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

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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 (TNFα) 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 TNFα 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 μg/mL. Furthermore, OLE exhibited antibacterial activity against some gram positive bacterial strains; however, gram negative bacterial strains were resistant to OLE. Downregulation of TNFα 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 • TNFα

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/person1 back to ancient times. The average annual consumption of farming practice in the Mediterranean region, which dates 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.
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.
PMNCs only $56.2 \pm 1.2$
PMNCs treated with $997.3 \pm 3.0$
1 $\mu$g of LPS
OLE concentration $80 \mu$g/mL $160 \mu$g/mL $320 \mu$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$\alpha$, 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 content of polyphenols, which gives OLE a unique approach to study the effects of polyphenolic in vitro, including anti-inflammatory and antioxidants effects. 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. Studies have shown that oleuropein exhibits many pharmacological activities in vitro, including anti-inflammatory and antioxidants effects. 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. 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 compared with OLE overall. We found a significant inhibition of TNF$\alpha$ secretion from PMNCs upon OLE treatment at concentration of $80 \mu$g/mL after LPS stimulation. TNF$\alpha$ level has reached $0.6 \pm 0.1$ pg/mL when high concentration of OLE ($320 \mu$g/mL) was used, which is consistent with what have been reported before. Oleuropein is the only individual component that exhibited a significant level of TNF$\alpha$ secretion once we tested OLE components separately at $20 \mu$g/mL. When higher concentrations were used ($50 \mu$g/mL), gallic acid showed a significant inhibition of TNF$\alpha$ 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 ($13 \mu$g/well) had a similar antibacterial effect against gram positive bacteria (S. aureus and S. epi-
dermidis), 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.

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**AUTHOR DISCLOSURE STATEMENT**

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

**REFERENCES**