

## THE EFFECT OF *OLEA EUROPAEA* MOUTHWASH ON C-REACTIVE PROTEIN , ANTIOXIDANT IN CHRONIC PERIODONTITIS

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### ABSTRACT

**Introduction:** a chronic inflammatory illness with damaging of tooth supporting structures is called periodontitis **Aim:** to evaluate the overall effects of *Olea europaea* mouthwashes as an helpful to non surgical periodontal therapy (NSPT ) on inflammation ,plaque and oxidative stress (OS) compared with chlorhexidine ( 0.2%) mouthwashes for managing of chronic periodontitis

**Material and methods :** A study will occurred at Periodontics Department, College of Dentistry, university of Duhok .The research composed from 72 participants with chronic periodontitis 39males, 33 females ranging from 25-55 years old, both NSPT and data collection were performed by single dentist . Randomly participants were divided into three categories , each category included 24patients,first group receiving NSPT and olive mouthwash, the second group receiving NSPT and CHX mouthwash and control group getting NSPT only. Clinical attachment loss (CAL) and Plaque Index (PI) were measured . Salivary CRP, Catalase(CAT) , eight hydroxy deoxyguanosine (8-OHdG) would be measured

**Results:** The concentration of salivary CRP and CAT for both groups were highly significant after 2 weeks , while other parameters were non significant

**Conclusion** Both mouthwash have a significant reduction on inflammation CRP , oxidative stress and consider as powerful antioxidant properties

**KEY WORD:** mouthwash, CRP, catalase( CAT), clinical attachment loss(CAL), (CHX mouthwash)

### INTRODUCTION

Mouthwash are fundamentally recognized to influence the supragingival plaque, while influence on the subgingival plaque is restricted, due to firm gingival attachment with tooth in well hygienic.[1].Many herbaceous extract and herbaceous mouthwash were experimented *in vivo* and *in vitro* in look for a appropriate aide to mechanical treatment for prolonged utilize.[2,3,4,5] .

*Olea europaea L.* leaves were vastly utilized in routine cures in Italy, France , Spain , Morocco, Turkey ,Israel, Greece , and Tunisia. They have been applied as an extract within the human nourishment and they incorporate few bioactive mixture that will have hypoglycemic, antihypertensive, anti-inflammatory, hypocholesterolemic, antiatherogenic in addition to antioxidant feature. Secoiridoid Oleuropein is bioactive mixture form about 6–9% of dry matter interior of the leaves. Triterpenes, flavonoids and Incorporate associated secoiridoids, are further bioactive mixtures present in olive leaves [6]. CHX digluconate is

regarded to be one of the first routinely applied compounds; Long ago ,from1950, it has been used as a powerful antiseptic factor expert in pharmaceutical with a powerful antimicrobial affect on both Gram negative and Gram positive microbes.[7,8].

In response to various inflammatory stimuli , CRP which is acute reactant plasma protein is made [9]. In serum of periodontitis patients, CRP has been identified and level were drastically greater than those of healthy individual [10]. A growing prove explained effectual root planning treatment can decrease CRP level [11]. Particles come from oxygen if not balanced by anti-oxidant compounds , can chiefly create cellular damage ( lipid, proteins and DNA). Their outcome is an fundamental structure of the entertainer reply to various damage , like microbes [12] and burns /trauma [13].

Cytochrome P-450 reactions , mitochondria, NADPH oxidase activity and peroxisomal fatty acid metabolism are major makers of ROS, , [14, 15]. Oxidative stress come from no equilibrium between redundant ROS generation and anti-oxidant mechanisms, elevated amount of ROS create a situation for OS which embroiled within pathogenesis of several illness

, counting diabetes and cardiovascular diseases [16, 17, 18]. OS has been connected with the couple onset for the breakdown of periodontal tissue [19] and systemic inflammation [20]. To evaluate the influence of *Olea europaea* gargles in comparison with CHX on salivary CRP levels on chronic periodontitis is aim of our research.

### Study design

A Flow up study was carried out at university of Duhok, College of Dentistry, Periodontology department from May 2020 to April 2021. Our study composed from 72 sick individual with chronic periodontitis (mild, moderate), both 39 males and 33 females ranging from 25-55 years old, collection of data and NSPT were performed by single dentist. randomly Patients were divided into three categories, each category composed from 24 patients, each participant were given tooth brush and tooth paste plus oral instruction

Group 1: patients who would given *olea europaea* mouth wash (20ml) two times each day about two weeks plus NSPT, for 30 second, the patients will advise to swish mouthwash

Group 2: patients who will receive CHX mouth wash (20ml) two times each day about two weeks plus NSPT and the participant would inform to gargle mouthwash for 30 second

Group 3: patients who would rinsing with normal saline plus NSPT.

Salivary antioxidant and periodontal parameters and inflammatory marker measurement would be done at base line and after two weeks. The ethical considerations was approved by the Ethical Committee in Duhok

### Exclusion criteria

- 1- Systemic disease
- 2- Consuming anti-inflammatory, antibiotics drugs for about 2 months,
- 3- Smokers
- 4- Any shape of surgical or non periodontal therapy for last period of 6 months, lactating mothers and pregnant

### Inclusion criteria

- 1- Individuals belonging to age category of 25–55 years
- 2- Individuals from mild-moderate chronic periodontitis.

### Periodontal assessment :

#### Clinical attachment loss (CAL) index

Estimating of CAL done by recording distance from cemento-enamel junction (CEJ) to

the base of the probing pocket depth by using periodontal probe [21].

**Plaque index (PI):** by Silness definition : Score 0 - no plaque, Score 1 - a film of plaque attaching to the free gingival margin and neighboring areas of tooth, Score 2 - moderate aggregation of soft deposits within the gingival pocket or on the tooth and gingival margin which can be seen. Score 3 - abundance of soft matter inside pocket of gingiva or on the tooth [22]

### Material and Methods

Participants were advised not to drink, eat, about 30 min prior gathering samples, wait 10 minutes after rinsing with water. 5ml of unstimulated saliva was gathered from patients from 9 to 11 a.m [23]. The participants were asked to be relaxed and let saliva to pool in base of their mouth then drain saliva in plastic tube about 5 minutes and store on ice, centrifuged at 4000 rpm for 10 min. The supernatant were transformed to laboratory and frozen at -20°C until recording for CRP, 8OHdG and CAT. Ethical Considerations of the study protocol was approved by the Scientific committee – College of Dentistry (16222022-1-13) from each participants written informed consents were obtained

#### Human catalase enzyme (CAT)

By Aebi's method, CAT was measured [19]. 100 µL of saliva was diluted with 4.9 mL of 50 mM (millimolar) phosphate buffer at a pH of 7. Then 1 mL of 30 mM hydrogen peroxide was mixed with 2 mL of the diluted saliva. Finally recorded at 240 nm by a spectrophotometer [24]

#### 8-Hydroxydeoxyguanosin (8-OHdG)

8-hydroxy-2'-deoxyguanosine assay No. ab201734 Abcam®, by ELISA kit was done [25]

**C-Reactive protein (CRP) :** ELISA way (Salimetric kit, USA, code number 1-3302) was done to assess spit CRP [26]

#### Preparation of plant extract

##### Cold aqueous extract preparation

*Olea europea* Rhizome was obtained from local Duhok market started from (8<sup>th</sup> April to 8<sup>th</sup> June 2020). Authentication of plant was done at Agr. Eng. Coll., Dept. of Forestry. Cleaning and peeling of rhizome were done, where peels being fresh were minced to very delicate pieces for extraction. : 200 ml of distilled water was mixed with fifty gram of (peeled rhizomes) manipulating electrical blender and the mixture then stirred magnetically for 24 h at 37°C. The residue will be removed by using filter paper (Whatman No. 1), and the filtrate was

concentrated using a rotary evaporator at 40°C [27]

**Preparation of 2%herbal mouth wash solution**

2 gram of plant watery extract will put in 100 ml volumetric flask and adding 100 ml distal water the volume will complete [28]

**Statistical Analyses :** Data were inserted in SPSS software (Ver.18). By using two tests: independent t test, unpaired t test, analyses of data were done. Significance level was set at 0.05 .

**Results :** according to table below, for both groups, there was a significant differences in CAT and CRP after two weeks the p-value  $\leq 0.0001$ . On other hand ,although there was a big changes in level of 8OHdG but it was insufficient to be significant the p-value  $\leq 0.06$ . For periodontal parameter( CAL and PI) no significant changes occur after two weeks for both groups.

**Table (1):** Comparisom between two groups after 2 weeks

After 2 weeks	<i>Olea europea</i> M±SD	Chlorohexidine M ±SD	p-value
CALmm	1.47 ± 0.61	1.45 ± 0.61	0.87
PI	1.79 ± 0.72	1.61± 0.59	0.36
CAT	0.38 ± 0.08	0.28 ±0.09	0.0001*
8-OHdG	2.32 ±1.02	1.90 ± 0.36	0.06
CRP	3075.63±524.86	5921.89±988.46	0.0001*

For second table ,there were a significant decline of (CAL,PI,CRP) and significant rise of CAT after two weeks of mouthwash and periodontal therapy

**Table (2):** Comparison within group chlorohexidine after 2 weeks

chlorohexidine	Baseline M±SD	2 weeks M ±SD	p-value
CALmm	2.05± 0.60	1.52±0.64	0.0001
PI	2.38± 0.46	1.38± 0.41	0.0001
CAT U/L	0.26 ± 0.09	0.30 ±0.10	0.003
8-OHdGng/ml	2.02 ± 0.49	1.90 ± 0.36	0.022
CRP pg/ml	6187.7 ±836.3	5921.8 ±988.4	0.022

For third table , for all parameter there were drastically reduction including (CAL,CRP,PI)

and significant increase of CAT, the p-value for all  $\leq 0.0001$

**Table (3):** Comparison within group *Olea europea* after 2 weeks

<i>Olea europea</i>	Baseline M±SD	2 weeks M ±SD	p-value
CALmm	1.69 ± 0.61	1.47± 0.61	0.0001
PI	2.18± 0.69	1.46± 9.58	0.0001
CAT U/L	0.27± 0.09	0.38± 0.08	0.0001
8-OHdG ng/ml	2.96± 1.16	2.32± 1.02	0.0001
CRP pg/ml	6345.5± 856.7	3064.4± 533.7	0.0001

**DISCUSSION**

This research is the first one to assess the influence of *Olea europea* gargles which utilized as helper to NSPT in chronic periodontitis CP. For many years ,the elemental therapy of periodontitis has been steady by elimination of calculus and plaque by NSPT. It has been observed that mechanical therapy points at eliminating all plaque rather than target-specific periopathogens,

so all the patients may not response to treatment . For protected from mechanical treatment and recolonization of periodontal pockets, certain periopathogens counting P. gingivalis may invade the tissues of gingiva .[29] Consequently, control of plaque by chemical method is pushed as an aid treatment for tissue such attacking periopathogens The out comes appeared that both periodontal treatment and mouthwash are successful in decreasing salivary CRP level inside each group in addition to

gingival inflammation . These outcomes are steady with past researches that appeared a diminish in CRP level after management of periodontitis by NSPT.[30,31,32-34] .On the contrary ,other research did not appear a alter in level of CRP after periodontal treatment[35,36].The changeability within outcomes among various studies may be differences in characteristics ,standard values of inflammatory parameters, severity of periodontitis and susceptibility of the participant . The time interval between spit collection for CRP level and periodontal therapy evaluation varies among diverse researches .The *Olea europea* show critical wide range antimicrobial properties. *Oleuropein* and byproduct from its hydrolysis example elenolate, a salt arise from elenolic acid consider as the highest active compound .All have attributes to the antimicrobial, anti inflammatory actions,[37, 38] that was capable for diminishment in plaque and gingivitis.[39, 40–42]

#### **Periodontal parameter**

The outcomes of the current research appeared that in all groups ,a noteworthy betterment in all clinical parameters of the periodontitis. This could be clarified by the truth that NSPT has an affect on the clinical discoveries, independent of the adjunctive treatment strategies. These outcomes relating to the clinical parameters of periodontitis are consistent with the past study.<sup>[19]</sup>

#### **Olive, Antioxidant and Oxidative Stress**

Concurring to our result , there was a high noteworthy changes of CAT and 8-OHdG parameter after two weeks of consuming *Olea europea* mouthwash and periodontal therapy, the p-value  $\leq 0.0001$ .,The researcher could not discover any similar studies relating to the utilize of *olive* leaf extract to be compared with this study. Activity of antioxidant of phenolic hydroxyl structure in *olive* leaf extract because of existence of hydroxyl groups in their compound for instance luteolin7-O-glucoside acid ,hydroxytyrosol and oleuropein [44].The unique mouthwash as anti-plaque is CHX for having a wide spectrum antimicrobial action and bacteriostatic or bactericidal impact (according to concentration). [45,46]

In any case, for long period of time utilize of CHX mouthwash may result in discoloration of tooth, tongue and restorative material, dryness ,soreness and taste annoyance. [47,48].CHX is cationic avoid arrangement of pellicle and is

bactericidal at higher concentrations and bacteriostatic at lower concentrations[49]

This had driven to frequent and broad examinations, looking for elective agents. Herbal gargles with less side effect and down taken a toll are presented to control the downsides of CHX. [50,51]

No contrasts was watched between *Oleuropein* and chlorohexidine mouthwash with respect to noteworthy diminishment of 8-OHdG, CAL and PI and noteworthy increment of CAT. Study done by Lalitha et al 2017 who watched way better in diminishing gingival inflammation after utilizing of chlorohexidine which accept with our study [52]

### **CONCLUSION**

Both mouthwash have a significant reduction of inflammation CRP ,oxidative stress and powerful antioxidant properties

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