

THE EFFECT OF *Pepper Nigrum*, *Olea Europaea* MOUTHWASH WITH NON SURGICAL PERIODONTAL THERAPY ON CLINICAL OUTCOME AND OXIDATIVE STRESS IN CHRONIC PERIODONTITIS PATIENTS

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ABSTRACT

Introduction: gargles are liquid topical drugs that action by gargling and rinsing into mouth. Many mouthwashes are existences and by prescription with a broad level of active components which can be manipulated as a helper to aid in the multifactorial therapy of complicated oral situation, consisting of gingivitis, halitosis, and periodontal illness. Newly (*Pepper Nigrum*, *Olea Europaea*) are herbal with antibacterial, anti-inflammatory, antioxidant and antifungal influences, using plant extracts as mouthwash, in addition to being safe and inexpensive.

Aim :To measure the effect of herbal mouthwash (*Pepper Nigrum*, *Olea Europaea*) and non-surgical periodontal therapy in comparison with periodontal therapy alone on oxidative stress, total antioxidant capacity, antioxidant enzyme activities in all spit of sick person with chronic periodontitis. To investigate association amidst oxidative stress parameter, antioxidant enzymes, total antioxidant capacity and periodontal parameter

Material and ways : A flow up study was conducted on a randomly selected sample of 72 patients (39male and 33femal) aged 25-55 years from patients attending college of dentistry, department of periodontology from December 2020, to April 2020. participants were assigned into three categories, every category consist of 24 patients: first group receiving non-surgical periodontal treatment NSPT and *Pepper Nigrum* mouthwash, second groups receiving *Olea Europaea* and NSPT while the third group control receiving NSPT only. Clinical attachment loss (CAL) and Plaque Index (PI) were recorded. Salivary oxidative stress eight hydroxy deoxyguanosine (8-OHdG), salivary Catalase (CAT) and salivary Total antioxidant capacity (TAOC), were measured

Result : The concentration of salivary antioxidants (CAT) significantly increase p-value <0.006, the periodontal parameter (CAL, PI) and oxidative stress 8-OHdG significantly decrease p-value <0.0001 and <0.001 respectively after two weeks following the NSPT in group of *Pepper Nigrum*, while TAOC was not changed. Moreover, for *Olea Europaea* group the clinical parameters of CAL, PI showed a significant decrease and p-value was (P<0.0001). Also CAT and TAOC express a significant increase (P<0.0001). In addition to oxidative stress 8-OHdG marker showed a significant decrease (p <0.0001)

Conclusion: According to our research, *Pepper Nigrum*, *Olea Europaea* mouthwash with NSPT reduced OS and bettering periodontal status and elevated salivary antioxidant. So, it is advised that *Pepper Nigrum*, *Olea Europaea* mouthwash with NSPT could be further efficient in control of inflammation in sick people with CP positive and negative correlations were found between biochemical and clinical parameters in *Pepper Nigrum* group while more positive correlation was found in *Olea Europaea* group. It indicates that periodontitis can be one of the factors for decreased antioxidant level in our body. It indicates that periodontitis can be one of the factors for decreased antioxidant level in our body

INTRODUCTION

An inflammatory course which influences defensive and steady tissues surrounded tooth is called periodontal illness. Bacterial aggregation on surface of the tooth results peripheral tissue aggravation, called gingivitis. Gingivitis is clearly usual and is show in more than 90% of united state

people[1]. If gingivitis is remain without therapy, it could advance to periodontitis, that is described by absence of periodontal connection support clinical attachment loss and resorption of bone, lately lead to movement of tooth and lack. A common characteristic feature of chronic periodontitis are slow progression and painless. It could happen in most age categories, but is more predominant amidst older age and

seniors around the world, precisely 35% of older age (30–90 years) within the US being influenced by at fewer one location with probing depth (PD) ≥ 4 mm and CAL ≥ 3 mm and [2]. The therapy of periodontitis still remains a difficult errand because of its multifactorial causes and complex illness process. In people medication, natural items have been utilized for a few a long time. Home grown drugs or herbal are the drugs which are originated from the plant have been utilized to treat illness or keep up the condition of progressed health [3]. These medications are an active alternative to antibiotics for therapeutic strategies and in the prevention of infections of oral cavity, in addition to treatment of systemic illnesses [4]. The utilize of black pepper (*pepper nigrum*) isn't only for human nutrition but can be used in therapeutic purposes [5]. As biological pest control agents and as preservatives [6]. The watery extraction of *Pepper nigrum* L. (black pepper), *Laurus nobilis* S., *Pimpinella anisum* L. and *Coriandrum sativum* L. show antibacterial activity against diverse sorts of microbes taken from oral cavity of two hundred people. Compare to *Pimpinella anisum* and *Laurus nobilis* black pepper appeared most powerful antibacterial action at the concentration of 10 μ L/disc [7]. Black pepper could be a risk nutritious origin of natural antioxidant as shown by Su et al [8].

Study design : A comparative research was done at College of Dentistry, university of Duhok, Periodontology Department. The study population consisted of 72 patients with chronic periodontitis (mild, moderate), both males and females attending college of dentistry during period of study ranging from 25-55 years old, the scaling and root planning (periodontal therapy) were performed by single dentist and data collection also done by same dentist. Selected patients were randomly assigned into three groups, each group consist of 24 patients giving each patient the same tooth paste and brush, all mouth wash occur after tooth brushing:

Group 1: patients who will receive NSPT and *Pepper Nigrum* mouth wash (20ml) 2 times per day for 2 weeks, the patients will advise to swish mouthwash for 30 second with oral hygiene instruction,

Group 2: : patients who will receive NSPT and *Olea Europaea* mouth wash (20ml) 2 times per day for 2 weeks, the patients will advise to

swish mouthwash for 30 second with oral hygiene instruction

• Group 3: patients who received NSPT, with rinsing with normal water and oral hygiene instruction only. The level of salivary antioxidant parameter and periodontal measurement were be measured at base line and after 2 weeks. Ethical accepted for the study was granted by the Scientific Committee of College of Dentistry, from all participants, the written consent was getting prior to starting of the research after the purpose of the study and oral instruction were describe to the participants who had including all of the involvement criteria. **Exclusion criteria :** History of systemic disease, manipulating antibiotics, anti-inflammatory medicines or any other drugs for at least 2 months, smokers (cigarette and nergela), vitamin supplement, patients who had experienced any type of surgical or non periodontal treatment in the last 6 months, lactating mothers and pregnant

Assessment of Periodontitis : The oral examination was carried on all teeth excluding 3rd molars in each visit. The periodontal probe was used to examine 4 sites of each tooth (mesial, buccal, distal and lingual). Buccal portion of interproximal area of tooth were probed with tip of the probe parallel to length axis of tooth and placement inter proximally near probable to touching point, measurement was done to nearest millimeters [9]

Clinical attachment loss : CAL was evaluated by measuring space from cemento-enamel junction (CEJ) to the bottom of probing pocket depth by manipulating Williams periodontal probe. [10]. Criteria of CAL included: 1-2mm mild periodontitism, 3-4mm moderate periodontitis, ≥ 5 mm sever periodontitis

Periodontal Status Examination : The dental examination was done by one of the authors who is specialist dentist under standardized environment manipulating a calibrated periodontal probe a disposable mouth mirror, masks, gloves tweezer and tray. In the current study, the periodontal status of participant is determined by the following indices. Dental PI was examined on the basis of an analysis of the four surfaces (buccal, mesial, distal and lingual), to assess the presence or absence of signs the mentioned indices. The sites were probed with a calibrated periodontal probe to confirm the presence or absence of gingival bleeding, having to wait 10 seconds. The presence of dental plaque was assessed whether

it was visualized with naked eye or there was a accumulation of soft matter inside gingival margin and /or on tooth and gingival pocket (score 2 and 3 in accordance with PI and considered to be existing if the characteristic sign is seen at least one site [11]

Material & methods (Sample Gathering) : Patients were informed not to drink, eat or clean teeth about 30 min before sampling, after rinsing with water (to remove any food residue), wait 10 minutes before collection of saliva to avoid dilution of it. 5ml of unstimulated saliva was collected from participants from period of 9 to 11 a.m to minimize diurnal variation [12]. The participants were requested to sit in relax and were informed to permit saliva to pool in base of their mouth then drain saliva inside plastic tube for 5 minutes and store on ice, then to partition squamous cells of the oral mucosa and impurities, then centrifuged of saliva at 4000 rpm about 10 min. Then supernatant were transferred to laboratory and frozen at -20°C until estimation for TAOC, 8OHdG, CAT later.

Total antioxidant capacity (TAOC) : TAOC measurement on saliva samples was done by the antioxidant commercially accessible kit (Rel Assay Diagnostics, MEGA TIP group, Turkey). Measurements were done as shown by the manufacturer. [13]

Human catalase enzyme (CAT) : Catalase was measured by Aebi's method [19]. 100 μL of spit was diluted with 4.9 mL of 50 mM (millimolar) phosphate buffer at a pH of 7. After that 2 mL of the diluted spit was mixed with 1 mL of 30 mM hydrogen peroxide. Kinetically

the reaction was followed at 240 nm by a spectrophotometer [14]

8-Hydroxydeoxyguanosin (8-OHdG) : Assay 8-hydroxy-2'-deoxyguanosine No. ab201734 Abcam®, United Kingdom, was performed. This competitive ELISA kit was done [15].

Cold aqueous extract preparation of plant : Rhizome of plant were obtained from local Duhok market / City of Duhok –Iraqi Kurdistan Region from (8th April to 8th June 2020). Authentication for plant was performed at Agr. Eng. Coll., Dept. of Forestry, Duhok University. Rhizome were cleaned and peeled where peels being fresh were minced to very fine pieces for extraction. : Fifty gram of (peeled rhizomes) was mixed with 200 ml of distilled water using electrical blender and the mixture will then stirred magnetically about 24 h. The residue will be taken out by filtration manipulating filter paper (Whatman No. 1), and the filtrate was concentrated manipulating a rotary evaporator at 40°C [16]

Preparation of 2%herbal mouth wash solution : Two gram of plant water extract will put in 100 ml volumetric flask and the volume will complete to 100 ml using distilled water [17]

Statistical Analyses : information were inserted in SPSS software (Ver.18). Analyses of data were done by manipulating two tests: independent t test, unpaired t test. Significance level was set at 0.05.

Results : Our results showed that (CAL, PI) indices, 8-OHdG, CAT and TAOC parameter between baseline and after 2 weeks was extremely significant p-value ≤ 0.0001 as in table 1

Table (1): General characteristic feature of study population

Variable	M \pm SD	p-value
age	44.05 \pm 6.94	
CAL baseline	1.96 \pm 0.59	0.0001
CAL 2weeks	1.63 \pm 0.57	
PI baseline	2.29 \pm 0.55	0.0001
PI 2weeks	1.56 \pm 0.53	
TAOC baseline	0.41 \pm 0.13	0.0001
TAOC 2 weeks	0.46 \pm 0.14	
CAT baseline	0.29 \pm 0.10	0.0001
CAT 2 weeks	0.33 \pm 0.11	
8-OHdG baseline	2.48 \pm 0.94	0.0001
8-OHdG 2 weeks	2.09 \pm 0.76	

Pepper Nigrum : after 2 weeks there was a significant difference in dental parameter (CAL, PI) p-value < 0.0001 . On other hand no any different for TAOC during this period, while for

CAT, it was a drastically increase p-value < 0.006 . In contrast 8-OHdG was significantly decreased p-value < 0.001 as shown in table 2

Table (2): Comparison within category (*Pepper Nigrum*) after 2 weeks

Variables	Pepper Base	Pepper 2 Wks	P- value
CALmm	1.99 ± 0.62	1.65 ± 0.53	< 0.0001
PI	2.19 ± 0.54	1.52 ± 0.50	< 0.0001
TAOC mmol / L	0.41 ± 0.12	0.41 ± 0.12	1
8-OH ng/ml	2.47 ± 1.06	2.22 ± 0.81	< 0.001
Cat u/L	0.26 ± 0.09	0.28 ± 0.09	< 0.006

According to pearson correlation between CAL and TAOC for (*Pepper Nigrum*), it was negative correlation ($r = - 0.14$) also it was non significant ($p\text{-value}=0.49$) as in figure 1

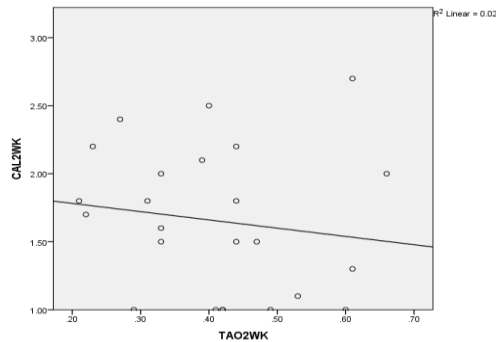


Fig. (1): Correlation between CAL and TAOC after 2 weeks

There was a strong positive correlation ($r = 0.55$) between CAL and 8-OHdG after two weeks, the result was highly significant ($p\text{-value} = 0.005$) as shown in figure 2

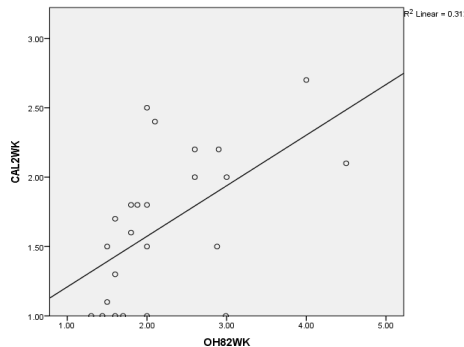


Fig. (2): Correlation between CAL and 8OHdG after 2 weeks

While for CAL and CAT, the pearson correlation was negative ($r = - 0.005$) also the data was non significant ($p\text{-value} = 0.98$) as in figure 3

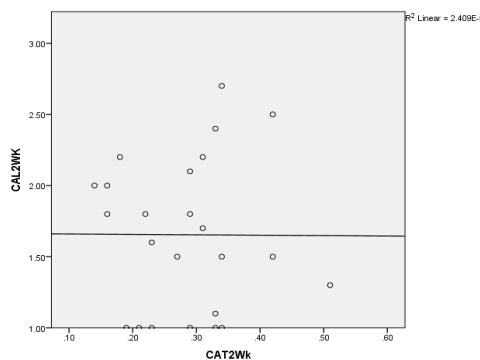


Fig. (3): Correlation between CAL and CAT after 2 weeks

For PI and TAOC, the correlation was positive ($r = 0.01$) and (p -value was $=0.96$) non significant as in figure 4

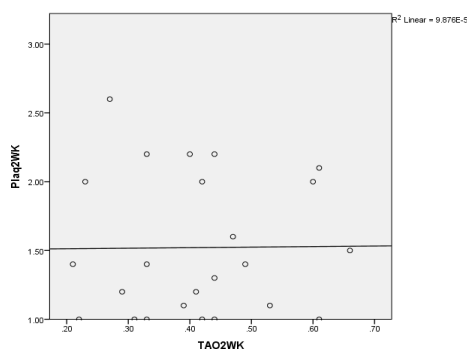


Fig. (4): Correlation between PI and TAOC after 2 weeks

A positive association among PI and 8-OHdG correlation ($r = 0.16$) and the data show non significant (p -value= 0.43) as in figure 5

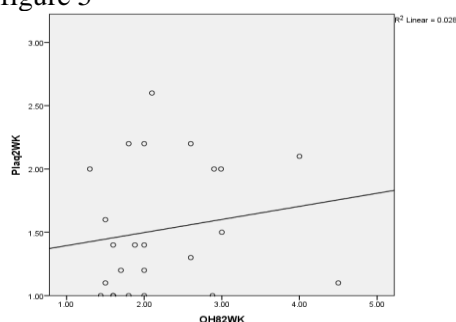


Fig. (5): Correlation between PI and 8OH-dG after 2 weeks

Also, a negative association among PI and CAT after two weeks ($r = - 0.28$), (p -value= 0.17) was non significant as in figure 6

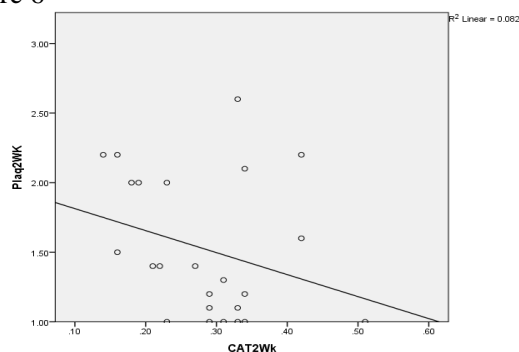


Fig. (6): Correlation between PI and CAT after 2 weeks

Olea Europaea : Inside category comparison in the intervention group explained there was a noteworthy differences in all parameter p -value < 0.0001 as shown in table 3

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

Variable	<i>Olea Europaea</i> Baseline	<i>Olea Europaea</i> 2weeks	P- value
CAL mm	1.69 ± 0.61	1.51 ± 0.63	< 0.0001
PI	2.18 ± 0.69	1.46 ± 0.58	< 0.0001
TAOC mmol / L	0.43 ± 0.13	0.56 ± 0.12	<0.0001
8-OH ng/ml	2.96 ± 1.16	2.32 ± 1.02	<0.0001
Cat u/L	0.27 ± 0.09	0.38 ± 0.08	< 0.0001

There was a positive correlation ($r = 0.02$) between CAL and TAOC after two weeks of treatment but it was non significant (p -value= 0.91) as in figure 7

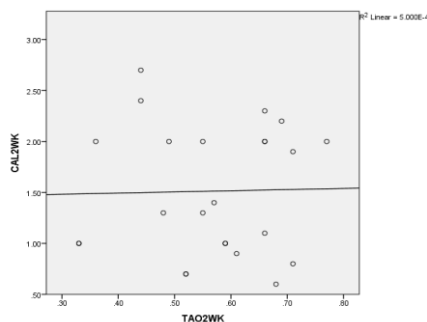


Fig. (7): Correlation between CAL and TAO after two weeks

There was a strong positive relation ($r = 0.62$) between CAL and 8-OHdG and it was noteworthy ($p\text{-value} = 0.001$) as in figure 8

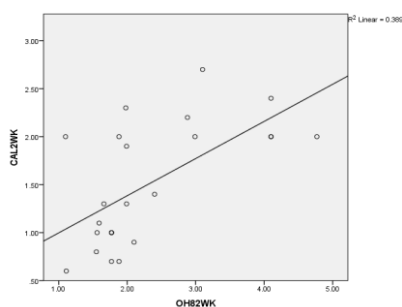


Fig. (8): Correlation between CAL and 8OHdG after 2 weeks

For CAT and CAL there was a negative correlation ($r = -0.17$) but the result was non significant ($p\text{-value} = 0.41$) as in figure 9

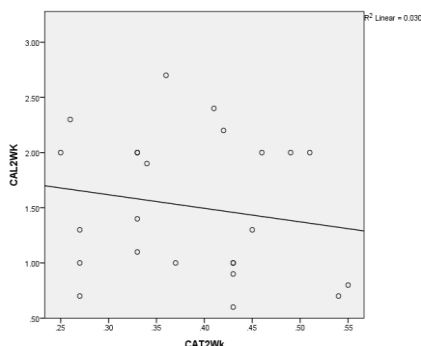


Fig. (9): Correlation between CAL and CAT after 2 week

A positive association between PI and TAO ($r = 0.14$), the data was non significant ($p\text{-value} = 0.48$) as shown in figure 10

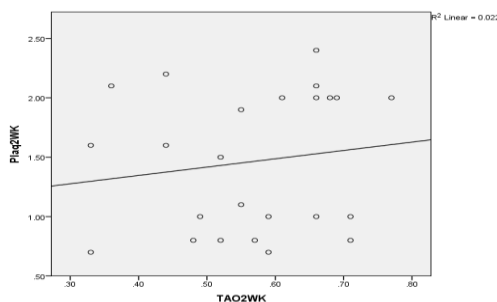


Fig. (10): Correlation between PI and TAO after 2 weeks

Also a strong a positive relationship was found between PI and 8-OHdG (r =0.42) and result was significant (p value =0.03) as shown in figure 11

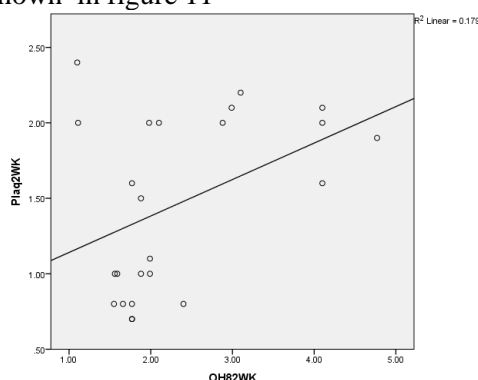


Fig. (11): Correlation between PI and 8OHdG after two weeks

Finally correlation between CAT and PI was positive (r=0.27) and(p-value=0.19) but was non significant as in figure 12

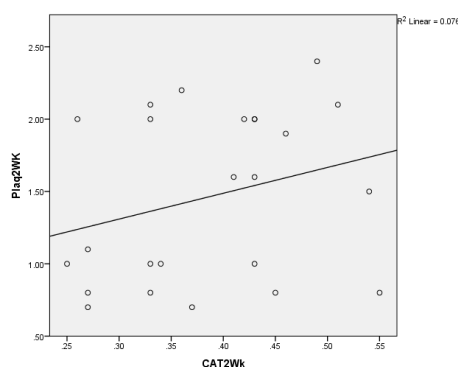


Fig. (12): correlation between PI and CAT after 2 weeks

Comparison between two groups after 2 weeks (*Pepper Nigrum* & *Olea Europaea*)

no noteworthy difference in CAL index, PI and 8-OHdG for both groups. Mean of CAT showed a significant difference between two groups 0.28 ± 0.09 and 0.38 ± 0.08

respectively, p-value < 0.0001 respectively. Patients under *Olea Europaea* and *Pepper Nigrum* mouthwash showed a significant differences in TAOC p-value < 0.0001 respectively as shown in table 4

Table (4): Comparison between *Pepper Nigrum* and *Olea Europaea* after two weeks

Variables After 2 weeks	<i>Pepper Nigrum</i> (M± SD)	<i>Olea Europaea</i> (M± SD)	P- value
CAL mm	1.65 ± 0.53	1.51 ± 0.63	NS
PI	1.52 ± 0.50	1.46 ± 0.58	NS
TAOC mmol / L	0.41 ± 0.12	0.56 ± 0.12	< 0.0001
8-OH- ng/ml	2.22 ± 0.81	2.32 ± 1.02	NS
CAT u/L	0.28 ± 0.09	0.38 ± 0.08	< 0.0001

Discussion: : As reported by our study , the postponing or avoidance cellular harms imitated by pathogenesis of free radical is conceivable because of oxidative defense ability of cell by admissions of antioxidant .Hence, most consideration has been committed to wholesome impact of common cancer prevention agents(natural antioxidant) in organic systems[18]. Radical scavenging actions and antioxidant of

black pepper (Piper nigrum Linn.) seeds have been well done both of ethanol and water extract of black pepper showed powerful antioxidant action [19]. piperine is main component of the most common spice Piper, been traditionally manipulated as diet for period of time and does not excited any danger upon subject consumption.Additional studies have revealed the safety of its consumption by

reporting a lack of piperine genotoxicity in Ames tests and in micronucleus tests [20]. Main phenolic component of *Olea europaea* L is Oleuropein has motivated attention for its many health useful characteristics example antioxidant, cardio- and neuro protective, , anti-inflammatory and anticancer.[21]According to general characteristic feature of our study population, there were a significant reduction of clinical parameter (CAL, PI) where p-value < 0.0001 .In addition to diminished of oxidative stress parameter 8-OHdG, p-value < 0.0001, while there were a significant elevated for antioxidant parameter (CAT and TAOC) , p-value < 0.001respectively.To the leading of our information, there's no consider investigates the effects of *Pepper nigrum* and *Olea Europaea* mouthwash in conjunction with NSPT on inflammation , antioxidant, and periodontal markers in sick people with CP..

***Piper nigrum* and periodontal parameters :** a study done by Jaya shankar et al reported that cleaning of teeth by a herbal toothpaste with *Pepper nigrum*, *Zinziber officinale* as consider one of main ingredient for about a 12 weeks ,showed a significant decreasing in , spit anaerobic microorganism number , gingival bleeding and better of oral hygiene [22]Yona Siddhartha et al explained that pepper extracts influence on *S.mutans* growth in vitro therefore improving it's antibacterial abilities [23] because of presence of volatile oil, alkaloids, resins , mono and polysaccharides in pepper [24] Other study on rat periodontitis model showed that Piperine clearly prevented loss of alveolar bone which agree with our study . Histological staining reveled that piperine notably decreased infiltration of inflammation in soft tissues [25].Absence of clinical study on human assessing impacts of *Pepper nigrum* mouthwash beside NSPT on periodontal parameters in CP patients . Data of the our research show that that utilization of *Pepper nigrum* mouthwash for 2 weeks beside with NSPT notably diminished mean of PI and CAL

Correlations between parameters :Relationship between clinical periodontal and biochemical parameters were assessed in *Pepper nigrum* group after two weeks .Interests, there were a negative relationships between biochemical, and clinical periodontal parameter concerning CAL. Only a strong significant positive correlation between CAL and 8-OHdG while others were non significant .On other hand , salivary 8-OHdG and TAOC were

positively correlated with dental PI while CAT was adversely correlated with it, Whereas in *Olea Europaea* group , noteworthy positive relationship was found between clinical parameter CAL and 8OHdG. Non significant positive and negative correlation between CAL and TAOC and CAT respectively .On other hand relation of dental PI with TAOC, and CAT were non significantly positive except strong positive significant between PI and 8OHdG Hence, correlation among antioxidant, OS and severity of periodontal infection watched here could be un equilibrium among antioxidants and ROS. No past investigates managed with the relationship between OS, antioxidant enzyme and TAOC of spit and clinical parameters in CP patients after periodontal treatment and using of mouthwash.

***Pepper nigrum* , antioxidant &OS :** Piperine have several anti-inflammatory characteristics as seen by Sarvesh et al . It has been confirmed [26] in-vitro experiments that it defend against oxidative harm by free radicals, hydroxyl radicals and ROS. Influence on lipid peroxidation was also occur . Piperine at little concentrations function as a hydroxyl radical scavenger [27]. It functions as strong superoxide scavenger with IC₅₀ of 1.82 mM and a52 %prevention of lipid peroxidation was seen with an IC₅₀ of 1.23 mM. Data explained that piperine have antioxidant action against many free radicals [27]Piperine shows antioxidant action in experimental situations both *in-vitro* and *in-vivo* environments by inhabiting GSH depletion and through its radical quenching influence .In our study ,intake of *Pepper nigrum* was associated with highly significant reduction of OS and increased of CAT while TAOC was not changed.The ingredients of pepper could directly initiate CAT actions as shown by Dhuley et al.[28] who have also observed that piperine prevents lipid peroxide generation and decreases elevated in acid phosphatase in rats injected with carrageenin,a structure obvious to initiate lipid peroxide generation which agree with our study. LPS in animal model , initiated mice when managed with piperine showed decreases in nitrite range and reduction TNF-a level. This research confirm free radical scavenging action of piperine [29]There was no report of any adverse effects by mouth washing with piper nigrum

***Olea Europaea* and periodontal parameter**

:No known of interactions between pharmaceuticals and OLE have been done [30] lack of data regarding studies to evaluate their dental effectiveness and role of it in OS lead to done of this study. Oleuropein is the most active compound and products from its hydrolysis example elenolate, a salt derived from elenolic acid. All have attributes to the antimicrobial and inflammatory jobs [31] which was responsible for decreasing in gingivitis and plaque .The OLE consist of natural flavonids and esters that form a structural complex that microorganisms may be unable to develop a resistance .[32] The OLE 7 has proved that it makes better the mouth's periodontal condition.[33, 34–36] which agree with our study ***Olea Europaea* , antioxidant & OS:** Oleuropein which is the main phenolic component in the *Olea europaea* L has antioxidant effect which comes from its capacity to scavenge ROS, resulted by ethanol, which stimulated peroxidation of lipid [37,38] peroxidation of lipid is a mechanism of cellular damage is widely accepted [39 - 42] El-Akabawy and El-Sherif showed that MDA, a commonly used indicator of OS and lipid peroxidation , was increased in acetic acid induced rats, while the enzymatic activity of antioxidants example CAT, SOD and GSH were decreased [43]. Ebrahimi and Hajizadeh who reported that management with olive leaf methanolic extract returned the action of antioxidant enzymes SOD, GPX, GRX, and CAT and also reduced MDA [44]. This suggests that oleuropein has a great radical scavenging activity and antioxidant which agree with our study. Also Muhammed et al study showed the capacity of oleuropein to counteract OS and inflammation ,Oleuropein caused a notably elevated in CAT ,SOD, and GPX level and a notably reduced colon MDA, and NO levels[45]

Conclusion : *Pepper Nigrum* , *Olea Europaea* mouthwash beside with NSPT reduced OS and improved periodontal status and elevated salivary levels of antioxidant enzymes.

Recommendation

1- More longitudinal research to establish the findings of our study and also to assess the efficacy of periodontal therapy with plant mouthwash in different systemic diseases related to periodontitis.

2- The tested (*Pepper Nigrum* , *Olea Europaea*) mouthwash can be regarded a good alternative for chlorhexidine in contraindicating situations.

3-The effect of the combination of antioxidants and periodontal therapy with in terms of antioxidant/oxidative stress parameters needed further investigation with longer follow up and bigger sample could further strengthen the study .

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