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# Effects Of Lavender Essential Oil On Interleukin-1B In Rat Periodontitis Model.

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**ABSTRACT**

Periodontal diseases are worldwide oral diseases which affect the supporting tissues of teeth (gingiva, periodontal ligament, cementum and bone). The most common etiological factor that contribute to initiation and progression of periodontal diseases is the dental plaque .host immune response is also a critical factor in disease progression. Many immunological cytokines have been improved to have their key effect in the destruction of periodontal tissue. Among these immunological factors are cytokines like interleukin 1-B (IL-B) .IL-1B is a well-known proinflammatory cytokine which has its rule in periodontitis. It induces inflammation process and bone resorption. lavender is a well-known aromatic plant that cultivated at many countries worldwide . Lavender essential oil is distilled from the fresh flower of lavender .It is well known for its sedative and anxiolytic effects. Many researchers have revealed an anti-inflammatory effect and antibacterial effect of lavender essential oil. In this study the effect of systemic lavender essential oil on (IL-1B) were investigated in an experimental periodontitis rat model .The researchers have induced periodontitis in rat for the first time in Iraq , as periodontitis does not affect rat teeth naturally .The periodontitis was induced experimentally . A 3.0 silk ligature was ligated around the upper second molar of the right side, to facilitate plaque build-up, accumulation and induction of periodontitis . And the periodontitis was induced after 11days of the experiment. Eighteen (18) rats were divided into 3 groups; control group, ligated only group and ligated & lavender treated group in which lavender essential oil were given systemically per oral rote once daily. After 11 days, rats were sacrified and specimens aspirated. IL-1B were measured in serum by ELISA. The results showed non significant effect of lavender essential oil on the level of IL-1B.

**Keywords**: lavender essential oil , chronic periodontitis , IL-1B .

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## INTRODUCTION

Lavender plants are a type of aromatic evergreen sub-shrubs that can reach to approximately 1 m in length. [1]. These plants are commonly found at the Mediterranean region, the Arabian peninsula, Russia, and Africa. Also, many types of lavender is found in the United States, the United Kingdom, southern Europe, and Australia.[2].The plants' flower are blue or purple in color and small . The leaves are narrow in shape, and they are gray and fuzzy when young and turn green as they mature.[3] .For a long time , Lavender has been used as an analgesic, antidepressant, antispasmodic, antibacterial agent, antifungal agent, carminative, cicatrizant, and sedative [2] . lavender extracts have been used to deal with many cases ranging from ache to migraines [1] .

The antibacterial properties of lavender essential oil and its anti-inflammatory effects has been described by different studies, but the mechanism of LEO on inflammation response is not well understood. [4]. some inflammatory processes, such as lipopolysaccharide-induced inflammatory reaction has been suppressed by Lavender [5].In a study by GABRIELA L. DA SILVA et al . 2015, searched about the anti-inflammatory effect of lavender essential oil treatment on inflammation induced by carrageenan in one model and by croton oil in another model.

Chronic periodontitis is a bacterial-induced chronic inflammation within the supporting structures of the teeth, that resulting in progressive bone and attachment loss . The clinical presentation of chronic periodontitis is determined by many host-derived and environmental risk factors, so chronic periodontitis is classified as a multifactorial disease. These factors include microbial biofilm composition, and genetic background sensitivity or systemic debilitating disorder . Host behavior can also affect the capacity of the disease like, oral hygiene habits or smoking [6,7] .
Controlling the bacterial biofilm and other risk factors, are the main strategy for periodontitis prevention and arresting progressive disease, and restoring lost tooth support are the main strategy for periodontitis treatment . [8]

The chronic inflammatory response is a complex process that consists of both innate and adaptive immune cells and their released molecules within the periodontal tissue. It is globally agreed that the locally produced proinflammatory cytokines have a major rule in periodontitis progression . these cytokines are produced by the inflammatory immune cells and by periodontal tissue cells . [6,9] .

Interleukin-1β [IL-1β] is an active pro-inflammatory cytokine that plays an essential rule in the host-defense mechanisms against infection and injury . It is also the most studied member of the 11 interleukin IL-1 family members. The most of researches have interested on its secretion by the innate immune cells like monocytes and macrophages, although it is secreated and produced by a variety of cell types [10,11] .TheIL-1 has a major role in the pathogenesis of periodontitis, through its activity in the up regulation of the host's inflammatory stimulation and bone destruction .

The IL-1B and other cytokines has major rule in the progression of periodontal disease by stimulation of the hosts immunity, the inflammatory cells recruitment, the production of lytic enzymes, and the osteoclasts activation which produce inflammation in response to the presence of periodontal pathogens [12,13,14].

## MATERIALS AND METHODS

### Animal sample

18 new Zealand rats [ albino rats]were used in the experiment . All were two to three months old age , males and females .The female should be not pregnant . All animals were subjected to the same food and same conditions , like : heat, weather, and should be healthy and free from any apparent illness , and should had normal physical activity. All animal subjected to 12 hours of day light and to 12 hours of night time [15] . All animals had been provided and reproduced by the animal house at Babylon university , the college of sciences .

**Experiment design**

The animals had been divided into 3 groups:

First group : non ligated , non treated.[control].
Second group : ligated and non treated
Third group : ligated & treated with lavender essential oil systemically per oral .

The tooth chosen for ligation was the maxillary second molar. [15].It is the most easier to be accessed through mouth opening during ligation procedure , and it had two neighboring teeth which facilitated ligature retention through the experiment time.

**Method**

The animals were divided and distributed on the cages ,in which two animals in each cage . male and female rats should be isolated and never be putted together in the same cage , otherwise the pregnancy was inevitable . The animals remained for 7 days together in the cages for acclimatization . All animal had ad libitum access to water , and one daily meal of cheese and sweet cake until the end of the experiment . After acclimatization , the second group [ ligated and non treated] and third group [ligated & treated without lavender essential oil ]were anaesthetized and ligated around the maxillary second molars with 3.0 surgical black suture . All the ligated animals should be monitored each day for ligature stability until the day of sacrificing[15] . The lavender essential oil are given orally systemically at dose of 150 mg per KG [16] . The third group [ ligated and treated without lavender essential oil ] should have received the dose once daily at morning for ten days until sacrificing.. [15]

**Lavender essential oil**

Lavender essential oil was purchased from the J.L.P.L.Panadora. Sri Lanka. As a 100% pure lavender essential oil . And a specimen of the oil have been sent to the university of science and technology in Baghdad , for characterization of the oil components. The examiner used the Agilent J&W Hps Ms ultra inert columns to characterize lavender oil sample using single quadrupole GC/MS identification to establish a sample profile . The dose were calculated according to the animal weight as 600 mg/Kg [16],and the dose were given systemically per oral through gastric tube and disposable syringe to inject the oil . The dose was given once daily until the scarifying day

In the 11 day , after the day of ligation , all animals were sacrificed . Each animal was anaesthetized by the Ketamin / xylazine mixture. The blood was aspirated from the heart of the animal by using 5 cc medical syringe and poured into yellow cap gel tube for serum isolation lately by centrifuge.

**Measurement of serum interleukin -1B**

Elabscience kit was used for serum interleukin -1B assay . 100 µl of serum was added to each well and kept in incubator at 37C° for 90 minutes. After 90 minutes the wells' liquid removed and 100 µl of of Biotinylated antibody was added to each well and incubated for 1 hour . After 1 hour of incubation with Biotinylated antibody, wells undergo three times of . aspiration and washing with wash buffer . After that each well was incubated for 15 minutes with 90 µl of substrate. Finally, a 50 µl of stop solution is added and results red at 450 nm and results calculated .

**Statistical analysis**

Data were analyzed using SPSS [statistical package of social science] software version 19. In this study the following statistics were used:

Descriptive statistics: including Shapiro-Wilk test, means, standard deviations, minimum and maximum values, frequency (No.), percentages, and statistical tables and figures.

Inferential statistics: including:

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One-way ANOVA test: to compare the measured variables among the groups. Tukey's HSD test: to test any statistically significant difference between each two groups.

## RESULTS

### Oil sample identity

The lavender essential oil was analyzed with single quadrupole GC/MS identification and the sample profile was as mentioned in table (1).

**Table 1: The compounds that consists the oil sample**

| Peak | Retention time | Compound name | Area | CAS number |
|---|---|---|---|---|
| 1 | 5.738 | Alpha-pinene | 0.51 | 000080-56-8 |
| 2 | 10.630 | D-limonene | 0.45 | 005989-27-5 |
| 3 | 10.748 | Ecalyptol | 6.63 | 00470-82-6 |
| 4 | 16.860 | 1,6-Ocatadien-3-ol, 3,7-Dimethyl | 39.61 | 000078-70-6 |
| 5 | 19.242 | Camphor | 6.10 | 000076-22-2 |
| 5 | 19.242 | (+)-Bornanone | 6.10 | 000464-49-3 |
| 6 | 20.881 | Endo-borneol | 0.57 | 000507-70-0 |
| 6 | 20.881 | Bicyclo [2.2.1}heptan-2-ol, 1,7,7-trimethyl-, (1S-endo) - | 0.57 | 000464-45-9 |
| 7 | 25.976 | 1,6-octadien-3-ol, 3,7-dymethyl-,2-amenobenzoate | 44.01 | 007149-26-0 |
| 7 | 25.976 | Linalyl acetate | 44.01 | 000115-95-7 |
| 7 | 25.976 | 1,5-dimethyl-1-vinyl-4-hexenyl butyrate | 44.01 | 000078-36-4 |
| 8 | 31.485 | Caryophyllene | 2.13 | 000087-44-5 |

The results were calculated and the values of serum IL-1B were presented as mean ±SD .The ligated &lavender showed the highest value between the three groups 316.067 ±14.192 . This were comparable with that of the ligated group 305± 14.331. While the control group showed the least values of serum IL-1B 164.717±10.733.(table.2) and (figure 1).

**Table 2: The Descriptive statistics of serum interleukin-1B [pg/ml] in different groups**

| Groups | N | Mean | S.D. | Min. | Max. |
|---|---|---|---|---|---|
| Control | 6 | 164.717 | 10.733 | 154.7 | 179.3 |
| Ligated | 6 | 305 | 14.331 | 280.3 | 320.4 |
| Ligated and lavender | 6 | 316.067 | 14.192 | 295.3 | 332.5 |

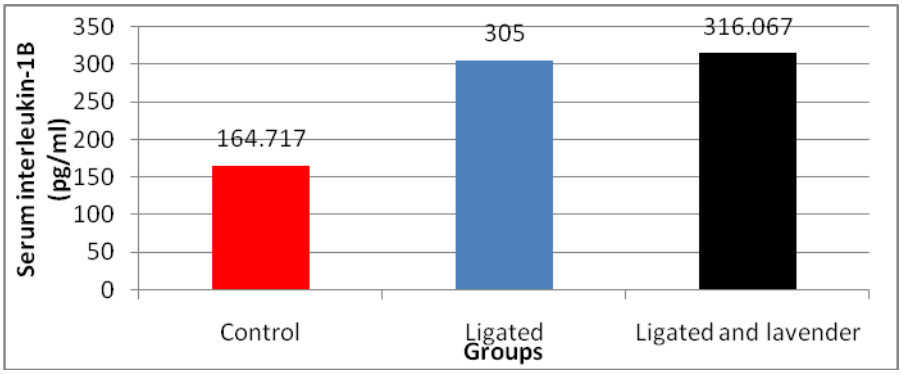


**Figure 1: The mean values of the three group [control , ligated and ligated & lavender].**

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Anova test were used to found the difference of the serum IL-1B among the three groups . Serum IL-1B showed a highly significant difference among groups .As in table (3).

**Table 3: The comparison of the serum IL-1B among the three groups. ANOVA test**

| ANOVA | Sum of Squares | d.f. | Mean Square | F-test | p-value |
|---|---|---|---|---|---|
| Between Groups | 85417.414 | 2 | 42708.707 | 245.460 | 0.000 [HS] |
| Within Groups | 2609.922 | 15 | 173.995 | | |
| Total | 88027.336 | 17 | | | |

The Tuhey's HSD test were used to found the intergroup comparison of mean difference of serum IL-1B between each pair of groups .Serum IL-1B showed a highly significant difference between control group and both of ligated group and ligated & lavender treated group . While there were non significance difference between ligated group and ligated &lavender treated group. As in table(4).

**Table 4: The Tukey's HSD test for intergroup comparison of mean difference of serum IL-1B between each pair of groups**

| Groups | | Mean Difference | p-value |
|---|---|---|---|
| Control | Ligated | -140.283 | 0.000 [HS] |
| | Ligated and lavender | -151.350 | 0.000 [HS] |
| Ligated | Ligated and lavender | -11.067 | 0.340 [NS] |

## DISCUSSION

Periodontitis is an inflammatory disease that cause destruction of the collagen fibers and alveolar bone. That destruction is a protective inflammatory mechanism of the tissue to make a space for the inflammatory cells recruitment to combat the invading microorganisms .[17].

This study revealed a highly significant increase of IL-1B in the ligated group and in the ligated & lavender treated group .Histopathologically, these two groups were presented with a grade 3 inflammation of periodontal disease .so the increase in IL-1B level in these two groups suggested its role in periodontal inflammation . IL-1B is a well known proinflammatory cytokine and it is responsible for inflammation initiation and progression with other types of inflammatory cytokines [18, 19].

In the ligated group there was a highly significant elevation of IL-1B as a result of periodontal disease inducement and microbial plaque buildup [20].Bacterial plaque will induce immune system and specially macrophage through pattern recognition receptors (PRR's) which senses[PAMP][pathogen associated molecular pattern] on the pathogen surface , and that will trigger intracellular signal to transcript the protiens responsible for IL-1B release.[10]. This result was in line with many studies[15, 21,22].

In the ligated & lavender treated group , there was a highly significant elevation of IL-1B as a result of periodontal disease inducement and microbial plaque buildup [20] as the same as ligated group .

IL-1B is a cytokin that responsible [with other inflammatory cytokines] about defensive inflammatory mechanism induction and maintenance to combat and restrict the cause of inflammation [23]. In this study, the cause of periodontitis was bacteria and its toxins [20]. So, when bacteria reduced in number the IL-1B will reduced [21] . or if there was a substance with an antagonist effect on IL-1B , IL-1B will reduced[22]or anti-inflammatory effect [24,25]. The non significant difference of IL-1B levels between ligated group and ligated & lavender treated group suggested that systemic chronic lavender dose has neither antagonistic effect on periodontal bacteria nor antagonistic effect on IL-1B.

Although in a previous study proved that lavender essential oil had a anti-inflammatory effect on IL-1B [16] but the difference was that lavender essential oil were used locally at the site of inflammation and

there was no continuous induction of inflammation , while in this study lavender essential oil was used systemically and there was a continuous induction of inflammation .And as it was mentioned above there was many other problems for the oil when used systemically:

a. It was a crude oil and the researcher did not know which material had the great effect against inflammation progression , in order to calculate its half-life and dose pattern .
b. It was impossible to determine the half-life of the oil because it was a mixture of many chemicals and not a single drug [26] . So it was used as an alternative medicine treatment , a single dose daily. So the effective chemicals may hadn't reached to the therapeutic serum level .
c. There was a difference in the constituents of the oil from one plant to another , and in the concentration of the same constituents between the plants genus's and types [27].
d. There could be a difference in the method of extraction or in the standardization of product by different companies [28,29].

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