INTERNATIONAL BACCALAURATE PROGRAMME

BIOLOGY EXTENDED ESSAY

Topic: Investigating the local antioxidant and anti-inflammatory properties of oleuropein, the main active compound in olive leaf, in human saliva in a placebo-controlled study.

Research Question: Does the use of an oleuropein lozenge (500 mg containing 40% oleuropein) locally reduce oxidative stress markers in saliva (malondialdehyde) and increase the concentrations of antioxidant enzymes (catalase and superoxide dismutase) in the oral cavity as compared to placebo lozenge without oleuropein?

Word Count: 3992

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Introduction

Saliva, similar to other body fluids, has antioxidant enzyme systems that are active to combat various sources of stress (Valko et al., 2007). Saliva, contained in the oral cavity, is a biological fluid that is composed of several serum-derived substances (Humphrey & Williamson, 2001). Investigating saliva as a medium to assess the effects of oxidative stress and anti-oxidant markers is a potentially intriguing area of research (Helmerhorst & Oppenheim, 2007).

Background Information

The structure and functions of saliva

The inside of the mouth is lined by saliva which serves as a barrier between the internal body and external environment (Lamont, Koo, & Hajishengallis, 2018). Saliva is secreted by the salivary glands and contributes to the integrity of the oral mucosa by a variety of mechanisms, as outlined below (Tenovuo, 1998).

Saliva contains several enzymes and antimicrobial peptides that have antimicrobial activity (Dawes, 2004). Its rich supply of lysozyme, lactoferrin, and histatins offers a frontline defense against harmful pathogens entering the oral cavity (Helmerhorst & Oppenheim, 2007). Secretory immunoglobulin A (SIgA) in saliva helps neutralize toxins and prevent microbial adhesion to mucosal surfaces (Brandtzaeg, 2013).

Oral pH balance is maintained by saliva and this buffering action protects enamel from acid erosion, a phenomenon that can potentially lead to cavities (Featherstone, 2004). Maintaining a balanced pH within the oral cavity is essential for the preservation of oral microbiome, the collection of microorganisms in the mouth (Wade, 2013; Han & Wang, 2013). These useful microrganisms help in digestion, metabolism, and further counteract sources of opportunistic unwanted microorganisms (Humphrey & Williamson, 2001). However, lack of oral hygiene, change of dietary habits and and systemic illnesses lead to a change in the composition of microbiome (called dysbiosis) and this altered environment provides the ground for various gum diseases, dental problems, and even systemic health issues (Kilian et al., 2016).

Saliva contributes to neutralizing free radicals and minimizing oxidative damage to gum tissues, thanks to its enzymatic and non-enzymatic antioxidants (Battino et al., 2002). These include salivary peroxidase, superoxide dismutase, glutathione peroxidase, catalase, uric acid, and albumin (Moore et al., 1994).

The exact role of saliva in the pathogenesis of several diseases including mucositis, gingivitis, and oral cancer have not yet been elucidated (Han & Wang, 2013). This study aims to highlight the changes in saliva in response to oral administration of an antioxidant extract through the measurement of certain oxidative stress biomarkers.

Antioxidant Role of Saliva

Antioxidants act by neutralizing harmful free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS contribute to oxidative stress, cellular damage, tissue injury, and DNA mutations (Valko et al., 2007). Research has also shown that antioxidants play a role in wound healing and help reduce the production of certain inflammatory proteins (Schafer & Werner, 2008). Saliva contributes to neutralizing free radicals and minimizing

oxidative damage to gum tissues, thanks to its enzymatic and non-enzymatic antioxidants (Battino et al., 2002). These include salivary peroxidase, superoxide dismutase, glutathione peroxidase, catalase, uric acid, and albumin (Moore et al., 1994). It is well known that oxidative stress plays a significant role in the development of chronic inflammatory conditions in the oral cavity (Chapple et al., 2001).

Periodontal disease is the most common inflammatory disorder of the oral cavity. The role of oxidative stress and antioxidant defence mechanisms in chronic periodontal disease has been the subject of several studies (D'Aiuto et al., 2004). In this regard, assessment of the salivary antioxidant biomarkers is essential for understanding the mechanisms of periodontal disease mechanisms and developing new preventive strategies (Tsai et al., 2005).

Factors Contributing to Periodontitis:

Inadequate oral hygiene (Löe, 1967)

Use of smoked or chewed tobacco products (Johnson & Guthmiller, 2007)

Hormonal shifts, such as those occurring during pregnancy or menopause (Laine, 2002)

Recreational drug use, including marijuana or vaping (Cho, Hirsch, & Johnstone, 2005)

Obesity (Saito, Shimazaki, & Sakamoto, 1998)

Low vitamin C intake and other poor dietary habits (Leggott et al., 1986)

Genetic factors (Kinane & Hart, 2003)

Certain medications that cause dry mouth (Guggenheimer & Moore, 2003)

Immune-compromising conditions, such as leukemia, HIV/AIDS, or ongoing cancer treatment (Epstein et al., 2001)

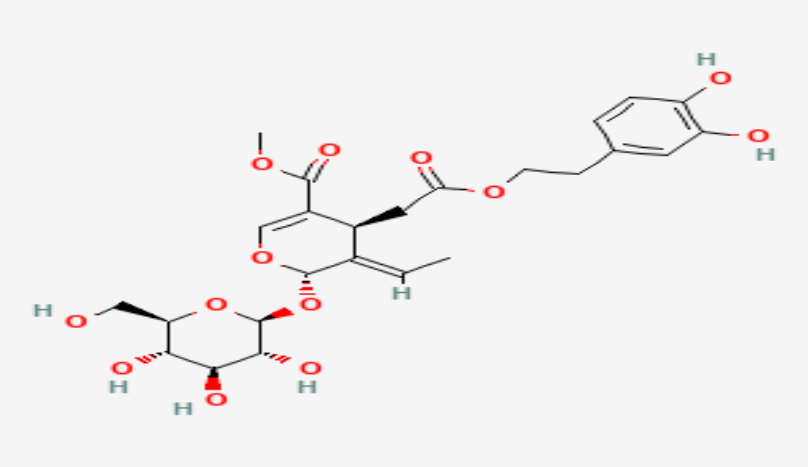
Uncontrolled chronic diseases, including diabetes mellitus, rheumatoid arthritis, and Crohn's disease (Mealey & Oates, 2006)

Oleuropein

Oleuropein is the predominant phenolic compound found in the olive fruit and leaves of Olea europaea L. (Oleaceae) (Omar, 2010). It is a glucosidic ester of elenolic acid and hydroxytyrosol. Recent studies have shown that oleuropein possesses beneficial effects on human health, such as antiatherogenic, anticancer, anti-inflammatory and antimicrobial properties.

In this essay, it is aimed to assess how applying an oleuropein lozenge affects salivary levels of malondialdehyde (MDA), a byproduct of lipid peroxidation and a key indicator of oxidative stress (Janero, 1990). Changes in the concentrations of salivary antioxidant enzymes, specifically superoxide dismutase (SOD) and catalase, before and after oleuropein use are also measured (Kankofer et al., 2002). If oleuropein is effective as an antioxidant, it is expected to observe reduced MDA levels along with increased SOD and catalase activity in saliva (Visioli et al., 2002). The results from this study will shed light on the local antioxidant effects of oleuropein.

Figure 1. The molecular structure of Oleuroplein



Methodology

Variables

Independent Variables: Oleuroplein extract (If the lozenges contain Oleuroplein or not)

Dependent Variables: Catalase activity, malondialdehyde (MDA), superoxide dismutase activity

Controlled variables: Participats health status, sampling times, sample storage conditions, laboratory methods, measurement methods.

Table 1. Controlled Variables

Controlled Variable	Reason for Control	Method for Control
Participants Health Status	Oxidative stress markers and antioxidant enzyme activites in the saliva are biological markers that can be very easily influenced by the subjects age. Participants who have systemic or oral diseases ar excluded as this can naturally increase oxidative stress in the body. 40 participants are chosen to improve reliability and statistical validity. Choosing participants that are in the 18-60 age range, reduces the risk of age-related physiological differences.	Healthy subjects who do not have any systemic or oral diseases such as diabetes, infections, inflammation, or periodontal diseases are chosen. The participants are all in the 18-60 age range.
Sampling times	Biological stress markers that are used to measure malondialdehyde (MDA), superoxide dismutase (SOD), and catalase activity can naturally change throughout the day. Same sampling times ensure that these natural daily variations do not influence the results.	All saliva samples were colleced at the same specific times. The baseline samples are collected at 8:30 a.m., and the second samples are collected at 9:30 a.m. This is exactly 1 hour after lozenge administration. The same sampling times are used on both the placebo and oleuropein administration days.

Sample storage conditions	The saliva contains enzymes and biochemical compounds that can easily change or degrade after collection if not stored properly. The MDA levels, SOD activity, and catalase activity are all sensitive to environmental conditions such as temperature, time, and bacterial activity.	The samples are stored at temperatures between +4 °C and +8 °C after collection. Keeping them at the same temperature ensures that the differences observed are caused by the effect of the Oleuroplein lozenges.
Laboratory Methods:	To ensure that the measurements of MDA, SOD, and catalase activity are accurate, consistent, and comparable across all samples. These standardized procedures ensure that samples are analyzed using the same reagents, equipments, and measurements.	The protocols were constant for all samples. This way, the experimental error and measurement variability were reduced.
Measurement Methods	To ensure that the results obtained from the saliva samples are accurate, consistent, and comparable. Measurements of MDA, SOD, and catalase activity depend on precise conditions.	Standardized analytical protocols are used including the Aebi, TBARS, and NBT methods. All measurements are performed using the same spectrophotometer wavelenghts (240nm for catalase activity, 632nm for MDA, 560 nm for SOD activity).

Risk Assesment

Direct contact with hydrogen peroxide can cause irritation, damage the skin, eyes, and the respiratory system. Ensure that proper protection equipment are worn at all times. These include googles, lab coats, and gloves. Phosphate butter is generally considered low hazard but gloves and lab coats should be used to avoid contamination. Gloves should be used for saliva samples and any direct contact should be restricted as it is considered potential biohazard because it contains microorganisms and pathogens.

Materials and Methods

Forty healthy subjects aged between 18 and 60 years participated in the study. Those with a history of oral and/or systemic diseases were excluded. IRB (investigational review board) approval was obtained. The oleuropein lozenges (500 mg containing approximately 40% oleuropein) and placebo lozenges without oleuropein were prepared at the Department of Pharmaceutical Technologies, Ankara University Faculty of Pharmacy (Figure 2).

Saliva Sample Collection Protocol

1) Participants instructed to refrain from consuming any food or beverages for at least two hours before sample collection (Navazesh, 1993). Chewing gum and smoking not also allowed during this time.

2) Collect all samples under these standardized conditions. The same rules of not eating, smoking or drinking also applied during the next 1-hour period until the second saliva collection.

3) Analyze the samples collected before and after placebo and oleuropein lozenge ingestion for MDA, superoxide dismutase (SOD), and catalase levels.

Collection of Samples

4) Collect saliva samples from the same participants over two consecutive days.

5) According to the study protocol, collect two saliva samples from each participant on each day of the study, resulting in a planned total of 160 saliva samples.

Placebo Administration

6) On the first day (placebo administration day), collect baseline saliva samples starting at 08:30 a.m. using sterile urine containers.

7) Following the initial sampling, give participants a lozenge that does not contain any pharmacological or herbal active substances, and instruct them to allow the lozenge to dissolve in the mouth.

8) One hour after lozenge administration (starting at 09:30 a.m.), collect the second saliva samples. Figure 2 demonstrates the oleuropein containing (brown) and placebo (colorless) lozenges.

Olive Leaf Extract-Oleuropein Administration

9) On the second day (olive leaf extract-oleuropein administration day), collect baseline saliva samples from the same participants starting at 08:30 a.m., after which a lozenge containing oleuropein is to be administered.

10) One hour after lozenge administration (starting at 09:30 a.m.), collect the second saliva samples, completing the sampling procedure.

Sample Storage

11) Store all saliva samples (Figure 3) at temperatures between +4 and +8 °C after collection in order to preserve biological stability until analysis. Maintain the samples under these conditions until laboratory analyses are performed (Figures 4 and 5).

12) To minimize the effects of diurnal rhythm and short-term biological variations, conduct all sampling procedures in the same individuals within the same time intervals.

Catalase Activity Measurement Method

Catalase is an important enzyme that acts to dissociate hydrogen peroxide (H_2O_2) into molecular oxygen (O_2) and water (H_2O).

Aebi Method (Measure catalase activity)

The Aebi method used to measure catalase activity was published in 1984. This method is based on the principle that the catalase enzyme breaks down hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2). As the reaction progresses, the absorbance of H_2O_2 decreases at 240 nm when measured spectrophotometrically. This decrease indicates catalase activity. High levels of hydrogen peroxide (H_2O_2) immediately lead to inhibition of the catalase enzyme by altering its active site structure, although there is variation in the extent to which this occurs.

Required Reagents:

Hydrogen peroxide (H_2O_2): Freshly prepared, usually 1:100 dilution of a 30% solution.

Phosphate buffer (50 mM, pH 7.0): Levels the reaction media.

Saliva sample: Samples stored in the refrigerator were centrifuged and the supernatant portion is used for the assay.

Spectrophotometer: Should be set at 240 nm.

Method (Modified protocol according to Aebi):

The reaction mixture is prepared to include the following substances:

2.9 mL phosphate buffer

50 μL H_2O_2 solution

50 μL saliva sample (enzyme source)

The mixture is stirred, and the change in absorbance at 240 nm is monitored (typically for the first 30–60 seconds). The rate of decrease in absorbance (ΔA240/min) is directly proportional to catalase activity.

Calculation of Catalase:

Catalase activity can be calculated using the following formula:

Activity (U/mL) = (ΔA240/min × Total Volume) / (Molar absorptivity coefficient × Sample Volume)

Molar absorptivity coefficient (ε): 39.4 $M^{-1}cm^{-1}$ (for H_2O_2 at 240 nm) (Aebi, 1984).

Total volume and sample volume should be entered in mL.

MDA Measurement Method

Malondialdehyde (MDA) is considered as the main marker in lipid peroxidation. Thiobarbituric acid (TBA) is reacted with MDA, which results in a colour compound, which can be determined spectrophotometrically, chromatographically, or through image processing techniques. Due to the reactivity of TBA with several reactive substances in the biological sample, a more widely accepted terminology called thiobarbituric acid reactive substances (TBARS) is now commonly used. TBARS is now considered as a standard marker for the lipid peroxidation induced oxidative stress.

The TBARS Method (Thiobarbituric Acid Reactive Substances) is the most common and classical method used for MDA measurement (Ohkawa, Ohishi, & Yagi, 1979).

Principle:

MDA (malondialdehyde) reacts with thiobarbituric acid (TBA) to form a pink-colored complex. This complex is measured spectrophotometrically.

Sample Protocol:

Saliva sample is centrifuged (3000 rpm, 10 min), and the supernatant is used.

200 µL sample + 800 µL TBA solution (e.g., 0.67% TBA in acetic acid solution).

The mixture is heated at 95°C for 60 minutes.

After cooling, the measurement is done spectrophotometrically at 532 nm.

A standard curve is prepared using 1,1,3,3-tetramethoxypropane (TMP) solution (used as an MDA equivalent) (Jentzsch et al., 1996).

NBT Method for Measuring SOD Activity

In the presence of superoxide radical (O_2^-), nitroblue tetrazolium (NBT) is reduced to blue-colored formazan (Beauchamp & Fridovich, 1971).

The SOD enzyme detoxifies these radicals and thus inhibits the reduction of NBT. Therefore, as SOD activity increases, formazan formation decreases. The absorbance change is measured at 560 nm.

Results and Statistical Analysis

This study was conducted on a total of 40 healthy volunteers. Of the participants, 22 were male (55%) and 18 were female (45%), with ages ranging from 22 to 30 years (mean ± SD: 25.3 ± 2.4 years; median: 25 years). Table 1 summarizes the demographics of the study participants. None of the participants had any known systemic diseases. In addition, a cursory oral examination was made to rule out active gingivitis, active oral infection, or any pathology that could affect salivary composition and alter the test results. Only individuals who were non-smokers and did not use any tobacco products were included in the study.

Prior to sample collection, all participants were instructed to refrain from consuming any food or beverages for at least two hours, and all samples were collected under these standardized conditions.

Table 2. Demographic features of the participants

Variable	Value
Number of participants	40
Male, n (%)	22 (55%)
Female, n (%)	18 (45%)
Age range (years)	22-30
Mean age ± SD (years)	25.3 ± 2.4
Median age (years)	25

On the first day, malondialdehyde (MDA), superoxide dismutase (SOD), and catalase levels were analyzed in the saliva collected before the oral administration of placebo lozenges without the extract. On the second day, the same measurements were made in the saliva collected before and and after the oral administration of lozenges containing olive leaf extract. However, saliva sample could not be obtained from 2 participants and 2 samples collected from another participant proved to be of insufficient volume for analysis. Therefore, these 4 samples were excluded from the analysis (Figure 6). The data were analyzed using paired t-tests to determine the effect of the oleuropein extract on each biomarker.

Oxidative stress parameters measured before and after treatment in the placebo and oleuropein groups are summarized in Table 2. A statistically significant reduction in MDA levels was detected following absorption of the oleuropein lozenge ($p = 0.016$). No statistically significant difference was observed in MDA levels before and after absorption of the lozenge without oleuropein extract, as expected ($p = 0.675$).

A significant increase in SOD levels was observed after absorption of the oleuropein lozenges compared to placebo ($p = 0.021$). The difference between pre- and post-absorption measurements in the non-extract group (placebo) demonstrated borderline statistical significance that can possibly be attributed to biological variability ($p = 0.051$).

Catalase activity did not change significantly following absorption of the lozenge without extract ($p = 0.399$). However, a pronounced and statistically significant decrease in catalase activity was observed after absorption of the oleuropein lozenge ($p = 0.002$).

These findings indicate that treatment with olive leaf extract oleuropein significantly modulated oxidative stress parameters, whereas no significant changes were observed in the placebo condition. The decrease in the catalase levels after absorption of oleuropein lozenges was unexpected and further analyzed in the Discussion Section.

Table 3. Oxidative stress markers measured before and after treatment in the placebo and olive leaf extract (oleuropein) groups

Parameter	Pre-Placebo	Post-Placebo	p-value**	Pre-Olive Leaf Extract	Post-Olive Leaf Extract	p-value**
MDA (nmol/mL)*	2.14 ± 0.76	2.30 ± 1.33	0.675	2.43 ± 0.95	2.05 ± 1.11	0.016
SOD (nmol/mL)*	3.86 ± 1.63	2.85 ± 1.43	0.051	3.84 ± 2.01	5.22 ± 2.50	0.021
Catalase (nmol/mL)*	91.88 ± 97.18	134.60 ± 193.11	0.399	150.06 ± 124.45	82.96 ± 89.93	0.002

MDA: malondialdehyde; SOD: superoxide dismutase; *: data presented as mean ± standard deviation; **: statistical analysis was performed using the t-test on paired samples. P value <0.05 was considered to be statistically significant.

Discussion and Evaluation

The aim of the study was to compare MDA, SOD, and catalase levels in the saliva before and after oral administration of lozenges containing oleuropein and placebo lozenges. Since MDA is a reliable biomarker of oxidative stress and lipid damage; increased MDA levels are associated

with increased lipid peroxidation (Halliwell and Gutteridge, 2015). The study showed that oleuropein containing lozenges led to a marked decrease in MDA levels, clearly demonstrating the antioxidant effect of oleuropein. Olife leaf is rich in phenolic compounds including oleuropein, hydroxytryrosol, and various flavinoids and has been shown to inhibit lipid peroxidation in previous studies (Visioli and Galli, 1998; Owen et al., 2000; Bulotta et al., 2014). Consistent with previously published findings in the literature, our results show that olive-leaf derived polyphenolic compounds contribute to a measurable reduction in oxidative stress marker MDA in the saliva (Owen et al., 2000; Botsoglou et al., 2010).

The increase in SOD activity in the oleuropein lozenge group suggests an increase in the body's own antioxidant defence systems after olive leaf extract administration. SOD plays a key role in keeping the redox balance by a chemical reaction that involves turning superoxide anions into hydrogen peroxide and oxygen (Marklund, 1992). The polyphenols contained in the olive leaf extract are associated with upregulation of antioxidant enzymes. Further, the polyphenols may also be involved in activating antioxidant signalling pathways like Nrf2 leading to the production of antioxidant enzymes including SOD (Brigelius-Flohé and Flohé, 2011). Therefore, the upregulation of the antioxidant system after oral olive leaf extract administration may be due either to increased production of SOD or activation of the existing enzyme armamentarium.

Catalase activity dropped significantly after oral use of oleuropein, which was an unexpected observation. Catalase is the second most important antioxidant enzyme after SOD responsible for the detoxification of hydrogen peroxide, preventing its accumulation and subsequent conversion into highly reactive hydroxyl radicals (Halliwell and Gutteridge, 2015). The decrease in catalase activity constrasts sharply to several previously published studies in the literature where catalase activity shows increase or stays unchanged following antioxidant supplementation. Hydrogen

peroxide detoxification in saliva, as in other biological systems, is not solely dependent on catalase; salivary peroxidase, glutathione peroxidase, and direct detoxification by polyphenolic compounds also have significant contributions. Oleuropein may enhance peroxidase-mediated detoxification of hydrogen peroxide or directly eliminate reactive species, thereby reducing the need for catalase activity. Interindividual variability, or metabolic and hormonal interactions may also be effective (Brigelius-Flohé and Flohé, 2011; Halliwell and Gutteridge, 2015). As a consequence, selective activation of a certain enzyme may be the end result, rather than a complete upregulation of the entire enzymatic system.

The body has a complex antioxidant defence system. Antioxidant enzymes are continually produced; however, their quantity depends on several factors including substrate availability, post-translational enzymatic modifications, change in body hormones, and metabolic status (Halliwell and Gutteridge, 2015; Prior and Cao, 1999). Another interesting point is that polyphenolic compounds can exert contradictory effects depending on their dose or duration of exposure to the compound (Bulotta et al., 2014; Rahman, 2007). Our study evaluated short-term outcomes. Therefore, the decrease in catalase levels may represent a transient response rather than a sustained long-term effect. The validity of these observations need to be confirmed with longer-term studies.

Additionally, potential confounding factors such as baseline dietary antioxidant intake, metabolic status, physical activity, and hormonal influences were not fully controlled. These variables are known to influence oxidative stress biomarkers and antioxidant enzyme activity (Prior and Cao, 1999; De Bock et al., 2013) and may have contributed to interindividual variability in our measurements.

Results:

Our results show that oral administration of oleuropein lozenges is associated with a reduction in salivary MDA and a reciprocal increase in SOD activity, pointing out the potential antioxidant effects of this compound. By lowering the oxidative burden in the oral and gingival tissues, oleuropein lozenges may provide a protective effect in the oral cavity that positively affects gum health.

The placebo group, in contrast to the oleuropein lozenge group, did not demonstrate a decrease in MDA levels. This result shows that placebo did not exert antioxidant effects and MDA levels did not consequently decrease. Catalase levels were essentially unchanged and SOD levels showed a slight statistically non-significant decrease in SOD activity. On the antioxidant enzyme side, no significant increases were observed, attesting to the lack of olive leaf extract effect in the placebo lozenges. While not statistically significant, the decrease in SOD levels in the placebo group may be due to the small sample size or normal biological variation. The fact that an increase in SOD was observed only in the oleuropein lozenge group strongly supports the real antioxidant effect in the olive leaf extract.

Limitations:

This study has certain limitations that need to be considered. First, the sample size was small, limiting the validity of the observations to disclose small effects. The sample size of 40 participants was determined based on feasibility considerations rather than a formal a priori statistical power calculation. With a sample size of 40, our study has approximately 80% power to detect medium effect sizes in paired comparisons at $\alpha=0.05$. Significant results suggest that our study was sufficiently powered for the observed effects. However, small or unexpected effects may have gone undetected. Second, variability in enzyme measurements may also affect the

validity of our results. Third, our study looked at only short-term changes after oral administration of oleuropein lozenges and long-term effects are still unknown. Long-term changes may alter the biomarkers evaluated in this sudy depending on several factors including dietary changes and antioxidant intake, metabolic status, physical activity and hormonal influences.

Conclusions

The study showed that the oral application of olive leaf extract oleuropein led to an increase in SOD and a decrease in MDA levels in the oral cavity, attesting to its antioxidant effects. Such changes were not observed in the placebo group. The polyphenolic compounds contained in the olive leaf extract exhibited an antioxidant effect in the saliva. Similar changes were not observed in the placebo group. Although an increase in catalase level was also expected, such an effect was not observed in the study. The decrease in catalase activity may reflect the adaptive and dynamic regulation of the body's antioxidant defense system, whereby other enzymatic and non-enzymatic mechanisms may come into play.

Oleuropein containing oral lozenges or similar medications may exert antioxidant effects in the oral cavity and provide protection for the oral cavity including the gingiva and teeth. Future research involving larger intervention and placebo cohorts with inclusion criteria for other factors controlling for dietary intake, metabolic and hormonal status, more robust statistical designs, and longer observation and re-assessment periods are needed to confirm these findings and to identify the mechanisms by which olive leaf-derived antioxidants exert their effects in the oral cavity.

Appendix

Figures

Figure 2. Photograph shows lozenges containing oleuropein (brown) and placebo (colorless).

Figure 3. Footage from inside the refrigerator at the laboratory

Figure 4. Saliva samples collected from the participants.

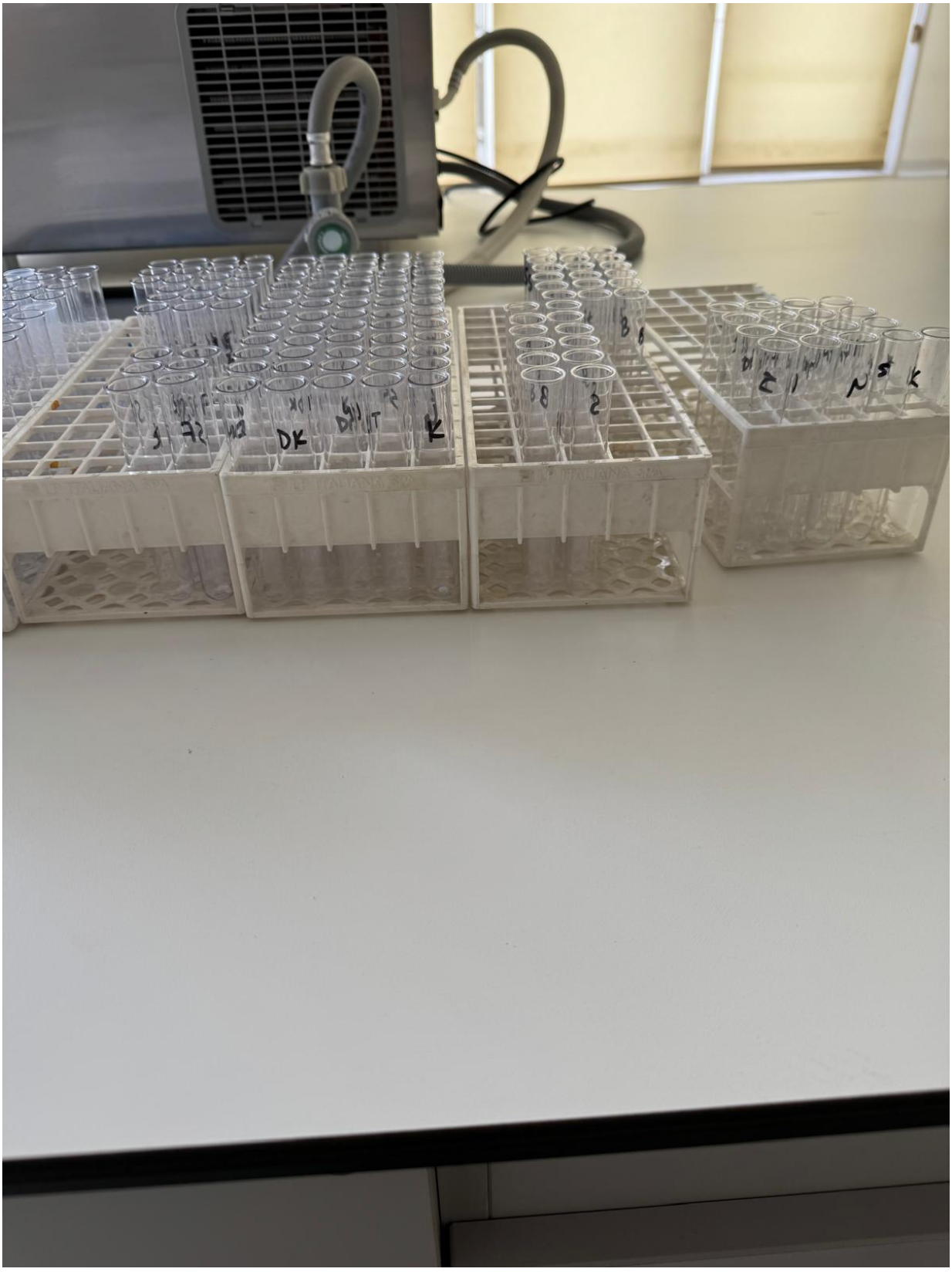


Figure 5. Testing for the study markers.

Figure 6. Excel flowsheet showing the catalase, SOD, and MDA levels obtained at baseline and after oral administration of oleuropein and placebo lozenges

Patient NO	MDA					SOD					KATALAZ							
	1	2	3	4		1	2	3	4		1	2	3	4				
1	2,5	1,21	1,53	1,45		2,31	3,6	2,02	5,12		6,59	7,32	65,88	58,56		1: Day 1, before using any lozenges		
2	3,55	1,85	2,34	1,53		7,32					58,56					2: Day 1, after oral administration of placebo lozenge		
3	1,61	1,61	1,85	1,69		2,82	4,72	3,5	5,32		87,84	7,32	541,68	336,72		3: Day 2, before using any lozenges		
4																4: Day 2, after oral administration of oleuropein lozenge		
5	2,02	2,02	1,78	1,61		2,52	2,06	3,78	6,52		36,63	7,32	21,96	14,64				
6	3,71	1,78	2,1	1,77		5,48	2,16	5,82	4,82		95,16	29,28	73,21	21,96				
7	1,53	2,18	1,93	2,42		3,42	3,98	7,04	4,62		146,43	58,56	87,84	73,22				
8	1,77	1,61	1,77	1,61		5,92	3,18	2,08	3,74		21,96	124,44	124,44	21,96				
9	2,51	1,45	1,77	1,13		5,34	5,28	4,68	3,46		307,44	43,92	183,04	29,28		Subjects 5 and 26: no saliva sample		
10	1,61	2,42	2,02	1,69		3,38	2,98	4,Mar	8,14		43,92	204,96	161,04	73,23		Subjects 3 and 24: inadequate saliva sample		
11	1,45	2,98	1,77	1,45		1,12	0,72	2,38	2,14		14,64	58,56	87,84	7,32				
12	2,11	1,77	2,18	1,77		4,82	5,38	2,82	3,64		21,96	475,81	183,02	58,56				
13	1,21	1,45	1,93	1,77		1,48	2,52	4,38	3,76		7,32	183,04	204,96	109,81				
14	2,42	1,69	2,34	1,61		3,04	3,56	3,94	8,52		21,96	175,68	248,88	102,48				
15	1,61	6,13	2,82	1,45		5,16	0,66	1,64	7,Mar		58,56	14,64	87,84	36,62				
16	2,5	1,45	2,42	1,77		3,44	2,66	1,4	5,6		95,16	87,84	285,48	241,56				
17	2,1	5,32	2,5	1,61		4,Jan	2,48	2,88	1,72		146,4	95,16	73,2	21,96				
18	1,77	3,06	4,76	5,97		6,58	3,14	5,58	6,04		190,32	761,28	58,56	197,64				
19	1,13	2,66	4,76	3,06		2,66	1,28	2,3	2,42		29,28	14,64	14,64	7,32				
20	3,48	1,11	3,71	3,63		6,19	0,96	9,06	11,62		321,82	72,74	199,2	80,03				
21	1,07	1,96	2,94	3,98		1,06	2,11	3,01	4,05		4,3	4,95	0,74	5,08				
22	2,47	1,23	1,6	1,39		2,34	3,58	2,0	5,15		5,17	9,23	59,15	61,17				
23	3,6	1,86	2,38	1,49		7,35					58,63							
24	1,57	1,6	1,86	1,63		2,74	4,74	3,5	5,35		89,11	6,09	551,18	338,08				
25																		
26	2,06	2,03	1,72	1,63		2,41	1,96	3,79	6,55		41,22	7,96	18,08	17,81				
27	3,66	1,82	2,11	1,84		5,4	2,03	5,86	4,43		93,99	29,1	70,57	24,88				
28	1,53	2,31	1,96	2,35		3,44	3,91	7,19	4,59		148,95	86,22	81,54	69,01				
29	1,75	1,51	1,73	1,62		5,96	3,19	2,11	3,69		20,76	123,92	118,56	18,53				
30	2,5	1,37	1,83	1,2		5,36	5,32	4,62	3,42		308,56	41,95	175,03	31,51				
31	1,51	2,52	1,97	1,68		3,32	2,94	3,96	8,12		44,98	203,34	159,7	72,06				
32	1,39	2,92	1,68	1,51		1,28	0,82	2,4	2,19		15,56	52,55	87,17	12,7				
33	2,08	1,76	2,23	1,71		4,95	5,37	2,81	3,57		17,11	484,75	180,27	54,92				
34	1,25	1,51	1,92	1,7		1,44	2,5	4,53	4,03		9,12	179,18	196,14	112,66				
35	2,42	1,74	2,33	1,64		2,95	3,55	4,08	8,61		20,2	181,26	258,31	103,52				
36	1,62	6,23	2,79	1,43		5,16	0,67	1,61	7		61,28	16,88	81,05	35,54				
37	2,54	1,53	2,35	1,81		3,55	2,71	1,35	5,58		84,75	98,97	288,78	246,31				
38	2,07	5,38	2,56	1,5		4,02	2,53	2,63	1,71		139,23	98,69	68,01	24,5				
39	1,79	3	4,73	6,01		6,62	3,08	5,53	5,93		191,78	749,28	64,31	192,66				
40	1,11	2,7	4,84	3,22		2,63	1,39	2,21	2,41		37,81	1,57	22,2	9,68				
41	3,41	1,13	3,75	3,63		6,23	0,88	9,03	11,66		325,76	86,13	193,69	86,16				
42																		

The results of the statistical analysis that was performed

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	MDA_1	2.1351	38	.76481	.17546
	MDA_2	2.3049	38	1.33074	.30529

Paired Samples Test

		Paired Differences			
		Mean	Std. Deviation	Std. Error Mean	95% Confidence .. Lower
Pair 1	MDA_1 - MDA_2	-.16979	1.73765	.39864	-1.00731

Paired Samples Test

		Paired ...			
		95% Confidence ... Upper	t	df	Sig. (2-tailed)
Pair 1	MDA_1 - MDA_2	.66773	-.426	18	.675

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	MDA_3	2.4363	38	.95313	.21866
	MDA_4	2.0543	38	1.11867	.25664

Paired Samples Test

		Paired Differences			
		Mean	Std. Deviation	Std. Error Mean	95% Confidence ... Lower
Pair 1	MDA_3 - MDA_4	.38196	.62638	.14370	.08005

Paired Samples Test

		Paired ...			
		95% Confidence ... Upper	t	df	Sig. (2-tailed)
Pair 1	MDA_3 - MDA_4	.68386	2.658	18	.016

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	SOD_1	3.8689	36	1.63990	.38653
	SOD_2	2.8500	36	1.43735	.33879

Paired Samples Test

		Paired Differences			
		Mean	Std. Deviation	Std. Error Mean	95% Confidence .. Lower
Pair 1	SOD_1 - SOD_2	1.01889	2.05725	.48490	-.00416

Paired Samples Test

		Paired ...				
		95% Confidence ... Upper	t	df	Sig. (2-tailed)
Pair 1	SOD_1 - SOD_2	2.04193	2.101	17	.051

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	SOD_3	3.8489	36	2.01898	.47588
	SOD_4	5.2289	36	2.50236	.58981

Paired Samples Test

		Paired Differences			
		Mean	Std. Deviation	Std. Error Mean	95% Confidence .. Lower
Pair 1	SOD_3 - SOD_4	-1.38000	2.31236	.54503	-2.52991

Paired Samples Test

		Paired ...				
		95% Confidence ... Upper	t	df	Sig. (2-tailed)
Pair 1	SOD_3 - SOD_4	-.23009	-2.532	17	.021

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	KATALAZ_1	91.8660	36	97.18807	22.90745
	KATALAZ_2	134.6067	36	193.11713	45.51814
Pair 2	KATALAZ_3	150.0600	36	124.45900	29.33527
	KATALAZ_4	82.9600	36	89.93214	21.19721

Paired Samples Test

		Paired Differences			
		Mean	Std. Deviation	Std. Error Mean	95% Confidence .. Lower
Pair 1	KATALAZ_1 - KATALAZ_2	-42.74067	209.69816	49.42633	-147.02111
Pair 2	KATALAZ_3 - KATALAZ_4	67.10000	76.12345	17.94247	29.24470

Paired Samples Test

		Paired ...			
		95% Confidence ... Upper	t	df	Sig. (2-tailed)
Pair 1	KATALAZ_1 - KATALAZ_2	61.53978	-.865	17	.399
Pair 2	KATALAZ_3 - KATALAZ_4	104.95530	3.740	17	.002

Date: February 16, 2026

Subject: Oleuropein Study

To Whom It May Concern,

Melissa has conducted her IB Diploma Programme "Extended Essay" experiments by herself under the supervision of our staff and me at the "Cebeci Campus Central Medical Laboratory" facilities of the "Ankara University School of Medicine, Ankara, Turkiye".

Best Regards

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Head of Central Medical Laboratory, Cebeci Campus
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