and W based on the provided data (fig. 8).

TABLE (1) The comparison of the inter- and intra-group differences for distance of L and W based on the provided data

	Groups	15 days		30 days		paired T test	P value
		Mean	SD	Mean	SD		
<b>L</b>	<b>Control</b>	1115.2 <sup>a</sup>	40.0	1273.6 <sup>a</sup>	62.7	6.905	0.001**
	<b>Diabetes</b>	981.8 <sup>b</sup>	68.9	730.9 <sup>b</sup>	51.0	12.81	<0.001**
	<b>Olive</b>	1163.9 <sup>a</sup>	38.9	1330.6 <sup>a</sup>	72.8	4.84	0.005**
<b>ANOVA test</b>		<b>20.34</b>		<b>166.76</b>			
<b>P value</b>		<b>&lt;0.001**</b>		<b>&lt;0.001**</b>			
<b>W</b>	<b>Control</b>	217.8 <sup>a</sup>	37.0	266.6 <sup>a</sup>	46.8	1.577	0.176 ns
	<b>Diabetes</b>	189.7 <sup>a</sup>	34.8	154.9 <sup>c</sup>	15.0	2.066	0.094 ns
	<b>Olive</b>	210.7 <sup>a</sup>	28.9	220.4 <sup>b</sup>	21.8	0.531	0.618 ns
<b>ANOVA test</b>		<b>1.12</b>		<b>19.61</b>			
<b>P value</b>		<b>0.35 ns</b>		<b>&lt;0.001**</b>			

\*\* and different superscript letters at the same column mean significant differences at  $P<0.05$  ns; mean no significant

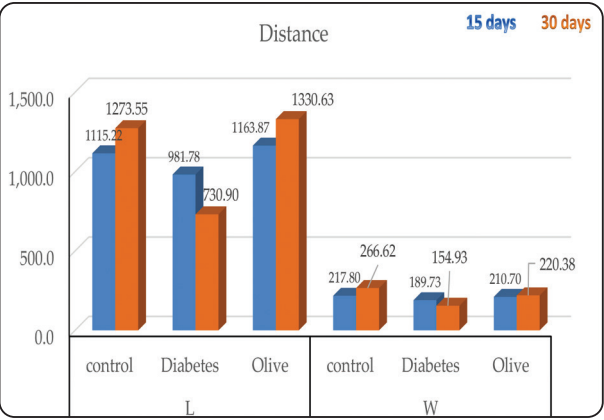


Fig. (7) Shows the comparison of the inter- and intra-group differences for distance in L and W based on the provided data

### (Length)

For inter groups, there was statistically significant difference between groups regarding width at 15 and 30 days ( $P<0.001$ ), the Olive group displayed the highest mean width at both intervals,

followed by the Diabetes group, while the control group had the lowest value.

For intra groups the control and olive groups showed a significant increase in mean value of Width measured after 30 days ( $P<0.001$ ). While in diabetes groups there are significant decrease in mean value of Width after 30 days times.

### (Width)

For inter groups, there was statistically significant difference between groups regarding width at 15 and 30 days ( $P<0.001$ ), the control group displayed the highest mean width at both intervals, followed by the Diabetes group, while the olive group had the lowest values.

For intra groups the control showed a significant increase in mean value of Width measured after 30 days ( $P<0.001$ ). While in diabetes and olive groups there is significant decrease in mean value of width after 30 days.



TABLE (2) The comparison of the inter- and intra-group differences for width of L and W based on the provided data

Groups		15 days		30 days		paired T test	P value
		Mean	SD	Mean	SD		
L	Control	32.40 <sup>c</sup>	5.16	138.52 <sup>b</sup>	22.37	10.53	<0.001**
	Diabetes	974.48 <sup>b</sup>	37.90	162.72 <sup>b</sup>	39.83	30.87	<0.001**
	Olive	209.64 <sup>a</sup>	15.47	1163.26 <sup>a</sup>	21.65	92.43	<0.001**
ANOVA test		2650.14		2408.87			
P value		<0.001**		<0.001**			
W	Control	190.28 <sup>a</sup>	11.95	264.38 <sup>a</sup>	9.94	11.04	<0.001**
	Diabetes	188.42 <sup>a</sup>	10.59	153.42 <sup>b</sup>	13.46	5.729	<0.001**
	Olive	150.81 <sup>b</sup>	24.39	62.93 <sup>c</sup>	4.31	7.975	0.001**
ANOVA test		10.51		614.13			
P value		0.001**		<0.001**			

\*\* and different superscript letters at the same column mean significant differences at  $P < 0.05$

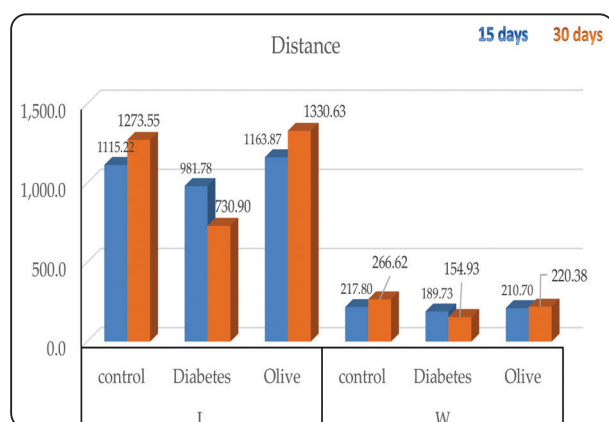


Fig. (8) Shows the comparison of the inter- and intra-group differences for width in L and W based on the provided data

## DISCUSSION

An increased blood glucose level brought by compromised insulin secretion or function is a hallmark of diabetes mellitus, a chronic illness. It's critical to manage hyperglycemia in these people to lessen issues with the macrovascular and microvascular systems. However, the usefulness of hyperglycemic medications as antidiabetic

treatments has been hampered by their negative effects. Thus, ongoing research into novel treatment molecules that can mitigate the negative consequences of diabetes has been undertaken.<sup>(18)</sup>

Many people believe that the tongue, particularly the filiform papillae, is a reflection of their general health. These filiform papillae have a high metabolic activity, which makes them especially vulnerable to atrophy when there are enzymatic imbalances, vascular changes, or nutritional deficits. As a result, they suffer damage and atrophic alterations sooner. As the tongue was selected for this study, filiform atrophy could be caused by diabetes, cancer, anticancer treatments, chemical or metal toxicity, or it could be a side effect of several medications, such as antibiotics.<sup>(19)</sup>

Filiform lingual papillae were found in the anterior two-thirds of the dorsal lingual surface in the current investigation. Rats have a single vallate papilla posteriorly in the midline of the tongue; hence vallate papillae were not seen in this study. Foliate papillae are also restricted to the rabbit tongue's



dorsolateral borders close to the base, which is why they were not seen in our investigation.<sup>(20)</sup>

In control group, samples at 15 and 30 days were taken to assess the length of filiform papillae. At fifteen days, the length of filiform papilla appeared large and regular. At thirty days, the length of filiform papilla appeared larger than the length at fifteen days. This can be explained by growth and maturation of tongue filiform papillae with time.

The group II data showed that the rats' tongues had undergone histological alterations. Significant lingual filiform papillae shortening, thinning, and atrophy were seen; this was most likely brought about by a decline in the quantity and size of the tongue's covering epithelial cells. The thickness of the dorsal surface's epithelial coating clearly decreased. The keratin layer that covered it looked thin, irregular, and had lost its integrity. Most likely, Alloxan's oxidative stress is to blame for these alterations.<sup>(21)</sup>

It has been suggested that oxidative stress is a key factor in numerous routes of alcohol-induced harm to various organs, including the liver, testes, and central nervous system. Reactive oxygen species (ROS) are produced by the metabolism of ethanol and can damage a number of macromolecules in the cell, including proteins, DNA, and lipids. This is consistent with research conducted by several researchers.<sup>(22)</sup>

Long conical filiform papillae with tapering keratinized ends are regularly oriented in one direction in this scanning electron micrograph of a group III rat's tongue. Olive oil is an excellent source of natural antioxidants and a strong supply of protein,  $\beta$ -carotene, and vitamin C, which can explain these results. The oxidative stress linked to diabetes, which can cause damage to keratinocytes and muscles, is prevented by olive oil. Its capacity to activate transcription factors and antioxidant content, which includes flavonoids, is responsible for this protective effect. Olive oil's antioxidants promote blood flow to tissues, improve circulation, and speed up salivation.<sup>(23)</sup>

Additionally, Olive oil lowers blood sugar because it contains terpenoids, which help to stimulate the liver's B-cells. Diabetic albino rats' blood glucose levels are hyperglycemic when olive oil is used. Some of hyperglycemia's negative consequences can be reversed by using olive oil.<sup>(24, 25)</sup>

The length of the tongue filiform papillae at both days 15 and 30 of olive oil treated group (group III) appeared greater than the normal control group. This new finding conducted that anti-oxidant natural material (olive oil) taken as a prophylactic agent against diabetes where olive oil prevented diabetic induction. This can be considered as a future point of research.

The results align with a study conducted by researchers who looked at the anti-diabetic benefits of ginger extract in albino rats with streptozotocin-induced diabetes mellitus. The filiform papillae in group I, according to histological investigation, looked normal and were distinguished by thread-like, keratinized structures with consistent thickness of the muscle layer, lamina propria, and lingual epithelium. In group II, the filiform papillae completely lost their conical structure, and inflammatory cells significantly infiltrated the muscle layer and lamina propria. The filiform papillae started to resemble themselves normally in group III.<sup>(26)</sup>

## CONCLUSION

Diabetes with chronic hyperglycemia had damaging effects on the lingual filiform papillae papillae. On the other hand, olive oil had a reparative and protective role towards these effects.

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