Research Article

Anti-inflammatory effects of manuka honey on salivary cytokines (clinical study)

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Abstract: Background: Manuka honey (MH) is a mono-floral honey derived from the Manuka tree (Leptospermum scoparium). MH is a highly recognized for its non-peroxide antibacterial activities, which are mostly related to its unique methylglyoxal content (MGO) in MH. The beneficial phytochemicals in MH is directly related to their favorable health effects, which include wound healing, anticancer, antioxidant, and anti-inflammatory properties. Aims: The purpose of this study was to evaluate the effect of MH on pro-inflammatory cytokines (IL-8 and TNF-α) in patients with gingivitis and compare it with chlorhexidine (CHX) and distilled water (DW). Materials and Methods: This study was a randomized, double blinded, and parallel clinical trial. Forty-five young participants aged (20–40) years were randomly selected and allocated into three groups: MH, CHX, and DW mouthwash groups. Each participant was given a random bottle. Five milliliters each of honey-based mouthwash formulation, CHX mouthwashes (0.2%) and DW were used twice daily for 21 days. All the participants were examined twice, once on the zero day (base line) and once after 21 days. Before and after each participant's mouthwash use IL-8 and TNF-α were measured using enzyme-linked immunosorbent assay (ELISA). Results: The results revealed a drop in the level of interleukin-8 in the manuka honey group which was statistically significant, but the decrease in the same biomarker in the chlorhexidine group was insignificant statistically. TNF-α levels were found to be insignificantly reduced in both the MH and CHX groups ($P>0.05$). The DW group, on the other hand, obtained the opposite outcome in both biomarkers. Conclusion: Mouthwash containing MH had an anti-inflammatory impact, indicating an immunomodulatory action. These signs may be encouraging and promising for the use of MH in treating gingivitis.

Keywords: Manuka honey, interleukin-8, tumor necrosis factor -α, gingivitis.

Introduction

Biofilm-induced gingivitis is the most common periodontal condition affecting mankind, with high potential to progress into more destructive periodontal disease if not managed at early stages. Although gingivitis is considered as the simplest form of periodontal disease, it is relatively easy to reverse. However, treatment of this condition is crucial as a preventive measure against more progressive periodontal disease, hence preserving the periodontal support and minimizing the need for more complicated and costly treatment [(1)]. Cytokines are small, low molecular weight, soluble proteins that are produced in response to an antigen and function as chemical messengers for regulating the innate and adaptive immune systems [(2)]. Pro-inflammatory cytokines and chemokines, comprising IL-1, IL-6, IL-8 and TNF-α create an environment that helps disease progression. These cytokines and chemoattractant are secreted by immune regulatory cells, tumor cells, tumor-associated macrophages, and stromal cells [(3)].

TNF-α has the potential to stimulate the production of secondary mediators, including chemokines or cyclooxygenase products, which consequently amplifies the degree of inflammation [(4)]. Recently TNF-α regarded as the most important cytokine in the periodontitis pathogenicity could be explained by their role in the destruction and erosive reaction of periodontal tissue, It was manifested, there was none cor-

relation among concentration of salivary TNF-α and explanation for the chronic, degenerative changes, like indices of plaque and gingival in addition to the probing pocket depth, therefore, TNF-α considered as a good indicator inflammatory process [5]. IL-8 is a cytokine /chemo attractant protein/chemokine which is produced by a variety of immune inflammatory cells in response to inflammation. IL-8 functions primarily to activate neutrophils, and plays a role in PMN recruitment to the inflammatory site. The unique coordinated expression of IL-8 facilitates the transit of neutrophils from the highly vascularized gingival tissue to the gingival crevice [6]. It is known that IL-8 plays a role in angiogenesis by stimulating the formation of new blood vessels through inducing the proliferation of endothelial cells [7]. Microorganisms and toxins in periodontal tissues stimulate the formation of IL-8, which induces a signal for the collection of neutrophils in local sites. IL-8 is identified to be a key molecule in the localization, collection and activation of neutrophils [8]. Because of its pro-inflammatory and neutrophil chemotactic properties, IL-8 may play a significant role in the pathogenesis of periodontitis [9].

The European Workshop on Periodontology in 1996 defined, the agents used in chemical supragingival plaque control as antiplaque, anti-gingivitis, plaque reducing, and antimicrobial agents. All of them have been shown to benefit gingivitis by altering the quantity/quality of plaque [10]. CHX is one of the most effective antimicrobial agents for plaque control and anti-inflammatory. but unfortunately have several adverse effect, the serious side effects of CHX are most often associated with its' prolonged use are: oral mucosa ulcer, white patches or sores and desquamative lesions, swelling of salivary glands, signs of an allergic reaction which may include difficulty in breathing or swelling of face, lips, tongue and throat [11].

Manuka honey contains a high level of potent antioxidant phenolic compounds i.e., flavonoids, methyl syringate, methoxylated benzoic acid, and syringic acid as well as non-phenolics including vitamin C, vitamin E, and β-carotene. Methyl syringate in MH is known to possess scavenging activity against superoxide anion radical, and to protect middle-aged Sprague-Dawley rats from oxidative damage after 30 days of daily supplementation confirming MH antioxidant activity. MH has been used as an anti-inflammatory remedy since ancient times, mostly attributed to its phenolics [12]. Moreover, MH improved cellular respiration and glycolysis via the activation of phosphorylated 5′ AMP activated protein kinase (p-AMPK), sirtuin 1, and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) which protect macrophage cells from LPS [13].

Interestingly, MH was found to decrease inflammation in LPS- or fMLP-(N-formylmethionine-leucyl-phenylalanine, chemotactic peptide)-treated neutrophils through the inactivation of nuclear factor-κB (NF-κB) signaling pathway and the reduction in superoxide release. Moreover, the recruitment of neutrophils to fMLP was decreased indicating the beneficial use of MH in the treatment of wounds and suggestive for its dual effect of antimicrobial and anti-inflammatory actions [14]. The purpose of this study was to evaluate the effect of MH on pro-inflammatory cytokines (IL-8 and TNF-α) in patients with gingivitis and compare it with CHX and DW.

Materials and Methods

Experimental Design: This study was a randomized, double blinded parallel clinical trial. It was carried out at the Department of basic sciences, College of Dentistry, University of Baghdad, between November 2021 and April 2022. The study was approved by the ethical committee at Dentistry College/ University of Baghdad (Project no 381821 in November 21-2021).

Subjects: 45 people with gingivitis were included in this study. Each of the three groups had 15 participants. The participation of each participant in the three study groups was randomly assigned. The first group included 15 people who used MH mouthwash; the second group included 15 people who used mouthwash with 0.20 percent CHX; and the third group included 15 people who used a placebo (DW) mouthwash. All participants evaluated were asked to complete a systematic questionnaire containing questions about their age, gender, and purpose for attending the dental clinic. In addition, the subject's medical history and past periodontal treatment history were documented.

Sample Size: The sample size was determined using G power 3.1.9.7 (Program written by Franz-Faul, Universitatit Kiel, Germany), with a 80 % power of study, an alpha error of probability of 0.05, a correlation between time points of 0.5, an effect size of F of 0.2526 (medium effect size), three major groups, and two time points.

Inclusion criteria: Participants in this research who were deemed eligible must have satisfied the following criteria: Subjects aged 20 to 40 years old, in good overall health, with more than 20 teeth, and gingivitis patients. Exclusion criteria included active cavity caries and/or periodontal disease, ongoing orthodontic treatment, a history of antibiotic intake within the previous 4 months, the need for prophylactic antibiotic coverage, the need for systemic and/or topical nonsteroidal anti-inflammatory drugs within the previous 4 months, pregnant and lactating mothers, having heart valve replacement and/or any systemic disease, smokers, and using mouthwash within the previous month.

Saliva Sample Collection**:** Unstimulated saliva samples were taken for study participants between 9:00 am and 1:00 pm, samples were collected before oral examination using a spit technique [(15)]. Then a letter and a number (before B and after A) were assigned to the sample. Each participant was given a plastic cup and instructed to allow saliva flow into the cup for five minutes in a quiet. After collection, the samples were immediately centrifuged at 3000 rpm for 10 minutes and the resulting supernatant were stored at -80˚C in eppendorf tubes until further immunological analysis by ELISA.

Oral examination**:** Periodontal health status was recorded through the examination of clinical periodontal parameters (PLI and BOP) by using a periodontal probe of William's graduation [(16, 17)].

Preparation of MH mouthwash: Based on a prior study done in New Zealand, a Manuka honey (514+ MGO; 15+UMF) mouthwash was created [(18)]. The dilution ratio was (1:3) (V/V) percent, which means that for every 250 ml of MH, there was 750 ml of DW. The solutions MH, CHX, and DW were placed in similar canisters to prevent prejudice between them. The examiner was provided a number-coded intervention sequence list created by a random number generator (Microsoft Excel 2016) to allocate the blinded intervention. Because of this, everyone had the same chance of being put in the intervention sequence.

Randomization and Blinding: This trial was double blinded in which examiner and participants were unable to identify the corresponding intervention. Decoding was done at the end of the study. The process of randomization, blindness and intervention allocation were carried out as described in a previous study [(19)]. Simple randomization and coding were performed by a third party not involved in the study. In order to ensure blinding of both participants and the examiner, the mouthwashes were dispensed into identical white opaque bottles, measuring 300 ml and each mouthwash group was assigned a random sequential letter (A, B, C) and decoding was performed at the end of the study. Microsoft excel (Microsoft Office 2016, Microsoft Corporation, USA) was used to generate random numbers that were used to resort the order of the mouthwash groups (A, B, C) and the participants (N=45) so that each group was received equal number of participants (N=15) with 1:1:1 allocation. Then, the coded bottles with the mouthwashes were given to the participants together with instructions of use.

Oral Hygiene Education: Each participant was given oral hygiene instructions. The examiner instructed the participant to brush and paste his / her teeth twice a day, and after finishing brushing for 15 minutes, all the participants were instructed to rinse twice daily (every 12 hours) with 5 ml of the assigned mouthwash (undiluted) for 30 seconds. They were also provided with measuring cups with 5 ml marking in order to use the correct volume of mouthwash.

Clinical Trial: Participants attended the dental clinic twice during the study period: once on the zero day (baseline) and once after 21 days. An ELISA test for IL8 and TNFα was performed on each participant before and after they used mouthwash.

Measurement of Salivary Cytokines**:** TNF-α and IL-8 concentrations in saliva were quantified using an ELISA according to the instructions in the kit's instruction booklet (Shanghai, China).

Calibration

A single examiner recorded all participants' PLI and BOP scores. The calibration of the scoring system in particular was carried out under the supervision of a senior periodontist. The examiner and supervisor reviewed the protocol, the case report form, and the PLI and POB criteria one week before the clinical calibration session.

1-Inter-examiner assessment: The all surface of all teeth except the third molars for BOP and two surfaces except third molar and caries or tooth with filling for PLI were examined by the examiner and the supervisor for five randomly selected subjects.

2-Intra-examiner assessment: The all surface of all teeth except the third molars for BOP and two surfaces except third molar and caries or tooth with filling for PLI for five subjects were examined and repeated after two hours by the same examiner.

Statistical analysis: Data description, analysis and presentation have been performed using computerized software statistical package for social science (SPSS version-22). Shapiro Wilk test was used to test the normality distribution of the quantitative variable. One-way analysis of variance (ANOVA) parametric test was used to determine and find difference between 3 or more independent groups, and Tukey honestly significant difference (HSD)/post hoc test was used to determine if the relationship between two sets of data is statistically significant. Paired t-test was used to compare the variables at baseline and after using. The percentage change quantifies the change between the old value and the new one and expresses the change as an increase or decrease.

Results

The mean age of the MH mouthwash group was 30.94 ±7.38 years, the CHX group was 30.80 ±8.32 year, and the DW group was 32.4±5.76 years. Distribution according to sex, MH group male was 9 (39.1%) and female was 6 (27.3%), CHX group male was 5(21.7%) and female was 10(45.5%), whereas, DW group male was 9(39.1%) and female was 6(27.3%). In the MH group, 6 (40%) were in high school and 9 (60%) were post-high school, In the CHX group, 6 (40%) were in high school and 9 (60%) were post-high school, while in the DW group, 7 (46.7%) were in high school and 8 (53.3%) were post-high school. The distribution of employment is as follows: In the MH group, 10 (66.7%) were employees and 5 (33.3%) were not. In the CHX group, 9 (60%) were employees and 6 (40%) were not, while in the DW group, 7 (46.7%) were employees and 8 (53.3%) were not, as shown in table (1)

Table 1: Demographic characteristic of participants in three groups of the study.

Demographic characteristics	**study groups** MH n=15	CHX n=15	DW n=15	***P*-value**
Age (years)				
Range	(20-40)	(20-40)	(22-40)	0.830^{NS}
Mean ± SD	30.9±7.38	30.8±8.32	32.4±5.76	
Gender				
Female, f (%)	6 (40)	10 (67)	6 (40)	1.000^{NS}
Male, f (%)	9 (60)	5 (33)	9 (60)	
Education				
High school, f (%)	6 (40)	6 (40)	7 (47)	0.182 ^{NS}
Post high school, f (%)	9 (60)	9 (60)	8 (53)	
Employment				
Employee, f (%)	10 (67)	9 (40)	7 (47)	0.528 ^{NS}
Non-employee, f (%)	5 (33)	6 (60)	8 (53)	

f: frequency, %: percentage, MH: manuka honey; CHX: chlorhexidine; DW: distilled water

Tumor Necrosis Factor-Alfa

Tumor necrosis factor-α levels were measured in the study groups at baseline and after 21 days, as shown in table (2) and plotted in figure (1). The mean salivary level of TNF-α in the MH group was (22.29±9.57) at baseline and (19.28±7.15) at 21 days, with a (13%) percentage change that was non- significant (P>0.05). The CHX group's mean was (19.17±8.07) at baseline and (16.26±5.91) at 21 days, the reduction was statistically insignificant (p<0.01) with a (15%) percentage change as seen in figure (3-6). While the DW group's mean was (19.43±5.57) at baseline and (20.71±8.25) at 21 days, the mean was raised and statistically insignificant (P>0.05) with a (6%) percentage change in the negative direction. The TNF-α change among all groups of study between two-time intervals; the mean difference was statistically insignificant (P>0.05).

Table 2: mean value of TNF-α in study groups before and after mouthwash use.

TNF-α	Study groups MH n=15	CHX n=15	DW n=15	ANOVA (*P*-value)
Before				
Range	(4.83-41.35)	(9.00-33.57)	(12.35-29.30)	
Mean± SD	22.29±9.57	19.17±8.07	19.43±5.57	0.717^{NS}
After				
Range	(7.57-33.77)	(3.75-24.22)	(5.17-32.97)	
Mean± SD	19.28±7.15	16.26±5.91	20.71±8.25	0.233^{NS}
T-test (*P*-value)	0.169^{NS}	0.135^{NS}	0.310^{NS}	
Percentage change	13% (decrease)	15% (decrease)	6% (increase)	

MH: manuka honey; CHX: chlorhexidine; DW: distilled water.

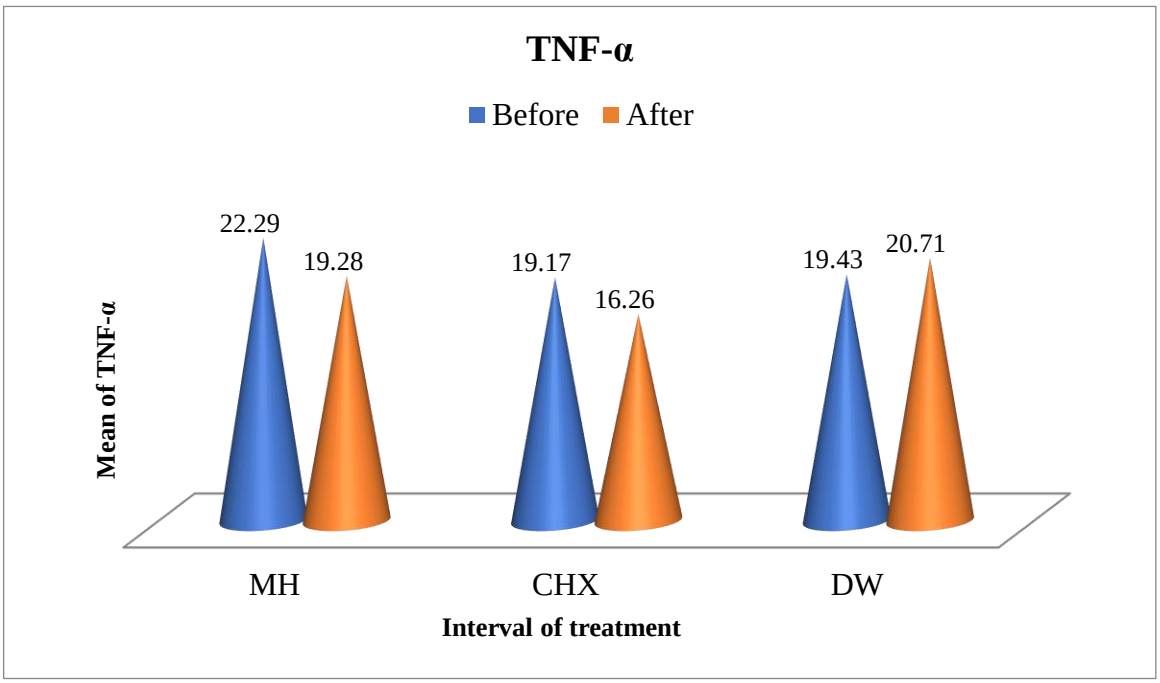

Figure 1: Mean values of TNF-α in study groups before and after mouthwash use

Table (3) illustrates the mean difference and intergroup comparisons of mean values of TNF-α for all pairs of groups at day 21. Statistically, the comparisons between all groups were non-significant (P>0.05).

Table 3: Intergroup comparisons of mean values of TNF-α between all pairs of groups after mouthwash use.

Grouping	Mean difference	Tukey's HSD (*P*-value)
TNF-α		
MH vs. CHX	3.02	0.487^{NS}
MH vs. DW	1.43	0.848 ^{NS}
CHX vs. DW	4.45	0.217^{NS}

MH: manuka honey; CHX: chlorhexidine; DW: distilled water.

The present findings revealed that the salivary mean level of IL-8 in MH group was (53.23±13.80) at baseline and (42.88±12.53) at 21 days, the reduction was statistically significance (P<0.05) with a (19%) percentage change. The mean of IL-8 in CHX group was (45.79± 9.61) at baseline and (43.42±11.89) at 21 days, the reduction was statistically not significance (P>0.05) with a (5%) percentage change. While the mean in the DW group was (39.69± 10.42) at baseline and (49.30±12.37) at 21 days, the mean was increase and statistically significance (P<0.05) with (24%) percentage change in negative direction. The IL-8 change among all groups of study between two-time intervals; the mean difference was statistically non- significant (P>0.05), as described in table (4) and figure (2).

Table 4: Mean value of IL-8 in study groups before and after mouthwash use.

IL-8	Study groups MH n=15	CHX n=15	DW n=15	ANOVA (*P*-value)
	Before			
Range	(25.24- 69.54)	(31.43- 62.04)	(22.90- 59.62)	
Mean± SD	53.23± 13.79	45.79± 9.61	39.69± 10.42	0.008**
	After			
Range	(24.55-65.28)	(17.24-62.18)	(32.27-71.95)	
Mean± SD	42.88±12.53	43.42±11.89	49.30±12.37	0.293^{NS}
T-test (*P*-value)	0.020*	0.276^{NS}	0.014*	
Percentage change	19% (decrease)	5% (decrease)	24% (increase)	

MH: manuka honey; CHX: chlorhexidine; DW: distilled water.

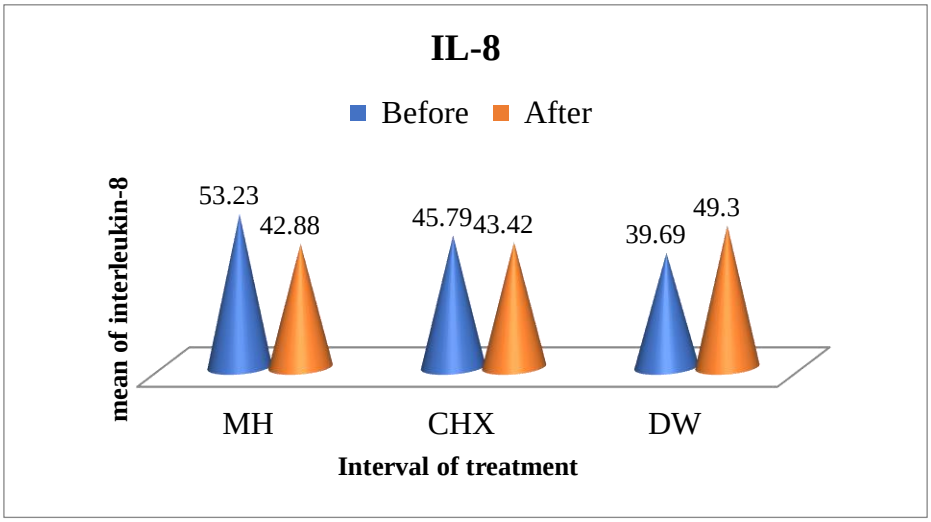

Figure 2: Mean values of IL-8 in study groups before and after mouthwash use.

Additionally, table (5) shows the mean difference and intergroup comparisons of IL-8 mean values at day 21 for all pairings of groups. Statistically, all group comparisons were insignificant (P>0.05).

Table 5: intergroup comparisons of mean values of IL-8 between all pairs of groups after mouthwash use.

Grouping	Mean difference	Tukey's HSD (*P*-value)
	IL-8	
MH vs. CHX	0.54	0.992^{NS}
MH vs. DW	6.42	0.333 ^{NS}
CHX vs. DW	5.88	0.395^{NS}

MH: manuka honey; CHX: chlorhexidine; DW: distilled water.

Discussion

Regarding salivary IL-8, the findings of this study showed a decline in the salivary level of IL-8 in the MH mouthwash group before and after use, and the difference was statistically significant. This reduction in IL8 levels was seen in the MH group because MH contains high levels of phenolic acid and flavonoids, which are the two most powerful antioxidants and anti-inflammatory agents by quenching reactive free radicals, which are directly related to the hydroxyl level of both compounds and are thought to be responsible for lowering IL8 levels [20].

It is noteworthy that the effect of MH mouthwash on IL-8 levels in gingivitis patients has not been examined before, however there are other studies in which the effect of MH on IL-8 levels has been measured in another kind of inflammation. Keenan et al. investigated the possibility of using MH internally to treat gastritis caused by Helicobacter pylori. It showed significant activity and enhanced the anti-inflammation impact of isothiocyanate-rich broccoli sprouts. Reduced cytokine production (e.g., IL-8 release from macrophages), induced ROS generation, and/or activity in stomach mucosal tissue are all possible. Additionally, cyclodextrin-encapsulated MH has been found to decrease inflammation by inhibiting neutrophil TNF release [21].

On the other hand, the CHX mouthwash also showed decrease in the mean level of salivary IL-8 but not statistically significant, this is consistent with previous research on the effect of CHX in lowering IL-8 levels in gingivitis patients. Türkoğlu et al. study found that using CHX mouthwash for four weeks reduced the level of IL-8 in gingivitis patients [22]. This does not imply that CHX is ineffective against this biomarker; the cause might be that the research sample was small, the participant did not fully comply

with the instructions, or the participant experienced other inflammation throughout the clinical study. In a comparison of MH with CHX mouthwashes, the present findings revealed that MH mouthwash had the highest effect in lowering IL-8 levels when compared to CHX mouthwash. Moreover, these results revealed that there was no statistically significant difference between MH mouthwash and CHX in reducing of IL-8 levels at day 21.

Tumor necrosis factor-α is a pro-inflammatory cytokine with several immunoregulatory roles. TNF has the ability to trigger the creation of secondary mediators such as chemokines or cyclooxygenase products, which increases the degree of inflammation [23]. TNFα and IL-8 are secreted by the same cell types, they frequently interact, and many of their pathways are similar, including inflammatory bone resorption. In the development ofa Th17 response TNF-α and IL-1β are found to amplify the response induced by TGF-β and IL-6, but unable to replace any of these cytokines [24].

In the current study, salivary level of TNF-α was decrease in the MH and CHX mouthwash groups but the difference from baseline was not statistically significant. This can be attributed to the small sample size, compliance to instruction, and may be the participant have another type of inflammation.

As with the IL-8, no clinical studies have been performed on the efficacy of MH on TNF-α in patients with gingivitis. However, several research (in vivo and in vitro) have been conducted to investigate the effect of honey or its constituents on TNF expression. In an RCT study done by Tartibian and Maleki observed an increase in IL-1, IL-6, IL-8, and TNF- in male road cyclists who did not consume honey compared to the group that consumed 70 g of honey for 8 weeks. Their findings indicate that honey intake during long-term exercise can lower seminal plasma cytokines while increasing antioxidant levels [25]. Moreover, Ahmed colleagues' study was performed on the Sprague-Dawley rats, and the results showed that systemic administration of Tualang honey (TH) and MH increases the susceptibility to expression of proapoptotic proteins (Apaf-1, Caspase-9, IFN-γ, IFNGR1, and p53) and decreases the expression of antiapoptotic proteins (TNF-α, COX-2, and Bcl-xL 1) in its mechanism of action. It suggests that, TH and MH may have a novel role in alleviating breast cancer [26]. Other authors, however, have indicated that manuka and other types of honey can reduce TNF-α expression in vitro studies (Candiracci et al., 2012; Song et al., 2012; Majtan et al., 2013; Safi et al., 2016; Al-Abd et al., 2017) [27].

Similarly, Majtan et al. investigated the influence of flavonoid content in honey on inhibiting TNF-α-induced MMP-9 expression in human keratinocytes, and the results showed that flavonoid content in honey suppressed TNF-α-induced MMP-9 expression in immortalized human keratinocytes (HaCaT) [28].

All of the previous investigations support findings that MH has the ability to lower the amount of TNF-α. Honey's anti-inflammatory effect may be explained by several mechanisms of action, including; inhibition of the classical complement pathway, inhibition of reactive oxygen species formation [23], inhibition of leucocyte infiltration [29] and inhibition of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase expression (iNOS) [30].

Honey's anti-inflammatory action is mostly attributed to phenolic constituents, especially flavonoids. Chrysin has been demonstrated to be an effective anti-inflammatory chemical. It reduces the production of NO and pro-inflammatory cytokines such as TNF-a and IL-1b and inhibits LPS-induced COX-2 expression by inhibiting nuclear factor for IL-6 DNA-binding activity [30, 31].

On the other hand, the third (placebo) group in both biomarkers showed negative results. This is a logical finding given that DW lacks anti-inflammatory properties like MH and CHX.

Conclusion

Manuka honey revealed an anti-inflammatory activity by significantly decreasing IL-8 levels. It also decreased TNF levels. All of the evidence points to MH being a safe agent that might be used as a component in mouthwash formulations. To promote oral health, this study recommend utilizing MH. According to the findings of thisstudy, IL8 might be a useful immunological marker for gingivitis.

Conflict of interest: The authors have disclosed no potential conflicts of interest.

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العنوان: تقييم آثار عسل مانوكا على IL8 و TNFα (دراسه سريرية)

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المستخلص:

الخلفية: عسل مانوكا (**MH**) هو عسل أحادي الأزهار مشتق من شجرة مانوكا **MH .(Leptospermum scoparium)** معترف به بشكل كبير لأنشطته غير البيروكسيدية المضادة للبكتيريا، والتي ترتبط في الغالب بمحتواها الفريد من ميثيل جليوكسال (**MGO**) في **MH**. ترتبط المواد الكيميائية النباتية المفيدة في **MH** ارتباطًا مباشرًا بآثارها الصحية الإيجابية ، والتي تشمل التئام الجروح ، ومضادات السرطان ، ومضادات الأكسدة ، وخصائصها المضادة للالتهابات. الأهداف: الغرض من هذه الدراسة هو تقييم تأثير **MH** على السيتوكينات المؤيدة للالتهابات (**IL-8** و **TNF-α**) في مرضى التهاب اللثة ومقارنتها بالكلورهيكسيدين (**CHX**) والماء المقطر (**DW**). المواد والطرق: كانت هذه الدراسة عبارة عن تجربة سريرية عشوائية مزدوجة التعمية ومتوازية. تم اختيار خمسة وأربعين مشاركًا شابًا تتراوح أعمارهم بين (20-40) عامًا بشكل عشوائي وتم تقسيمهم إلى ثلاث مجموعات: مجموعات غسول الفم **MH** و **CHX** و **DW**. تم إعطاء كل مشارك زجاجة عشوائية. تم استخدام خمسة مليلتر من كل من غسول الفم القائم على العسل وغسول الفم **CHX** (0.2٪) و **DW** مرتين يوميًا لمدة 21 يومًا. تم فحص جميع المشاركين مرتين ، مرة في يوم الصفر (خط الأساس) ومرة بعد 21 يومًا. قبل وبعد استخدام غسول الفم لكل مشارك تم قياس **IL-8** و **TNF-α** باستخدام مقايسة الممتز المناعي المرتبط بالإنزيم (**ELISA**). النتائج: أظهرت النتائج انخفاضًا في مستوى **interleu-kin-8** في مجموعة عسل مانوكا كان ذا دلالة إحصائية ، لكن الانخفاض في نفس المرقم الحيوي في مجموعة الكلورهيكسيدين كان ضئيلًا من الناحية الإحصائية. تم العثور على أن مستويات **TNF-α** انخفضت بشكل ضئيل في كل من مجموعات **MH** و **CHX (P>0.05)**. من ناحية أخرى ، حصلت مجموعة **DW** على النتيجة المعاكسة في كل من المرقمات الحيوية. الاستنتاج: غسول الفم الذي يحتوي على **MH** له تأثير مضاد للالتهابات ، مما يشير إلى وجود تأثير مناعي. قد تكون هذه العلامات مشجعة وواعدة لاستخدام **MH** في علاج التهاب اللثة.