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EFFECT OF LICORICE EXTRACT GEL ON THE LEVELS OF INTERLEUKIN-1β IN GINGIVAL CREVICULAR FLUID IN CHRONIC PERIODONTITIS

Dental Science

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ABSTRACT

Background-Licorice herb has shown to have anti-inflammatory and antibacterial properties. Also interleukin-1β being a principle mediator of inflammation. This study was conducted to assess the efficacy of licorice extract gel on the level of interleukin-1β in gingival crevicular fluid along with the clinical parameters in chronic periodontitis. **Methods-** 20 patients having chronic periodontitis were randomly divided into two groups into control and experimental group. The experimental group received the local application of licorice extract gel. The changes in the levels of Interleukin-1β was measured through the gingival crevicular fluid collected which was then subjected to enzyme linked immunosorbent assay for the determination of levels of interleukin-1β. **Results-** Interleukin 1β levels were higher in the control group as compared to the experimental group. There was a greater reduction in the probing pocket depth and gain in clinical attachment level in the experimental group when compared to the control group where value of p = 0.01 was considered to be significant. **Conclusion-** The local application of licorice extract gel caused a greater reduction on the levels of interleukin-1β in the experimental group when compared to the control group. These results suggest licroice extract gel as a therapeutic potential i the treatment of chronic periodontitis.

KEYWORDS

INTRODUCTION

Periodontitis is characterized by a destructive inflammatory process affecting the supporting tissues of the tooth, resulting in formation of periodontal pockets, resorption of alveolar bone and eventually tooth loss.[1] The periodontal treatment involves the management of the periodontal pathogen along with the integrated host response.[2] The host factors that determine the susceptibility are the pro inflammatory cytokines, matrix metalloproteinase's, arachidonic acid derivatives and tissue inhibitors of matrix proteinases, etc.[3]

Licorice herb is a dry root extract most frequently used in traditional Chinese medicine (TCM).[4] Licorice have been reported with antitumor, antimicrobial, antiviral anti-inflammatory, antidiabetic , immunoregulatory , hepatoprotective , neuro-protective properties and adrenal cortical hormone kind functions .[5]

Recent studies on licorice reveals that most important bioactive compounds in licorice are triterpenes, flavonoids and polysaccharides which contains antibacterial and anti-inflammatory properties with reduction in the levels of interleukin-1β (IL- 1β) (Charles Bodet et al 2008).[6]

Bacteria and their products such as lipopolysaccharides (LPSs) can induce the production of pro-inflammatory cytokines in periodontal pockets.[7]

The etiologic correlation of cytokines with periodontitis has been extensively studied and is well established (Bascones A, 2005).[8]

IL-1β is a multifunctional cytokine with diverse biological activities which is a principal mediator of the inflammation which has been implicated in the pathophysiology of periodontal disease.[9]

In 1982, Charon et al was the first to demonstrate the increase level of IL-1β in case of inflamed sites in gingivitis patients.[10]

Hence this was the first clinical study conducted to assess efficacy of licorice on IL-1β levels with clinical parameters in chronic periodontitis.

MATERIALS AND METHOD

Patient Selection

To evaluate the changes in levels of IL-1β along with clinical parameters between before and after scaling and root planing (SRP) procedure with the application of licorice extract gel.

This clinical study was conducted in the Department of Periodntology and Implantology.

Patients both male and female of age group 25-55 years having moderate chronic periodontitis with probing pocket depth of ≥5 with radiographic evidence of bone loss, patients with good oral hygiene and without any history of systemic disease were included in the study.

Patients with history of smoking or use of tobacco in any form, pregnant and lactating women, immuno- compromised patients were exclud Ethical clearance for the study was obtained from ethical committee and reviews board of the institution.

Written informed consent was taken from selected patients explaining the nature of the study design and procedure of local drug delivery.
Randomization was done and 20 patients were included in the study which were divided into two groups (control and experimental group) with toss of coin.

Control group was treated only with SRP.
Experimental group was treated with SRP followed by application of licorice extract gel at baseline and at 7th day (reapplication of gel). (Figure i and Figure ii) After the placement of licorice extract gel, the treated sites were given periodontal pack to isolate the area and restrict the effect of licorice extract gel. Patients were instructed not to use any other anti-plaque agents other than normal brushing and mouth rinsing.

The following clinical parameters were evaluated before and 1 month after the SRP procedure. The gingival index(GI) , PPD, clinical attachment level (CAL) and bleeding on probing(BOP) were assessed at the site of GCFsampling.PPD and CAL were measured to the nearest millimeter using a straight periodontal probe (PCP UNC15, Hu-Friedy, Chicago,IL, USA).

As a reference for CAL measurements, the occlusal stent was used . GI, PPD, CAL and BOP were measured at four sites around the tooth. A reevaluation was done 1 month after the SRP procedure. All clinical parameters were assessed by one examiner.

The samples were collected on the baseline (Before SRP) and on 15th day.

The sites to be sampled were isolated with cotton rolls and supragingival plaque was removed to avoid contaminations and blocking of micropipettes. After the sites were gently dried by blowing with air, GCF samples were collected with micro capillary pipettes obtained from Sigma-Aldrich Chemical Company, Bangalore, India.

The samples were collected by placing the tip of pipette extra crevicular (unstimulated) until it gently touches the marginal gingival, a standardized volume of 2 µl gingival crevicular fluid was collected in 5-20 minutes from each test site.

After collection of gingival crevicular fluid the sample was assigned to particular group with the toss of a coin.

The test site which did not express standard volume (2 µl) of gingival crevicular fluid was excluded and micropipette contaminated with blood and saliva was discarded.

Only one site per patient was selected as sampling site in periodontitis patients.

The gingival crevicular fluid collected was immediately transferred to plastic vials and sealed with sterile paraffin tapes then stored at -70ºC (within 2-3 hrs of sample collection) till the time of assay.

The samples collected were assayed for Interlukin-1β levels using commercially available Human Enzyme-Linked Immune Sorbent Assay (ELISA) (Krishgen Biosystems LTD, Mumbai).

The assay was conducted according to the manufacturer's instructions using ELISA Reader (Biorad Laboratories, California,USA).

Highly sensitive ELISA kit was used to detect the Interleukin-1β levels in the samples.

The graph was plotted between Human IL-1β and values of absorbance observed at 450 nm of the standards. The graph obtained was used for the determination of various values of absoranbne of interleukin 1 beta in 40 samples.(Graph 1 and Figure iii)

Statistical Analysis

Data analyses were performed using version 21.0 of the Statistical Package for Social Sciences (IBM Corporation, Armonk, New York, USA).Shapiro-Wilk test showed that values of plaque index scores, gingival bleeding index scores, probing pocket depth, relative attachment levels and absorbance value of IL-1β followed normal distribution hence parametric tests were applied for further data analysis.

RESULTS

The mean ± S.D. of Plaque Index level in Experimental Group was 1.71±0.29 before the starting of the treatment. After 30 days the value of the Plaque Index decreases with mean ± S.D. of 0.74±0.18 and the result was statistically significant (t value = 19.99, p = 0.01). The mean ± S.D. of Plaque Index in control Group was 1.67±0.34 before the starting of the treatment. After 30 days the value decreases with mean ± S.D. of 1.02±0.15 and the result was statistically significant (t value = 9.43, p value =0.01).(Table 1 and Graph 2)

The mean ± S.D. of Gingival Bleeding Index in Experimental group was 96.81±4.71 before the starting of the treatment. After 30 days the value of the Gingival Bleeding Index decreases with mean ± S.D. of 39.42±5.11 and the result was statistically significant (t value = 50.35, p = 0.01). The mean ± S.D. of Gingival Bleeding Index score in Control Group was 97.11±4.83 before the starting of the treatment. After 30 days the value decreases with mean ± S.D. of 60.45±4.56 and the result was statistically significant (t value = 21.65, p value =0.01).(Table 2 and Graph 3)

The mean ± S.D. of PPD level in Experimental group was 6.90±0.44mm before the starting of the treatment. After 30 days the value of the PPD decreases with mean ± S.D. of 3.70±0.57 mm and the result was statistically significant (t value = 23.24, p = 0.01). The mean ± S.D. of Probing Pocket Depth level in group A was 6.70±0.59 mm before the starting of the treatment. After 30 days the value of the Probing Pocket Depth decreases with mean ± S.D. of 4.90±0.71 mm and the result was statistically significant (t value = 11.05, p value =0.01). (Table 3 and Graph 4)

The mean ± S.D. of Relative attachment level in Experimental Group was 11.00±0.97 before the starting of the treatment. After 30 days the value of the relative attachment level decreases with mean ± S.D. of 7.60±0.68 mm and the result was statistically significant (t value = 17.22, p = 0.01) . The mean ± S.D. of relative attachment level in Control Group was 10.55±1.05 mm before the starting of the treatment. After 30 days the value of the relative attachment level decreases with mean ± S.D. of 8.85±0.81 mm and the result was statistically significant (t value = 10.55, p value =0.01). (Table 4 and Graph 5)

The GCF samples subjected to commercially available HUMAN IL-1β ELISA kit (Krishgen Biosystem, Mumbai) and the absorbance of value of the sample was compared. The mean ± S.D. of Value level in Experimental Group was 183.80±38.67 before the starting of the treatment. After 15 days the Value decreases with mean ± S.D. of 56.80±20.22 and the result was statistically significant (t value = 46.17, p = 0.01) The mean ± S.D. of Value level in Control Group was 134.25 ± 30.91 before the starting of the treatment. After 15 days the value decreases with mean ± S.D. of 79.10±8.65 and the result was statistically significant (t value = 6.29, p value =0.01).(Table 5 and Graph 6)

DISCUSSION

Nonsurgical periodontal therapy (NSPT) is the cornerstone of periodontal therapy which aims to eliminate inflammation, arrest progression of periodontal disease, improve esthetics and create an environment conductive to maintenance of health.[11]

However, with Non-Surgical Periodontal Therapy (NSPT) it is difficult to remove all the pathogenic microflora residing in areas of deep pocket, furcation and grooves which are inaccessible through SRP alone. These pathogenic bacteria become more complex with time thus requiring the use of systemic antimicrobials .[12]

Despite the benefits of reduction in inflammation, reduction in probing pocket depth, gain in clinical attachment level, these systemic antimicrobials expose an individual to potential risks of development of microbial resistance, emergence of opportunistic fungal infections, allergic reactions and other drug-dependent systemic side effects.[13]

Licorice a perennial herb with active constituents like saponins, flavonoid, isoflavones which are responsible for its anti-bacterial,anti-inflammatory, anti-viral, hepato-protective and anti-oxidative activities .[4]

The continuous high secretion of bacteria and their end products induces a host mediated inflammatory response through the secretion of various cytokines including IL-1β, IL-6, IL-8 and TNF-α.[14]

IL-1β is a multifunctional cytokine with diverse biological activities and has been implicated in the pathophysiology of periodontal disease.[9]

Also, Charles bodet et al (2008) found that licorice has demonstrated anti-inflammatory activity against lipopolysaccharide induced by P.gingivalis, leading to the reduction in the level of IL-1β.[6]

Gingival Crevicular Fluid (GCF) being serum transudate, inflammatory exudates have its composition similar to blood plasma as the fluid in the GCF is derived from the post capillary venule thus can easily be collected through the gingival crevice as its non-invasive.[15]

Also, the volume of Gingival Crevicular Fluid (GCF) alters with the change in inflammation and can be evaluated for the therapeutic intervention and its outcome.[16]

Therefore, this clinical study was designed with an intention to assess the efficacy of licorice as a local drug by quantitatively relating it to the levels of IL-1β in the gingival crevicular fluid of patients with

periodontitis along with assessment of clinical parameters.

The commercially available ELISA kit (Krishgen Biosystem, Mumbai) was used to assess the levels of IL-1β.

In this clinical study a convenient sample of 20 patients diagnosed as chronic periodontitis based on clinical examination, with classifications determined according to criteria proposed by the 1999 International World Workshop for a Classification of Periodontal Diseases and Conditions[17] were randomly divided into two groups as control and experimental group.

Control group was treated with SRP only while ExperimentalGroup was treated with SRP and local application of licorice extract gel.

The local application of licorice extract was done at baseline and at 7 day following SRP, the clinical parameters were measured at base line and 1 month after the SRP

In this study the clinical parameters included the plaque index (Sillness and Loe, 1964) gingival bleeding index (Ainamo and Bay, 1975), Probing Pocket Depth and relative attachment level.

P.gingivalis is considered a major factor along with other factors to be associated with the etiology of periodontitis. Licorice extract has demonstrated antibacterial activity against Pg with and MIC and MBC of 62.5µg/ml and 25µg/ml respectively. Also, licorice has demonstrated a reduction in the levels of IL-1β at conc of >10µg/ml.[18-20]

Therefore, in this clinical study a 20% w/v (mg/ml) extract of licorice was prepared by Pansila' M Impact with the procedure as explained by More et al (2008). [21]

In this present study 2µl of GCF volume was taken in both the groups from most inflamed siteat baseline and on 15th day using colour coded micro capillary pipettes procured from Sigma Aldrich, Bangalore, India. The micro capillary pipettes were taken in order to avoid the adsorbance of polypeptide to cellulose [22] and were stored at -70 c.which was then subjected to commercially available ELISA kit for assessment of interleukin 1β.

The present study showed a significant reduction in the plaque index in Experimental Group when compared with Control Group which was significant (p value = 0.01) which could be attributed to the conclusions drawn from the study done by R Segal et al(1985)where they found saponins a constituent of licorice inhibited the bacterial plaque formation.[23]

In this clinical study, there was significant reduction in Probing Pocket Depth, slight improvement in gain of Clinical Attachment Level and reduction in bleeding score correlating with the results drawn from Charles bodet (2008) where he found licorice contributing its anti-inflammatory effect against LPS induced by bacterial end products leading to reduction in inflammation thereby reducing its clinical signs.[6]

The reduction in the quantitative values in control group was from 134.25 ± 30.91 to 79.10 ± 8.65 following SRP where as in Experimental group values reduced from 183.80±38.67 to mean value of 56.80±20.22 following SRP and local application of licorice extract gel, indicating a decrease in the level of IL-1β correlating with the study of Yukirohieda et al (2017) in which they found a reduction in the level of IL-1β following SRP which can be explained by the reduction in the LPS induced influx of pro inflammatory cytokine as both the groups underwent SRP following randomization. [24]

Also, Matsuki et al (1993) demonstrated an increased level of IL-1β in inflamed sites when compared to healthy sites , Konopka et al (2012) found a decrease in the values of IL-1β 4 weeks after SRP along with improvement in the clinical parameters.[25-26]

However, Yoshinari N et al (2004) found no significant difference in the levels IL-1β following SRP which could be attributed to small number of sites along with the time between the two GCF samples (one month) which could be insufficient to detect significant trends in IL-1 levels before and after SRP.[27]

In this clinical study a greater reduction in the levels of IL-1β in experimental group as compared to the control group was observed which could be attributed to the application of licorice extract gel as Vu Dang La et al (2011) demonstrated that the presence of active licorice constituents licoricidin and Licorisiflavan A reduced the inflammatory mediators by inhibiting the LPS signaling pathway which inhibited the NF-κ b p-65 phosphorylation.[28]

Thus, based on our results on effect of licorice on levels of IL-1β, licorice can be used a potential therapeutic compound in the management of periodontitis.

Table 1 - Comparison Of Plaque Index At Baseline And 30 Days Among Both Groups

Groups	Time Period	Mean	N*	Std. Deviation	T value	P value
Experimental group	Before	1.71	10	.29	19.99	0.01
	After	.74	10	.18		
Control group	Before	1.67	10	.34	9.43	0.01
	After	1.02	10	.15		

* Number of individuals
P= 0.01 considered to be statistically significant

Table 2 Comparison Of Gingival Bleeding Index At Baseline And 30 Days Among Both Groups

Groups	Time Period	Mean	N*	Std. Deviation	T value	P value
Experimental Group	Before	96.81	10	4.71	50.35	0.01
	After	39.42	10	5.11		
Control Group	Before	97.11	10	4.83	21.65	0.01
	After	60.45	10	4.56		

* Number of individuals
P= 0.01 considered to be statistically significant

Table 3 Comparison Of Probing Pocketdepth At Baseline And 30 Days Among Both Groups

Group	Time Period	Mean	N*	Std. Deviation	T value	P value
Experimental Group	Baseline	6.90	10	.44	23.24	0.01
	30 days	3.70	10	.57		
Control Group	Baseline	6.70	10	.59	11.05	0.01
	30 days	4.90	10	.71		

* Number of individuals
P= 0.01 considered to be statistically significant

Table 4 Comparison Of Mean Attachment Level At Baseline And 30 Days Among Both Groups

		Mean	N*	Std. Deviation	t value	P value
Experimental Group	Before	11.00	10	.97	17.22	0.01*
	After	7.60	10	.68		
Control Group	Before	10.55	10	1.05	10.77	0.01*
	After	8.85	10	.81		

* Number of individuals
P= 0.01 considered to be statistically significant

Table 5 Comparison Of Absorbance Values At Baseline And 15 Days Among Both Groups

Groups	Time	Mean	N*	Std. Deviation	T value	P value
Experimental group	Baseline	183.80	10	38.67	46.17	0.01
	15 days	56.80	10	20.22		
Control group	Baseline	134.25	10	30.91	6.29	0.01
	15 days	79.10	10	8.65		

* Number of individuals
P= 0.01 considered to be statistically significant

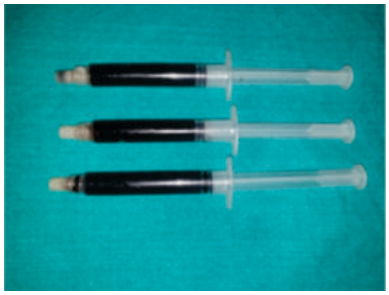


Figure I – Licorice Extract Gel Prepared.

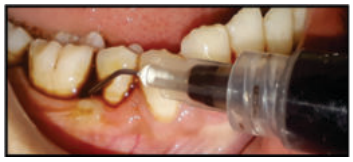
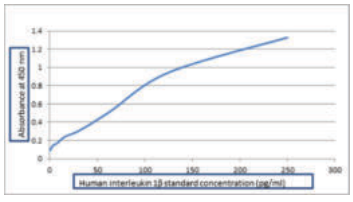
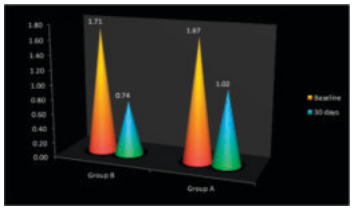


Figure II - Application Of Licorice Extract Gel

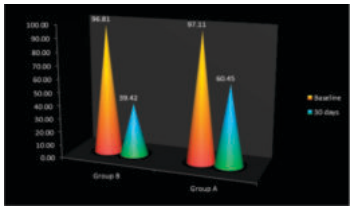
Figure III- Standards Obtained After The Completion Of Assay



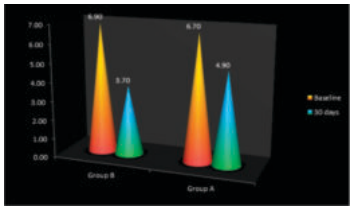
Graph 1



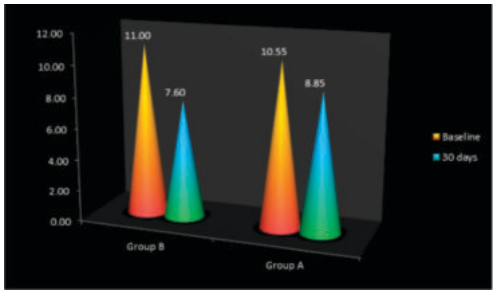
Graph 2



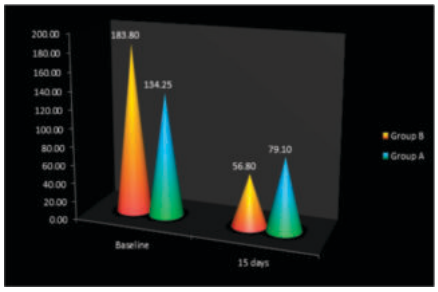
Graph 3



Graph 4



Graph 5



Graph 6

Conflict Of Interest – Nil

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