EXPERIMENTAL STUDY

Investigation of the effects of oleuropein rich diet on rat enteric bacterial flora

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ABSTRACT

OBJECTIVES AND BACKGROUND: Oleuropein is a phenolic compound of olive leaves. Enteric bacterial flora is very important for human health and diet is a directly affecting factor on enteric bacterial flora composition. In this study, it was hypothesized that oleuropein could reduce total aerobic bacterial count in rat caecal flora.

METHODS: Twenty adult, male, Wistar albino rats were randomly divided into two groups. Group C (n=10) was fed with standard rat chow and water for 30 days. Group O (n=10) received olive leaf extract 20 mg/kg/day by intragastric gavage in addition to standard rat chow and water for 30 days. One gram of caecal content was collected from each rat and then consecutive 10-fold serial dilutions were prepared with a final concentration of 10^-8. Then 0.1 ml of each dilution were spread onto the surfaces of Plate Count Agar and Violet Red Bile Glucose Agar to enumerate the aerobic enteric bacteria.

RESULTS: Total aerobic bacterial counts of Group O were significantly lower than of Group C in all agar plates inoculated with caecal samples for every dilution (p<0.05).

CONCLUSION: Adding oleuropein to enteral feeding solutions of critically ill patients may be advantageous in the presence of clinical conditions predisposing to bacterial translocation by reducing enteric bacterial counts (Tab. 1, Ref. 32). Text in PDF www.elis.sk.

KEY WORDS: enteric bacteria, intensive care, oleuropein, rat.

Introduction

Oleuropein is the main constituent of the leaves and olive drupes of Olea europaea and known as a rich source of polyphenols (1). Olive leaf (Olea europaea L.) has been used traditionally in Mediterranean countries, particularly as an antimicrobial and cardioprotective agent (2). Phenolic compounds such as oleuropein, have beneficial health effects with antioxidant, anti-inflammatory properties. These compounds are mostly concentrated in the leaves of olive plant (3). Oleuropein is rapidly absorbed after oral administration, with maximum plasma concentration occurring in two hours (1). It is reported in in vitro studies that oleuropein is considered to have antimicrobial activity against gram-positive and gram-negative bacteria (4).

Enteric bacterial flora is essential for the health of the host and composed of about 100 trillion bacteria. Enteric bacterial flora has a very important role in the development and homeostasis of immune cells and protection of the host from infectious diseases. Although it is stable over time, several factors including diet, lifestyle, age, probiotic and antibiotic use, infections, and chronic conditions can alter the diversity and abundance of enteric bacterial flora (5). Food is a directly affecting factor on enteric bacterial flora composition (6). Oleuropein and other biophenols decrease pathogenic bacteria and regulate enteric bacterial flora (7, 8). Bacterial translocation (BT) is defined as enteric bacterial presence in various organs by spreading through the epithelial mucosa (9). Although it is not clear whether the process of BT involves live bacteria or bacterial products, it is definitely known that the increase of enteric bacterial count (bacterial overgrowth) presents a risk for BT (10). BT is frequently discounted in critically ill patients and may lead to the development of sepsis, multiple organ dysfunction syndrome and death (11). In a previous experimental study, it was reported that olive leaf extract reduced the incidence of BT in obstructive jaundiced rats (12). Previous studies in critically ill patients have reported that early enteral feeding decreases mortality rates and decreases the risk of complications (13–15).

In this experimental study, we hypothesized that oleuropein obtained from olive leaf could reduce the total aerobic bacterial count in rat caecal flora.
Materials and methods

After ethical approval of Çanakkale Onsekiz Mart University, Local Ethics Committee for Animal Experiments (decision number: 2015/08-25), twenty adult, male Wistar albino rats with weight ranging from 250–350 grams were housed in a clean facility, 2 per cage under a 12:12 h light/dark cycle with a constant room temperature of 22±1 °C. Standard rat chow and water was provided ad libitum. Rats were randomly divided into two groups:

Group C (n=10) was the control group and rats were fed with standard rat chow and water for 30 days.

Group O (n = 10) was the study group. Rats received olive leaf extract 20 mg/kg/day by intragastric gavage in addition to standard rat chow and water for 30 days.

Olive leaves were harvested from olive tree (*Olea europaea var Ladolia*) grown in Gökçeada (Imbros), Çanakkale, Turkey. Leaves were dried at 80 °C for 4 hours. Dried leaves were ground using DeLonghi coffee grinder KG49 and then filtered using 850 mm laboratory sieve. Olive leaf powder was extracted 1:5 (w:v) with hot water in water bath (Memmert WNB 10, Germany) at 80 °C for 10 min. The mixture was filtered using cotton fabric and transferred to falcone tubes (15 mL). Olive leaf extracts were stored at −20 °C to prevent degradation of oleuropein during the experimental procedure.

At the end of the study period all rats were sacrificed with high dose anesthesia by intramuscular 80 mg/kg ketamine (Ketalar, Pfizer, Turkey) and 10 mg/kg xylazine HCL (Rompun, Bayer, Turkey). Laparotomy was performed and one gram of ceacal content collected from each rat under sterile conditions. Sterile physiological saline solution of 9 mL was added to all ceacal contents. Subsequently, they were homogenized by using a stomacher (Interscience-Bag Maxer 400) for 1–2 minutes and consecutive 10-fold serial dilutions were prepared with a final concentration of 10⁻⁸. Then 0.1 mL of each dilution were spread onto the surfaces of Plate Count Agar (Oxoid, UK) (PCA) and Violet Red Bile Glucose Agar (Oxoid, UK) (VRBG) to determine the total number of aerobic enteric bacteria. After incubation at 37 °C for 48 hours, bacterial counts from all plates were recorded as CFU/g (colony forming unit/gram).

### Materials and methods

#### Statistical analysis

The statistical analyses were performed using the SPSS 16.0 for Windows. Group comparisons were performed by the Mann–Whitney U test corrected with Bonferroni and p<0.05 were accepted to be statistically significant.

#### Results

Total aerobic bacterial counts of Group O were significantly lower than Group C in both PCA and VRBG agar plates inoculated with ceacal samples for every dilution (p<0.05) (Tab. 1).

#### Discussion

Intestinal microbiota is estimated to comprise over 10¹⁴ bacteria from more than 1000 species (16). The composition of the human intestinal microbiota has an important role in health (17). The majority of mammalian intestinal microbiota is constituted of four bacterial phyla and among these, the *Bacteroidetes* and the *Firmicutes* are two predominating phyla (16). The concentration of aerobic flora is much lower and *Escherichia coli* species are dominant among the gram-negative bacilli. Among the aerobic gram-positive cocci, the enterococci, staphylococci and streptococci are dominant (10). Most of the members are obligate anaerobes and many of them have not been cultivated. The rat intestinal microbiota is similar to human intestinal microbiota in terms of bacterial content, therefore the rat is an ideal model to study the microbial composition. The composition of intestinal microbiota is influenced by host environment and diet and is a very important affecting factor (16). Currently, the modification of intestinal microbiota has become an important objective of researches (17).

Normally, the intestines provide a barrier to invasion by pathologic microorganisms. This barrier function impairs during critical illnesses and microbial compounds may easily cross the mucosa, reach to mesenteric lymph nodes, portal blood system and other organs. It refers to BT and is a very life-threatening clinical tableau (9, 10). It is known that critically ill patients are predisposed to alterations in enteric bacterial flora that lead to complications. The disruption of the balance of enteric bacterial flora alter defense mechanisms and lead to overgrowth of potentially pathogenic
bacteria such as *Salmonella*, *Yersinia* and *Pseudomonas aeruginosa* (18). Normal enteral feeding is a factor affecting intestinal functions positively such as mucosal mass maintenance, cellular proliferation and brush border enzyme production. Considering that most endogenous infections are caused by aerobic flora, provision of enteral nutrition maintains the predominant, anaerobic flora of the intestines (10).

Today, there is an increasing interest in medicinal plant extracts that are easily attainable. Olive tree leaf is one of these plant extracts and has been used traditionally in Mediterranean countries. Olive leaf contains triterpenes, flavonoids, chalcones and tannins. Among these, oleuropein, one of the iridoide monoterpenes, is the main constituent of olive leaf and is responsible for its pharmacological effects (2). The proven effects of oleuropein such as antioxidant, antiatherosclerotic, hypoglycemic, antihypertensive, anti-inflammatory and antimicrobial were reported in various experimental studies (19–22). In this study, it was found that intragastric administration of oleuropein during 30 days reduced the total aerobic bacterial count in rat enteric bacterial flora.

Malnutrition is a very important issue in critically ill patients and delays in enteral feeding initiation are a common problem worldwide (23). Total parenteral nutrition (TPN) is widely used for critically ill patients. Various studies reported that early enteral nutrition is an improving factor over patient outcome compared with TPN (24–27). There are various studies about enteral nutrition enriched with different compounds in critically ill patients and although obtaining conflicting results, some of them reported beneficial effects (21, 28–30). It may be due to different patient populations, study designs, timing, dosing regimens and outcome measures evaluated. A previous study reported that in critically ill patients, enteral nutrition compared to parenteral nutrition decreased infectious complications and length of hospital stay (31). Today, it is known that mortality rates will decrease by starting enteral feeding earlier in critically ill patients (32). Overgrowth of enteric bacterial counts. Bioavailability of oleuropein is heterogeneous and highly dependent on various factors such as gender. Adjustment of the most beneficial dose may be found by further experimental studies with longer periods.

References


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