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## The usage of orally gavage and topically applied omega -3 in the treatment of experimental gingivitis in rats.

Qayssar Riyadh abdulrazzaq \* B.D.S, M.Sc\*

Athraa M. AL Waheb\*\* B.D.S, M.SC.,Ph.D\*\*

\*Ph.D student at College of Dentistry, Baghdad University, Department of Pedodontic and Preventive Dentistry, Iraq, B.D.S, M.Sc. in Preventive Dentistry, E-mail: [qaissarr@yahoo.com](mailto:qaissarr@yahoo.com) , phone:07901641393

\*\*Prof. Dr. at College of Dentistry, Baghdad University, Department of Pedodontic and Preventive Dentistry , Iraq, B.D.S., M.Sc., Ph.D. in Preventive Dentistry, E-mail: [drathraamustafa@codental.uobaghdad.edu.iq](mailto:drathraamustafa@codental.uobaghdad.edu.iq) , Phone: 009647905995268.

### Abstract

**Background:** Gingivitis is a reversible form of periodontal disease in which the inflammation is limited to the gingiva and no other supporting tissues are destroyed. Omega-3 PUFA have the most effective immunomodulatory properties of all the fatty acids. Few studies have investigated topical usage of omega 3 poly unsaturated fatty acid in the treatment of gingivitis

**Aim of the study:** This study aimed to evaluate effects of omega 3 polyunsaturated fatty acids on ligation induced gingivitis in rats through the immunological analysis for rat's gingival tissue level of tumor necrotic factor- $\alpha$ ( TNF- $\alpha$ ) .

**Materials and methods:** The gingivitis was induced for rats by ligation around lower central incisors. Fifty animals with the induced gingivitis were divided into: five animals were sacrificed at the first day after removal of ligature, and fifteen animals as positive control group. Fifteen animals were used as 1<sup>st</sup> treatment group with orally gavage omega 3 polyunsaturated fatty acid and fifteen animals as 2<sup>nd</sup> treatment groups with topically applied omega3 a Five animals were used as negative control groups (-ve) group.

Animals were sacrificed and tissue sections were evaluated for Tumor necrotic factor - $\alpha$  (TNF- $\alpha$ ) levels, this biomarker were measured by ELISA method for four times, first after ligation removal (day zero), three days, one week and two weeks for immunological analysis.

**Results:** The result showed that level of TNF- $\alpha$  were significantly lower in systemic treatment groups in comparison with positive control groups in all duration of study, and there were significant difference between topical treatment groups on the 3<sup>rd</sup> and 7<sup>th</sup> days and no significant differences on the 14<sup>th</sup> day after removal of ligature in comparison with (+ve) ,also there was no significant

difference between these two treatment groups in all duration of study. T-test used for statistical analyses, with  $p < 0.05$  regarded as significant and  $p < 0.001$  as high significant.

**Conclusion:** orally gavage and topical application of omega-3 FAs is effective treatment for reduction of inflammation in gingivitis, resulting in lower level of TNF- $\alpha$ .

**Key words:** omega 3, TNF- $\alpha$ , rats gingivitis

## Introduction

Gingivitis is a reversible form of periodontal disease in which the inflammation is limited to the gingiva and no other supporting tissues are destroyed. Gingivitis is a complex disease caused by the combination of pathogenic bacteria invasions and various degrees of host immune response. The most common kind of gingivitis is plaque-induced gingivitis, which is caused by the deposition of microbial plaque comprising more than 300 different bacterial species and is characterized by gingival redness and edema<sup>(1)</sup>. However, as a result of breakdown of symbiosis between host's immune inflammatory response and biofilm, and the development of an incipient dysbiosis, gingivitis will be developed if dental plaque accumulates for days or weeks without disruption or eradication<sup>(2)</sup>.

IL1, TNF-, and IL8 were the most common cytokines found in normal healthy gingiva as well as in induced gingival tissue inflammation in rats.

In response to infection, monocytes and macrophages produce IL-1, but too much of it can cause irreversible tissue damage<sup>(3)</sup>.

Polyunsaturated fatty acids (PUFAs) are fatty acids that have more than one double bond between carbon and carbon. In human cells, both omega-3 and omega-6 have anti-inflammatory effects via effects on synthesis of nuclear transcription factors, enzymes, and cytokines<sup>(4)</sup>.

Omega-3, omega-6, and omega-9 fatty acids are three main types of omega FAs. Omega-3 is found in foods like fish, walnuts, and green

leafy vegetables, whereas omega-6 is found in grains and vegetable oils, and omega-9 is found in animal fat and vegetable oil<sup>(5)</sup>.

Omega-3 PUFA have the most effective immunomodulatory properties of all the fatty acids, and those from fish oil, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), are more biological potent than -linoleic acid (ALA). DHA is more beneficial than EPA, according to dose-response studies, and diets combining the two omega-3 fatty acids are synergistic<sup>(6)</sup>.

In periodontics, host modulatory therapy (HMT) refers to medications that are administered systemically or locally as a supplement to traditional periodontal treatments<sup>(7)</sup>.

There have been a few studies on the effects of topical using of omega 3 in the treatment of periodontal disorders in both humans and animals, but the results have been contradictory<sup>(8)</sup>.

As a result, the purpose of this study was to show the effects of orally gavage omega-3 PUFAs (60 mg/kg) and topically applied omega-3 on ligature induced gingivitis in rats using immunological analysis for tissue levels of TNF- $\alpha$ .

## Materials and methods

The research protocol was approved by the Research Ethics committee of college of dentistry, Baghdad University (number 331, date 18-4-2021). The experiment was carried

out in accordance with the ethical code for the handling and use of animals.

### **Rats and housing:**

Fifty-five male Sprague Dawley rats (8–9) weeks old (weighting 175–200 g) and cared in the animal house. One week before the experiment began, the animals were housed for acclimation. One rat was kept in each wire cage, with access to conventional rat food pellets and water, in a room that was kept at a constant temperature and humidity level ( $23 \pm 1$  °C and  $60 \pm 5$  % relative humidity).

### **Induction of experimental gingivitis:**

An intramuscular injection of 0.12 ml /100 g body weight of a solution of 10% Ketamine and 2% Xylazine (2:1) was used to establish general anesthesia.

After administration, anesthesia was installed in 4-5 minutes. Each subject's body weight was determined. Non resorb able 4/0 sterile silk thread were placed in figure “8” in the inferior frontal group.

This ligature worked as a gingival irritant for 7 days, promoting plaque accumulation and the progression of periodontal disease. The rats were kept in the same condition after the ligatures were placed, with one rat in each cage. The rats were transferred to a soft diet consisting of commercial food biscuits soaked in warm water for 10 minutes and then drained. Rats were monitored for seven days. The animals were assessed for proper diet and body weight on a daily basis and performed ligatures control<sup>(9)</sup>.

### **Experimental procedures :**

A total of 55 males rats were divided randomly into two main groups as follow:

**1<sup>st</sup> negative control** group consist of (5 rats ) without induced gingivitis and without any treatment just brushing the gingiva of the lower

frontal teeth with distal water were fed with the normal food Scarified after 7 days.

### **2<sup>nd</sup> Study groups**

Fifty rats with induced gingivitis is divided into following groups:

5 anesthetized animals were sacrificed at the day of removal of the ligature for tissue TNF- $\alpha$  analysis and histological examination.

The (45 rats) was subdivided into 3 subdivision groups as figure 1.

### **Cytokines analysis**

These proteins' antigens were identified using enzyme-linked immunosorbent assay (ELISA) techniques. Tissue samples were obtained and coded from lower labial gingiva of central incisor teeth. Then placed in sterile tubes and preserved at -70 C for one month to prevent bioactivity loss and contamination until TNF- $\alpha$  concentrations were determined using an immunoassay. TNF- $\alpha$  concentrations were measured using rat-specific enzyme-linked immunosorbent assay kits (CSB-E08055r) according to the manufacturer's instructions. Tissue were homogenized in 1 ml of Phosphate-buffered saline (PBS), the homogenates were centrifuged for 5 minutes at 5000rpm. The supernatant was removed and assayed immediately.

Data were analyzed using SPSS software version 23. The data were summarized using means and standard deviations. The T- test was performed to assess individual pair of groups for statistically significant finding. A p-value less than 0.05 was considered statistically significant and P-value less than 0.001 was considered highly significant.

### **Results**

Tissue TNF- $\alpha$  level Statistical analysis showed high significant increase in tissue tumor necrotic factor- $\alpha$ (TNF- $\alpha$ ) level ( $p < 0.01$ ) in the( +ve )control group and topical omega3

treatment groups in relation with the (-ve) control groups in all durations studied, and high significant increase in TNF- $\alpha$  levels in systemic omega3 treatment group on 3<sup>rd</sup> and 7<sup>th</sup> days, and significant increase on the 14<sup>th</sup> day after removal of the ligatures in comparison with (-ve) control groups (Table 1).

The systemic groups show significant decrease ( $p \leq 0.05$ ) in TNF- $\alpha$  tissue levels in comparison with (+ve) control groups in all durations of the study.

The topical groups show significant decrease ( $p \leq 0.05$ ) in tissue TNF- $\alpha$  level in the 3<sup>rd</sup> day and 7<sup>th</sup> day and non-significant decrease on the 14<sup>th</sup> days in comparison with (+ve) control groups.

The systemic groups show non-significant differences in the TNF- $\alpha$  tissue levels in comparison with topical groups on all durations of the study (Table 2).

## Discussion

Gingivitis is one of the most common diseases in humans, and many studies have looked at its pathogenesis using experimental animals. The use of ligatures in the teeth has been recommended as a way to achieve an experimental gingivitis condition faster than natural gingivitis<sup>(10)</sup>. Because of their morphological and histological similarities to the human cohort, rats are the most extensively researched animals for the development of periodontal disorders<sup>(11)</sup>.

Most rodent studies have used ligatures in the molar teeth's gingival sulcus to induce periodontal disease and increase biofilm buildup<sup>(12,13)</sup>.

Because of the complexity and difficulty of performing experiments on rats and placing ligatures around molars, the present study proposed a modification of the existing model

by placing ligatures around lower incisors instead of around molars. This modification was done to produce a repeatable experimental model for the development of periodontal disease in Wistar rats, and this method was used in a recent study<sup>(7)</sup>.

The study showed that gingivitis causes a high significant increase in TNF- $\alpha$  in all durations studied in comparison with the negative control groups. Araghizadeh et al, found that level of TNF- $\alpha$  in negative control group was ( $169.59 \pm 23.45$  pg/ml) and these level was increased significantly in the experimental gingivitis group to ( $409.84 \pm 110.02$  pg/ml) after ligation<sup>(8)</sup>.

Jalal et al, showed that periodontitis causes a significant increase in TNF- $\alpha$  during all durations of the study in comparison with the negative control/water treatment group<sup>(4)</sup>.

## Influence of orally gavage omega 3 PUFAs on TNF- $\alpha$ in rats with gingivitis

The present study showed a high significant ( $P < 0.01$ ). differences present between the tissue TNF- $\alpha$  level of the systemic omega 3 treatment group and that of negative control group in all durations of the study.

The systemic groups show significant decrease ( $p < 0.05$ ) in TNF- $\alpha$  level on all duration of the study in comparison with (+ve) control groups.

Boram found that the mean levels of TNF-increased in both the placebo and fish oil groups from baseline to four months in his study. This could be the result of an acute short term of systemic inflammation<sup>(14)</sup>.

## Influence of topical omega 3 PUFAs on TNF- $\alpha$ in rats with gingivitis

The present study showed a high significant ( $P \leq 0.01$ ). differences present between the tissue

**TNF- $\alpha$**  level of the topical omega 3 treatment group and that of negative group in all durations of the study.

The topical groups show significant decrease ( $p < 0.05$ ) in TNF- $\alpha$  level in the 3<sup>rd</sup> day and 7<sup>th</sup> day, and no significant difference on the 14<sup>th</sup> days 1 in comparison with (+ve) control groups.

Local application of omega-3 and omega-6 PUFAs can prevent or treat experimental gingivitis in humans, according to a study by Eberhard et al. In the test group, the main effect

Change activation of important transcription factors involved in controlling expression of genes encoding inflammatory proteins, such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-B), may be a mechanism through which omega-3 reduces IL-1<sup>(17,18)</sup>.

The metabolism of omega-3 FAs produces "pro-resolving lipid mediators," or SPMs, such as resolvins and protectins, which have anti-inflammatory and immunoregulatory characteristics and limit immune cell passage while inhibiting the synthesis of pro-inflammatory cytokines<sup>(19,20,21)</sup>.

## Conclusion

The results of the current study indicated that the use of Omega 3 PUFAs topically and gavage orally can reduce the gingival inflammation. because of its anti-inflammatory effects.

Omega 3 also showed reduced level of cytokines (TNF- $\alpha$ ) in gingival tissue.

## Conflicts of Interest

The authors reported that they have no conflicts of interest.

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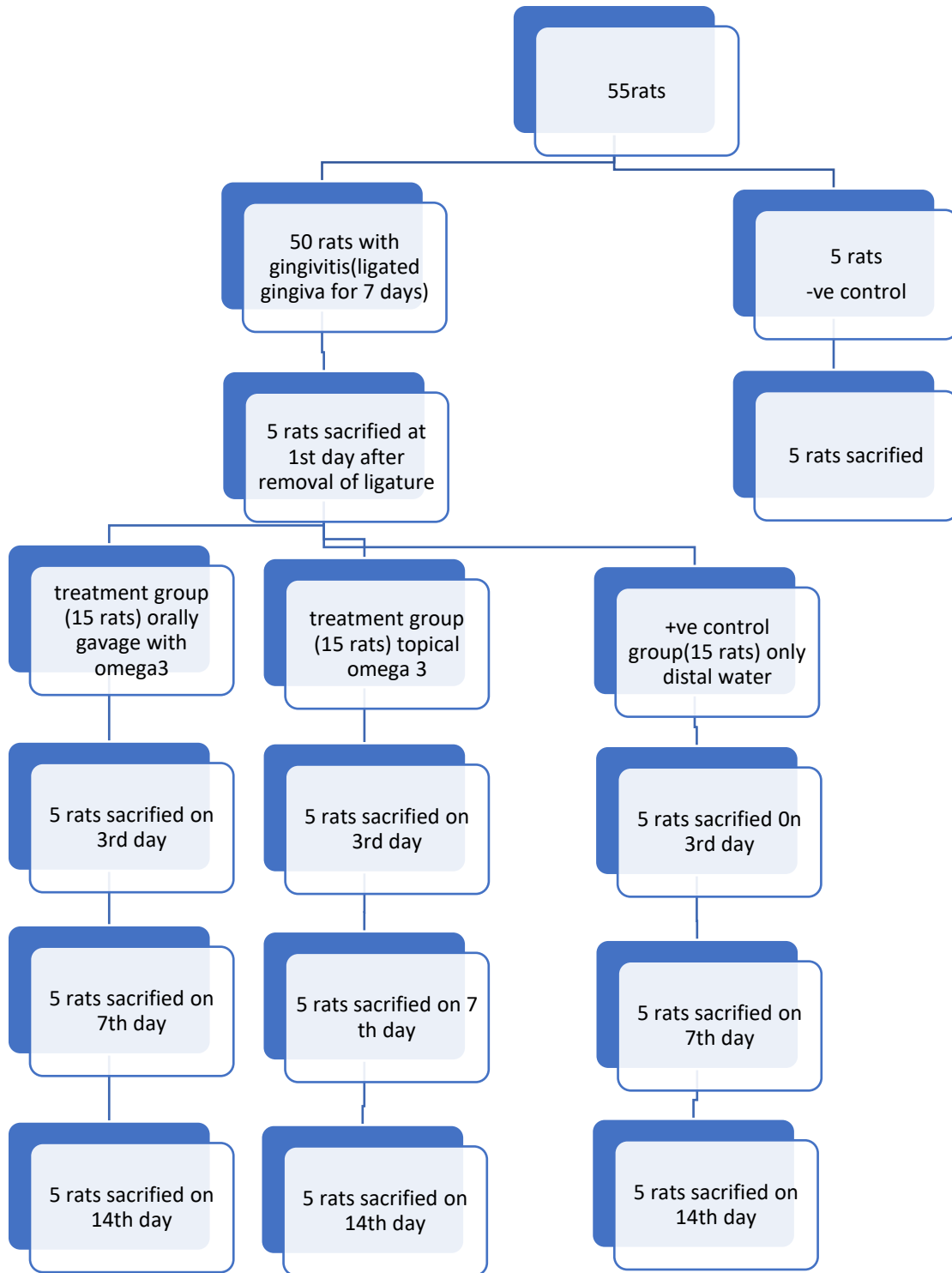


Figure 1 groups of rats



**Table 1: The difference in TNF- $\alpha$  tissue levels in different groups with difference periods**

Groups	1 <sup>st</sup> day	P value	3 <sup>rd</sup> day	P value	7day	P value	14 day	P value
-ve/+ve	206.47 $\pm$ 11.48  409.7 $\pm$ 15.06	0.0001 **	206.47 $\pm$ 11.48  387.6 $\pm$ 14.22	0.0036 **	206.47 $\pm$ 11.48  339.6 $\pm$ 12.75	0.0035 **	206.47 $\pm$ 11.48  311.2 $\pm$ 12.09	0.0042 **
-ve/systemic	-	-	206.47 $\pm$ 11.48  330.5 $\pm$ 16.84	0.0029 **	206.47 $\pm$ 11.48  290.6 $\pm$ 13.57	0.0091 **	206.47 $\pm$ 11.48  264.3 $\pm$ 10.95	0.0075 *
-ve/topical	-	-	206.47 $\pm$ 11.48  342.9 $\pm$ 23,94	0.0046 **	206.47 $\pm$ 11.48  294.4 $\pm$ 17.54	0.0057 **	206.47 $\pm$ 11.48  283.6 $\pm$ 15.63	0.0096 **
* (P<0.05). ** (P<0.01).								

**Table 2: The Effect of group in TNF- $\alpha$  tissue levels with difference period**

Groups	3 <sup>rd</sup> day	P value	7 days	P value	14 day	Pvalue
+ve/systemic	387.6 $\pm$ 14,.22  339.5 $\pm$ 16.84	0.0367 *	339.6 $\pm$ 12.75  290.6 $\pm$ 13.57	0.042 *	311.2 $\pm$ 12.09  264.3 $\pm$ 10.95	0.0376*
+ve/ topical	387.6 $\pm$ 14.22  342.9 $\pm$ 23.94	0.0487 *	339.6 $\pm$ 12.75  294.4 $\pm$ 17.54	0.049 *	311.2 $\pm$ 12.09  283.6 $\pm$ 15.63	0.598 NS
topical/Systemic	342.9 $\pm$ 23.94  339.5 $\pm$ 16.84	0.337 NS	294.4 $\pm$ 17.54  290.6 $\pm$ 13.57	0.408 NS	283.6 $\pm$ 15.63  264.3 $\pm$ 10.95	0.066 NS
* (P<0.05), NS: Non-Significant.						