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Efficacy of Moringa Leaf Extract in Management of Bone Healing after Implant Placement

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ABSTRACT

Purpose: This study aimed to evaluate the effect of using topical Moringa leaf extract on healing after delayed dental implant placement in posterior maxilla. **Subjects and methods:** A total of twelve patients with missing maxillary premolars and molars were involved in this study. They were randomly divided into two groups; Group I (test group; n = 6) patients that received delayed implant placement with the use of topical Kaempferol (Moringa olifera leaf extract) gel and Group II (control group; n = 6) patients that received delayed implant placement only for replacement of missing teeth. Clinically, pain was evaluated for each patient by using visual analog scale (VAS) and swelling was assessed in form of mild, moderate and severe. Assessment of healing progress was conducted by modified gingival index and probing depth at 1st, 3rd and 6th month after implant placement. **Results:** A statistically significant difference was recorded in VAS assessment for 1st week follow up in both groups (P=0.026), regarding swelling no statistically significant difference between studied groups (P=0.065). Considering the values of modified gingival index and probing depth, the study group showed lower values than of control group during the follow up periods of the study however, this difference was non-significant (P= 0.240) for both parameters. **Conclusion:** The use of Kaempferol topical gel with delayed dental implant placement offer a new promising natural, safe and effective remedy for management of healing process.

KEYWORDS

*Dental implant, Moringa Leaf
Extract, Bone healing*

- Paper extracted from Master thesis titled “ Efficacy of Moringa Leaf Extract in Management of Bone Healing after Implant Placement”
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INTRODUCTION

During most recent years, dental implantology has ended up as an indispensable portion of standard dentistry, helping dentists to improve the quality of life of large patient populations. While implant treatment could often be a helpful alternative to conventional treatments, in certain cases, it is the primary choice for the restoration of serious functional, anatomical or aesthetic issues occur as a result of missed teeth ⁽¹⁾. Among healthy patients, the successfulness of almost dental implants within range 90% -95% at 10 years ⁽²⁾. Titanium implant has been frequently used for endosseous implant materials for the reason that it has opportune physical properties, chemical resistance, and biologically compatible ⁽³⁾.

The principal prerequisite for this therapy is to connect titanium dioxide implant to vital bone without the organization of fibrous tissues this procedure is named osseointegration. The treatment period for endosseous implants requires more time than needed for other treatments, since a few months are required to get appropriate osseointegration⁽⁴⁾. So, a shortened dental implant treatment term would be helpful for patient. Delayed or immediate loading requests a high level of osseointegration at the early healing phase ⁽⁵⁾. Dental implants are outlined to attain primary mechanical stability and to promote a potent bone-implant interaction over time through osseointegration. Fundamentally, the process of osseointegration reflects an anchorage mechanism whereby non-vital components can be reliably incorporated into living bone which remain under all standard states of loading. After dental implants are secured, an arrangement of immune-inflammatory responses followed by angiogenesis and in the long run osteogenesis take place to attain osseointegration ⁽⁶⁾.

This is often impacted by the implant surface characteristics owing to the capability for protein adsorption based on implant surface topography and hydrophilicity. In like manner, thrombin and

fibrinogen adhere to the implant surface. Afterward, neutrophils populate the implant recipient site before the monocytes and macrophages penetrate the area. These occasions fulfill a key part on the early homeostasis as they discharge the cytokines and growth factors that stimulate collagen matrix deposition around the titanium oxide layer driving to newly-formed woven bone (as a rule happens 5 days afterward). In a matter of 8 to 12 weeks, lamellar bone starts the biological stability, that is to say osseointegration ⁽⁷⁾.

Enhancements to the morphology and properties of the dental implant surface have been inspected to get more strong and prior osseointegration, inclusive control of the surface topography and hydroxyapatite coating. These physical and chemical alterations of the titanium dioxide surface are as of now utilized in clinical treatment. Even so, these alterations are not sufficient to abbreviate the time demanded for osseointegration ⁽⁸⁾. In spite of the fact that the bioactive molecule immobilization and genetic engineering strategies enhanced the new bone arrangement around titanium dioxide implants, the safety of these strategies has not been built up; such as, their pharmacodynamics and drawbacks effects are obscure. Furthermore, these strategies are too expensive for common use. Furthermore, the effects of these bioactive molecule include only one course, such as cell attachment, cell adhesion, or osteogenic induction ⁽⁹⁾.

Natural drugs can decrease the side effects, toxicities of synthetic counterparts and will maximize therapeutic consequences with most effective and dynamic healing effects. WHO has stated that herbal or therapeutic plants are the excellent source to get an assortment of drugs ⁽¹⁰⁾. Among these plants, *Moringa oleifera* has its great contribution from ancient time, which is also known as "miracle tree" because of its remarkable healing properties on various diseases and some chronic diseases ⁽¹¹⁾. *Moringa oleifera* leaf has various compounds as amino acid, fatty acid, beta carotene, minerals, vitamin E and flavonoids ⁽¹²⁾. These flavonoids

counting kaempferol (KP) and quercetin have been definitely related with better skeletal wellbeing in humans ⁽¹³⁾. KP is mentioned to as a natural supplement though to its beneficial effects on human health already demonstrated scientifically, which incorporated as cardioprotective, antineurotoxicity, antianxiety, pain relieving, antioxidative and has the ability to prevent allergy, platelet aggregation, cancer, microbial infection, obesity, hyperglycemia, hypertension, hyperlipidemia, aging, inflammation and osteoporotic affects ⁽¹⁴⁾.

KP has been integrated with titanium dioxide habitually used as a biomaterial for therapeutic implants, especially in dental and orthopedic fields. An in vivo study at 2018 was performed to examine the bone-regenerating effects. They uncovered that the placement of KP-immobilized TiO₂ in rat tibia displayed absence of osteonecrosis, inflammation, laxation and separation, illustrating that the implants were well associated to the bones. The comes about from histological finding were furthermore fixed by quantitative examination, whereby the bone-implant contact in the tibia of KP-immobilized implant group was significantly higher than the group with TiO₂ implant without KP. These assemblages reiterated the reality that implant placement outcome in better cell proliferation and osteogenic differentiation, in this way improving bone formation around the implants ⁽⁹⁾. Thus the purpose of this case study was to assess the effect of using topical moringa leaf extract (KP) on healing after delayed dental implant placement.

MATERIAL AND METHODS

Patients selection:

This study was a randomized controlled clinical trial including a total number of twelve patients with missing maxillary posterior teeth who planned to replace the missing teeth, they selected from the outpatient clinic of Oral Medicine, Periodontology, Oral Diagnosis and Radiology Department, Faculty of Dental Medicine for Girls, Al-Azhar University.

Prior to any procedure, all subjects were informed about the nature, benefits and / or risk of being involved in the present study. All patients had undergone an adequate pre-surgical preparation consisting of detailed case history and radiographic examination. Research ethic committee approval of the Faculty of Dental Medicine for Girls was obtained. The code number isOMPDR-103-2F.

All patients were chosen agreeing to the undermentioned inclusion criteria; Patients with lost premolar and molar maxillary teeth, age range from 30 up to 50 years old, have no systemic diseases that affect the bone healing according to Modified Cornell Medical Index or interfere with the periodontal treatment, no history of taking systemic antibiotics or non-steroidal anti-inflammatory agents during the preceding 6 months. Smokers, bad oral hygiene, medically compromised patients, pregnant women and patients with para functional habits, variation in anatomical landmarks were excluded.

Edentulous space considerations; mesiodistal dimension and buccolingual dimension not less than 5mm and bone height (ridge crest to nearest anatomic land mark) not less than 10mm, adequate inter-occlusal space at implant site, ridge bone density in range between D3 to D4, fully healed surgical site (from 3 to 6 months after tooth extraction).

Sample size:

Sample size calculations achieved using <http://biomath.info/power> according to the results of the previous study in 2016 ⁽¹⁵⁾. Considering that $\alpha=0.05$; power at 0.8; distribution ratio (1:1) and the significance = 1.12. Hence, a total sample size of 12 patients (6 patients in each group) was sufficient to identify the difference. Sample was calculated using EPI INFO program with confidence level 95% and power 80%.

Study design and randomization method

The involved twelve patients were randomly allocated by means of preoperative envelope

drawing, to be managed in the diverse study groups. The patients were shared out equally into two groups. In Group I (test group; n = 6) patients were under-went delayed implant placement with the use of topical Moringa leaf extract. Group II (control group; n = 6) involved patients were under-went delayed implant placement only.

Materials:

1. Neo Biotech implant system (Neo Biotech Co, Seoul, Korea) was used in this study. Implants were made from commercial pure titanium with length ranging from (7.3 – 13 mm) and diameter ranging from (3.5-5.5 mm), which has sandblasting with large grit and acid etching surface that make rough surface by blasting on machined implant surface with hydroxyapatite particle smaller than $50\mu\text{m}$ and dual acid etching. Also, it has wide cutting edge at the apex which improve fixation and increase operator comfort when placing implant.
2. Kaempferol (Moringa olifera leaf extract) gel: This gel was applied topically in group I by plastic syringe covered with aluminium foil, each one had $10\mu\text{gm}$ of kaempferol. It was purchased from ²Tocris Bioscience (Ellisville, MO, USA).which had a purity of over 98%.

Surgical phase:

The surgical steps were formed under aseptic conditions; All patients were operated under infiltration local anesthesia (1:100,000 Epinephrine) sub-periosteal buccal and palatal infiltration at the site of operation then a scalpel #15 was used to incise the mid-crestal tissues. At least 1.5 mm of keratinized tissue should be left on the buccal to this incision line. A Molt elevator was utilized to make a full thickness mucoperiosteal flap reflection to expose crestal bone and a few millimeters of the lateral aspects of the implant site. Then sequential drilling according to the procedures recommended by manufacturers. The osteotomy is made with an electric motor at a preferred speed of 1500 rpm under copious amounts of chilled saline irrigant to

lubricate and clean the bone tap and osteotomy site of debris during this process.

The implant was held by the fixture adapter from the vial and inserted into the prepared socket and screwed manually with apical pressure until there is resistance. The implant was rotated into the position by hand ratchet, not require a torque greater than 35 N-cm until the implant reached the final insertion depth slightly below the crest of the bone. The implant should be rigid upon placement, with no observable mobility under slight compressive forces. After implant insertion, the smooth healing abutment was screwed to the implant and tightened using implant screw driver. Two interrupted sutures approximated the soft tissue for primary closure. A 4-0 vicryl suture material was used. Slight pressure to the approximated tissues for 4 -6 minutes decreased bleeding and improved the adaptation of the tissue to the bone.

Test group:

The same steps were followed except: after complete preparation of the osteotomy site, Kaempferol gel in prepared plastic syringe was inserted in the site and apply the gel until fill the all osteotomy site, then the implant inserted and screwed within the site with placement of healing abutment, then return the flap and suturing. Figure (1)

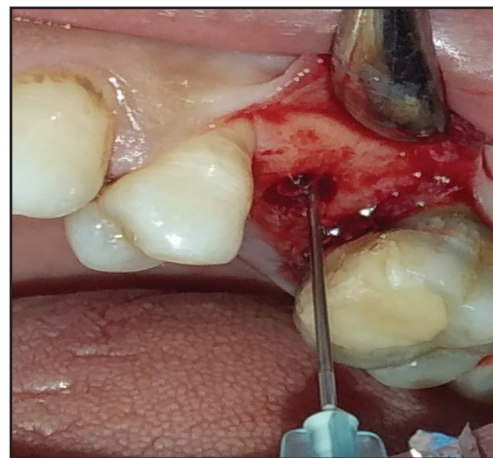


Figure (1) A photograph showing kaempferol gel application in the prepared socket

Post-surgical phase:

All cases had been given instructions to put cold bag extra orally periodically every 2 hours for 10 minutes on the 1st day to minimize postoperative edema and swelling. Chlorhexidine 0.12% mouth wash started after the day of surgery twice per day for 2 weeks to enhance plaque control, an Antibiotic (Curam, Novartis Co., Egypt) every 12 hours for 5 days and Ibuprofen 400 mg tablet used according to patient need (Kahira Co., Egypt) drugs. If there was remaining suture material after 10 days, would be removed.

Clinical evaluation:

1. Presence of pain and Swelling: All patients in the study groups were evaluated using the visual analogue scale (VAS) for pain assessment involving a straight line of 10-cm among ends, with 0 indicating no pain and 10 for intolerable pain was performed. While swelling was assessed as mild, moderate and severe. Both were performed for each patient at 1st, 3rd, 5th and 7th day. Tenderness and discomfort were evaluated according to the signs and symptoms of the patients
2. Modified gingival index (MGI) and Probing depth (PD): The MGI is noninvasive approach (visual examination only without any probing). Even though, the depth of the peri-implant sulcus was evaluated using a calibrated periodontal probe with light force to avoid tissue damage and overextension into healthy tissue. Measurements were taken at four points all over each implant (mesial, distal, buccal and palatal) after implant placement at 1st, 3rd and 6th month.

Prosthetic phase:

After 6 months post operatively, the healing abutment was removed and the abutment was tightened, a condensation silicone impression was utilized to take the impression, and final ceramometal crowns were cemented to the all patients.

Statistical Analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) The Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation, median and interquartile range (IQR). Significance of the obtained results was judged at the 5% level.

RESULTS

In these study there were twelve implants inserted in posterior maxillary arch (8 females and 4 males) of mean age 41.33 (± 5.68 SD) years. All cases were undergone follow up for six months and the results were registered as regards clinical evaluation.

1. Presence of pain: pain was evaluated at 1st, 3rd, 5th and 7th day following surgery utilizing visual analogue scale (VAS), there was significant difference between the different studied periods in the study group and the control group ($P=0.001$). For the control group there was decrease in the mean at 7th day from 1st day by 2.5 (± 0.55 SD) with percentage of 100%, while in the study group the mean decrease in 7th day from 1st day was 1.5 (± 0.55 SD) with percentage of 100%. Also, there was difference between study group and control group as account VAS score at 1st, 3rd, 5th day and 7th day ($P=0.026$) which is statistically significant. (table1)
2. Presence of Swelling: swelling evaluated according to swelling score mild, moderate and severe. There was significant difference between the different studied periods in the control group and study group ($P=0.003$), but there was no statistically significant difference between studied groups as regard swelling score ($P=0.065$). (table 1)
3. Modified Gingival Index: at 1st month, study group showed a lower MGI mean (0.50 ± 0.84)

than control group (0.67 ± 0.82), however this difference was statistically insignificant ($P=0.699$). At 3rd month, study group showed a lower MGI mean (0.0 ± 0.0) than control group (0.50 ± 0.55), however this difference was statistically insignificant ($P=0.180$). At 6th month, study group showed a lower MGI mean (0.17 ± 0.41) than control group (1.33 ± 1.51), however this difference was statistically insignificant ($P=0.240$) (table 2).

4. Probing Depth: at 1st month, study group showed a lower PD mean (0.96 ± 0.68) than that of control group (1.75 ± 0.27), however this difference was statistically insignificant ($P=0.818$). At 3rd month, study group showed a lower PD mean (1.83 ± 0.26) than control group (2.0 ± 0.55), however this difference was statistically insignificant ($P=0.240$). At 6th month, study group showed a lower PD mean (1.92 ± 0.20) than control group (2.42 ± 0.66), however this difference was statistically insignificant ($P=0.240$) (table 2).

Table (1) VAS and swelling changes of both groups through the study period:

Clinical Parameter		1 st day post-operative	3 rd day	5 th day	7 th day	Change in 7 th day from baseline	P (change by time within the same group)
VAS	Mean±S.D Group I	1.50±0.55	0.50±0.55	0.0±0.0	0.0±0.0	1.50 ± 0.55	0.001(S)
	Mean±S.D Group II	2.50±0.55	1.33±0.52	0.83±0.41	0.0±0.0	2.50 ± 0.55	0.001(S)
	P (between groups)	0.026(S)	0.065(NS)	0.015(S)	1.00 (NS)		
Swelling	Mean±S.D Group I	0.0±0.0	1.0±0.63	0.17±0.41	0.0±0.0	0.0 ± 0.0	0.003 (S)
	Mean±S.D Group II	0.67±0.52	1.67±0.52	0.33±0.52	0.0±0.0	0.67 ± 0.52	0.003 (S)
	P (between groups)	0.065(NS)	0.321(NS)	0.699(NS)	0.06 (NS)		

U: Mann Whitney test

$P > 0.05$: Non Significant (NS), $P \leq 0.05$: Significant (S), $P < 0.01$: Highly Significant (HS)

Table (2) MGI and PD changes in both groups through the study period

ClinicalParameter			At 1 st month	At 3 rd month	At 6 th month	Change from 1 st month to 6 th month	P (change by time within the same group)
Modified Gingival index (MGI)	Mean±S.D Group I		0.50±0.84	0.0±0.0	0.17±0.41	0.0 ± 0.0	0.368(NS)
	Mean±S.D Group II		0.67±0.82	0.50±0.55	1.33±1.51	0.67 ± 0.52	0.444(NS)
	P (between groups)			0.699(NS)	0.180(NS)	0.240(NS)	
Probing Depth (PD)	Mean±S.D Group I		1.83±0.61	1.83±0.26	1.92±0.20	0.08 ± 0.66	0.607(NS)
	Mean±S.D Group II		1.75±0.27	2.0±0.55	2.42±0.66	0.67 ± 0.68	0.196(NS)
	P (between groups)			0.818(NS)	0.818(NS)	0.240(NS)	

U: Mann Whitney test

$P > 0.05$: Non Significant (NS), $P \leq 0.05$: Significant (S), $P < 0.01$: Highly Significant (HS)

DISCUSSION

Titanium dioxide is now the main material for dental implant that is surgically inserted into hard and soft tissues which provide a superstructure for esthetics and function purposes. The ancient medicinal science describes different herbal preparations that improve bone healing. One of the herbs that have shown beneficial effects on bone belongs to the kaempferol (KP) which is *Moringa olifera* leaves extract. Kaempferol is considered an important flavonoid that found in different fruits and vegetables, nutrients fertile in flavonols (a subcategory of flavonoids inclusive of kaempferol and quercetin) have been invariably correlating with major health of skeleton in humans⁽¹³⁾. Thus the current study was a clinical trial to estimate the efficacy of kaempferol (KP) on bone healing around TiO₂ implants done by clinical assessment and its effect on pain and inflammation after implant placement.

There was study in 2016 that reported that kaempferol (10 µg) stimulated the duplicational activity and induction of osteoblast demarcation biomarkers, including ALP, which advocated the osteoblasts mineralization⁽¹⁶⁾. Thus, in this study Kaempferol (*Moringa olifera* leaf extract) gel was applied topically in study group with 10µgm concentration of kaempferol by using plastic syringe following the method of application of melatonin gel with dental implant⁽¹⁵⁾.

In the present study, the results revealed a decrease in the pain score assessed by using VAS along the different observational times within each study group, where on first postoperative days, the experience of all patients ranged from mild to moderate pain at the surgical site, scoring between one and three on visual analogue scale. For the control group the mean decrease in 7th day from baseline was 1.3 (±0.52 SD) and the study group the mean decrease in 7th day from baseline was 0.50 (±0.55 SD). Comparing the two study groups regarding VAS score there was statistically significant difference at baseline (1st day), 3rd, 5th day and 7th day (P=0.026).

In 2017, pain measurement in oral and maxillofacial surgery study was performed and stated the effects of unidimensional scales, as the Visual Analogue Scales (VAS), to evaluate oral maxillofacial acute pain. In this study, the peak of pain perception occurred on 1st day following surgery. The mean average of pain reduced significantly during the follow up (P =0.001), from a VAS score at 1st, 3rd and 7th day. The most unfavorable pain was on the first postoperative day; it also reduced to the half of maximum level at the 2nd or 3rd day postoperative. Self-evaluation by the patient suggested that implant insertion is a mild to moderately painful and apprehension-annoying procedure. Restriction of several daily activities and other symptoms are predictable to be happened, mostly through the three postoperative days⁽¹⁷⁾.

These VAS results are in accordance with the study performed in 2017, which suggested that the decrease of VAS score was significant after the three days postoperative (P < 0.05). Although there is no severe pain was reported at any time, the pain score range from mild to moderate for all patients. They also were able to show that the postoperative pain increase is not an indication of implant early failure.

Swelling was evaluated according to swelling score mild, moderate and severe. The difference between the different studied periods in the study and control group was significant (P=0.003), but there was no significant difference between studied groups as regard swelling score (P=0.065).

The significant results in decreasing the swelling and pain scores in test group may be explained by the reports of variety researchers where kaempferol has considerable anti-inflammatory effects by means of several mechanisms as inhibition of the NF-κB binding activity of DNA and myeloid differentiation factor 88⁽¹⁹⁾ which, suppressed the liberation of IL-6, IL-1β, IL-18 and TNF-α⁽²⁰⁾ and deactivation of the toll-like receptor- 4 (TLR4)⁽²¹⁾.

Additionally, an in vitro study had demonstrated the inhibitory effect of kaempferol

on cyclooxygenase enzymes COX1 and COX2. The pathway is stimulated when a chemical, mechanical or physical injury happens for the different body parts. The phospholipids' membranes are ruptured, and produce arachidonic acid, that one is furthermore transformed to prostaglandin alternatives by cyclooxygenase enzymes, that causes inflammation⁽²²⁾.

Another, clinical assessments to evaluate the dental implants success are modified gingival index and probing depth. Modified gingival index by (modified gingival index for application around dental implant), it serves to describe disease activity and degree of gingival inflammation around dental implants. These assessments were recorded at four positions for the implant (mesial, distal, buccal and palatal)⁽²³⁾.

Measurements were taken after implant placement at 1st, 3rd and 6th month. According to MGI, study group showed a lower MGI mean (0.50 ± 0.84) than control group (0.67 ± 0.82) at 1st month, but this difference was statistically insignificant ($P=0.699$). at 3rd month, study group showed a lower MGI mean (0.0 ± 0.0) than control group (0.50 ± 0.55), however this difference was statistically insignificant ($P=0.180$). In addition, at 6th month, study group showed a lower MGI mean (0.17 ± 0.41) than control group (1.33 ± 1.51), however there was no statistical significant difference ($P=0.240$).

Also, probing depth at 1st month, study group showed a lower PD mean (0.96 ± 0.68) than that of control group (1.75 ± 0.27), however this difference was statistically insignificant ($P=0.818$). At 3rd month, study group showed a lower PD mean (1.83 ± 0.26) than control group (2.0 ± 0.55), however this difference was statistically insignificant ($P=0.240$). At 6th month, study group showed a lower PD mean (1.92 ± 0.20) than control group (2.42 ± 0.66), however this difference was statistically insignificant ($P=0.240$).

The lower values of both MGI and PD in study group in comparison with control group in accordance with results of study illustrated in 2019, which reported that the flavonoids as kaempferol suggested a favorable scope treatment for some bacterial infections, which have potential properties against bacteria, with more inhibition on gram-positive bacteria than gram negative one⁽²⁴⁾.

Also these results supported by study in 2016, that explained the effects of moringa olifera extract on periodontal pathogens like Porphyromonas gingivalis (Pg), Aggregatibacter actinomycetemcomitans (Aa), Fusobacterium nucleatum (Fn) and Prevotella intermedia (Pi) through subgingival plaque samples which obtained from chronic periodontitis patients, cultivated, and incubated anaerobically as per the standard procedure. The sub cultured strains of Aa, Pg, Pi and Fn are tested with the prepared extracts of Moringa. Their results showed data supporting the use of the Moringa. as a natural antimicrobial agent in periodontal therapy and so, prevention of progression of periodontal diseases⁽²⁵⁾.

This results were in line with the previous trial in 2019, which concluded that the topical application of KP, which was considered to have anti-inflammatory and antioxidant effects. So it has direct effects on healing of incisional and excisional injuries in diabetic and non-diabetic rats; these effects did by elevating the hydroxyproline and collagen amount at the wound site, enhancing the resistance of the wound (tensile strength), assisting wound healing and hastening re-epithelialization⁽²⁶⁾.

CONCLUSION

The use of Moringa olifera extract gel with delayed dental implants may be able to decrease post-operative pain and swelling in addition to improvement healing around dental implants by the anti-inflammatory and antibacterial properties.

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