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The potential of honey to promote wound healing in periodontology: a pilot randomized clinical trial

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Abstract

Background: Honey has been used as a nutraceutical in many traditional and ancient remedies. Considering well documented benefits of honey to accelerate wound healing, for the first time we aimed to assess intra oral surgical wound healing process with honey.

Methods: We designed a pilot randomized placebo controlled cross-over clinical trial. Patients who required bilateral Modified Widman Flap (MWF) surgery randomly assigned to receive either 15 cc topical Persian *Thymus Vulgaris* concentrated honey three times a day or normal saline as placebo with the same amount at the site of the surgery for seven consecutive days. After a 35-day wash-out period the study groups were crossed. The primary efficacy outcome was changes in healing index (Landry index) and the secondary efficacy outcome were changes in gingival and plaque indices (Loe & Sillness, Sillness & Loe indices). It also includes safety issues consisting of any allergic reaction, delayed healing or wound dehiscence.

Results: Ten patients enrolled with the mean age of 36 (±1.5) ranged between 35-40 *yrs*. There was a significant improvement in wound healing considering time and treatment effects in both groups, although faster wound healing observed in honey treated patients ($p<0.001$). In both groups gingival indices were noticed to be improved by the time during the first phase of the study. Both groups displayed aggravated Plaque formation; nevertheless it was merely statistically significant in the control group [$F(3, 27) = 12.88$, $p < 0.001$]. All wounds healed normally and no adverse events recorded.

Conclusion: Our study established the safety, efficacy and feasibility of topical honey to promote periodontal surgical wound healing. (IRCT138901192547N2)

Keywords: Dental plaque, Gingivitis, Honey, Periodontal surgery, Wound healing.

Introduction

Benefits of honey as a nutraceutical substance have an ancient history back to 2000-2200 BCE in an Egyptian text [1]. Avicenna (980-1037 ACE), foremost Persian philosopher-scientist, also introduced honey as a natural remedy to cure and deodorize wounds [2]. Many early traditional medicines such as Ayurvedic (Indian), Chinese and Persian also used honey to treat wounds [1,2]. Honey is supersaturated nectar processed by honeybee, *Apis Mellifera,* contains 40% fructose, 30% glucose, 5% sucrose and about 20% water [3]. Many researchers have studied the effect of honey

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on wound healing process in acute or chronic settings. Subrahmanyam evaluated the honey as a biologic dressing for burns [1]. Honey also has been an interesting topic for negotiation considering its safety [4,5], anti-oxidative [6,7], bactericidal [8-10], anti-nociceptive [11] and wound healing properties [12-14]. A recent study discovered a 5.8 KDa particle within honey that stimulates peripheral monocytes to release cytokines and chemokines like TNFα, IL-6, IL-1β and TGFβ; thus promoting tissue healing [15]. Moreover, its low pH helps oxygen release from haemoglobin in damaged tissue and neutralizes bacterial ammonia production which may induce further damage [16]. High osmolar honey (named Hygroscopicity) absorbs water out from the surrounding bacteria [17]; whereas glucose oxidase would be activated within more diluted honey, which further forms peroxide hydrogen. These two mentioned features are the most believed antibacterial mechanisms for honey [16,18]. Honey may also exert as an anti nociceptive factor by means of NMDA antagonism of peripheral GABA receptors with its Kynurenic acid (KA) derivatives, 3-pyrrolidinyl-kynurenic acid (3-PKA) and gamma-lactamic derivative (gamma-LACT-3-PKA), respectively [11]. It has also been shown that honey may prevent plaque formation and lessen gingival inflammation [19]. Usually it takes a few weeks to months for wound to be completely healed after intraoral surgeries. Besides, this lag time may be disturbing and prolonged in the cases of locally and systemic illnesses which decelerate surgical site healings especially after periodontal surgeries. Collectively, based on superb mentioned honey characteristics so better to be called *Liquid Gold* [20], we aimed to investigate honey plausible role in accelerating wound healing after periodontal surgery using concentrated Persian *Thymus Vulgaris* honey.

Methods

Eligibility Criteria and Randomization

We conducted a pilot single blinded (examiner and biostatistician) placebo controlled cross-over clinical trial investigating the safety, feasibility and efficacy of concentrated Persian *Thymus* honey for periodontal surgical site wound healing. Randomization performed using permuted block method with 4 intervals with 1:1 ratio in each arm prepared within closed envelopes. All referral patients to periodontology ward who required bilateral Modified Widman Flap (MWF) enrolled. Among those who had probing pocket depth (PPD) of 4- 6 *mm* included and excluded if they had systemic illness, Antibiotics consumption within last month, taking Corticosteroid or any honey products at the time of randomization, smoking during last 2 years and pregnancy. All patients approached regarding written informed consent and randomized participants provided with scaling, root planning (SRP) and oral hygiene instruction (OHI). Besides, routine laboratory tests and panoramic graphs were ordered for all of them. Finally, thirty two patients who required bilateral MWF surgery were assessed for eligibility and inclusion criteria to be enrolled in this study. A few participants were excluded by more than one exclusion criteria. They were mostly excluded due to Antibiotic consumption within last month.

Study Design and Protocol

After enrolment, written informed consent and randomization, all patients were subjected to receive either 15cc topical honey TDS on the site of the surgery or normal saline with the same amount and frequency (phase I). The patients were asked to apply honey with sterile micro brushes given to them in packages and to rinse normal saline. To avoid early elimination of honey, they also instructed to pick up the lips in the affected area for 5 minutes each time and not to suckle of the area during application of medicaments. The trial individuals were instructed to rinse 30 minutes after application of topical honey with 15cc normal saline to prevent probable provoked dental caries. Both group partici-

pants were asked to do not brush on the site of surgery for 7 days following surgery. They also instructed to do not eat or drink within 30 minutes after each application of topical honey. We designed our study as a 7-day trial or placebo treatment followed by a 35-day wash-out period and the study groups would be then crossed (phase II). This designation was decided as the healing process varies widely individually and thus each patient would serve as his or her own control. All patients underwent MWF on either jaw looked for recall having another surgery on the other side of the same jaw after predetermined period. They instructed to use only acetaminophen as an analgesic and not to use any other medications or oral rinses during the study period.

Assessments

At the beginning, each participant was thoroughly examined for oral health condition in terms of presence of any hard or soft tissue disorders in the mouth. Also they are asked to report any side effects assumed to be the consequence of active or passive treatment. Primary outcome was healing index changes and the secondary outcomes were gingival and plaque indices changes besides safety issues including any allergic reaction (itching, hyperaemia and inflammation) and disturbance in healing process (non healing wounds and wound dehiscence). At the day of the surgery, gingival and plaque indices were recorded and scheduled to be followed up in the days 1, 3 and 7 after surgery. Moreover, healing index measured at the days 1, 3 and 7 after surgeries. We performed our measurements using Loe & Sillness, Sillness & Loe and Landry indices for gingival, plaque and healing indices, respectively [21-23]. All surgeries and index recordings were performed solely by an experienced staff periodontologist in a blinded manner in order to lessen measurement biases. Institutional ethics committee assigned an external staff specialist to monitor the blindness and study protocol process and to insure about the safety issues which was also responsible to prepare randomized numbers. Besides, this person would be in charge to either suspend or request for unplanned sequential analysis in the case of unpredicted or frequent dangerous side effects.

Honey Preparation

Briefly, the *Thymus Vulgaris* honey which is mostly gathered from *Damavand* mountain in *Polour* area around *Lar* dam transported to Chashtgah Company (Shahdavaran engineering cooperation, Babolsar, Iran). They were examined to obtain minimum recommendations dictated by Iranian Standard Institute including humidity, reducing sugar, acidity, diasthetic function, fructose to glucose ratio, pH, mineral content and solid non-dissolved particles (table 1) [24]. Processing first started via mixing of the liquid honey with crystallized honey in a 1:9 ratio and then centrifuging with 350 *rpms* for 14 minutes. Further concentration achieved by means of refrigeration in 8-14 ºC for seven consequent days and underwent Gamma irradiation for sterilization [25]. More concentration was performed for more stability of honey sample on surgical site.

Statistics

The sample size of 28 (14 in each group) was calculated to provide a power of 80 percent and a maximum of 0.05 for type I error by two-sided test to detect a standardized difference of one and a half (based on an expert opinion). Continues data are expressed as mean (±SD) and nominal data showed as frequencies. A Kolmogrov Smirnov test was used to determine normal distribution of the continuous data. Considering the cross-over design for the study; carry-over effect (Treatment* time interaction), treatment effect and the time effect were evaluated. Data for the second period would be ignored if a significant carry-over effect observed [26]. Grouped continuous data were compared with T-test or One-way ANOVA where appropriate in which homogeneity of variances were tested with Levene test. There was Browns-Forsythe

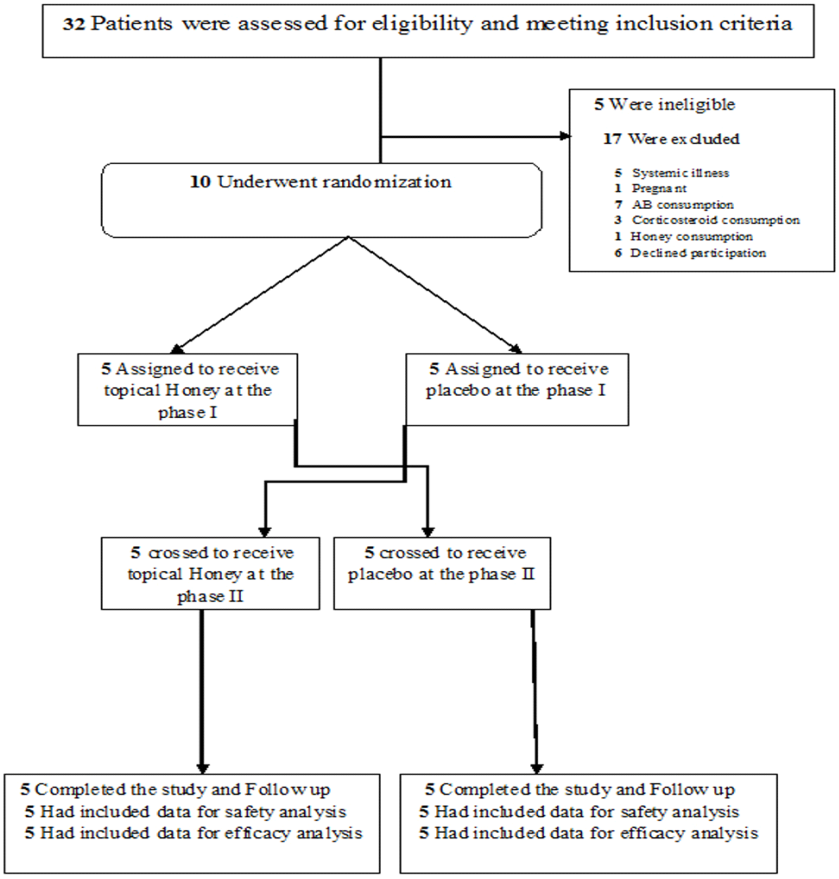

Fig.1. Study design from screening and randomization to completion of the trial.

adjustment in the case of homogeneity of variances violation (one of ANOVA statistics assumption). Comparison of less than three-dependent-scale variables performed with paired T-test, otherwise with repeated-measure ANOVA. Data would be interpreted with Greenhouse-Geisser adjustment in the case of sphericity violation (one of repeated measure ANOVA statistics assumption). A pooled data of trial or control individuals of each arm were computed in order to overall treatment effect comparison by means of repeated-measure ANOVA in the absence of carry-over effect. The authors declare no conflict of interest in relation to the work described. This study has been accepted by the ethics committee of Babol University of Medical Sciences and all researchers undertook Helsinki's treaty. This study was financially supported by the deputy of research, Babol University of medical sciences.

Results

Study population and demographic characteristics

Detailed patients randomization till study completion demonstrated in Fig. 1. Enrolment of the first 10 participants took about 23 months and External Data and Safety Monitoring Board (DSMB) recalled for an interim analysis to check for possible superiority, inferiority, harm or futility. The results established as an early termination rule due to superiority of new medicament to treat periodontal wound healing. A total of 10 participants were finally enrolled and 5 were randomized into each group. All participants were female with the age ranged between 35-40 *yrs* [36 (1.5±)]. Baseline characteristics of indices are shown in Table 2.

Table1. Sample honey measures and acceptance standard limits.

Characteristics	*Tested honey sample measures*	*Acceptance limits by Iranian Standard Institute*
Humidity	15	20
Reducing Sugars	85	Minimally 65
Sucrose	2.33	Maximally 5
pH	3.6	Minimally 3.5
Acidity(meq/kg free acids)	25	Maximally 40
Diasthehitic function	5	Minimally 3
Fructose/Glucose ratio	0.9	Minimally 0.9
Mineral content (%)	0.5	Maximally 0.6
non-dissolved solid particle	0.05	Maximally 0.1

Outcome measures

There was a significant improvement in wound healing considering time and treatment effect in both groups although faster wound healing observed in Honey treated group (Fig. 2). Due to the observed carry-over effect for gingival index, this parameter was evaluated and compared only at the phase I of the study before wash-out period. Regarding the case, data were not pooled for this index. As shown in Table 2, in both groups gingival indices were noticed to be improved by the time, though with the superiority of honey treated group. The results of paired T-test showed that by 1 day after the surgery, a significant rise in gingival indices were observed in both trial and control groups (p=0.03 and p=0.004, respectively). However, the indices were declined until day 3 which was only significant in trial group (p=0.03 and p=0.07, respectively) and with further insignificant decrease till day 7 *(*p=0.11 and p=0.47, respectively). On the other hand, and as it is illustrated in Fig. 3, the change-trend of gingival index in the phase I of the study was significant in both trial and control groups (demonstrated by repeated-measure ANOVA test p=0.008 and p=0.001, respectively). Both groups displayed aggravated plaque formation; nevertheless it was merely statistically significant in the control group (F (3,27) =12.88, p<0.001). The plaque indices in trial and control groups increased toward day 1 (p=0.19 and p=0.006 respectively) which continued to aggravate till day 3 (p=0.28 and P=0.54 respectively), along with final declination in both groups (p =0.002 and p= 0.13 respectively; Fig. 4). Effect size for outcomes calculated for

Table 2. Comparison of Healing Index, Gingival Index and Plaque index changing trends during the study in Trial (Honey) and control (Normal Saline) groups.

Index	*Intervention Group*	*Measurement schedule*				*Statistics*	*Mean Difference ($day_{0/1-7}$)*	*Estimated Effect size (95% CI)*
		Day 0	Day 1	Day 3	Day 7			
Healing Index	Trial		3.02(±0.65)	3.62(±0.42)	4.24(±0.35)	F(1.25,11.28)=23.52 P value<0.001[a]	1.22(0.73)	0.69(0.011 to 1.27)
	Control		2.61(±0.33)	2.85(±0.45)	3.14(±0.32)	F(2,18)=5.17 P value=0.12[a]	0.53(0.48)	
p value			0.09	0.001	<0.001	<0.001[b]	t(18)=2.5, P=0.02[e]	
Gingival Index	Trial [c]	1.54(±0.29)	2.02(±0.27)	1.61(±0.35)	1.31(±0.44)	F(3,12)=6.28 P value=0.008[a]	-0.26(0.38)	-0.68(-1.06 to -0.30)
	Control [c]	1.18(±0.19)	2.35(±0.38)	1.97(±0.34)	1.77(±0.54)	F(3,12)=12.46 P value=0.001[a]	0.42(0.42)	
p value		0.05	0.15	0.13	0.17	0.95[d]	t(18)=-3.81, P=0.001[e]	
Plaque Index	Trial	1.72(±0.44)	1.93(±0.56)	2.16(±0.44)	1.87(±0.50)	F(1.75,15.82)=2.14 P value=0.12[a]	0.15(0.63)	-0.38(-0.86 to 0.08)
	Control	1.58(±0.42)	2.23(±0.38)	2.32(±0.45)	2.23(±0.26)	F(3,27)=12.88 P value<0.001[a]	0.54(0.33)	
p value		0.47	0.17	0.44	0.19	0.34[b]	t(18)=-1.71, P=0.1[e]	

[a] Within group analysis, [b] Between group comparison of total change-trend by repeated measurement ANOVA test, [c] Data are not pooled in this table due to a significant carry-over effect, [d] Between group comparison of change-trend in phase I of the study by repeated measurement ANOVA test, [e] statistical significant difference between two groups compared for changing trends during study calculated by t-test.

mean difference of each index for either groups measured by mentioned schedule. Detailed descriptive and analytic data are shown in Table 2.

Safety Assessment

All wounds healed normally and no adverse events regarding wound healing disturbance and allergic reaction were noticed.

Discussion

In the work described, we assessed different honey characteristics which may apply in periodontology. For the very first time, honey superb healing accelerating properties in periodontal surgical flap was evaluated using topical concentrated Persian *Thymus Vulgaris* honey. In comparison to the control group, honey significantly augmented and accelerated the wound healing process besides its anti-inflammatory effects with notable improvement in gingival index. No allergic or other side effects related to honey application was recorded either objectively or subjectively. Our key outcome (wound healing) was in commitment with earlier studies which proved beneficial application of honey to promote and accelerate wound healing [1,3-5]. Since mechanical manipulation in surgical site may increase the inflammation, primary increased gingival indices were logical in both groups. Honey soon hampered this inflammation while the control group showed elevated gingival index till the final period of the intervention. This excellent honey property is in commitment with previous study by English et al in which honey proved to possess anti-inflammatory effect by about 30% reduction in gingival index [19]. This research displayed disparate antiplaque effect from English et al study. They figured out that Manuka honey reduced plaque index by 35% taking honey contained gums for a period of 21 days, three times a day [19]. This superficial observation may be challenged with more judicious look at the results. As previously shown both groups displayed increasing pattern of plaque formation during each study arm,

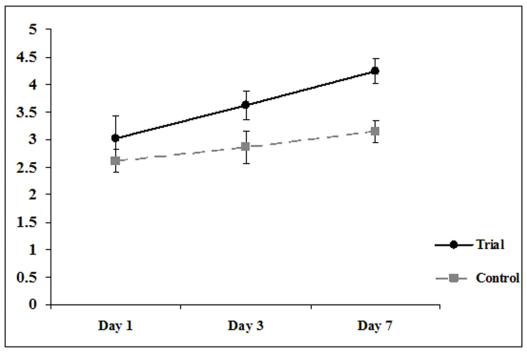

Fig. 2. Trend of changes in healing index after 7 days of study in trial and control groups (* Statistical significant difference between group comparison)

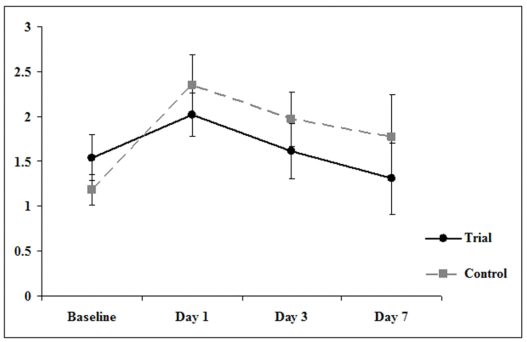

Fig. 3. Trend of changes in gingival index after 7 days of study in trial and control groups in phase I of the study (Data are not pooled in this figure due to a significant carry-over effect)

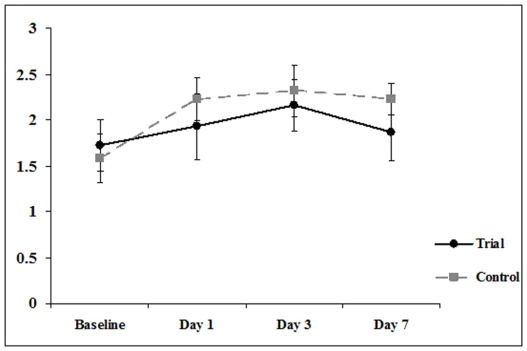

Fig. 4. Trend of changes in plaque index after 7 days of study in trial and control groups (None of the between group comparisons were statistically significant)

yet not significant rise was seen in honey treated groups [F(1.75,15.82)=2.14, p=0.12]. Our contradictory findings may be speculated with increasing trends, as the patients were asked not to brush for 7 consequent days after the surgery; hence anti plaque benefits of honey were hampered. Shorter period of our study (7 vs. 21 days) may explain more of these differences. All recruited

patients we within the range of 35-40 year-old and were female. No proven document exists upon gender- or age-dependent manner of wound healing, hence it seems that this uncontrolled bias had minimal effect on our outcome.

Hadika et al reported an inhibitory effect of honey on oral calcium phosphate precipitates [27]. This newly proposed anti-calculus characteristic of honey may even more expand the promising horizons of nutraceutics in modern medicine; in particular honey may be suitably honoured as *Liquid Gold* in current era [20]. To explain the mechanism and whether honey prevents calculus formation or it can also dissolve formed calculi, further research is necessary. The emergence of antibiotic or even multiple antibiotic resistant strains of certain bacteria has complicated the treatment of involved area with such microbes that sometimes are life threating. Honey as a natural product has been negotiated for various clinical relevance. Manuka honey is best known for its antibacterial activity possesses Unique Manuka Factor (UMF) [19]. There are some supporting evidences for the usage of honey even in doughy refractory venous leg ulcers or Epidermolysis Bollusa skin lesions not responding to conventional remedies [28-30]. Moreover various honey products like honey plus Royal jelly (exclusive queen nutritional source) may have improved anti-pseudomonal synergism [31]. A recent Cochrane metha-analysis demonstrated some benefits in favour of honey to treat superficial and partial thickness burn healing. These results do not support the recommendation for larger, deeper and/or other types of wounds to be cured solely with honey [1]. Based on brilliant properties of this bio- dressing, patients may benefit from honey to treat refractory or slough wounds as an adjuvant. Honey failed to cure chondrotitis as a deep tissue infection compared to mephenamic acid in another study from Iran [32]. It seems that plausible deficient anaerobic activity of honey and its products may rationalize failed results of some researches investigating the superiority or efficacy of honey to treat large or deep wounds [1,32,33]. More periopathogens oriented bactericidal activity culturing of various honey products from different honey types may indisputably reveals the blind spots for this contradiction. Beside previously demonstrated mechanism of antibacterial and antioxidant activities of honey, it has been also speculated that this properties may rise from endotoxin within honey and it is not related to monocyte excitation immunomodulatory pathways [34]. A future in vivo research upon changes of periodontal pathogens within plaques measured with the BANA test (N-benzoyl-DL-arginine-2-naphthylamide) may be a more precise index of periodontal disease activity [35]. Our study lacked concomitant biochemical assessment of inflammatory cytokine and further researches are necessary to clarify active biologic components of various Persian honeys, propolis and royal jelly in particular phenol compounds. Additionaly, with more precise tracing of growth factors such as Vessels Endothelial Growth Factor (VEGF) within whole saliva or Gingival Crevicular Fluid(GCF) is sugessted. Phenol and flavoniods compounds are widely responsible for different biological activities such as, antibacterial, antiviral, anti-inflammatory, anti-allergic, vasodilatory, antioxidant, anti-ulcerous and anti-calculus actions [36]. Moreover, our honey sample was not tested for exact measure of antioxidants in terms of MDA, catalase and superoxide desmotase (SOD) measures. In an unpublished very recent research, intentional mandibular defects were treated with a Persian honey at the surgical site in comparison to placebo in rats (unpublished data) and showed higher remineralisation and angiogenesis in the honey treated group in comparison to the placebo group. Besides the potential osteoclastic inhibitory effect of honey shown from a previous study [37], this novel outcome may strengthen the desire to apply honey in order to accelerate both soft and hard tissue wounds healing. However,

further researches with larger sample sizes and paraclinics aided reports are required. Pattern of wound healing may be adversely affected by advancing age besides common problems in elderly such as systemic illness (e.g. Diabetes) and local infections (e.g. Candida albicans).The lesser time between surgery and healing, the more convenient and desirable remedial course. Many of oral health problems (mainly derived by aging) need surgical interventions to some extent. These include crown lengthening for fixed restorations, implantology and oral biopsies and cancers. Such oral rehabilitation may shorten the remedial courses especially in aged group and whom the natural process of healing is not satisfactory. Therefore, it improves quality of life and lessen disability adjusted life year (DALY) and years of life lived with disability (YLD).

Conclusion

It may be concluded that honey is a safe and low cost medicament and may feasibly apply to promote wound healing for intra oral surgeries, especially in whom underlying systemic and local diseases that further disturb healing processes.

Putatively, this relative newly approached remedy with ancient background is recommended.

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