ISSN: 0975-833X

*Available online at http://www.journalcra.com*

*International Journal of Current Research*
*Vol. 11, Issue, 02, pp.1151-1156, February, 2019*

**DOI: https://doi.org/10.24941/ijcr.34284.02.2019**

**INTERNATIONAL JOURNAL OF CURRENT RESEARCH**

## RESEARCH ARTICLE

# POTENTIAL THERAPEUTIC EFFECT OF MORINGA OLEIFERA ON TONGUE PAPILLAE OF DIABETIC ALBINO RATS

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**ARTICLE INFO**

***Article History:***
Received 19th November, 2018
Received in revised form
27th December, 2018
Accepted 24th January, 2019
Published online 28th February, 2019

***Key Words:***
Diabetes, ROS, Moringa Oleifera, Alloxan, Tongue, Mucosa, Papilla, Hyperglycemia.

**ABSTRACT**

***Background:*** The tongue is the second most commonly affected oral site after periodontal tissues in diabetes. Diabetic complications are mainly attributed to oxidative stress and side effects from long term use of drugs to treat diabetes. MO has been reported to be a valuable source of natural antioxidants. ***Materials and methods:*** this study comprised three groups; control, diabetic and MO treated groups. The experiment was terminated after fourteen days. Evaluation of the rats was done by measuring blood glucose levels, as well as, histological assessment of the tongue tissue by H&E and SEM. ***Results:*** There was a significant reduction in blood glucose levels and histological evaluation revealed improvement in the condition of the tongue mucosa in the MO treated group compared to the diabetic group. ***Conclusion:*** MO has a hypoglycemic effect on the blood glucose level of diabetic albino rats. MO may partially reverse some of the detrimental effects hyperglycemia has on the tongue mucosa of diabetic rats.

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**Citation:** ***Hala El-kammar, Raneem F. Obeid and Radwa T. El-sharkawy.*** **2019.** "Potential Therapeutic Effect of Moringa Oleifera on Tongue papillae of Diabetic Albino Rats", *International Journal of Current Research*, 11, (02), 1151-1156.

## INTRODUCTION

The incidence of diabetes is increasing rapidly (Eltokhey, 2013). The tongue is the second most commonly affected oral site after periodontal tissues in diabetes (Gandara, 2011). Under normal physiological conditions saliva that is sufficient in quantity and quality offers protection to the tongue mucosa. Lower salivary flow rates, expressions of s-IgA and epidermal growth factor are commonly observed in diabetics. It is well established that hyperglycemia alters several signalling pathways, resulting in oxidative stress and the liberation of Reactive oxygen species (ROS) and advanced glycation end products (AGEs) (Volpe, 2018). AGEs impair skin keratinocytes function in vivo and in vitro, through the activation of NF-κB (Tian, 2012). ROS decreases tissue bioavailability of, a potent vasodilator. The resultant vasoconstriction reduces the flow of nutrients to and the elimination of wastes from the tongue tissues. This results in an increased susceptibility to infection and some degenerative changes (Eltokhey, 2013). The impact of impaired blood flow is especially prominent in the nerve tissue and is one of the causes of the taste deficits frequently associated with diabetes. Researchers, have also reported that a high glucose level was linked to variations in cellular morphology, in addition to decreased proliferation rates and aberrations in the $Ca^{2+}$-dependant keratinocyte differentiation. Furthermore, in the presence of high glucose, ligand-induced insulin like growth factor receptor is decreased resulting in a reduction of glucose uptake and subsequently proliferation (Spravchikov, 2001; Hirsch, 2008; Hu, 2016). Keratinocyte gap junction abnormalities, chronic inflammation, impaired angiogenesis, and abnormal expression of matrix metalloproteinases, are also frequently reported in diabetic patients (Hu, 2016). The tongue skeletal muscles are also affected by hyperglycemia. Hyperglycemia causes a long-standing elevated activity of the ubiquitin-proteasome, autophagy-lysosome and caspase-3-mediated proteolytic signalling pathways. These pathways increase the degradation of contractile muscle protein, eventually resulting in muscular atrophy. The primary regulator of protein synthesis in skeletal muscle is the mammalian target of rapamycin (mTOR), which is activated by Akt via insulin, this pathway is hence disrupted in diabetes, leading to muscle degeneration (Bodine, 2001). Recent studies have also indicated that Peroxisome proliferator-activated receptor alpha (PPARα) is a transcription factor that reduces fatty acid oxidation and lipid accumulation in skeletal muscles through increased sensitivity of tissue insulin. Moreover, findings suggest that hyperglycemia mediated down-regulation of PPARα has an significant role in diabetic neuropathy (Koh, 2003).

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Moringa oleifera (MO), is a widely cultivated tree. MO has been reported to be a rich source of protein, β-carotene and vitamin C and is a valuable source of natural antioxidants. MO has a protective effect on oxidative stress induced keratinocytes damage and muscle damage, associated with diabetes (Koh, 2003). This takes place through its` antioxidant content –as it contains flavonoids- and the stimulation of PPAR-α transcription factor. Anti-oxidants in MO improve circulation and blood supply to the tissues, as well as, the salivary flow rate. MO is also reported to reduce blood glucose levels due to the presence of terpenoids which have a role in stimulation of B-cells of the liver (Koh, 2003; Rani, 2018). This study was performed to evaluate the effect of MO on the tongue tissue of diabetic albino rats.

## MATERIALS AND METHODS

***MO aqueous extract preparation:*** MO aqueous extract was prepared by mixing ten grams of dried and powdered MO leaves with 100 mL of distilled water for twenty four hours that was then stored at 4°C. After that, the mixture was filtered two times through a two-µm pore filter paper. The aqueous extract stock solution (100 mg/mL) was stored at 4 °C. (Tuorkey, 2016).

***Animals care and treatments:*** Twenty-one male albino rats (200-250gm) were adapted in the laboratory for two weeks under identical natural environmental settings (temperature and photoperiod). This was done in the medical research center animal house, Future University in Egypt. All the procedures were in accordance with the protocol of the National Animal Care and Use Committee and Guidelines for the Care and Use of Experimental Animals. Rats were randomly divided into three groups (7 rats each) as follows: control group (Group I), diabetic untreated group (Group II), and diabetic group treated with 100 mg/kg of MO aqueous extract given by an oral gavage (Group III). The experiment was terminated after fourteen days of diabetes induction (Tuorkey, 2016). Diabetes induction was done with two alloxan intra-peritoneal injections (Sigma-Aldrich), that were dissolved previously in ice-cold ultra-pure water, pH 6.8 (Millipore system). The 1st dose was 150 mg/kg, and the 2nd dose was 100 mg/kg given 2 days after the 1st dose, to ensure rats remain diabetic throughout the experimental period, this was done in accordance to *Bromme et al., 2000*. Blood glucose threshold for diabetes was considered at blood glucose level >250 mg/dL (one touch ultra 2, China).

***Blood glucose and weight measurements:*** Random Blood glucose levels and weight were measured after two, seven and fourteen days of the 2nd Alloxan injection, (Balance Shimadzu Unibioc, TX423L, Japan & One Touch Ultra 2, China).

***Histological preparation:*** At the end of the experimental duration, rats were sacrificed by means of an anaesthetic overdose (chloroform, HPLC Sigma Aldrich), the tongue was excised right away, labelled and fixed in:

10% formaldehyde and prepared for staining with Haematoxylin and Eosin stain, for the detection of any structural changes of tongue tissue between the studied groups, by light microscope. Four different microscopic 40x fields were examined (inverted light microscope, Olympus, CKX41, Japan) and measurments were done with the help of image analysis software (image J, 1.41a, NIH, USA). The following parameters were evaluated; Papillae length, epithelial cells thickness without keratin and the keratin thickness. The length in mm was then tabulated and the ratio between the keratin thickness and the epithelial thickness was obtained and then all the values were statistically analysed (Davydova, 2017). b-2.5% glutaraldehyde in 0.1 M phosphate buffer for scanning electron microscope (SEM). SEM evaluation was done at the 'THE EGYPTIAN MINERAL RESOURCES AUTHORITY', Central Laboratories Sector. Using SEM Model Quanta 250 FEG (Field Emission Gun) attached with EDX Unit (Energy Dispersive X-ray Analyses), with accelerating voltage 30 K.V., magnification14x up to 1000000 and resolution for Gun.1n). FEI company, Netherlands

***Statistical analysis:*** The statistical evaluation, was done using, One-way ANOVA test for the simultaneous comparison of the studied groups and Tukey`s post-hoc test for multiple comparisons between each pair of studied groups, regarding; ratio (Keratin/epithelial thickness), keratin thickness and total thickness of papillae epithelium. Calculations were made using the SPSS 13.0 program pack. $P \leq 0.05$ was accepted as statistically significant.

## RESULTS

***Blood glucose and body weight assessment:*** High blood glucose was first noted in rats of groups II and III (445.2mg/dl and 458.2 mg/dl, respectively) after the 2nd Alloxan injection. After 14 days, blood glucose levels increased in group II and were considerably reduced in group III (593.4 mg/dl and 92 mg/dl, respectively). Body weight measurements did not differ much (236.6 grams and 248.6 grams, respectively). The blood glucose and the body weight readings remained more or less constant in group I throughout the experiment. The blood glucose and body weight records are summarized in (Fig. 1).

***Microscopic assessment***

***Inverted light microscopic assessment:*** The histological examination of group I showed the normal histological structure of both the filiform and fungi form tongue papillae. The filiform papillae appeared conical in shape with stratified squamous epithelium covered by uniform thin keratin layer. In between the filiform papillae, the fungiform papillae were observed with broad connective tissue core and covered by a relatively thin keratin layer, a single taste bud was observed on top of these papillae. The submucosal layer exhibited connective tissue with muscle fibers running in different directions (Figs 2A and 2B). The filiform papillae of Group II (diabetic rats) showed shortening and loss of their conical appearance and in some areas appeared flat. Moreover, their mucosa appeared thickened with poorly defined connective tissue papillae. Also, the fungiform papillae observed in most areas were dome shaped with narrowing of the connective tissue papillae. The thickness of the keratin layer covering both papillae appeared to be increased. The submucosa of this group showed separation between the muscle fibers and areas of fatty degeneration (Figs 3A and 3B). The lingual papillae of Group III (MO treated rats) revealed almost the normal appearance of the papillae. The filiform papillae appeared elongated and conical. The fungiform regained its normal form with the taste buds on top, their connective tissue papillae were noticibly wider than that of group II. The keratin layer showed apparently less thickening than the diabetic group, thickness similar to that of the control group. The underlying muscle fibers showed almost normal architecture in spite of the observation of few areas of fatty degeneration (Figs 4A and 4B).

**Table 1. Discriptive analysis, One Way ANOVA and Tukey`s post hoc test for the studied groups regarding ratio (Keratin/epithelial thickness), keratin thickness, and total thickness of papillae epithelium**

| Parameter | Descriptive | | | Tukey`s post hoc test | | | ANOVA (between groups) |
|---|---|---|---|---|---|---|---|
| | group | mean | standard deviation | Compared groups | | Sig. | |
| Ratio (Keratin/epithelial thickness) | Group I | .5771 | .23953 | Group I | Group II | .001 | 0.00 |
| | Group II | 1.5360 | .77359 | Group I | Group III | .854 | |
| | Group III | .6974 | .31733 | Group II | Group III | .002 | |
| Keratin thickness | Group1 | 1.410 | .8034 | Group I | Group II | .295 | 0.45 |
| | Group II | .980 | .5554 | Group I | Group III | .997 | |
| | Group III | 1.390 | .4864 | Group II | Group III | .328 | |
| Total thickness of the papillae | Group I | 3.730 | 1.5384 | Group I | Group II | .001 | 0.00 |
| | Group II | 1.770 | .8056 | Group I | Group III | .841 | |
| | Group III | 3.470 | .4270 | Group II | Group III | .001 | |

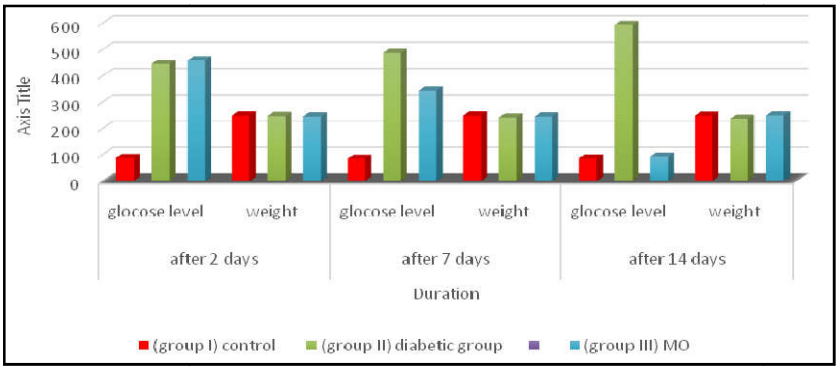
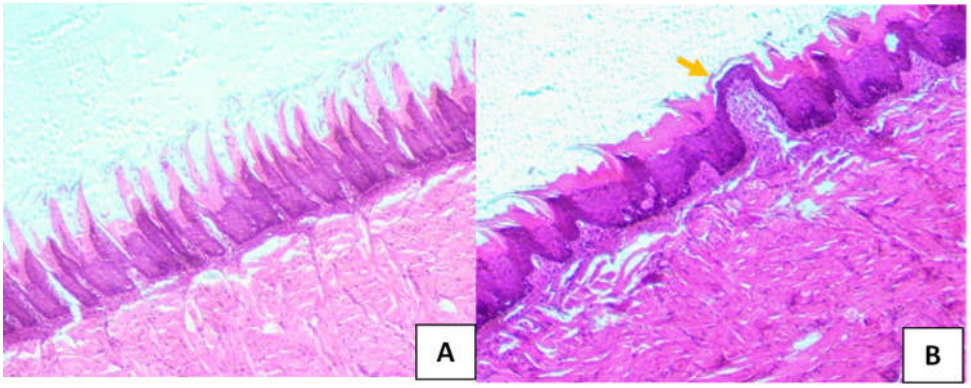
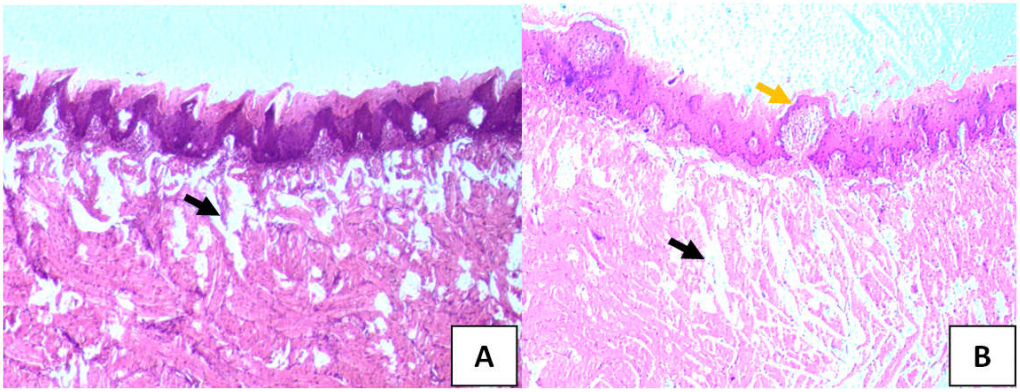


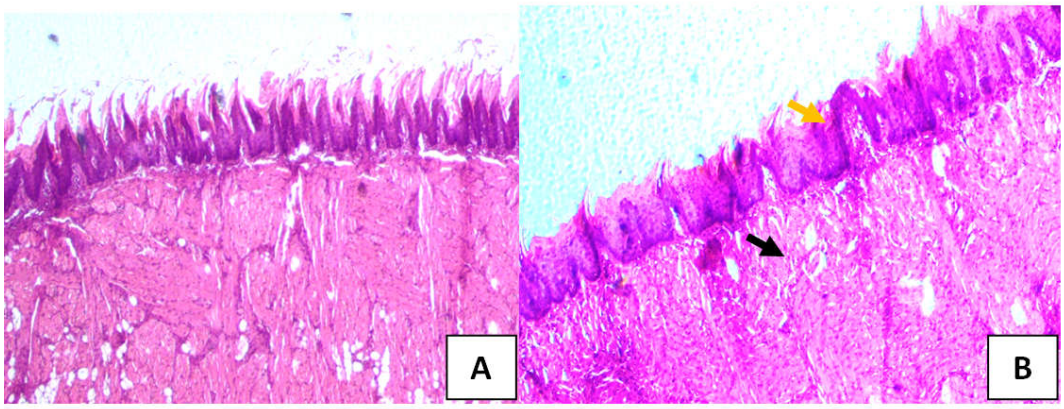

**Fig. 1. Bar chart showing the blood glucose and weight measurements at the beginning of the experiment, after 7 and 14 days of the induction of diabetes**




**Figs 2. A. Photomicrographic picture of group I (control group) showing conical filiform papillae covered by keratinized stratified squamous epithelium, B:the fungiform papillae in between the filiform papillae with a single taste bud on top, connective tissue. Note: muscle fibers running in different directions in the submucosal layer.(H&Ex 100)**




**Figs 3. A. Photomicrographic picture of group II showing short and blunt filiform papillae, B: fungiform papillae with a keratin layer covering (yellow arrow). The submucosa of this group showed separation and degeneration of the muscle fibers (black arrows). (H&Ex 100)**




**Figs 4. A. Photomicrographic picture of group III the moringa group showing long and conical filiform papillae, B: normal form fungiform papillae with the taste buds on the top, with a relatively normal keratin thickness (yellow arrow), few areas of degenerations in the muscle fibers (black arrow). (H&Ex 100)**

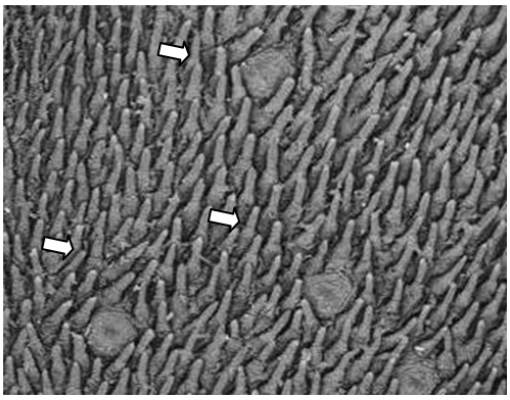
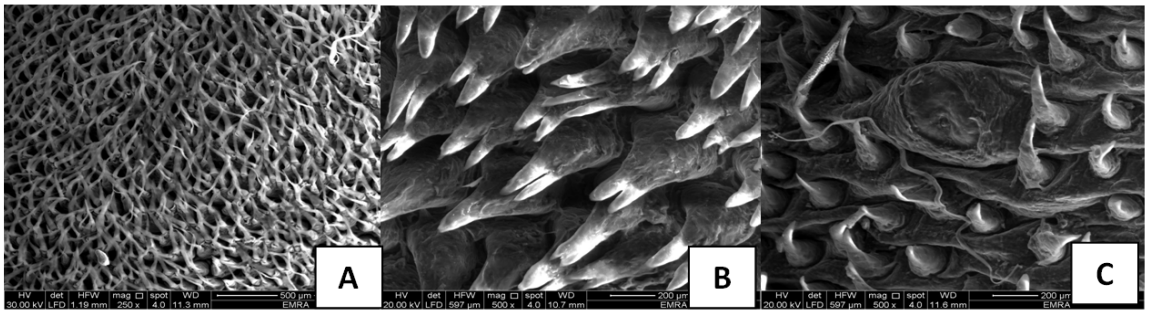
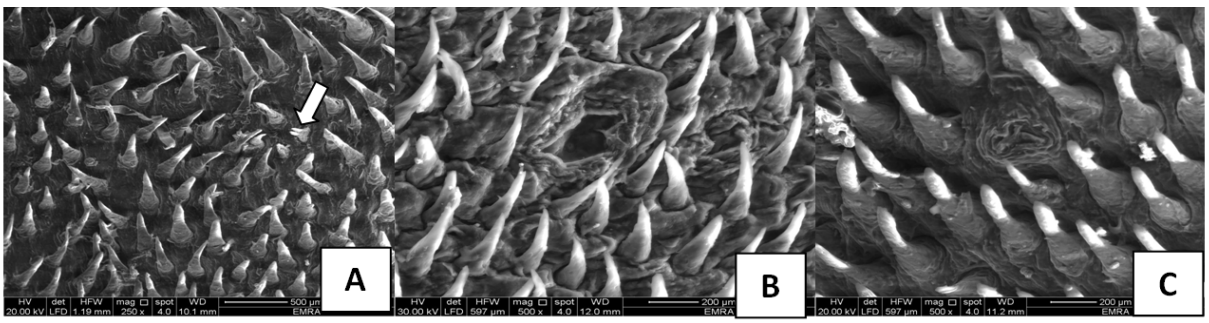


**Fig 5. A Scanning electron micrograph of tongue of the control group showing parallel rows of conical filiform papillae with tapering ends and mushroom-like fungiform papillae in between filiform papillae (white arrows). (SEMx250)**




**Figs 6. A: Scanning electron micrographs of tongue of group II showing scattered filiform papillae with different directions and inclinations (SEMx250).B: bifid tapering end filiform papillae and heavy keratin layer (SEMx500). C: Fungiform papillae appeared covered by heavy layer of keratin and the taste pore surrounded by deep irregular groove (SEMx500).**




**Figs 7 A. Scanning electron micrographs of tongue of group III ,the moringa treated rats showing nearly normal direction and inclination of the filiform papillae (SEMx250) , few papillae still showed bifid ends(white arrows). B: uniform keratin layer on the papillae tips. (SEMx500) C: Fungiform papillae showed less keratinization than group II. (SEMx500)**

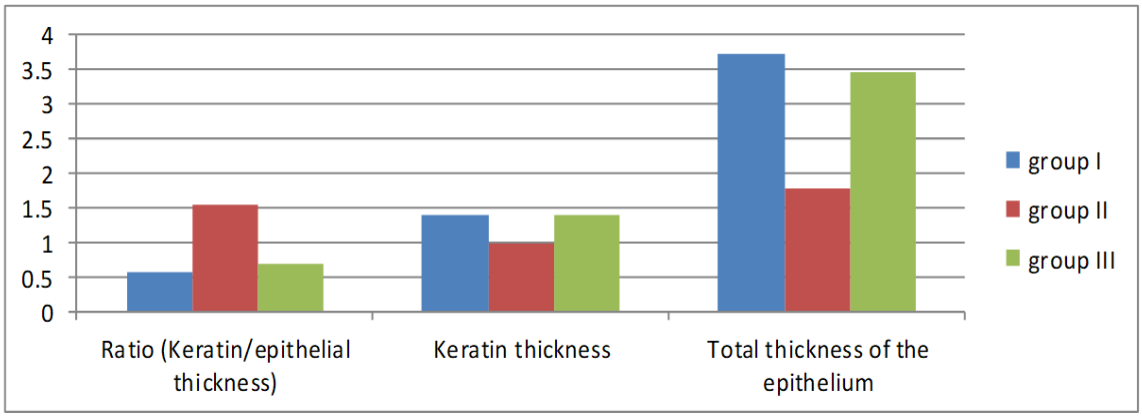



**Fig. 8. Bar chart showing the ratio (Keratin/epithelial thickness), keratin thickness, epithelial cells, total thickness of papillae epithelium among the studied groups**

***SEM assessment:*** Scanning electron microscope examination of the dorsal surface of the tongue of Group I revealed numerous parallel filiform papillae, their antero-posterior direction pointing towards the pharynx, they appeared tapered conical in shape with a uniform keratin layer on the tips. Fungiform papillae appeared like mushroom heads scattered in between the filiform papillae, a well-defined taste pore encircled by shallow depressions in its centre, were also noted. (Fig 5). In Group II, the dorsal surface of the diabetic rat tongues showed scattered filiform papillae with different directions and inclinations. They showed bifid tapering ends and thick keratin layers. Fungiform papillae appeared covered by heavy layer of keratin as well, the taste pore became surrounded by deep irregular grooves (Fig 6A, 6B and 6C). The MO treated group (Group III) revealed nearly normal direction and inclination of the filliform papillae. They appeared tapered conical in shape with a uniform keratin layer on the tips as in the control group, however few papillae still showed bifid ends. Fungiform papillae and their taste pores didn't regain their normal shape but they showed less keratinization than the diabetic group. (Figs 7A, 7B and 7C)

***Statistical assessment:*** The mean and standard deviation of the three studied groups regarding ratio (Keratin/epithelial thickness), keratin thickness, epithelial cells and total thickness of papilla epithelium are listed in Table 1 and Fig. 2. The maximum ratio (Keratin/epithelial thickness) (=1.5360) was seen in group II and, the least keratin thickness (0.980), and total thickness of papilla epithelium (1.770), was also noted in group II. One-way ANOVA test was used for the simultaneous comparison between the studied groups regarding ratio (Keratin/epithelial thickness), keratin thickness and total thickness of papilla epithelium. The differences between the means of all parameters in all the three studied groups was significant, except for the keratin thickness where there was statistically insignificant difference (p=.245) among the studied groups (Table 1). Tukey`s post-hoc test was used for multiple comparisons between each pair of study groups regarding; ratio (Keratin/epithelial thickness), keratin thickness and total thickness of papilla epithelium. The mean difference between group I and group III was always insignificant for all of the measured parameters ( p= .854, .997,.841, respectively), while, that between group I and group II and, between group II and group III, was always highly significant (p=0.002 or less) except for the difference in keratin thickness, where the difference between all the three groups was insignificant ( p= more than 0.05) (Table 1).

## DISCUSSION

Diabetes is one of the most common diseases of our modern-day life. The tongue is the second most prevalent intra-oral site to exhibit hyperglycemia induced complications. Diabetic complications are mainly attributed to oxidative stress and side effects from long term use of drugs to treat diabetes (Gandara, 2011; Spravchikov, 2001). MO extract is studied in this research as it possesses a known anti-oxidant property and can be used synergistically or even on its own to manage diabetes (Rani, 2018; Tuorke, 2016). Alloxan was administered to the rats to induce in this study using, as it is effective in inducing diabetes, is in-expensive and is easily available. Alloxan is selectively toxic to pancreatic beta cells (Macdonald Ighodaro, 2018). The results of this study demonstrate that there was a significant reduction in the blood glucose levels, when MO extract was given to the diabetic rats. These results are consistent with those of Paula PC, *et al*, 2017 (Paula, 2017). This is proposed to be due to the simple fact that fibers in MO reduce the uptake of glucose from the intestine. Added to this, Chlorogenic acid, a phytochemical present in MO, has also, been shown to inhibit glucose-6-phosphate translocase in the liver leading to a reduction of hepatic glycogenolysis and gluconeogenesis (Fard, 2015).

The results of this study also show that there was a significant reduction in the total height of the filiform papillae in the diabetic group, in spite of the fact that, there was an insignificant difference between the keratin thickness among all the studied groups. To avoid mis-interpretations, this study opted to calculate the ratio between the keratin and the thickness of epithelium -for standardization-. The ratio demonstrates that in the diabetic group, there was a significant increase in the ratio which indicates that the keratin thickness was increased on expense of the other epithelial layers. The H&E histological sections demonstrated similar findings, where there was flattening of the papillae with hyperkeratosis this may due to the fact that in diabetes there is a decreased activation of the protein synthesis signaling pathways. The SEM assessment of the diabetic group showed dis-orientation of the papillae where some papillae exhibited bifid or split tips and the fungiform papillae showed aberrations of the taste pores. These observations may be attributed to different factors, on top of which is the increased oxidative stress due to the hyperglycemic state of the diabetic rats. The resultant altered blood and nerve supply, give rise to atrophic changes and may explain the alterations depicted in the taste pores of

the fungi form papillae (Okano, 2016). Findings also suggest that hyperglycemia induces reduction of PPARα which has an important role in diabetic neuropathy (Koh, 2003). Researchers also reported that hyperglycemia was linked to decreased proliferation due to reduction of IGF-I receptor and thus decreased glucose uptake, as well as, keratinocyte gap junction abnormalities and chronic inflammation (Spravchikov, 2001; Hirsch, 2008; Hu, 20167). These findings may also be due to the alterations in salivary flow and composition, which compromises the nutritional supply to the tongue mucosa (Fard, 2015). Researchers also state that hyperkeratosis is a result of increased gene expression of keratin associated proteins and keratin complexes, in diabetic rats (Spravchikov, 2001; Koh, 2003; Macdonald Ighodaro, 2018). This study also reported that there were some alterations in the sub-mucosa where there was some degeneration of the muscle fibers, this is due to upregulation of pathways that cause degradation of contractile muscle protein (Bodine, 2001) and the PPARα which causes lipid accumulation in the muscle (Koh *et al*., 2003; Kinase *et al*., 2014; Rani, 2018; Tuorkey, 2016; Fard, 2015). There was no statistically significant difference, in this study between the control and the MO treated group, this indicates the ability of MO to, at least partly, reduce the alterations resulting from diabetes. This power of `healing` may be due to the fact that MO reduces blood sugar levels (as discussed earlier), is a huge source of antioxidants and causes stimulation of PPAR-α transcription (Paula, 2017).

**Conclusion**

MO has a hypoglycemic effect on the blood glucose level of diabetic albino rats. MO may partially reverse some of the detrimental effects hyperglycemia has on the tongue papillae of diabetic rats

**Conflict of interest:** None

**Funding:** None

## REFERENCES

Bodine, S.C., *et al*., Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nature cell biology*, 2001. 3(11): p. 1014.

Brömme, H., *et al.,* 2000. *Scavenging effect of melatonin on hydroxyl radicals generated by alloxan. Journal of pineal research*,. 29(4): p. 201-208.

Davydova, L., *et al*., 2017. Anatomical and morphological aspects of papillae, epithelium, muscles, and glands of rats' tongue: Light, scanning, and transmission electron microscopic study. Interventional Medicine and Applied Science, 2017. 9(3): p. 168-177.

Eltokhey, H.M. 2013. The Influence of Ozonized olive oil gel on the dorsal surface of the tongue ofstreptozotocin induced diabetic albino rats. *Journal of American Science*, 9(10).

Fard, M.T. *et al*., 2015. Bioactive extract from Moringa oleifera inhibits the pro-inflammatory mediators in lipopolysaccharide stimulated macrophages. *Pharmacognosy magazine*, 2015. 11(Suppl 4): p. S556.

Gandara, B.K. and Morton, T.H. 2011. Non-periodontal oral manifestations of diabetes: a framework for medical care providers. Diabetes Spectrum, 24(4): p. 199-205.

Hirsch, T., *et al*., 2008. Enhanced susceptibility to infections in a diabetic wound healing model. BMC surgery. 8(1): p. 5.

Hu, S.C.-S. and C.-C.E. Lan, High-glucose environment disturbs the physiologic functions of keratinocytes: focusing on diabetic wound healing. *Journal of Dermatological Science,* 2016. 84(2): p. 121-127.

Kinase, J.N.T., Moringa tea blocks acute lung inflammation induced by swine confinement dust through a mechanism involving tnf-α expression, c-jun n-terminal kinase activation and neutrophil regulation. *American Journal of Immunology*, 2014. 10(2): p. 73-87.

Koh, E.H., *et al*., Peroxisome proliferator-activated receptor (PPAR)-α activation prevents diabetes in OLETF rats: comparison with PPAR-γ activation. *Diabetes*, 2003. 52(9): p. 2331-2337.

Macdonald Ighodaro, O., A. Mohammed Adeosun, and O. Adeboye Akinloye, Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. *Medicina*, 2018. 53(6): p. 365-374.

Okano, J. *et al*. 2016. Hyperglycemia Induces Skin Barrier Dysfunctions with Impairment of Epidermal Integrity in Non-Wounded Skin of Type 1 Diabetic Mice. PloS one, 11(11): p. e0166215.

Paula, P.C., *et al*., 2017. A protein isolate from Moringa oleifera leaves has hypoglycemic and antioxidant effects in alloxan-induced diabetic mice. *Molecules*, 22(2): p. 271.

Rani, A., *et al*., 2018. Moringa genus: A review of phytochemistry and pharmacology. *Frontiers in Pharmacology*, 2018. 9: p. 108.

Spravchikov, N., *et al*., 2001. Glucose effects on skin keratinocytes: implications for diabetes skin complications. Diabetes, 2001. 50(7): p. 1627-1635.

Tian, M., *et al*., 2012. Effects of advanced glycation end-products (AGEs) on skin keratinocytes by nuclear factor-kappa B (NF-κB) activation. *African Journal of Biotechnology,* 2012. 11(50): p. 11132-11142.

Tuorkey, M.J., Effects of Moringa oleifera aqueous leaf extract in alloxan induced diabetic mice. *Interventional Medicine and Applied Science,* 2016. 8(3): p. 109-117.

Volpe, C.M.O., *et al*., 2018. Cellular death, reactive oxygen species (ROS) and diabetic complications. Cell death & disease. 9(2): p. 119.

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