Journal Homepage: - www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/14001
DOI URL: http://dx.doi.org/10.21474/IJAR01/14001

RESEARCH ARTICLE

A POSSIBLE ALTERNATIVE AND SAFE ADJUNCTIVE ROLE FORLOCALLY APPLIEDMANGIFERININ PERIODONTAL THERAPY; A CLINICAL AND MICROBIOLOGICAL STUDY

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Manuscript Info

Manuscript History
Received: 25 October 2021
Final Accepted: 29 November 2021
Published: December 2021

Key words:-
Mangiferin, Periodontitis, Alternative, Adjunctive, Periodontal Therapy

Abstract

Background: Periodontal health has great impact on overall oral conditions which reflects subject's health. With the radical change that happened to human diet in the past several decades specially the wide spread of finely processed diet over the raw complex diet, it led to increase in several health problems which among them is the periodontal diseases. The periodontal therapy had been relaying on oral hygiene where a lot of researches where implied on adjunctive products which it could promote tissue healing after scaling and root planning as the prim treatment for such conditions. Many of these products showed side effects on long term use. So, apparently the search of a safe product that fulfil this rule would be a wise choice.

Aim: The present study was designed to investigate the clinical and microbiological effectiveness of subgingivally delivered mango extract loaded on oxidized paper points (OPP) as an adjunct to scaling and root planing (SRP) in the treatment of periodontitis.

Patients and methods: Thirty subjects with periodontal pockets were selected. For each, one site was randomly assigned to receive SRP+mangiferin, SRP+chlorhexidine and a third site received SRP only. The evaluated clinical outcomes were plaque index, gingival index, sulcus bleeding index, and PPD at baseline, two weeks and one month. Microbial analysis was done to estimate the count of aerobic and anerobic counts and also to assess antimicrobial activities of mangiferin against Porphyromonasgingivalis and Prevotella intermedia as compared to well-known antibiotics.

Results: There was an intra-group significant reduction in each of the all clinical indices after 2 and 4 weeks of treatment. There were

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significant differences in each of PI, GI, and SBI between the negative control group and each of the study and positive control groups. There were no significant differences in these indices between the test and positive control groups. PPD showed no significant inter-group differences at any time points. There was intra-group significant reduction in each of the aerobic and anaerobic bacterial counts. There were no significant inter-group differences in these bacterial counts. Comparison in the inhibition zone of mangiferin and chlorhexidine showed that there was a significant difference favoring the mangiferin against tested organisms. There were significant differences between mangiferin when its inhibition zone was compared to tested antibiotics.
Conclusion: These results suggested that mangiferin could be used as a good adjuvant in periodontal therapy and this treatment might improve the clinical parameters and reduce the bacterial count as comparable to the gold standard chlorhexidine treatment.

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Introduction:-

Periodontitis is among the most prevalent periodontal disease as form of gingivitis continuation [1] and recognized as a major public health problem throughout the world [2]. This condition results in destruction of periodontal supporting connective tissue and alveolar bone loss, as well as formation of pathological pockets around the affected teeth [3]. Furthermore, it may result in exfoliation of teeth and even deterioration of systemic health if sufficient attention is not paid for its treatment [4].

The primary etiologic factor is considered to be dental plaque, which harbors pathogenic microorganisms and their byproducts [5]. However, periodontitis result from interaction between plaque bacteria and immune system in a susceptible host.This host susceptibility is partly hereditary but can be influenced by environmental and behavioral factors such as viral infections, smoking and stress [6]. In addition, the adherence of bacteria to mucosal cells is a critical step in the development of periodontal infection. Porphyromonasgingivalis is among the anaerobic bacteria which is able to adhere to cellular and acellular surfaces [7], form a biofilm [8], and function as a keystone pathogen, by elevating the virulence of the entire microbial community and interfering with host immunity [9].

Effective control of this bacterial etiology has been shown to be the most appropriate means of arresting the progression of periodontal disease [10]. However, mechanical treatment such as scaling and root planing (SRP) doesn't always give satisfactory results as manifested by remainingsigns of inflammation or residual periodontal pocket due to the tissue invasive nature of periodontal pathogens[1]. Thus, antimicrobial therapy as an adjunct to mechanical therapy has grabbed the attention in the treatment of periodontitis [4]. These adjunctive chemicals have antimicrobial effects, and are able to decrease the levels of inflammatory cytokines [11]. Nonetheless, overuse of these synthetic antimicrobials irrespective of their delivery system has led to the outburst of antibiotic-resistant pathogens, in addition to development of hypersensitivity reactions,and suppression or imbalance of normal oral flora [12].

Hence, the search for less harmful agents as or like natural herbal products or phytochemicals isolated from plants used in folk medicine are receiving extraordinary attention for using in medical remedy [13]. In this regard, the paradigm of treatmentconcept is also varying and it seems that plant extracts which don't have any adverse effects on the host may be useful for treating inflammatory diseases [14].

A number of plants and natural products from time immemorial had being used for their therapeutic benefits, such as antioxidant, anti-inflammatory, antimicrobial, antiseptic, and anti-collagenase properties [15]. Nowadays, many nutraceuticals are profited for human's health and play a crucial role in the prevention of human periodontal disease such as; aloe vera [16], neem[16,17], guava [18],curcumin[19], chamomile [20], tulsi[21], moringaoleifera[22, 23], grape seeds [24], cranberry [25], pineapple [26], pomegranate [27], mangosteen[28] and mango [29].

The mango belongs to genus “Mangifera”; sometimes; called “The king of fruits”, and consists of numerous species of tropical fruits in the family of Anarcardiaceae. The mango received the name "indica" as it is believed to originate in India [30]. Mangiferin, a C-glucosylxanthone with the formula 1,3,6,7-tetrahydroxyxanthone-C2-β-D-glucoside, is reported to be a principal constituent of Mangiferaindica L. and is found in various parts such as; leaves, bark, fruit, and roots. Chemical constituents of mangiferaindica are always of an interest, as it contains variable compounds, especially the polyphenolics, flavonoids, triterpenoids[31].

Mangiferin has a potential anti-hyperlipidemic and anti-atherogenic activity as it significantly reduces plasma total cholesterol, triglycerides, and LDL-C associated with a concomitant increase in HDL-C levels and a decrease in atherogenic index in diabetic rats [33].Polyphenolic compounds in the mango byproducts have shown to possess antioxidant and antibacterial activities, and may have caused disruption of microbial biofilm [32]. Some authors suggested that these phenolic compounds could lead to changes in the protein cell membrane, thus altering its permeability causing cell death [34].

In addition to reduction of reactive oxygen species (ROS) [35], mangiferin had been described as having an immunomodulatory effect [36] with an anti-inflammatory inhibition of COX-2 expression [37,38], as well as leukotriene B_4 $(LTB)_4$[39], nuclear transcription factor-kappa B (NF-κ B), tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1), which are very important cytokines in the inflammatory process and in bone resorption[40,41, 42].

Regarding the toxicity of mangiferin, it was indicated that longtime usage of active components of mango resulted in a higher percentage survival rate of human gingival fibroblasts and less toxicity when compared to chlorhexidinegluconate 0.2% and povidone-iodine-based mouth rinses[43]. In addition, Reddemanet al reported lack of mortality and toxic changes with 90-day repeated dose of a mango leaf extract containing 60% mangiferin[44].

An vitro study indicated that neem and mango stick extracts are inhibitory to oral streptococci which are responsible for various oral diseases [45]. Additionally, Carvalloet al. found that mangiferin derived from mango prevented periodontitis in Wistar rats [46].This plant species also showed antibacterial activity in vivo against specific periodontal pathogens including P. gingivalisand P.intermedia[47].Moreover, other studies clinically evaluated the effect of mango extract mouthwash against salivary microbial population, plaque inhibition and gingival health improvement [48, 49].

Based on all these activities already described for mangiferin, the aim of the present study was to evaluate its clinical and microbiologic effects as adjunctive local delivery therapy versus chlorhexidine for treatment of periodontitis.

Patients And Methods:-

Patients

A total of 30 periodontitis patients with an age ranging from 35 to 50 years were selected from the Department of Oral Medicine and Periodontology Outpatient Clinic, Faculty of Dentistry, Mansoura University. Those patients were diagnosed as having stage II periodontitis, according to the new classification of periodontal and peri-implant diseases and conditions 2017 [50], withprobing pocket depth (PPD) ≥ 5 mm and clinical attachment level (CAL) ≥ 3 mm.

Patients suffering from any systemic diseases/conditions, or received systemic antibiotic therapy in the last 3 months and/or periodontal treatment in the last 6 months were excluded from the study. In addition, smokers or pregnant and lactating patients were also excluded.

For each patient, three sites were selected with PPD ranging between 5-7 mm and one site was randomly assigned to represent the study site (SRP &mangiferin), whereas, another site was assigned to represent the positive control site (SRP &chlorhexidine). Additionally, a third site was assigned to represent the negative control site which received SRP only without local adjunctive treatment.

Clinical Assessment

Complete medical and dental histories were recorded for all patients. Periodontal examination was performed for each patient using periodontal probe (Williams graduated probe, Nordent, USA) including Plaque Index (PI) [51],

Gingival Index (GI) [52], Sulcus Bleeding Index (SBI)[53], and PPD.Periodontal assessment was performed at baseline, two weeks and one month following the treatment. All parameters were recorded by the same examiner.

Local drug delivery vectors preparation:

Paper points (Absorbents paper points # 40, Meta biomed Co. Ltd, Korea) were first oxidized following Kumar and Yang method [54]. A mixture 10 ml. of nitric acid (Nitric acid 65%, El-Gomhoria Co. for drugs and chemicals, Egypt) and phosphoric acid (Phosphoric acid 85 %, El-Gomhoria Co. for drugs and chemicals, Egypt) was made to prepare the oxidizing solution. Ten paper points were inserted in this acidic mixture and 0.12 gm sodium nitrite powder (Sodium nitrite, El-Gomhoria Co. for drugs and chemicals, Egypt) was added at once. This produced NO2 vapour which was cleared through extractor hood. This reaction was done at room temperature for 48 hours. The paper points were rinsed with 1L distilled water and 1L of phosphate buffered saline solution(Phosphate buffered saline solution (PH 7.4),El-Gomhoria Co. for drugs and chemicals, Egypt) to neutralize the residual acidity. Finally, the oxidized paper points (OPPs) were washed in soxhlet extractor with distilled water and air dried at room temperature overnight. For loading of the OPPs with mangiferin, a solution was made of mangiferin powder 100 mg (Mangiferin, My BioSource Inc., USA) and 2mL dimethyl sulphoxide (DMSO, Arab lab chemicals, UAE). DMSO was recommended as a solvent by mangiferin producer, and was known to be a carrier that help the drug in tissue penetration [55]. The prepared solution of mangiferin was of concentration 50 mg/mL, which is the minimum inhibitory concentration (MIC) of mangiferin[56]. The OPPs was dipped in the solution for 4 hours and then air dried at room temperature overnight. For the chlorhexidine, the same procedures were done with chlorhexidine hydrochloride 20% (Chlorhexidine, Sigma-Aldrich, Egypt) according to the method by Tabaryetal.[57].

Treatment

SRP was performed for all patients once weekly for one month. After finishing SRP, #40 OPPs loaded with mangiferin and chlorhexidine were cut into 5 mm pieces for standardization, then inserted using sterilized tweezers in the pockets assigned for study sites and positive control sites respectively. Finally, these sites were covered with periodontal dressing (COE-PAK, GC America Inc., USA). This procedure was repeated after 14 days.No antibiotics or anti-inflammatory agents were prescribed after treatment. Individuals were instructed to refrain from chewing hard or sticky foods.

Microbiological Assessment

Sample collection and processing

Sub-gingival microbial samples were collected from the selected pockets three times. The first time was at baseline, whereas, the second and third times were after 2 weeks and one month of treatment, respectively. The sampling area was isolated with cotton rolls, carefully cleaned with sterile cotton pellets, and then air dried. In each site, two sterile paper points were inserted to the bottom of the pocket for a 20 seconds period and then transferred into transport fluid medium (Enriched Thioglycollate Broth). The samples were processed in Microbiology Diagnostic and Infection control Unit (MDICU), Mansoura University within 24 hours[58]. Serial dilutions were performed with 1 ml and 99 ml pipettes. 100-fold dilutions were done 1:102, 1:104 and 1:106. After preparing the dilutions, they were incubated aerobically for 24 hours at 37°C and anaerobically for 2-3 days at 37°C using gas pack system. They were cultured on blood agar as nonselective media and on MacConkey agar as indicator media [59, 60]. Anaerobic strains were identified by micro-morphology, colony morphology and physical characters. After the incubation was done, the plate with 30-300 colony forming units CFU was selected and the colonies were counted. The bacterial counts were calculated using the following formula (CFU/plate × dilution factor) [61].

Mangiferin susceptibility testing

Mangiferin susceptibility was tested by disk diffusion method[62]. The test was performed by applying a bacterial inoculum of approximately 1-2 $\times 10^8$ CFU/mL to the surface of a large (150 mm diameter) Mueller-Hinton agar plate. Plates were incubated for 16–24 h at 35°C prior to determination of results. A well was dug into the plate with similar diameter of the standard commercially available antibiotic discs as there were no discs available for mangiferin. For comparison of results, clindamycin, tetracycline and metronidazole discs were used. The zones of growth inhibition around each were measured to the nearest millimeter. The diameter of the zone was related to the susceptibility of the isolate.

Statistical Analysis

Means and proportions pertaining to periodontal parameters were calculated for the study groups. Paired-sample T test was performed for comparison of periodontal parameters at different intervals within the groups. The

relationship among clinical parameters between the groups was performed through Tow-way ANOVA test. All data analyses were performed using a statistical software package for windows (SPSS, 18), Chicago, Inc. $P < 0.05$ was considered statistically significant.

Results:-

All patients have completed the study without any drop-outs. No untoward side effects were recorded by patients from using both mangiferin or chlorhexidine during the whole period of the study. The age of the selected patients ranged from35 to 50 years with mean age 39.8 ±6.941. The test subjects included 10 females and 20 males.

Clinical Assessment

In the three studied groups, the clinical parameters; PI, GI, SBI, and PPD; showed reduction in the mean values as shown in figures 1, 2, 3and 4, respectively. Furthering, there were also statistically significant differences between the baseline values and those after two weeks and one month of treatment, in addition, there was a significant difference between the 2 weeks' results and those after one month.

Moreover, the Two-way ANOVA test was used for inter-groups comparison between the three studied groups. The P values of PI in comparing between the test and positive control sites, the test and negative control sites, and the positive and negative control sites were 0.7122, 0.0001, and 0.0008, respectively. Additionally, the P values of GI in the comparison between the test and positive control sites, the test and negative control sites, and the positive and negative control sites were 0.7589, 0.0412, and 0.0449, respectively. Furthermore, the P values of SBI in comparing between the test and positive control sites, the test and negative control sites, and the positive and negative control sites were 0.7310, 0.0001, and 0.0007, respectively. Considering the PPD, the P values of inter-groups comparison between the test and positive control sites, the test and negative control sites, and the positive and negative control sites were non-significant at P values of 0.9558, 0.3822, and 0.0537, respectively.

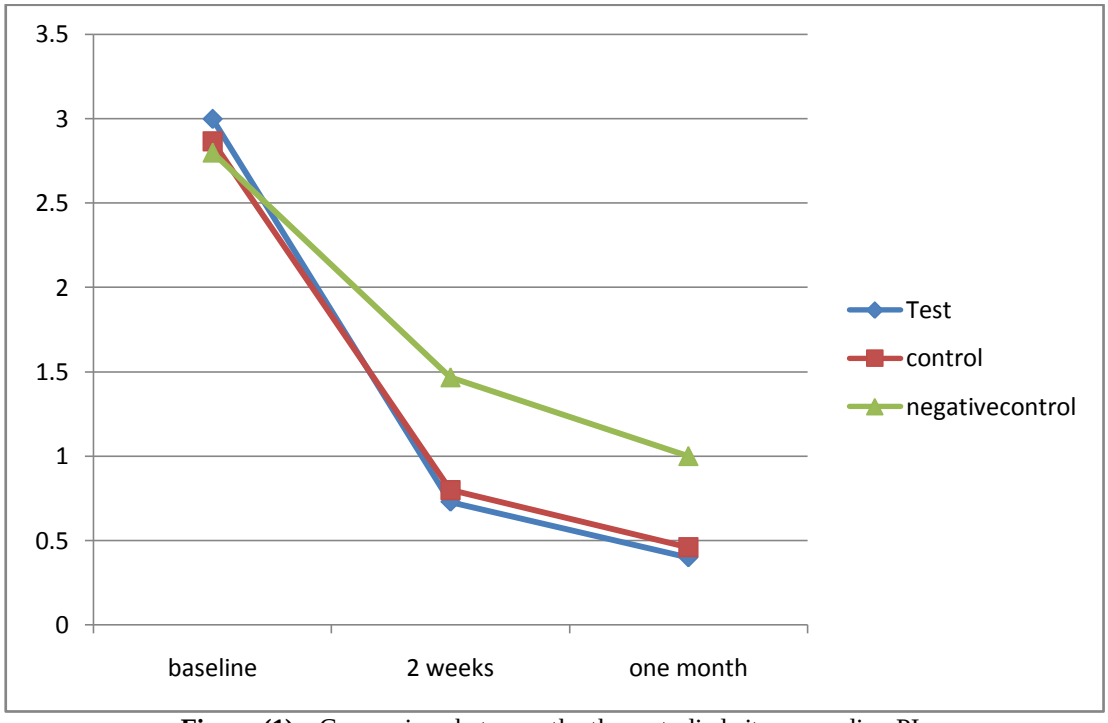

Figure (1):- Comparison between the three studied sites regarding PI.

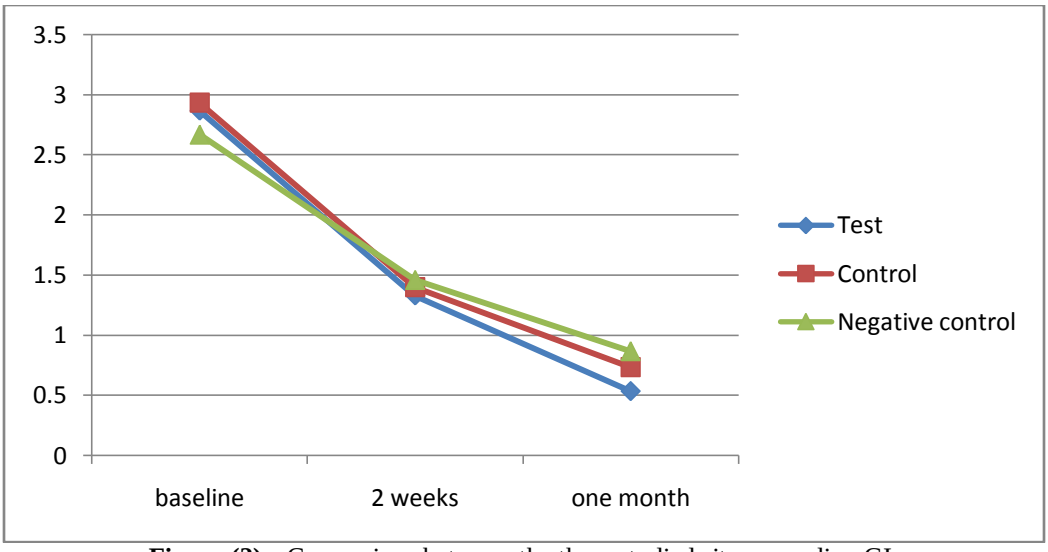

Figure (2):- Comparison between the three studied sites regarding GI.

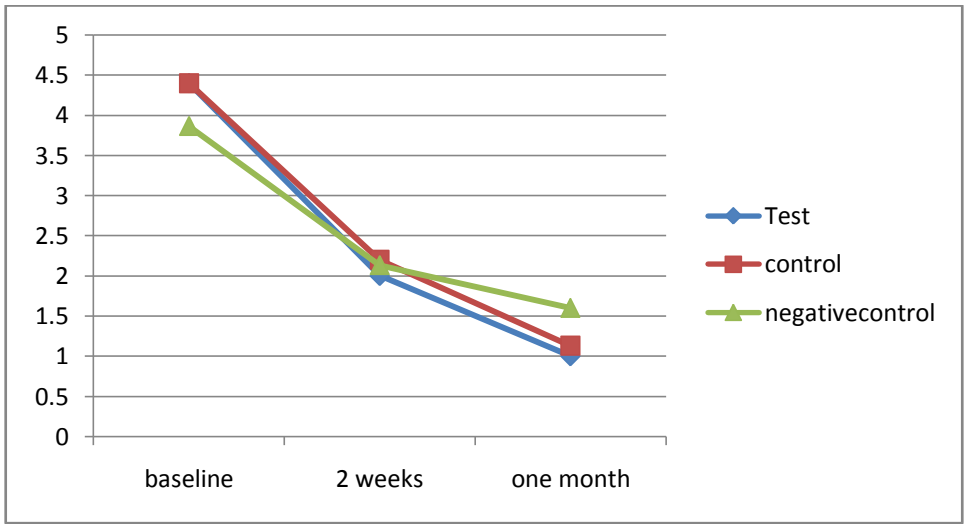

Figure (3):- Comparison between the three studied sites regarding SBI.

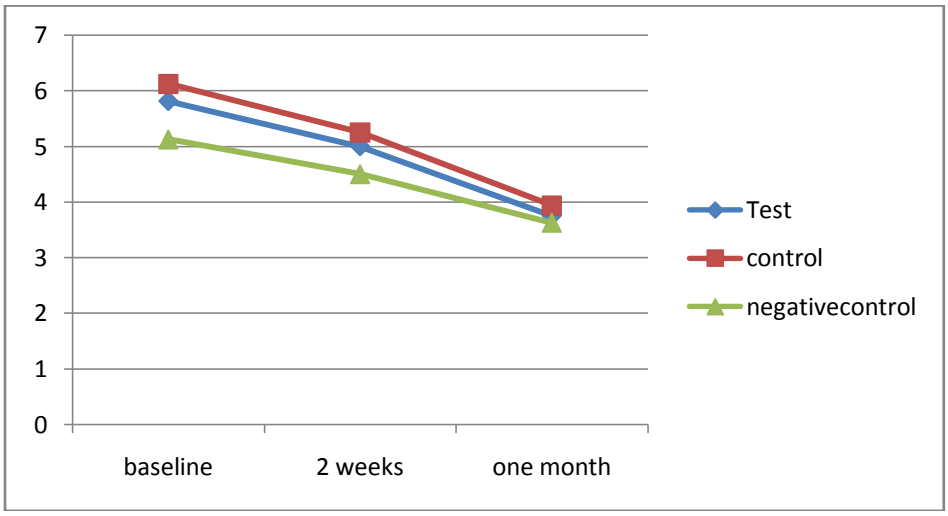

Figure (4):- Comparison between the three studied sites regarding PPD.

Microbiological Assessment

The qualitative microbiological assessment of all samples showed that 100% of all samples showed P. gingivalis, P.intermedia and alpha hemolyticStreptococci. While 86.67% showed Candida species and 13% showed either Pseudomonas species or Klebsiella species. The quantitative analysis was done based on the oxygen requirements into aerobes or anaerobes.

Aerobic Bacterial Counts:

The mean values of the aerobic bacterial counts are shown infigure 5. Concerning the test sites, the mean aerobic counts were 9.16×10^5 CFU ± 25.423×10^5 at the baseline and 7.54×10^5 CFU ± 2.57×10^5 after two weeks. Additionally, it was 9.574×10^3 ± 2.539×10^4 after one month. Intragroup comparison showed significant statistical differences when comparing the results after two weeks versus those of the baseline and after one month, in the meanwhile, there was a highly statistically significant difference for baseline results versus those after one month.

Regarding the positive control sites, the bacterial colony counts was 9.088×10^5 CFU ± 2.544×10^6 at the baseline, and, 7.234×10^4 CFU ± 2.566×10^5 after two weeks. At the end of the study, it was 3.82×10^3 CFU ± 4.536×10^3. Intragroup comparison showed significant statistical differences between all the evaluation time points.

In the negative control sites, the mean and standard deviation values of the aerobic counts were 1.509×10^7 CFU ± 3.463×10^7 at baseline and 9.63×10^5 CFU ± 2.53×10^6 after two weeks. After one month, it was 4.36×10^4 CFU ± 4.78×10^4. Moreover, there were statistically significant differences when comparing the results after two weeks with those at the baseline and the end of the study. Also, a highly statistically significant difference was found when comparing the results at the baseline with those after one month.

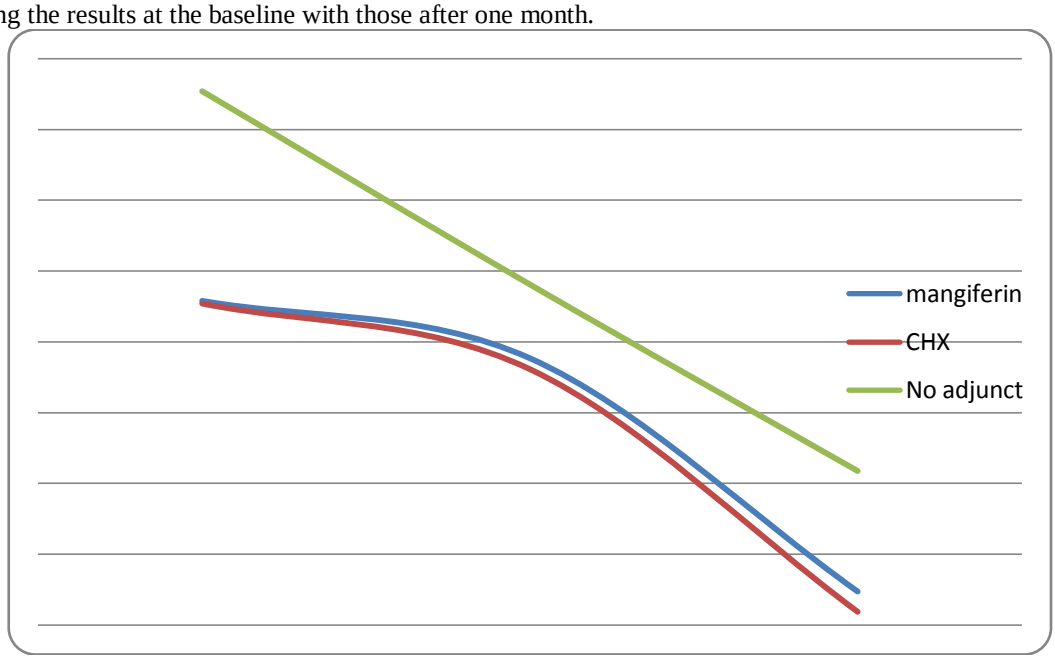

Figure (5):- Comparison between the three studied sites regarding aerobic bacterial counts.

Anaerobic Bacterial Counts:

The mean values of the anaerobic bacterial counts are shown in figure 6. As regards to the test sites, the mean values of anaerobic bacterial counts were 2.254×10^6 CFU ± 4.026×10^6, and 1.08×10^5 CFU ±2.51×10^5 at the baseline and after two weeks, respectively. After one month, this count was 7.63×10^4 CFU ±2.56×10^5. There was a highly statistically significant difference between the count recorded at the baseline and that after one-month results. In addition, a statistically significant difference was found on comparing the count of baseline with that after two weeks. However, no statistically significant difference was found when comparing the results after two weeks with those after one month.

Considering the positive control sites, the mean of anaerobic colony counts were 2.29×10^6 CFU ± 4.009×10^6, and 1.57×10^5 CFU ± 3.44×10^5 at time zero and after 2 weeks, respectively. After one month, this countdecreased to

1.39×10^5 CFU ± 3.44×10^5. Statistical analysis showed significant statistical differences between all-time points of the microbiologic assessment.In the negative control sites, the mean value of the anaerobic microflora was 2.781×10^6 CFU ± 4.51×10^6 at the baseline and changed to 2.5×10^5 CFU ± 3.91×10^5 and 3.34×10^4 CFU ± 4.16×10^4 after two weeks and one month respectively. Statistical analysis showed significant statistical differences between all-time points of the microbiologic assessment.

Regarding the inter-groups' comparison, both aerobic and anaerobic colony counts showed no statistically significant difference between the test and positive control sites, the test and negative control sites, and the positive and negative control sites.

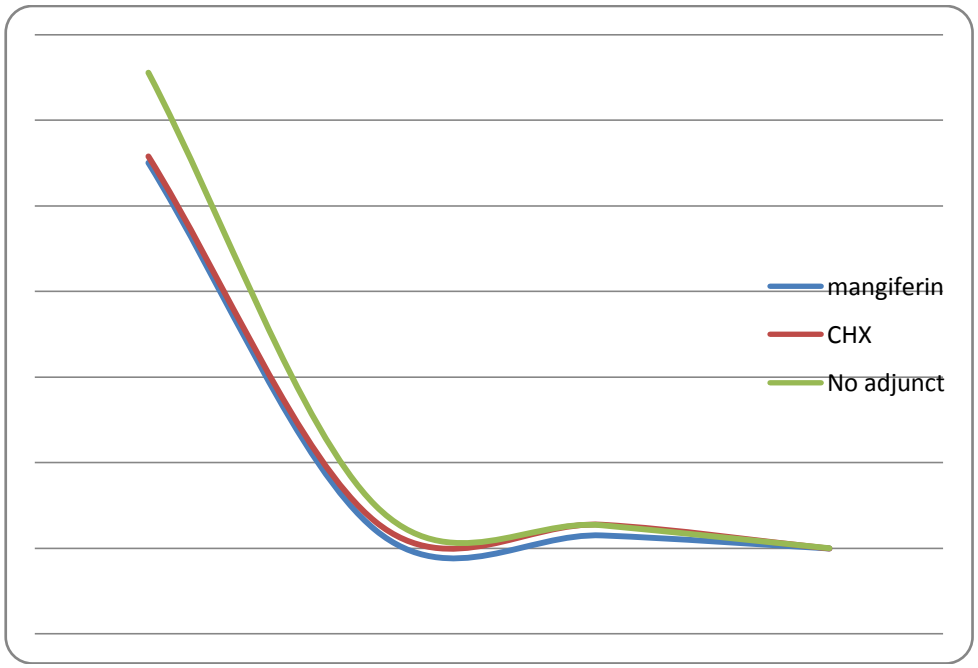

Figure (6):- Comparison between the three studied sites regarding anaerobic bacterial counts.

Susceptibility test

Inhibition zone diameters represent the antimicrobial activities of mangiferin and chlorhexidine againstP.gingivalis and P.intermedia. Regarding P. gingivalis, the mean inhibition zones were 17.25 ± 2.217 mm and10.75 ± 3.096 mm as caused by mangiferin and chlorhexidine, respectively. Interesting, there was a statistically significant difference favoring the mangiferin's inhibition zone at P=0.0021. On the other hand, P. intermedia growth was inhibited by both mangiferin and chlorhexidine and showing mean inhibition zones of 7 ± 4.583mm and 7.5 ± 3.536 mm, respectively with no significant difference between them (P= 0.9059).

Data recorded in figure 7 showed the antibacterial activity of mangiferin as compared to well-known antibiotics. The mean inhibition zone of mangiferin against P.gingivalis and anaerobic streptococci strains was 10.750 ± 1.753 mm. In the meanwhile, the mean inhibition zones were 5.875 ± 2.232 mm, 6.375 ± 1.847 mm and 6.25 ± 1.982 mm for clindamycin, tetracycline and metronidazole, respectively. Moreover, there were highly statistically significant differences between mangiferin inhibition zone when it was compared to those of clindamycin, tetracycline and metronidazole.

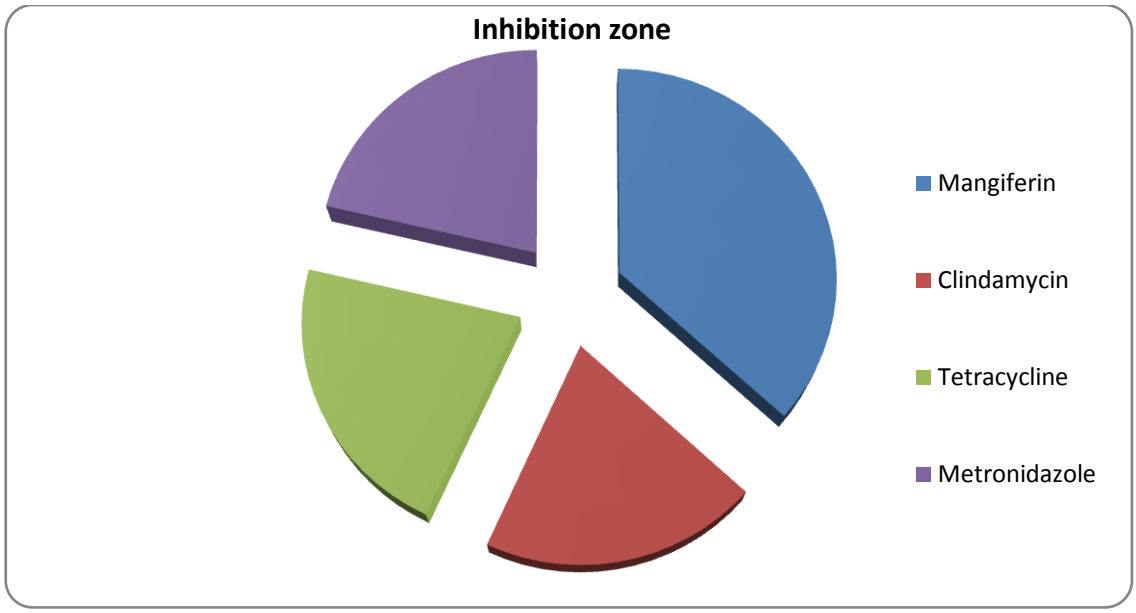

Figure (7):- The mean inhibition zone of mangiferin, clindamycin, tetracycline and metronidazole against P.gingivalis and streptococci Strains.

Discussion:-

Periodontitis is among the diseases that affect a large portion of adult population and resulting in tooth loss [29]. Traditionally, phase I therapy is the first line of treatment and preventing the progression of periodontitis. However, evidence suggests that this method may not give a good therapeutic result, as about 15% tooth loss may occur even with long term maintenance, especially in cases of residual periodontal pockets [63]. Therefore, an adjunctive therapy, such as antimicrobial agents, should be used; but unfortunately, this always demonstrate multiple adverse reactions [64]. So, it is reasonable to explore the clinical and antimicrobial efficacy of mangiferin as an alternative safe treatment modality.

A total of 30 patients with an age range 35-50years old were selected as having stage II stage periodontitis. Patients suffering from systemic diseases were excluded as their systemic conditions could alter the treatment outcome. Pregnant and lactating women were excluded as there is no available information whether mangiferin has toxic effect on developing or growing fetus/child or not. Patients who had periodontal treatment or antibiotic therapy in the preceding three months were excluded to avoid the possibility that the treatment/drug may to some extent alter the results of this study.

In the present study, the clinical and antimicrobial effects of mangiferin, as an adjunct to SRP, wereevaluated. This natural extract has been proven to have antimicrobial, anti-inflammatory and antioxidant efficacy due to the presence of Mangefira indica L. which is a phenoliccompound and thus, could be very useful in periodontal therapy [29]. In addition, chlorhexidine 0.2% widely used in plaque control[65] and considered as a gold standard in treatment of periodontal diseases[66]was selected for comparison with mangiferin.

Mangiferin was used in 50 mg/kg concentration in a bioresorbable delivery device. This concentation was recommended as the minimum inhibitory concentration to achieve antibacterial effect in addition to anti-inflammatory effect [47]. The trend of bioresorbable delivery device was not a new idea as it was used by several investigators to control the delivery of chlorhexidine [67, 68] and tetracycline [69] and it was mentioned that the maximum biodegradation of OPPs was 65% in the first 16 days. Therefore, OPP was used as a vector for the sake of controlled slow release in the periodontal pocket to make mangiferin more substantive and it was replaced with new one after 14 days to guarantee its sustained release during the study time interval. Furthermore, a periodontal pack was applied on the treated sites after thorough SRP and application of OPPs in order to restrict the effect of the locally applied drug to the treated site as possible [70] and aid in the retention of OPPs [71].

Considering the clinical assessment performed in this study, all cases of the mangiferin, chlorhexidine and negative control groups showed clinical improvement. The PI, GI and SBI showed a significant statistical difference between all-time points except the PPD which did not decrease significantly during the first two weeks concerning the study and negative control sites. Moreover, all clinical indices showed no statistical significant differences between mangiferin and chlorhexidine sites. When comparing the mangiferin and negative control groups, there were statistically significant differences in PI, GI and SBI, however, only PPD was non-significant. The comparison between chlorhexidine and negative control sites showed significant differences in PI, GI and SBI, in the meanwhile, no significant difference was shown regarding the PPD. The non-significant results related to the PPD may be due to the presence of deep pockets which usually do not respond to phase I therapy only and needs either prolonged treatment and/or surgical intervention.

As far as we know, mangiferin was evaluated as local treatment for periodontal diseases in only few studies. However; considering the difference in the periodontal condition and the manner of application of mangiferin; the results of the present study could be in accordance with the those of Sharma et al.[49], where there was no significant difference between mangiferin and chlorhexidine when they were used as a mouthwash twice daily for 21 days. Additionally, there were significant differences in the plaque and gingival scores in both groups after 21 days and 1 month. Moreover, another study showed that gargling with mango leafdecocation resulted in reducing the plaque index score on the lingual surfaces of mandibular incisors as effective as chlorhexidine [72].

In contrary, Bhat et al.[48] suggested that 0.12% chlorhexidine resulted in higher reduction in the plaque scores and better improvement in the gingival health in addition to more reduction in the streptococcus count than 2% mango leafmouth wash. This result might be due to the short period of thatclinical trial, as both chlorhexidine and mango were used as a mouth wash once daily for only 5 days.

As far as we know,no human in vivo studies examined the effect of mangiferin extract on periodontitis in the form of local delivery, however, fewstudiesevaluated its effect on ligature-induced periodontitis. One study showed significant reduction in the gingival index, probing depth and bone loss [73], in the meanwhile, the other study documented the effect of mangiferin on the alveolar bone loss by histologic techniques and immunohistochemistry [46]. Both studies attributed these results to the antioxidant effect of mangiferin.

Regarding the microbiologic results, qualitative assessment of the subgingival microbial samples of this study showed that 100% of these samples showed Porphyromonasgingivalis, Prevotella intermedia and alpha hemolytic Streptococci. While 86.67% showed Candida species and 13% showed either Pseudomonas or Klebsiella species. The quantitative analysis was done based on the oxygen requirements into aerobes or anaerobes. The aerobic bacterial counts of the mangiferin, chlorhexidine and negative control sites decreased significantly throughout the study period. These results indicated same efficacy for each of mangiferin and chlorhexidine as well as SRP on the studiedaerobic bacteria.

Both aerobic and anaerobic colony counts showed insignificant statistical difference between the treated sites in all groups sites at all-time points. Considering the anaerobic bacterial counts in the mangiferin sites, they decreased significantly after the first two weeks, however, these counts did not decrease significantly during the second two weeks. These results indicated a rapid antimicrobial efficacy formangiferin on the anaerobic bacterial count. This may be attributed to the role of the phenolic compounds present in the mango extracts[33].In the meanwhile, the anaerobic bacteria decreased significantly in the chlorhexidine andnegative control sites throughout all the study.

Regarding the susceptibility test, mangiferin showed significant difference in the inhibition zone for P. gingivalis not P. intermediagrowth when compared to chlorhexidine. Moreover, there were significant differences between mangiferin when its inhibition zone was compared to those of clindamycin, tetracycline and metronidazole. These results indicated that, the effectiveness of mangiferin against P. gingivalis was higher than the other tested antimicrobial agents.

These results could be coincided; to a least extent; with those of Subbiyet al.[74] who studied the antimicrobial effect of mangiferin and found a significant reduction in CFU (colony forming units) of Enterococcus faecalis biofilm even at a very low concentration (5mg/ml). Additionally, it was shown that 50% concentration of mango extract exhibited maximum zone of inhibition on Streptococcus mitisin comparison to neem. This study attributed its results to the astringent effect of tannins and resins contents in mangiferaindica[45]. In contrary, there was no significant

difference among plant extracts of Azadirachtaindica, Synzygiumaromaticum, Piper nigrum and Mangiferaindica in their antimicrobial effect on oral bacteria [75].Additionally, Masibo and He [76] established that leaf extracts of Chinese mangiferaindicaL possesses mild antimicrobial activity against Salmonella typhi, Escherichia coli, Staphylococcus aureus**,** and Bacillus cereus and it was suggested that this effect was owing to the presence of various polyphenolic compounds in mango, however, the geographic location was blamed to this relatively weak antimicrobial activity.

Phenolic compounds could be responsible for the antimicrobial activity of mango extracts as these compounds could cause changes in the membrane through its interaction with the carboxyl groups of the hydrophilic amino acids of the protein in the cell membrane, thus altering its permeability. Furthermore, phenolic compounds might cause an alteration of pH and electrical potential, leading to the release of protons to the outside. Thus, there would be a coagulation of cytoplasmic content in the bacteria, accompanied by a loss of normal cell metabolism, leading to cell death [33].

Moreover, Matsusaka and Kawabata [77] found that the non-edible parts (seed and peel) of mango,had a high radical scavenging capacity and possessed the major content of phenolics. It was also suggested that mango byproducts could be a potent source of phenolic compounds with high antioxidant capacity[78]. This high antioxidant performance is due to the ability of mangiferin to chelate the iron, so, avoiding its participation in the Fenton reaction. It can also prevent lipid peroxidation, thus, protecting against loss of mitochondrial membrane potential and mitochondrial swelling [79].

Furthering, it has the ability to scavenge O^{-2} due to its potent antioxidant action in the NADPH-dependent peroxidation system [80]. Mangiferin also contribute to maintaining the balance among the enzymes SOD, catalase and the GSH system [81]which has a key role in the cellular defense against free radical damage. Additionally, it was suggested that the extraction process from mango seed could affect the phenolic profile as well as the antioxidant and antimicrobial capacities of the extract [82].

Anothermangiferin's effect that could explain the results of the present study is its very marked inhibitory effect on the gene expression of the ICAM-1 [83]which is important forleukocyte trafficking during inflammation, thus preventing the progression of periodontal disease and destruction of periodontal tissues. Furthering, mangiferin has anti-chemotacticeffect on leukocyte rolling [46],therefore, maintain normal levels of lipoxin A4 (LXA4).

Some studies showed that mangiferin can modulate the pathway of NF-kB by decreasing its mRNA levels [84], thus, restoring transforming growth factor-β (TGF- β) [80], and downregulating pro-inflammatory cytokines (IL-1, IL-6, TNF-α, and IFN-Y) [85,80,86]expression. In addition, inhibition of TNF-α activated signal pathways reduced the expression of COX-2 as well as their end product PGE2 in gingival fibroblasts [87]. Additionally, the ability of mangiferin to upregulate TGF-β can inhibit the expression of cyclooxygenase 2 (COX-2) which is predominantly expressed during inflammatory reactions and produces various types of PGs.Lohinaiet al. found that administration of a selective inhibitor of COX-2 (NS-398) in rats with periodontal disease could reduce plasma extravasation in the gingivomucosal tissue[34]. So, it was expected that topical application of mangiferin, as a selective COX-2 inhibitor, presenting advantages over the systemic administration of nonsteroidal anti-inflammatory drugs.

Conclusion:-

The results of the present study implied that mangiferin could improve the clinical parameters and reduce bacterial growth in periodontitis and its action might be related to its beneficial antibacterial effect, in addition to modulating the oxidative stress on the periodontium during periodontitis. Moreover, it was indicated that the efficacy of mangiferin could be comparable and equally effective in inhibiting plaque microbiota as chlorhexidine and could be used as a promising and good adjuvant in periodontal therapy, especially in cases whichneed prolonged maintenance therapy or those with drug sensitivity.

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