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Effectiveness of Luteolin as an adjunct to Non-Surgical Periodontal therapy in Stage III Periodontitis Patients: A Randomized Controlled Clinical Trial with Biochemical Analysis

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Abstract

Exploring natural compounds to modulate inflammatory responses presents a promising treatment approach for periodontal disease. Herbal remedies are increasingly valued for being natural, cost-effective, and typically free from adverse effects compared to pharmacological options. This study aims to evaluate the impact of luteolin capsules on clinical outcomes and Interleukin-1β (IL-1β) biomarker levels in gingival crevicular fluid (GCF). The current study was conducted on thirty patients selected in this study with generalized periodontitis stage III and grade (A). Patients were randomly divided into two groups; with fifteen subjects in each. Group (I): (Test group) This group was treated with full mouth periodontal mechanical debridement at baseline, followed by oral hygiene measures and was prescribed 50mg luteolin capsules once daily for 45 days. Group (2): (Control group) they were treated with full mouth periodontal mechanical debridement at baseline, followed by oral hygiene measures only. Both groups demonstrated significant improvements in clinical parameters, including plaque index (PI), gingival index (GI), probing depth (PD), and clinical attachment level (CAL), over 3- and 6-month follow-ups. Additionally, IL-1β levels decreased significantly in both groups. The findings suggest that luteolin, when used as an adjunct to non-surgical periodontal therapy, yields clinical outcomes comparable to traditional treatment. However, further research is warranted to explore the efficacy of varying luteolin concentrations.

Keywords: Periodontitis; Non-surgical therapy; Luteolin, Interleukin 1-β.

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1. Introduction

Periodontal disease is one of the most prevalent oral health conditions, characterized by intense inflammation and damage to the periodontal structures that support teeth. This condition often leads to clinical attachment loss, bone destruction, and eventual tooth loss. Its progression is driven by a combination of microbial influences and host immune responses, which disrupt the equilibrium between bacterial virulence and the host's defenses, ultimately damaging periodontal tissues [1]. Periodontitis classification has evolved over time to align with advancing scientific understanding. The 2017 World Workshop identified three main types of periodontitis based on pathophysiology: necrotizing periodontitis, systemic disease-associated periodontitis, and a category encompassing previously known chronic or aggressive forms of the disease, now collectively termed "periodontitis." This modern classification also incorporates a multidimensional staging and grading system that considers disease severity, complexity, distribution, progression rate, and modifiable risk factors [2]. The pathogenesis of periodontitis includes antagonistic actions

between subgingival biofilm and host immune response causing a loss of balance between the virulence of bacteria and the host defense and leading to changes in the function and structure of the periodontium. The bacterial plaque has a major role in exacerbating these responses and causing the disease [3].

Among the critical mediators of this process is Interleukin-1β (IL-1β), a pro-inflammatory cytokine essential for host defense against infection and injury [4]. It is also the best characterized and most studied of the eleven IL-1 family members. As a pro-inflammatory cytokine, IL-1β participates in inflammation, immune regulation and bone resorption in periodontitis. IL-1β is a strong stimulator of periodontal tissue destruction. The properties of IL-1β include promoting bone resorption and inducing the production of tissue-degrading proteinases [5]. Patients with deeper pocket depths and more severe bleeding on probing (BOP) have increased levels of GCF IL-1β [6]. Although non-surgical periodontal therapy (NSPT) remains the cornerstone of periodontitis management, its inability to completely eliminate periodontal pathogens from the soft and hard tissue surfaces and may cause re-colonization leading to reinfection. To overcome these deficiencies, adjunctive use of systemic or local chemotherapeutic agents becomes an indispensable treatment modality (Ehizele et al., 2013). Recent studies suggest that natural compounds capable of modulating host inflammatory responses could offer a novel therapeutic avenue for managing periodontal disease [7]. Researchers have oriented herbals to be more natural, cheaper and safer products with no side effects in comparison to systemically or locally delivered drugs [8].

Flavonoids, a class of polyphenolic compounds, exhibit a wide range of biological activities, including antioxidant, anti-inflammatory, and antimicrobial effects (Middleton, Kandaswami and Theoharides 2000). Among them, Luteolin appeared to be an important member of the flavonoid family, with its proven anti-inflammatory and anti-osteoclastic activity [9]. Luteolin (3′,4′,5,7-tetrahydroxyflavone) has also been shown to possess anti-tumor, antioxidant, and anti-apoptotic effects, making it a versatile candidate for therapeutic applications [10]. It has been reported that luteolin has also an anti-osteoclastic property. Based on the above-mentioned data, it could be concluded that, despite the pleiotropic beneficial effects of luteolin, yet there is scarce evidence related to its clinical application in treatment of human periodontitis. To the best of the author's knowledge, this is the first randomized clinical trial that aimed to investigate the effect of luteolin as an adjunctive therapeutic modality in non-surgical periodontal treatment of stage III & IV periodontitis. Successful use of adjunctive luteolin in treatment of periodontitis could have a great contribution in reducing the need for periodontal surgery, thus facilitating a cost-effective management and decreasing patient's morbidity, which would certainly help to improve the oral health-related quality of life.

2. Subjects and Methods

2.1. Study Design

This study was designed to be a randomized, controlled, clinical trial; eligible participants were randomly allocated for one of the two comparative parallel-Groups; test and control groups using computer generated random tables.

2.2. Patient selection and grouping

The study involved 30 patients diagnosed with generalized stage III, grade A periodontitis, recruited from the Oral Medicine, Periodontology, and Oral Diagnosis Department at Ain Shams University. Participants were divided equally into two groups: Group I (Test group): included (15) patients with stage III, grade A periodontitis; treated with scaling and root surface debridement followed by oral hygiene measures and was prescribed 50mg luteolin capsules once daily for 45 days [11-12]. Group (2): (Control group) they were treated with full mouth periodontal mechanical debridement at baseline, followed by oral hygiene measures only.

- Patients were selected considering the following criteria:

(they were free from any systemic disease according to the modified Burkett's health history questionnaire, both genders with age range from 30-50 years, Generalized Stage III Periodontitis Patients; (Interdental CAL > 5mm, PD ≥ 6mm, radiographic vertical bone loss of ≥ 3mm extending to the middle or apical third of the root with ≤ 4 teeth lost due to periodontitis) [13] and must be able to return to recall visits). Exclusion criteria included smokers, pregnant or lactating women, prisoners, individuals with a history of allergies, uncooperative patients, or those who had undergone periodontal treatment or taken antibiotics/anti-inflammatory drugs within the previous six months. All patients in this study received non-surgical periodontal therapy followed by instructions in self-performed oral hygiene measures The study was conducted in accordance with ethical guidelines, and approval was obtained from the Research Ethical Committee of Ain Shams University Faculty of Dentistry. All eligible patients were thoroughly learned of nature, possible risks & their auxiliary aids in research, signed written informed consent documents. Whole study carried out from September 2021 to July 2023.

2.3. Methods

2.3.1. Drug preparation

Preparing a sodium hydroxide aqueous solution; weighing 8.1kg of sodium hydroxide, dissolving in 300L of water, and stirring until the solution is clear to obtain a sodium hydroxide aqueous solution; Weighing 10.0kg of rutin, adding into the prepared sodium hydroxide aqueous solution, heating in a water bath at 45-80˚C, and stirring until the solution is clear. Adding of 35.0kg of self-made reducing agent or sodium hydrosulfite into the solution, and stirring until the solution is clear; Keeping the solution at the temperature of 65-85˚C and continuing stirring for reaction, sampling every half hour for HPLC detection, and monitoring the residual amount of the raw material rutin; Finishing the reaction within 1-2 hours, then cooling the temperature of the reaction liquid to 10-20˚C, drop wise adding 7M hydrochloric acid solution until the pH value of the reaction liquid is 2-3, and stirring to separate out the solid. After stirring for 10 minutes, filtering the reaction solution, collecting a filter cake, leaching the filter cake with 200L of water, and then placing the filter cake at the temperature of 60˚C for vacuum drying until the LOD percent is less than or equal 5 percent to obtain a luteolin crude product; Mixing the luteolin crude product prepared in

the step 6) with methanol, where in the volume of the methanol is 10 times of the mass of the luteolin crude product, heating and stirring the mixture for 1 hour in a water bath at 65 ˚C. Then cooling to 20 ˚C, and standing for 1 hour; Filtering the solution, collecting filter cakes, leaching the filter cakes by using 50% methanol aqueous solution with the volume being 3 times of the mass of the filter cakes, and then placing the filter cakes at temperature of 60 ˚C for vaccum drying until the LOD % is less than or equal to 5% thus obtaining the luteolin capsules [14].

2.3.2. Gingival crevicular fluid sample collection

Gingival crevicular fluid (GCF) samples were collected at baseline and three months post-therapy using perio-paper strips inserted into the gingival sulcus or periodontal pocket for 30 seconds until resistance was felt. Contaminated strips were discarded. IL-1β levels in GCF were measured using an enzyme-linked immunosorbent assay (ELISA) [15].

2.3.3. Clinical assessment

Periodontal conditions were assessed at baseline, three months, and six months post-treatment. Plaque Index (PI) was used to evaluate plaque accumulation, while Gingival Index (GI) assessed gingival inflammation. Probing Depth (PD) and Clinical Attachment Level (CAL) were measured using a University of Michigan O' probe with Williams' graduations. Occlusal stents were utilized to ensure accurate placement of the periodontal probe [16].

3. Results and discussion

3.1. Results

This study involved a total of 30 periodontitis patients (generalized periodontitis stage III, grade A). The study involved 13 males and 17 females, their age ranged from 30 to 50 years with the mean age being 35 years. All the participants completed the study without allergic reactions or any adverse effects. Study participants were assessed throughout three time intervals; at baseline, after 3 months and after 6 months of therapy.

A. Clinical and radiographic evaluation

Regarding oral hygiene status, plaque index scores (PI) in both groups of the study showed significant improvement in each group after 3 months of the treatment. By comparing the two studied groups, there was no statistically significant difference between them at baseline, 3 months and after 6 months of therapy (Table 1, figure 1). Additionally, regarding the comparison between the luteolin group and the control group in GI after 6 months of follow-up, the results were statistically significant (p value <0.05) (Table 1, figure 1). Furthermore, When comparing between the Luteolin group and the control group concerning the PD after 3 and 6 months of follow-up findings non-statistically significant (p value > 0.05) (Table 1, figure 2). The current investigation discovered a statistically significant difference in CAL b/w the Luteolin group and control group after 3 and 6 months of follow-up (p-value <0.05) (Table 1, figure 2). Moreover, luteolin group showed a statistically significant better reduction in bone defect depth at 6 months follow-up interval than control group (p-value <0.05) (figure 3).

B. Biochemical evaluation

After 3 months of follow-up, the Luteolin group had a substantially reduced mean difference in IL-1β compared to the control group indicating statistical significance (p-value <0.05). The Luteolin group had a smaller mean percentage change than the control group indicating a statistically significant difference (p value < 0.05) (Table 2, figure 4).

3.2. Discussion

The primary aim of periodontal therapy is to inhibit destruction and replace the periodontal apparatus with its initial form and structure. Non-surgical periodontal therapy, including scaling and root surface debridement has been suggested as the ideal initial treatment for patients suffering from periodontitis. Although a consistent amount of evidence has indicated that this mechanical debridement is effective in controlling inflammation and reducing clinical parameters of disease, so-called phase I periodontal therapy alone cannot guarantee remission of the disease and achieve healing of tissue completely. Hence, root surface debridement should be augmented with adjunctive methods systemically or locally [17-19]**.** In past few years, appreciation of natural compounds as a potential innovative treatment for human health has grown considerably [20]. Among various available options for managing periodontitis, dental care products containing herbal compounds have been in spotlight owing to beneficial pharmacological properties of bioactive ingredients [21]**.**

In this context, the anti-inflammatory activity of luteolin has been harnessed in order to fight periodontal disease and promote the restoration of damaged bone tissue [22]. Luteolin is a powerful antioxidant with anti-inflammatory properties that can be used to treat diseases such as periodontitis [23]. Besides, Luteolin's antimicrobial properties have been well documented. In a recent study, the inhibitory effect of luteolin on the growth *of P.gingivalis* and hence biofilm formation was significantly proved [24]. With its proven anti-inflammatory and anti-osteoclastic activity, luteolin was expected to prevent periodontal disease by decreasing inflammation and bone loss and increasing osteoblastic activity. Despite this, no trials done to confirm its beneficial effect in treatment of periodontitis. Thus present randomized clinical trial conducted to evaluate effect of adjunctive use of luteolin 50mg capsule in treatment of stage III periodontitis among patients attending faculty of dentistry, Ain-Shams University. This clinical trial demonstrated that there was no statistically significant difference regarding plaque index (PI) between Luteolin group and control group at baseline, after 3 months, and after 6 months follow-up. While for intragroup comparison in PI scores, a statistically significant difference was detected at baseline, after 3, and 6 months of follow-up for both groups. These results were similarly coinciding with a study conducted by Tunkel J. et al., (2002).

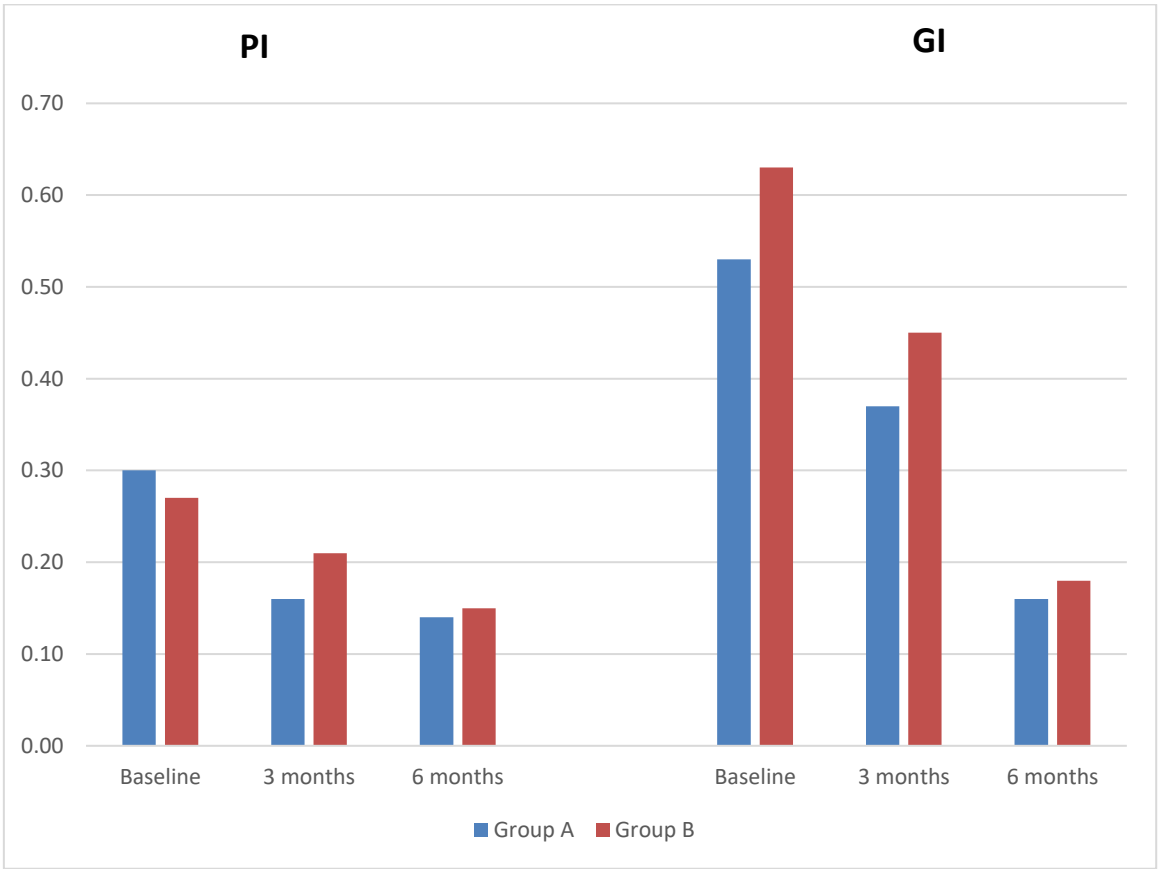

Figure (1): Bar chart showing PI and GI percentage changes for two studied groups at baseline, after 3 months, and after 6 months follow up

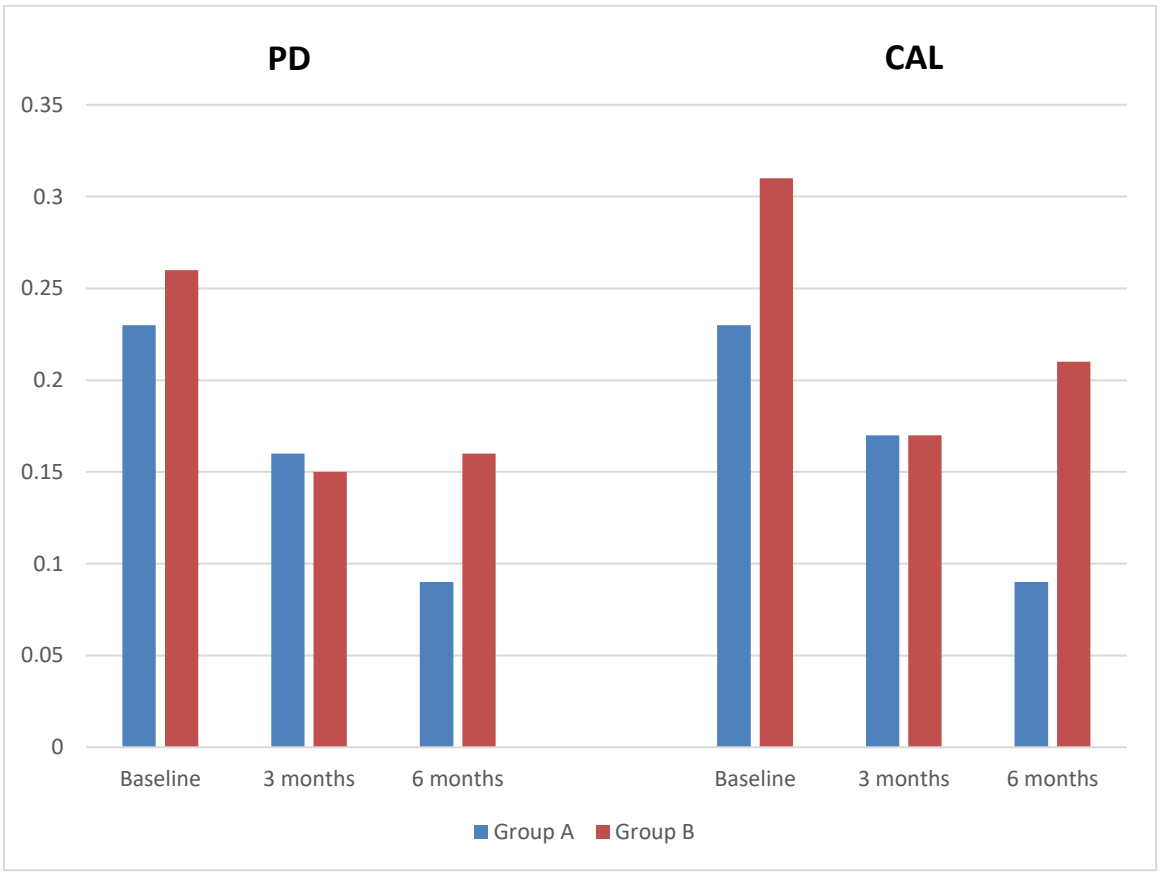

Figure (2): Bar chart showing PD and CAL percentage changes for two studied groups at baseline, after 3 months, and after 6 months follow up

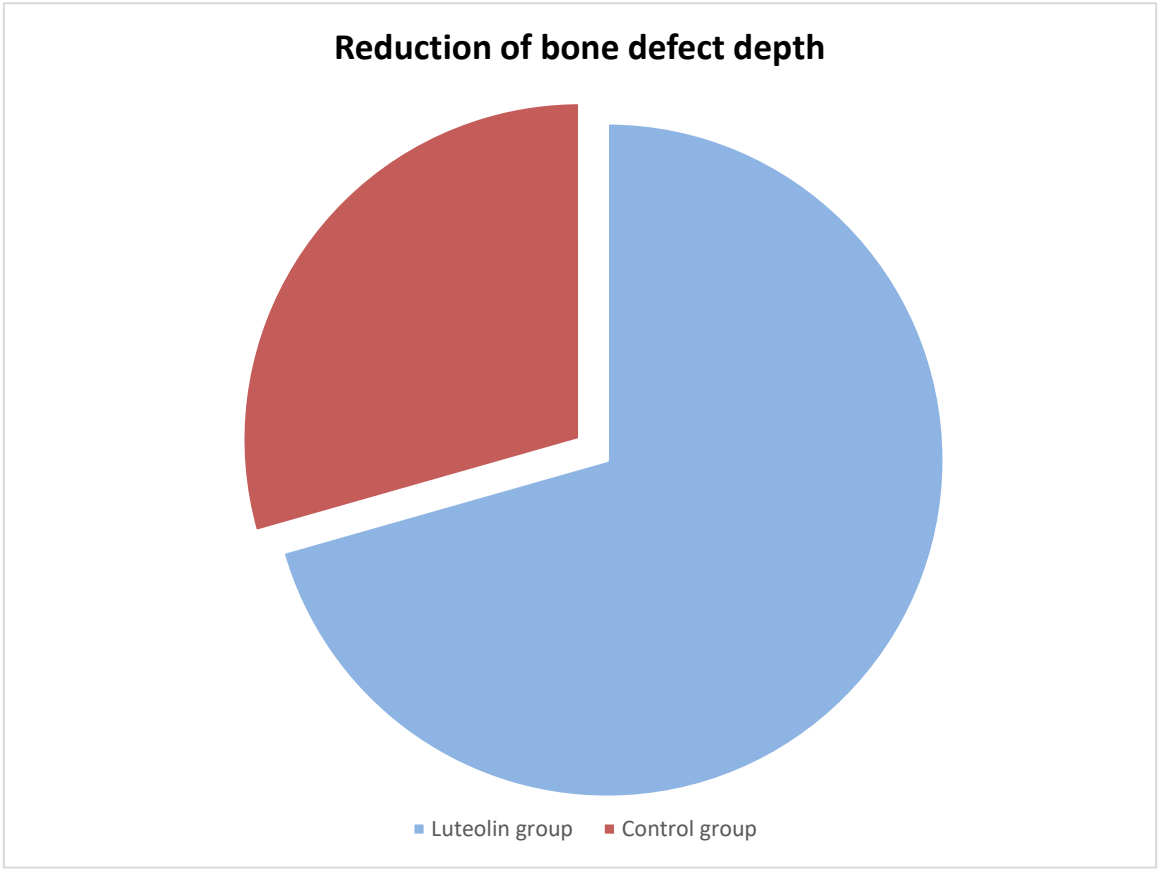

Figure (3): Pie chart showing percentage reduction in bone defect depth for 2 studied groups from baseline to 6 months follow up

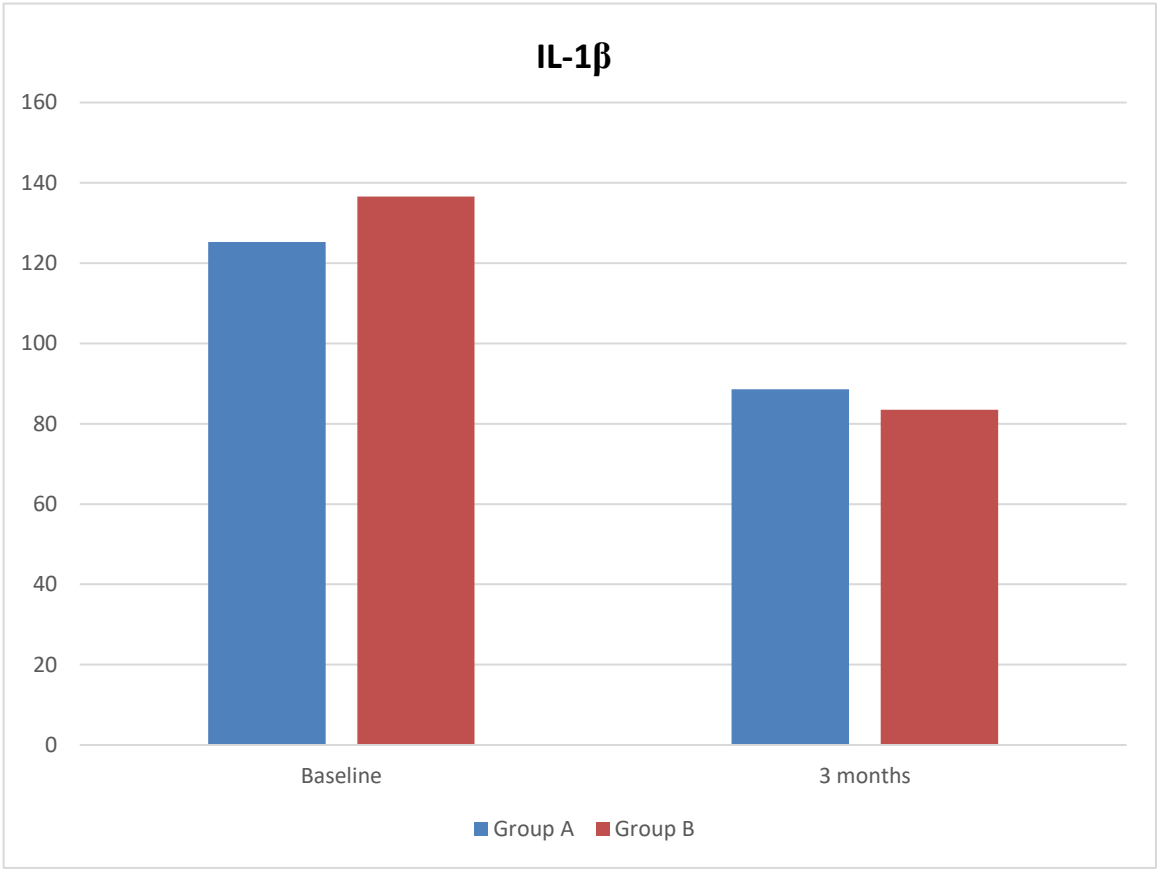

Figure (4): Bar chart showing IL-1β percentage changes for two studied groups at baseline and after 3 months follow up

Table (1): Mean and standard deviation (SD) for PI, GI, PD, and CAL percentage change for studied groups at baseline, after 3 months, and after 6 months follow up

Clinical Parameter	Time interval	Percentage change (%) (Mean±SD)		P value
		Group A (n=15)	Group B (n=15)	
PI	**Baseline**	0.30±0.08	0.27±0.09	0.339
	3 months	0.16±0.09	0.21±0.07	0.082
	6 months	0.14±0.09	0.15±0.07	0.542
GI	**Baseline**	0.53±0.09	0.63±0.15	***0.042**
	3 months	0.37±0.09	0.45±0.13	***0.05**
	6 months	0.16±0.04	0.18±0.06	0.303
PD	**Baseline**	0.23±0.09	0.26±0.05	0.251
	3 months	0.16±0.07	0.15±0.04	0.592
	6 months	0.09±0.11	0.16±0.09	0.111
CAL	**Baseline**	0.23±0.11	0.31±0.07	0.35
	3 months	0.17±0.09	0.17±0.06	0.927
	6 months	0.09±0.14	0.21±0.12	***0.031**

Group A (Luteolin group), Group B (control group).
Independent T Test for quantitative data between the groups for parametric data.
Significant level at P value < 0.05

Table (2): Median and Range for IL-1β percentage change for studied groups at baseline and after 3 months follow up

Time Interval	IL-1β percentage change (%) (Median±Range)		P value
	Group A (n=15)	Group B (n=15)	
Baseline	125.30±17.40	136.60±13.10	***0.001**
3 months	88.60±8.20	83.50±10.00	***0.001**

Data displayed as Median, interquartile rang (IQR)
Wilcoxon Signed Ranks Test for quantitative data between the groups for non-parametric data
Significant level at P value < 0.05.

Regarding Gingival index (GI); the current investigation revealed that there was no statistically significant difference among Luteolin group and control group at baseline, after 3 months, and after 6 months of follow-up. However, a statistically significant decrease was recognized at 3 months and 6 months follow-up intervals for both groups, indicating a better improvement in GI scores in luteolin group than control group. These results compatible with study demonstrated by [25]. As observed in our results, there was no statistically significant difference regarding probing depth (PD) among Luteolin group and control group at baseline, after 3 months, and after 6 months of follow-up. This is consistent with studies by [26-28]. While for intragroup comparison regarding PD among Luteolin and control groups, a statistically significant difference recorded at base line, after 3, 6 months of follow-up in each group. This result was in contrast with study done by Martande. et al. [29]. As regards the Clinical attachment level (CAL), no statistically significant difference was noticed among Luteolin group and control groups at baseline, and after 6 months of follow-up. However, a statistically significant lower CAL scores were observed in luteolin group at the 3 months follow-up period, which remained stable at the 6 months follow up interval, unlike for the control group where CAL scores re-increased at the 6 months interval. This was in harmonious with the result of Balci Yuce et al. [30]. On the contrary, Palmer et al. [31] reported no difference between scaling and root surface deberidement and systemic metronidazole with scaling and root surface deberidement. This might suggest a possible long-term effect of luteolin in maintaining a stable periodontium as reflected by a steady CAL scores. Furthermore, the quantitation of Human Interleukin 1β in GCF revealed that there was no statistically significant difference among Luteolin group and control group at baseline, and after 3 months of follow-up. However, a statistically significant difference detected in intragroup comparisons in each Luteolin and control group at baseline, and after 3 months of follow-up. These findings concurred with study done by Gutiérrez-Venegas et al. [32].This result is similarly coinciding with a study conducted by Zhang et al. [25] who reported that luteolin, with the doses of 20, 40, 80, and 160mg/kg, decreased the levels of IL-1β.

In contradiction with study done by Gong et al., [33] they found that there was no effect on level of IL-1β reduction after treatment by Roxithromycin therapy adjunctive to non-surgical periodontal debridement applied in cyclosporine-A-induced gingival overgrowth. That's can be explained mainly due to effectiveness of non-surgical periodontal debridement therapy that reduces overall bacterial load in oral cavity, further leading to resolution of inflammatory response. Regarding radiographic assessment, inter-group comparisons and reduction of the bone defect depth was significant in luteolin group but not for the control group. However, no differences detected b/w groups for both parameters. This was in accordance with study done by Elboraey et al. [34]. This was inconsistent with Kurian et al., (2018) [35], found that subgingivally-delivered 1%

metformin and Aloe vera gel in treatment of intrabony defects in periodontitis had no significant effect on bone. These results might be justified because of Luteolin reduce alveolar bone loss by inhibiting osteoclastogenic action and creation of markers of osteoclastogenesis such as MMP-9 and RANKL in a dose-dependent manner. Moreover, Luteolin resulted in an upregulation of osteogenic markers including tissue inhibitor of metalloproteinase led to elevation in osteoblastic activity.

4. Conclusions

Within the limitations of the current study, it can be concluded that: Systemic delivered luteolin drug can be considered an effective therapeutic agent on the clinical and biochemical parameters at time interval, showed significant reduction in probing depth and gain in clinical attachment level in adjunct to non- surgical periodontal therapy in the treatment of generalized stage III grade A periodontitis. Also, luteolin agent showed significant reduction in IL- 1β level in GCF at time intervals.

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