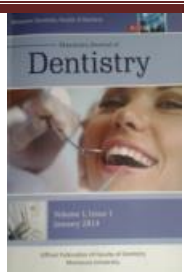
" The Anti-Inflammatory Effect of Garcinia Mongostana Extract in Treatment of Chronic Periodontitis clinical and laboratory study"



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Abstract:

Background:Patients with choric periodontal diseases have high levels of lactate dehaydrogenase(LDH) in gingival cervical fluid (GCF) . *Garcinia mangostana* (*MGA*), more popularly known as mangosteen can be used as an adjunct to SRP to provide a new dimension to periodontal therapy .

Aim:

1. To evaluate the effect of garcinia mangostana extract gel as a local drug delivery in treatment of chronic periodontitis patients.
2. Assessment of Lactate dehydrogenase enzyme levels in GCF of such patients.

Subjects and methods: Ten patients (*MGA* group) were treated with SRP, and the subgingival application of mangostana gel was used as local drug delivery. Ten patients (chlorhexidine gel) were treated with SRP and chlorhexidine gel. Ten patient treated with SRP only . Five group (control group).Clinical parameters were recorded and at the one month and samples of GCF were analyzed using ldh ELISA kit for all group.

Results: All periodontal parameters(PI,BOP,PPD,CAL) and Laboratory analysis results LDH were reduced significantly by local drug delivery treatment.

Conclusion: Scaling and root planning (SRP) is an effective treatment in chronic periodontitis patients associated with local drug delivery therapy (mangostana gel) can improve the GCF level of one of the inflammatory biomarkers (LDH) .

Introduction

Periodontitis is a chronic inflammatory disease of the supporting tissues of the teeth, characterized by resorption of the alveolar bone and loss of the soft tissue attachment.[1] The main etiologic factor is considered to be dental plaque, which harbors pathogenic microorganisms.[2] The elimination of these microorganisms is achieved through mechanical debridement, which is considered a gold standard procedure with the systemic administration of antimicrobials.[3] These antimicrobials have their own side-effects, including resistant strains and superimposed infections, as well as lack of patient compliance. Recently, local drug delivery (LDD) has gained importance in the field of periodontology.[3] Delivery of the drug for a longer duration of time into the periodontal pocket has been shown to reduce probing pocket depth (PPD), stabilize the clinical attachment level (CAL), and minimize bleeding, allowing better control of the disease.[4] LDD achieves bioavailability to surrounding tissues without any side-effects, as it can reach the base of the periodontal pocket and is maintained for an adequate amount of time for the antimicrobial effect to occur.[5] Although various microbial agents are being commonly used as LDD in periodontology, the need for safe, effective, and economical agents has always motivated researchers to move towards herbal products. Herbosomes are recently-introduced herbal formulations that are better absorbed, and as a result, produce better bioavailability and actions than the systemic administration of antibiotics, which are associated with the distribution of drug throughout the body, giving rise to toxicity.[6] Herbal medicines have been widely used all over the world since ancient times, as they are non-toxic and play a compatible role in pharmaceutical formulation.[7]

Garcinia mangostana (*MGA*), more popularly known as mangosteen, also called the "queen of fruits", belongs to the Guttiferae family. It is an evergreen tree native of South–East Asian countries, including India, Malaysia, the Philippines, and Sri Lanka.[8] Its reddish-to-dark purple fruit with a white juicy edible pulp has a long history of medicinal use to treat conditions, such as diarrhea, wounds, and inflammatory disorders. It contains various bioactive compounds, such as chrysanthemum; garcinones A, B, and C; sesquiterpenoids; gartanin; fructose; sucrose; and tannins in its pericarp.[9] Some of the important properties of mangostana include anti-inflammatory, antimicrobial, antioxidant, anticancer, antiproliferative, pro-apoptotic, and aromatase inhibitory properties.[10] Lactate dehydrogenase (LDH), an enzyme released extracellularly following cell death.[11] Previous investigational studies have confirmed that the activity of LDH in GCF is significantly correlated with chronic periodontitis.[12] Then, it has been estimated that LDH occur within the GCF as an indicator for monitoring chronic periodontitis.[13]

Chlorhexidine is a broad-spectrum biocide effective against Gram-positive bacteria, Gram-negative bacteria and fungi. Chlorhexidine inactivates microorganisms with a broader spectrum than other antimicrobials and has a quicker destruct rate than other antimicrobials (e.g. povidone-iodine). It has both bacteriostatic (inhibits bacterial growth)

and bactericidal (bacteria destroyer) mechanisms of action, depending on its concentration. Chlorhexidine destroy by disrupting the cell membrane. [14]

Subjects and Methods:

Patient selection: This study was carried on 35 patients. The patients were selected from those attending Oral Medicine and Periodontology Department,Faculty of Dentistry,MansouraUniversity .They were 18 males and 17 females. Their ages ranged from 35 to 55 years.The selected patients were divided into three groups .

Study groups: Group I (study group): This group includes ten patients with moderate chronic periodontitis .

Group II (chlorhexidine group): Consisted of 10 patients were chosen with moderate chronic periodontitis with (pocket depth, >4 mm).

This group were treated with scaling and root planning in addition to chlorhexidin gel subgingivaly as lcal delivary drug. .

Group III : Consisted of 10 patients were chosen with moderate chronic periodontitis with (pocket depth >4 mm). This group were treated with scaling and root planning only .

Group IV (control group): This group consisted of 5 subjects with clinically healthy periodontium and good oral hygiene status

Inclusion criteria: (1)Age 35- 55 years.(2)Presence of at least 20 teeth, excluding third molars. (3)Cooperative patients.

Exclusion criteria:(1)Periodontal treatment or antibiotic therapy in the past 3 months before the study.(2)Patients who are pregnant or breast feeding.(3) Any other systemic diseases such as cardiac disease,diabetes mellitus, rheumatoid artheritis, SLE and liver disease.(4)Mental disability

Clinical Assessment: Proper case history was taken from each patient, also the onset and duration of the patient's periodontal status was reported as well as any past dental treatment.Patients was exposed to thorough clinical oral and extraoral examination.

Periodontal Assessment:

Periodontal indices:

- Plaque index. (15)
- Bleeding on probing (BOP). (16)
- Probing pocket depth: Probing will be performed with a calibrated periodontal probe. (17)
- Clinical attachment level (CAL). (18)

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Gingival cervical fluid sample collection:

- GCF samples for all groups were obtained from one site in each patient,the site which showed the highest probing depth and CAL (range 4-6 mm) score was selected.
- To avoid contamination, the selected test site was air-dried and isolated with sterile cotton rolls. Supra gingival plaque was removed gently without touching the marginal gingiva to avoid bleeding from gingiva.
- The Gingival Crevicular Fluid samples were collected by insertion of sterile medium-sized endodontic paper points into the pocket for 30 seconds.
- The collected GCF was immediately transferred into plastic vials containing 300µ phosphate buffer saline (PH7.4)and the samples were frozen at -70° C till they were assayed for the levels LDH.

Treatment procedures:

All patients received a full mouth supra and sub gingival scaling and root planning as basic full mouth treatment, using ultrasonic and hand instruments under local anesthesia if needed. All the pockets in one side were ingected with hexetidine gelusing a syringe with blunt cannula which inserted gently to depth of the periodontal pockets to assure delivery of gel . [12]

This procedure repeated once weekly for five weeks. Patients were instructed not to rinse or drink any liquid for at least 30 minutes. Also they were given carful instruction of self-performed oral hygiene measures, twice daily brushing. No antibiotics or anti-inflammatory agent were be prescribed after treatment.

Laboratory analysis: Laboratory assessment for measuringLactate dehydrogenase Enzyme level using ELISA:

- The GCF samples were collected from all subjects at baseline.
- Recollection of GCF samples from all subjects in groups I,II and III one month after therapy .
- Absorbent paper were inserted by using the technique of superficial intracrevicular and were left for 30 seconds. [10]Sample which were contaminated by blood were not taken. Absorbent paper was taken and placed in Eppendorf tube that has been filled with 1 ml phosphate buffer solution. Specimens are labeled. The samples taken were stored at -20 C and will were analyzed using Enzyme linked immunsorbent assay ELISA.

I. Data analysis:

Numerical data were explored for normality by checking the data distribution, calculating the mean and median values, evaluating histograms and normality curves and using Kolmogorov-Smirnov and Shapiro-Wilk tests. Data were presented by mean, standard deviation (SD).Paired t test was used for comparison between before and after and between right and left

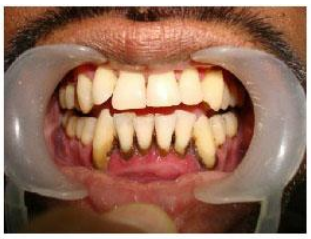
T test was used for comparison between healthy and study group.The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows

Results: This study revealed that there was statistically significant difference regarding LDH between groupI&groupII when measured .

The results of our study showed significant reduction in all clinical indices(PI,BOP,PPD,CAL) of studied groups when measured at 4weeks after non-surgical periodontal treatment . Also there was no statistically significant difference found in periodontal indices between groups.

Comparison Clinical periodontal parameter and LDH level in GCF of three study groups after treatment with control group.

Measure Mean ±SD	Group 1 n=10	Group 2 n=10	Group 3 n=10	Group 4 n=5	Test of significance ANOVA
GI	0.6±0.04^{abc}	0.7±0.05^{ade}	0.8±0.1^{bdf}	0.5±0.02^{cef}	F=66.9 p<0.0001**
PPD	3.6±0.3^{a}	3.8±0.3^{b}	3.9±0.3^{ac}	3.3±0.3^{bc}	F=6.2 p=0.002*
PI	0.7±0.07	0.6±0.2	0.7±0.03	0.5±0.09	F=2.4 p=0.09 *
GBI	0.3±0.07^{ab}	0.51±0.18^{ac}	0.6±0.06^{bd}	0.3±0.01^{cd}	F=14 p<0.0001**
LDH	117.5±8.1^{a}	106.5±5.3^{b}	169.4±49.8^{abc}	88.1±2.1^{c}	F=13.6 p<0.0001**
CAL	3.9±0.3^{a}	3.9±0.5^{b}	4.5±0.4^{ab}	------	F=6 p=0.007 *



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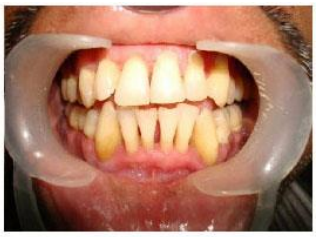

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Discussion

Chronic periodontitis is a chronic, microbial-induced inflammatory disorders that affect the structure supporting the teeth. The bacterial biofilm that forms on the surfaces of teeth provides a chronic microbial stimulus that elicits a local inflammatory response in the gingival tissues. However, long-term plaque accumulation at the dento-gingival niche results in the enrichment and maturation of the biofilm sustained inflammatory and an irreversible loss of the supporting tooth structures.[1] In chronic periodontitis, the host immune response is believed to play an essential role in the breakdown of connective tissue and bone.[85] The immune and inflammatory responses are critical for understanding the pathogenesis of periodontal diseases, which is orchestrated by a number of host-related factors.[25]

Although periodontitis can be diagnosed on the basis of the clinical parameters and radiographic findings, however, these measurements provide information about the past periodontal tissue destruction and do not elucidate the current state of the disease activity nor predict the future.[199] Moreover, it has been recently found that evaluation of various biologically specific proteins or markers in oral fluids (GCF and saliva) by using immunologic or biochemical methods have the potential to provide an insight much beyond the classical clinical and radiographic findings of the disease process. These biomarkers can be either released from soft tissue inflammation, alveolar bone loss, bacterial products or antimicrobial proteins associated with the periodontal destruction.[184, 200]

The clinical finding of the present study reflected significant reduction of periodontal inflammation in chronic periodontitis patients with non-surgical periodontal treatment as evident by improvement in the periodontal parameters, where the levels of PI, GI, BOP, PD, and CAL index after phase I therapy were significantly decreased versus their levels at baseline in study group (**Table 2)** (**Table4) (Table 6)**.

This may be related to the fact that non-surgical periodontal therapy is effective in reducing the bacterial load leading to improvement of clinical parameters and oral health. The in attendance study focused on the evaluation of changed of LDH in GCF chronic periodontitis patients and the potential involvement with it, and to determine the efficiency of local delivery drug (garsinia mongostana) compare with available local delivery drug as cholorohexidin gel and non-surgical periodontal treatment (NSPT) in regulating the LDH concentration (as monitor) towards health along with the improvement in the periodontal parameters.

Further, in this study, to control variables like age and sex, we chosen subjects with an one and the same number of males and females, who fell under the age range of 35-65 years, in all groups. The study design involved moderate chronic periodontitis patients (n = 30) and healthy subjects (n = 5) Lactate dehydrogenase (LDH), an enzyme normal limited to the cytoplasm of cells, is only release dextracellularly after cell death. Previous studies have demonstrated that the activity of LDH in GCF is significantly correlated with gingival inflammation and tissue destruction from periodontitis. Therefore, it has been proposed that LDH activity in the GCF is a potential marker for monitoring periodontal metabolism. The present study focused on the assessment of altered local LDH in GCF and used Garsinia mongostana as local delivery drugs inserted in gingival pocket of 10 patient have chronic periodontitis with pocket depth from 3into 5 mm after scaling and root planning in all one week for one month and take sample of GCF by paper point insert in gingival pocket of target area gently avoid blood in sample the time of insert 30 second then Put it down in eppendorf tube that has been filled with 1 ml phosphate buffer solution.measure the (PI, GI, GBI, PPD and CAL) and continuous insert garcinia gel all 1weak for one month after this take GCF sample to see LDH after treatment path by garsinia mongostana gel clinical parameters (PI, GI, GBI, PPD and CAL) of this group were found to be significantly decreased versus their levels at baseline .

The present study focused on the assessment of altered local LDH in GCF and used Garsinia mongostana as local delivery drugs inserted in gingival pocket of 10 patient have chronic periodontitis with pocket depth from 3into 5 mm

after scaling and root planning in all one week for one month and take sample of GCF by paper point insert in gingival pocket of target area gently avoid blood in sample the time of insert 30 second then Put it down in eppendorf tube that has been filled with 1 ml phosphate buffer solution.measure the (PI, GI, GBI, PPD and CAL) and continuous insert garcinia gel all 1weak for one month after this take GCF sample to see LDH after treatment path by garsinia mongostana gel clinical parameters (PI, GI, GBI, PPD and CAL) of this group were found to be significantly decreased versus their levels at baseline .

In the present study, the mean GCF of LDH was found to be higher at baseline in chronic periodontitis patients as compared to the after therapy in group one (garssinia mongostana) (395.9±78.1 vs 117.5±8.1), **(Table3),** and the difference between their means was found to be statistically highly significant (P2<0.001). This could be attributed to Lactate dehydrogenase is always confined within cell cytoplasm and becomes extracellular when a cell dies. So, its extracellular presence is always related to cell necrosis and tissue breakdown and release into the GCF ,after removal of the source of this inflammation which decreases the necrosis of the cells , tissue breakdown and LDH quantization in GCF ***Rad, Ali Banihashem, et al(2018)***.[19,18]

essential properties of mangostana include antimicrobial, anti-inflammatory, antioxidant, antiproliferative, anticancer, aromatase, and pro-apoptotic inhibitory properties. [13] There are previous studies confirmed the potential benefits of Garcinia mangostana in the prevention of many pathological disorders associated with oxidative stress and inflammation ***Chaverri, José Pedraza, et al(2019).***

Pregnant and lactating mothers were excluded from the study; as LDH are very elevated. [97] Lactation is characterized by a profound change in the levels of circulating hormones, including LDH , LDH levels during lactation are typically very low, linked to depleted fat stores and or active inhibition of LDH production by factors associated with the energy drain of milk withdrawal ***Kugahara, Tomoko, Yayoi Shosenji(2018)***. [30]

Moreover, all the individuals who participated in the study were non-smokers as smoking could affect LDH levels. Smoking appears to be among the direct modulators of LDH metabolism, smoking increases plasma catecholamines and adreno corticotrophic hormone concentrations which alter the sympathetic system activity and cause lipolysis which may decrease LDH concentrations who found that GCF LDH levels were significantly lower in smokers than in non-smokers, indicating that smoking may modulate LDH levels ***Rao, Kumuda, et al (2017)***. The clinical finding of the present study reflected significant reduction of periodontal inflammation in chronic periodontitis patients with non-surgical periodontal treatment as evident by improvement in the periodontal parameters, where the levels of PI, GI, BOP, PD, and CAL index after phase I therapy were significantly decreased versus their levels at baseline in study group **(Table 2)**. This may be related to the fact that non-surgical periodontal therapy is effective in reducing the bacterial load leading to improvement of clinical parameters and oral health ***Isola, Gaetano, et al(2018)***.

And in the same time other 10 patient used chlorohexidine as local delivery drug in the same rang of pocket depth and inserted the gel after first stage therapy and insert all one week for one month and take GCF by paper point insert in pocket for 30 seconds for LDH analysis before study and after month from used hexidin gel measure the (PI, GI, GBI, PPD and CAL) before and after .other 10 patient with same pocket depth and chronic periodontitis only do scaling and root planning and take GCF sample for LDH Analysis before and after one month from phase 1 therapy take GCF sample by paper point inserted in pocket gently .

Lactate dehydrogenase (LDH), a metabolic enzyme catalyzing the anerobic glycolysis has been a nonspecific indicator of diseases such as myocardial infarction, liver disease (being particularly high in toxic hepatitis with jaundice), megaloblastic anemia's, and renal disease ***Rad, Ali Banihashem, et al(2018)***.

LDH is an intracellular enzyme detectable in the cellular cytoplasm of all the cells in the human body, which becomes extracellular upon the cell death. Therefore, its extracellular presence is related to the cell death and tissue destruction. The LDH concentration in saliva, as an expression of cellular necrosis, could be a more specific indicator of the oral lesions that affect the integrity of the oral mucosa. This article thus reviews LDH as an enzyme marker of oral health and disease with a note on its isolation and storage.Raised LDH activity in saliva is often related to tissueinflammation and damage to oral tissues, commonly caused ***Rad, Ali Banihashem, et al(2018)***.

Lactate dehydrogenase (LDH), a metabolic enzyme catalyzing the anerobic glycolysis has been a non-specific indicator of diseases such as myocardial infarction, liver disease (being particularly high in toxic hepatitis with jaundice), megaloblastic anemia's, and renal disease. LDH is an intracellular enzyme detectable in the cellular cytoplasm of allthe cells in the human body, which becomes extracellular upon the cell death. Therefore, its extracellular presence is related to the cell death and tissue destruction. The LDH concentration in saliva, as an expression of cellular necrosis, could be a more specific indicator of the oral lesions that affect the integrity of the oral mucosa.

This article thus reviews LDH as an enzyme marker of oral health and chronic periodontitis with a note on its isolation and storage. Lactate dehydrogenase in oral health and chronic periodontitis ***Vijay, N. Yannawar, et al(2017)*** stated that LDH activity was reduced after an individual undergoes the periodontal therapy (with significant reduction with just phase 1 therapy). Thus, LDH in saliva has been a suitable indicator and diagnostic tool to assess the periodontal health. ***Vijay, N. Yannawar, et al(2017)*** in their study observed that the activities of LDH enzyme in saliva which was significantly increased in the patients with periodontal disease in comparison to those healthy patients .

In the present study, after four weeks of a non-surgical periodontal treatment of chlorohexidine gel as local delivary drug ; there was significant reduction in GCF LDH level from (395.9±78.1) at baseline to(117.5±8.1) after treatment **(Table 6)**.The findings of this study are in

accordance with***; Vijay, N. Yannawar, et al(2017).*** who investigated the effects of periodontal treatment on the GCF levels of LDH in patients with chronic periodontitis. He found that after non-surgical periodontal treatment, GCF levels of LDH was significantly decreased. Furthermore, ***Al-Khatieeb, Mustafa M., Reem (2018).***[183]investigated the role of non-surgical periodontal therapy (NSPT) on GCF levels in chronic periodontitis patients with normal body mass index. GCF concentrations were significantly higher in chronic periodontitis patients than healthy controls ***Akram, Zohaib, et al(2016)*** .

Nevertheless, in second study group (used chlorohexidin as local delivary drugs) GCF there was an increase in LDH levels from (459.7±197.3) at baseline to(106.5±5.3) after treatment (Table 5), and this was in agreement with ***Jeyasree Renganath Murugan (2018),*** chlorohexidin Chlorhexidine is a broad-spectrum biocide effective against Gram-positive bacteria, Gram-negative bacteria and fungi. Chlorhexidine inactivates microorganisms with a broader spectrum than other antimicrobials and has a quicker destruct rate than other antimicrobials (e.g. povidone-iodine). It has both bacteriostatic (inhibits bacterial growth) and bactericidal (bacteria destroyer) mechanisms of action, depending on its concentration. Chlorhexidine destroy by disrupting the cell membrane. ***Laugisch, Oliver, et al.(2016).***

Moreover, in this study, in the group 3 (only first phase therapy) GCF there was an increase in LDH levels from (415.6±49.1 vs 169.4±49.8), respectively **(Table 7)**. was in agreement with ***Al-Khatieeb, Mustafa M., Reem (2018).***

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