

Oleuropein Is Responsible for the Major Anti-Inflammatory Effects of Olive Leaf Extract

Khaled Qabaha,¹ Fuad AL-Rimawi,² Ahmad Qasem,³ and Saleh A. Naser³

¹Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, Arab American University, Jenin, Palestine.

²Department of Chemistry, Faculty of Science and Technology, Al-Quds University, Jerusalem, Palestine.

³Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, Florida, USA.

ABSTRACT Olive leaves are rich in polyphenolic compounds that are known to have antioxidant, antimicrobial, and anti-inflammatory activities. Therefore, olive leaf extract (OLE) is considered as a natural supplement. In this study we evaluated the antibacterial and the anti-inflammatory effect of OLE and its individual phenolic components *in vitro*. Polymorphonuclear cells (PMNCs) were isolated from the whole blood using Histopaque solution and cultured in RPMI-enriched medium. Tumor necrosis factor α (TNF α) level was determined by ELISA after 24 h of lipopolysaccharide stimulation. The antibacterial activity of OLE was determined by well diffusion assay. We found a significant decrease in TNF α secretion level in PMNCs culture treated with OLE. Oleuropein is the only OLE component that has shown anti-inflammatory effects at a concentration of 20 μ g/mL. Furthermore, OLE exhibited antibacterial activity against some gram positive bacterial strains; however, gram negative bacterial strains were resistant to OLE. Downregulation of TNF α secretion in PMNCs culture in response to OLE treatment indicates that this polyphenol-rich extract has an anti-inflammatory effect, and oleuropein is the major OLE component responsible for this effect. The antibacterial activity of OLE is limited to gram positive bacteria.

KEYWORDS: • gallic acid • OLE • oleuropein • olive leaf extract • phenolic compounds • TNF α

INTRODUCTION

THE CULTIVATION OF OLIVE TREES (*Olea europaea*) and the production of olive oil have been a very crucial farming practice in the Mediterranean region, which dates back to ancient times. The average annual consumption of olive oil in that area varies between 0.5 and 15 kg/person,¹ where the Mediterranean diet has been known to be protective against cancer and coronary heart disease.^{2,3}

Olive leaf extract (OLE) is a liquid obtained from the leaves of the olive tree, which has been marketed as a natural supplement for multiple health benefits, such as its antioxidant activity, which results in cardioprotective and chemoprotective effects.⁴ In addition to that, OLE has antimicrobial activities, which may help in treatment of various infectious diseases.^{5,6} Several compounds have been found in OLE, altogether known as olive biphenols, which are primarily responsible for its therapeutic activities. Oleuropein is the most abundant biphenol in OLE, whereas other biphenols are present in lower quantities.⁷

Reported epidemiological studies suggest that intake of olive products might influence disease intensity and prevalence.^{8–10} For instance, the mortality rate from colorectal

cancer is lower in countries such as Spain where olive oil intake is high than in countries such as England where the olive oil intake is low.¹¹ Owing to geographical location and variation in plant nutrition, OLE might have a different composition depending on where it is coming from. Therefore, it is essential to find out the connection between OLE individual components (Fig. 1) and their physiological therapeutic effects. In this study, the primary focus was on comparing between the anti-inflammatory and the antibacterial effects of OLE and its individual components.

MATERIALS AND METHODS

Individual compounds of OLE

Liquid solutions of biphenolic OLE compounds tyrosol, catechin, gallic acid, and vanillic acid were purchased from Sigma Aldrich (St. Louis, MO, USA). Oleuropein liquid solution was purchased from Merck & Co. (Kenilworth, NJ, USA).

Olive leaf crude extract

Olive leaves were obtained from Nabali cultivar (Ramallah, Palestine), dried at 30°C, then ground with a blender. Five grams of the ground extract was exposed to 50 mL of absolute ethanol for 3 h at 40°C. The liquid extract was filtered through suction filtration, followed by evaporation by using a rotary evaporator, and crude OLE was finally obtained.

Manuscript received 17 May 2017. Revision accepted 21 August 2017.

Address correspondence to: Khaled Qabaha, PhD, Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, Arab American University, Jenin 06610, Palestine, E-mail: khaledqabaha@yahoo.com

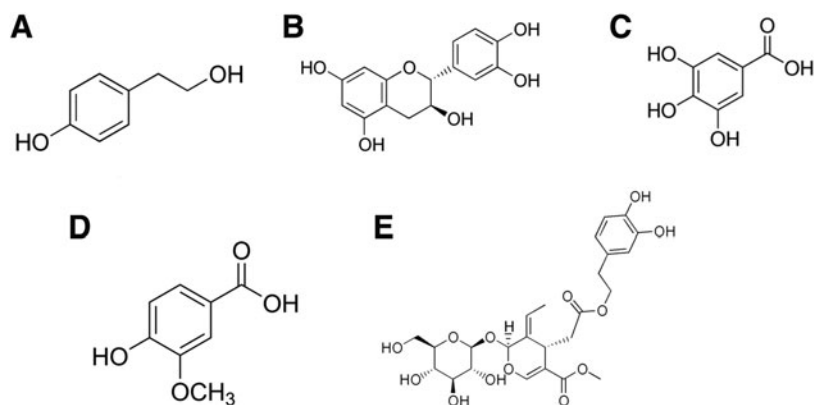


FIG. 1. Structures of individual olive leaf extract phenolic compounds. (A) Tyrosol, (B) catechin, (C) gallic acid, (D) vanillic acid, (E) oleuropein.

Medium preparation

Soybean–casein digest broth (TSB) has been prepared according to manufacturer instructions and autoclaved at 121°C for 15 min.

Evaluation of antibacterial activities

Clinical strains of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Escherichia coli* were kindly provided by the microbiology department of Al-Quds University (Jerusalem, Palestine). Mueller–Hinton agar plates were used to evaluate the antimicrobial activity of the OLE and results were compared with neomycin as a control. Well diffusion assay was performed according to National Committee for Clinical Laboratory Standards (NCCLS; 1993). Thirteen micrograms of both the OLE and neomycin was added to different wells, zone of inhibition of bacterial growth was measured after 24 h of incubation at 37°C (NCCLS, 1993).

Blood sample processing

Human blood sample was collected, after signing a consent form from the donor, in two separate sets (6 mL each) and saved in K2-EDTA tubes. Polymorphonuclear cells (PMNCs) were isolated from whole blood and cultured as described earlier.¹²

Cytotoxicity of OLE

Cell viability was tested by trypan blue exclusion assay.¹³ OLE was added to PMNCs culture at a concentration of 320 µg/mL for 16 h after stimulation with 1 µg/mL of lipopolysaccharide (LPS). Results were compared with control cell culture with or without LPS stimulation.

Evaluation of anti-inflammatory activities of OLE and its phenolic compounds

Cultured PMNCs were stimulated with 1 µg/mL LPS and exposed to different concentrations of OLE as well as its individual phenolic compounds. Secreted tumor necrosis factor α (TNFα) level was detected by TNFα detection ELISA kit (R&D Systems, MN, USA), according to manufacturer's instructions.

Statistical analysis

Values were analyzed for significance using paired *t*-test. SPSS software version 19 was used. *P*-value < .05 was considered significant.

RESULTS

OLE has no cytotoxic effects

OLE at a concentration of 320 µg/mL showed no significant effect on PMNCs cell viability compared with cell culture with or without LPS stimulation. Results are illustrated in Table 1.

Anti-inflammatory activity of OLE and its phenolic compounds

The OLE had a significant inhibitory activity on TNFα production followed by LPS stimulation at a concentration-dependent manner (Table 2). When we tested the individual OLE compounds separately, we found that only oleuropein has a significant inhibitory effect of TNFα production at a concentration of 20 µg/mL. The other compounds did not show any significant inhibitory effect at concentration as high as 50 µg/mL except gallic acid (Table 3).

Antibacterial activity

We compared the antibacterial activity of OLE to neomycin against four clinical bacterial strains. OLE has shown an antibacterial activity similar to neomycin against

TABLE 1. EFFECT OF OLIVE LEAF EXTRACT (320 µg/mL) ON POLYMORPHONUCLEAR CELLS VIABILITY

Contents	Viability	P
PMNCs only	96.1 ± 1.5	
PMNCs with LPS only	94.2 ± 2.1	>.05
PMNCs with LPS and OLE	93.4 ± 2.4	>.05

Results are expressed as average ± standard deviation (*n*=3).

LPS, lipopolysaccharide; OLE, olive leaf extract; PMNCs, polymorphonuclear cells.

TABLE 2. EFFECT OF OLIVE LEAF EXTRACT ON PRODUCTION OF TUMOR NECROSIS FACTOR- α BY POLYMORPHONUCLEAR CELLS

	<i>TNFα mean concentration pg/mL</i>		
PMNCs only	56.2 \pm 1.2		
PMNCs treated with 1 μ g of LPS	997.3 \pm 3.0		
OLE concentration	80 μ g/mL	160 μ g/mL	320 μ g/mL
	44.7 \pm 1.5	16.8 \pm 0.4	0.6 \pm 0.1

Results are expressed as average \pm standard deviation ($n=3$).
TNF α , tumor necrosis factor.

S. aureus and *S. epidermidis*. However, there was no antibacterial activity of OLE detected against *P. aeruginosa* and *E. coli*. Data are illustrated in Table 4.

DISCUSSION

Olive leaves contain a very limited amount of oleic acid with a significant quantity of polyphenols, which gives OLE a unique approach to study the effects of polyphenolic content of olive oil and olive products in general.¹⁴ One of the major phytochemical compounds present in large quantities in OLE is oleuropein, which can be hydrolyzed to hydroxytyrosol, oleuropein aglycone, elenolic acid, and glucose.^{15,16} Studies have shown that oleuropein exhibits many pharmacological activities *in vitro*, including anti-inflammatory and antioxidants effects.^{16,17} Interestingly, similar effects were noticed *in vivo*, wherein oleuropein and its major metabolite have enhanced nitric oxide production, decreased blood pressure, inhibited platelet aggregation, and reduced infarct size in animal models.^{18–20}

In this study, our aim was to determine which individual phenolic component of OLE will have the most anti-

TABLE 3. EFFECT OF THE FIVE PHENOLIC COMPOUNDS ON PRODUCTION OF TUMOR NECROSIS FACTOR- α BY POLYMORPHONUCLEAR CELLS

	<i>TNFα mean concentration pg/mL</i>	
PMNCs only	56.2	
PMNCs treated with 1 μ g of LPS	997.3	
Tyrosol (concentration is 0.14 and 0.35 mM at 20 and 50 μ g/mL, respectively).	20 μ g/mL	50 μ g/mL
	933 \pm 3.0	763 \pm 3.9
Catechin (concentration is 0.07 and 0.17 mM at 20 and 50 μ g/mL, respectively).	990 \pm 3.8	988 \pm 5.1
Gallic acid (concentration is 0.12 and 0.29 mM at 20 and 50 μ g/mL, respectively).	816 \pm 3.2	138 \pm 6.0
Vanillic acid (concentration is 0.12 and 0.29 mM at 20 and 50 μ g/mL, respectively).	999 \pm 8.3	756 \pm 7.0
Oleuropein (concentration is 0.04 and 0.09 mM at 20 and 50 μ g/mL, respectively).	159 \pm 3.2	26.5 \pm 1.4

Results are expressed as average \pm standard deviation ($n=3$).

TABLE 4. ANTIBACTERIAL ACTIVITY OF OLIVE LEAF EXTRACT COMPARED WITH NEOMYCIN

<i>Zone of inhibition (mm)</i>		
Bacteria	Neomycin (13 μ g/well)	OLE (13 μ g/well)
<i>Staphylococcus aureus</i>	15.5 \pm 0.3	13.1 \pm 0.1
<i>Staphylococcus epidermidis</i>	15.8 \pm 0.2	14.3 \pm 0.1

Results are expressed as average \pm standard deviation ($n=3$).

inflammatory effect compared with OLE overall. We found a significant inhibition of TNF α secretion from PMNCs upon OLE treatment at concentration of 80 μ g/mL after LPS stimulation. TNF α level has reached 0.6 \pm 0.1 pg/mL when higher concentration of OLE (320 μ g/mL) was used, which is consistent with what have been reported before.²¹ Oleuropein is the only individual component that exhibited a significant level of TNF α secretion once we tested OLE components separately at 20 μ g/mL. When higher concentrations were used (50 μ g/mL), gallic acid showed a significant inhibition of TNF α secretion, whereas the effect of other components was not significant. Furthermore, OLE did not show any cytotoxic effects to PMNCs.

Finally, we have shown that OLE at the same concentration as neomycin (13 μ g/well) had a similar antibacterial effect against gram positive bacteria (*S. aureus* and *S. epidermidis*), whereas gram negative bacteria (*P. aeruginosa* and *E. coli*) were resistant to OLE treatment.

In conclusion, this work has shown that OLE has anti-inflammatory and some antibacterial effects. Oleuropein is the major compound in OLE that is responsible for its anti-inflammatory effect. Thus, the purified major compound oleuropein from OLE can potentially be used for further pharmacological applications. In addition to that, a better understanding of how pathogens respond to OLE will contribute to using it as a preservation compound especially against foodborne pathogens.

ACKNOWLEDGMENTS

Our thanks are due to all laboratory members who participated in this study.

AUTHOR DISCLOSURE STATEMENT

The authors have no conflict of interest that could inappropriately influence this research article.

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