Comparative Clinical Study Of Standardized Gel Of Salvadora Persica Extract Containing 0.8% Benzyl Isothiocyanate Vs. 0.8% Hyaluronic Acid Gel As An Adjunct To Conventional Periodontal Treatment.

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Abstract:
Background: *Periodontitis is a multifactorial disease in which putative periodontal pathogens trigger chronic inflammatory and immune responses because of the large numbers of bacteria residing in subgingival sulcus in the presence of multiple risk factors.*
The local administration of adjunctive antimicrobial agents to the periodontal pockets showed better clinical improvements, especially in the sites that did not respond to mechanical periodontal therapy alone. A study reported on the effectiveness of local delivery antimicrobial agents (LDAs) in the treatment of periodontitis with the advantages of direct delivery to the site of the infection, reduction in patient complaints, and avoidance of systemic side effects of the antimicrobial agent.
In this study we compared the effect standardized methanolic extract of salvadora persica(sME-SP) (containing 0,8% benzyl isothiocyanate) gel with 0,8% hyaluronic acid gel in improvement of clinical periodontal parameter (plaque index, bleeding index, clinical attachment gain and probing depth) .
Material and method: *In this study a total number of twenty patients were diagnosed with stage I and stage II periodontitis according to new Classification of Periodontal and Peri-Implant Diseases and Conditions. Each patient received non-surgical periodontal treatment (scaling and root planning) and (sME-SP) on one side of his mouth and hyaluronic acid gel on the other side as (split mouth design). clinical periodontal parameters were assessed at baseline and 6 weeks after gel application.*
Result: *There was a statistically significant difference before and after treatment in periodontal clinical parameters (P<0.0001) in both groups but without significance difference between SP an HA group before and after treatment.*
Conclusion: *Both treatment modalities SP and HA helped in reducing periodontal inflammation in conjunction with full mouth debridement.*
Keywords: *Salvadora persica, benzyl isothiocyanate, hyaluronic acid*

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I.   Introduction

Periodontitis is one of the world's most common chronic human diseases, and it has a significant impact on oral health [1]. It is a biofilm-induced inflammatory disease involving a complex interplay between the immune-inflammatory pathways and symbiotic ecological changes, leading to periodontal attachment loss[2] The primary etiological factor for periodontal disease is bacterial plaque on the tooth surface that leads to marginal tissue inflammation known as gingivitis. Gingivitis is a reversible condition cured by the mechanical removal of the biofilm and may develop to periodontitis when left untreated [3]. Periodontitis is a multifactorial disease in which putative periodontal pathogens trigger chronic inflammatory and immune responses as a result of the large numbers of bacteria residing in subgingival sulcus in the presence of multiple risk factors [2].

The local administration of adjunctive antimicrobial agents to the periodontal pockets showed better clinical improvements, especially in the sites that did not respond to mechanical periodontal therapy alone. A study reported on the effectiveness of local delivery antimicrobial agents (LDAs) in the treatment of periodontitis with the advantages of direct delivery to the site of the infection, reduction in patient complaints, and avoidance of systemic side effects of the antimicrobial agent [4].

Salvadora persica (SP) also known as miswak is described as one of the oldest tooth-brushing tools in history and is usually obtained from the roots of an Arak tree (Salvadora persica) [5]. It has been popular in countries which are home to Muslims predominantly as well as other countries due to its therapeutic potential[6].

The antibacterial property of *S. persica* is mainly attributed to one active component called benzyl isothiocyanate (BITC). It penetrates the outer bacterial membrane and interferes with the bacterial redox systems. BITC is reported to be active against Gram-negative periodontal pathogens such as *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*.[7]

The antibacterial and anti-inflammatory activities of hyaluronic acid led to its introduction as a local chemotherapeutic agent with several clinical therapeutic properties for the treatment of periodontitis [8]

subgingival administration of HA gel as adjuvant to SRD has significant anti-inflammatory effects with a potential role for the treatment of periodontitis through its significant positive impact on clinical periodontal parameters, pro-inflammatory cytokines, and biochemical marker improvement[9].

II. Material and method

A total number of twenty patients were diagnosed with stage I and stage II periodontitis according to new Classification of Periodontal and Peri-Implant Diseases and Conditions 2018. They were selected from the department of Oral Medicine and Periodontology clinic, Faculty of Dentistry, Mansoura University. Complete medical and dental histories were taken from all participants. They also clearly understood the purpose, steps, benefits, and hazards of the study, and signed an informed consent.

Study design: split mouth study.

Study location: the department of Oral Medicine and Periodontology clinic, Faculty of Dentistry, Mansoura University.

Sample size: 20.

Sample size calculation: Sample size calculation was based on mean TNF-α among studied groups treated with hyaluronic acid chlorhexidine retrieved from previous research [9]Using G*power[10] version 3.1.9.4 to calculate sample size based on effect size of 1.0592222, 2-tailed test , α error =0.05 and power = 90.0% then total sample size will be 20 in each group at least.

Inclusion criteria:
1. Stage I periodontitis (mainly horizontal bone loss with maximum probing depth ≤ 4 mm and with 1-2 mm clinical attachment loss) and Stage II periodontitis (mainly horizontal bone loss with maximum probing depth ≤ 5 mm and with 3-4 mm clinical attachment loss).
2. Systematically free from any diseases.

Exclusion criteria:
1. Patients with systemic conditions that affect the periodontal supporting tissue according to new classification of periodontal and peri-implant disease and condition 2018.
2. Chronic smokers.
3. previous periodontal therapy within 6 months.
4. Patients with stage III and stage IV periodontitis (mainly vertical bone loss with probing depth ≥ 6 and with ≥ 5 clinical attachment loss).

Procedure methodology:

Recording periodontal indices, measurements, and collecting GCF samples for all participating individuals were done at baseline. Patients were subjected to meticulous full mouth debridement using ultrasonic scalers and manual specific Gracey curettes. This step was repeated weekly if indicated. Patients were instructed to maintain good oral hygiene measures throughout the whole treatment period. standardized gel of salvadora persica extract containing 0.8% benzyl isothiocyanate was applied in periodontal pocket of the affected tooth and 0.8% hyaluronic acid gel was injected in the contralateral side (split mouth design). The procedure was repeated after one week. The application of the gel was done by using plastic syringe with a needle of a flexible large gauge to facilitate easy and effective gel delivery.

Flexible needle tip was inserted into the base of the pocket then the gel was injected while pulling the needle outward to ensure that the gel reached the entire pocket depth.

The injection started from the base of the pocket top ward slowly to ensure that the gel reached the entire pocket depth. Patients were reevaluated in terms of periodontal indices, measurements, 6 weeks of treatment completion.

All patients get instructions of oral hygiene measures and instructed to refrain from chewing hard and sticky foods throughout the treatment period.

Statistical analysis:

Data analysis was performed by SPSS software, version 25 (SPSS Inc., PASW statistics for windows version 25. Chicago: SPSS Inc.). Quantitative data were described using mean± Standard deviation for normally distributed data after testing normality using Shapiro Wilk test. Significance of the obtained results was judged at the (≤0.05) level. Student t test was used to compare 2 independent groups for normally distributed data. Paired t

test was used to compare 2 paired readings distributed data. The Pearsons correlation is used to determine the strength and direction of a linear relationship between two normally distributed continuous variables.
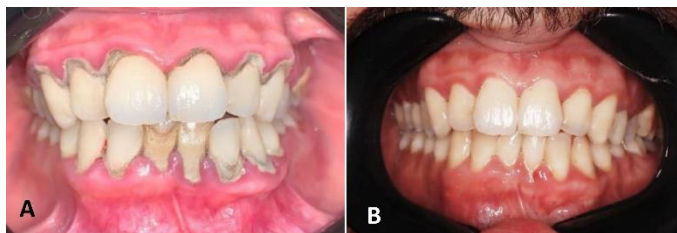

Figure (1): before and after scaling and root planning.

Figure (2): initial probing depth measurement on left side, initial probing depth measurement on right side.
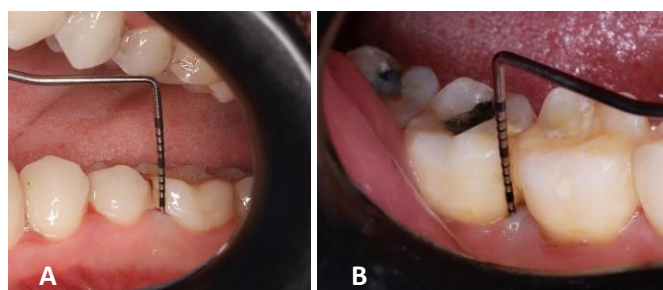

Figure (3): final probing depth measurement on left side, final probing depth measurement on right side.

III. Result:

Tables and charts:

Table (1): age of the studied cases

	Mean±SD (Min-Max)
Age / years	41.75±6.25 (26-53)

Table (1) shows that mean±SD age of the studied cases is 41.75±6.25 ranging from 26 to 53 years

Table (2): comparison of probing depth between studied groups before and after treatment

		Group 1 (right side) n=20	Group 2 (left side) n=20	test of significance (Student t test)
PD Mean±SD	Before	3.26±0.48	3.35±0.60	t=0.517 p=0.608
	After	2.46±0.65	2.43±0.61	t=0.157 p=0.876
Paired t test		t=7.89 p<0.001*	t=9.55 p<0.001*	

Table (2) shows that no statistically significant difference between studied groups as regard mean probing depth . For group 1; a statistically significant decrease is detected from 3.26±0.48 before treatment to 2.46±0.65 after treatment (p<0.001). For group 2; a statistically significant decrease is detected from 3.35±0.60 before treatment to 2.43±0.61 after treatment (p<0.001).
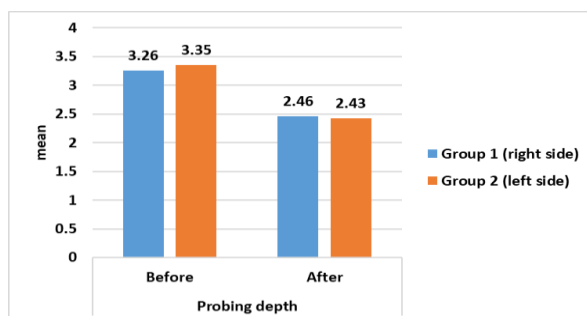
Figure (1): probing depth among studied sides before and after treatment.

Table (3): comparison of clinical attachment loss between studied groups before and after treatment

		Group 1 (right side) n=20	Group 2 (left side) n=20	test of significance (Student t test)
CAL Mean±SD	Before	2.68±0.57	2.84±0.56	t=0.923 p=0.362
	After	1.94±0.62	1.97±0.70	t=0.151 p=0.880
Paired t test		t=7.54 p<0.001*	t=6.79 p<0.001*	

Table (3) shows that no statistically significant difference between studied groups as regard mean clinical attachment loss. For group 1; a statistically significant decrease is detected from 2.68±0.57 before treatment to 1.94±0.62 after treatment (p<0.001). For group 2; a statistically significant decrease is detected from 2.84±0.56 before treatment to 1.97±0.70 after treatment (p<0.001).
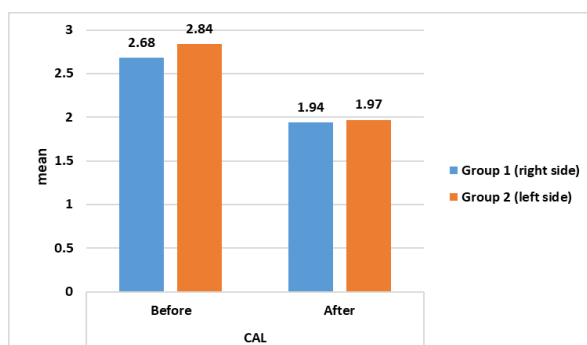

Figure (2): Clinical attachment loss among studied sides before and after treatment

Table (4): comparison of plaque index between studied groups before and after treatment

		Group 1 (right side) n=20	Group 2 (left side) n=20	test of significance (Student t test)
PI Mean±SD	Before	2.31±0.24	2.39±0.19	t=0.994 p=0.326
	After	1.09±0.36	1.07±0.34	t=0.235 p=0.815
Paired t test		t=13.19 p<0.001*	t=16.36 p<0.001*	

Table (4) demonstrates no statistically significant difference between studied groups as regard mean plaque index. For group 1; a statistically significant decrease is detected from 2.31±0.24 before treatment to 1.09±0.36 after treatment (p<0.001). For group 2; a statistically significant decrease is detected from 2.39±0.19 before treatment to 1.07±0.34 after treatment (p<0.001).
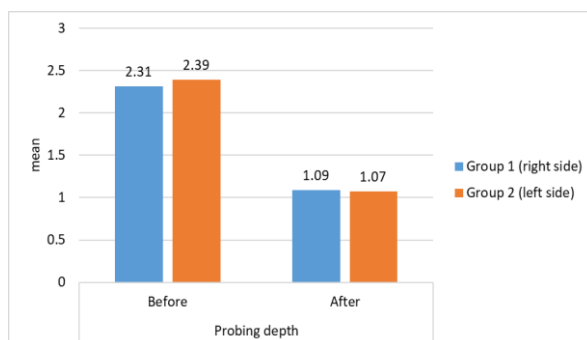
Figure (3): plaque index among studied sides before and after treatment

Table (5): comparison of bleeding index between studied groups before and after treatment.

		Group 1 (right side) n=20	Group 2 (left side) n=20	test of significance (Student t test)
BI Mean±SD	Before	2.61±0.18	2.65±0.13	t=0.899 p=0.374
	After	0.385±0.174	0.382±0.146	t=0.069 p=0.945
Paired t test		t=60.38 p<0.001*	t=83.01 p<0.001*	

Table (5) demonstrates no statistically significant difference between studied groups as regard mean bleeding index. For group 1; a statistically significant decrease is detected from 2.61±0.18 before treatment to 0.385±0.174 after treatment (p<0.001). For group 2; a statistically significant decrease is detected from 2.65±0.13 before treatment to 0.382±0.146 after treatment (p<0.001).
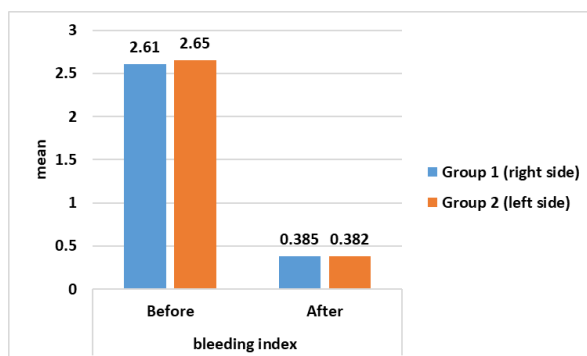

Figure (4): bleeding index among studied sides before and after treatment

IV. Discussion:

Periodontitis is a multifactorial disease in which putative periodontal pathogens trigger chronic inflammatory and immune responses as a result of the large numbers of bacteria residing in subgingival sulcus in the presence of multiple risk factors[11]

The local administration of adjunctive antimicrobial agents to the periodontal pockets showed better clinical improvements, especially in the sites that did not respond to mechanical periodontal therapy alone. A study reported on the effectiveness of local delivery antimicrobial agents (LDAs) in the treatment of periodontitis with the advantages of direct delivery to the site of the infection, reduction in patient complaints, and avoidance of systemic side effects of the antimicrobial agent[12].

Salvadora persica (SP) also known as miswak is described as one of the oldest tooth-brushing tools in history and is usually obtained from the roots of an Arak tree (Salvadora persica) [5]. It has been popular in countries which are home to Muslims predominantly as well as other countries due to its therapeutic potential[6].The antibacterial property of *S. persica* is mainly attributed to one active component called benzyl isothiocyanate (BITC). It penetrates the outer bacterial membrane and interferes with the bacterial redox systems. BITC is reported to be active against Gram-negative periodontal pathogens such as *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*.[7]

Hyaluronic acid, when introduced as a local chemotherapeutic agent, exhibited numerous clinical therapeutic properties and showed anti-inflammatory and antibacterial properties for the treatment of periodontal

disease[8]. HA demonstrates a protective role by limiting the damage that can occur during the inflammatory process. It is acknowledged for its antibacterial, antifungal, and anti-inflammatory effects in addition to its angiogenesis and osteoinductive properties that enhance wound healing in a variety of tissues of the human body, including the periodontal tissues[13].

Regarding our results, it has been showed that there was a significant difference in clinical and Laboratory assessment for all groups when comparing pre-treatment measurements vs post 6 weeks treatment measurements. While there was no significant difference in both clinical and Laboratory assessment after 6 weeks when comparing both treatment groups either salvadora persica with SRP or hyaluronic acid with SRP.

For group A, treated with salvadora persica gel contain 0.8% BITC in addition to SRP; there was a statistically significant improvement in pretreatment values compared to the post treatment values. This could be due to the anti-inflammatory and anti- oxidant activity besides the anti-bacterial activity of SP[14].

The clinical parameter (plaque index/bleeding index/probing depth/CAL) in this study there was a significant difference in group A treated with SP before and after treatment.

This results are agreed with study performed by *Niazi et al*[15]. who found A significant improvement in the BOP and PI was seen in the group treated with SP at both follow up visits when compared with other groups in their study (p < 0.05).

The results obtained regarding the reduction in the levels of bleeding on probing (BOP) and plaque index (PI) corroborate with the results of the previous studies. *Azaripour et al*.[5] reported significant reduction in plaque index levels where SP was used as a toothpaste ingredient. And according to *Agrawal et al*[16]there was a significant difference in probing depth after using meswak.

Regarding group (B) H.A group there was a significant difference between the clinical and laboratory values before and after treatment

Regarding the clinical parameters there was an improvement in (PI/BI/PD/CAL) before and after treatment with 0.8% hyaluronic acid gel application.

These finding came in accordance with *Mohammed et al*[9] showed in their study the effect of subgingival administration of 0.8% hyaluronic acid gel as an adjunct to SRP on periodontal parameters, pro-inflammatory cytokines and they found that These values significantly decreased from 1.74 ± 0.31 to 0.84 ± 0.21 in PI (p = 0.001), from 1.40 ± 0.24 to 0.92 ± 0.17 in GI (p = 0.001), from $51.60 \pm 24.72\%$ to $11.00 \pm 5.07\%$ in BOP (p = 0.001), from 4.16 ± 0.21 mm to 3.20 ± 0.25 mm in PPD (p < 0.001), and from 4.74 ± 1.05 mm to 3.74 ± 0.85 mm in CAL (p < 0.001).

Improvements have been observed in healing, periodontal indices, and clinical attachment level (CAL) following treatment with HA. In a split-mouth study, *Al-Shammari et al* [17]evaluated the effects of SRP + HA and SRP alone in patients with periodontitis (p < 0.05).

V. Conclusion
Both treatment modalities SP and HA are effective in reducing the periodontal clinical parameters before and after treatment without statistically significance difference between them.

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