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NUTRITIONAL INTERVENTION PROTOCOL – CLINICAL STUDY

"Study of the Postprandial Effects of Consuming Olive Leaf Products and Tea on Plasma Antioxidant Status and Metabolic Syndrome Markers in Healthy Individuals."

Research Team

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Funding Organization
This study will take place under the project "OLIHERB."

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✓ **Σύνοψη Πρωτοκόλλου**

1. Protocol Summary

This is the protocol of a randomized controlled nutritional intervention - clinical study to investigate the bioactivity of preparations (biscuits and tea) with dried olive leaves.

This is a nutritional intervention - clinical study with a cross-over design (cross over design: acute study). The main objective of the study is to investigate the postprandial effect of olive leaves on selected biomarkers and on the antioxidant status of plasma.

The participants of the study will be healthy volunteers, aged 18-65 years, who will come from areas of Greece and will be based on the island of Lemnos, in order to be able to attend the days of the study. The study will be carried out in the specially designed area of the Human Nutrition Unit, University of the Aegean, in collaboration with external doctors. The total duration of the study will be 15 days (3 axes).

Participants will be randomly divided into three groups. On the first day, the first group will consume a meal rich in carbohydrates and fats, which will consist of 2-3 biscuits made with butter, flour and sugar and drops of caramel coloring (E150a), along with a glass of natural mineral water with the addition of caramel coloring (E150a) at a refrigerator temperature of ~4°C (control group), the second group 2-3 biscuits made with butter, flour and sugar and dried olive leaf powder, along with a glass of natural mineral water with the addition of caramel coloring (E150a) at a refrigerator temperature of ~4°C (intervention group A) and the third group 2-3 biscuits made with butter, flour and sugar and drops of caramel coloring (E150a), along with a glass of dried olive leaf tea and natural mineral water at a refrigerator temperature of ~4°C (intervention group B). In all groups, the meal (control or intervention) will be consumed first and the liquid (control or intervention) will be consumed immediately afterwards. The total time for consuming the meal (biscuits) will be 15 minutes and in the next 10 minutes, the liquid (olive leaf tea or water with caramel coloring) must also be consumed. After 7 days (washout period), the same volunteers will come to the specially designed area and the procedure will be repeated, with the difference that the control group of the 1st day will belong to intervention group A, intervention group A will belong to intervention group B and intervention group B will belong to the control group. Finally, on the 15th day, the procedure will be repeated with the same volunteers, where now the control group of the 1st day will belong to intervention group B, intervention group A will belong to the control group and intervention group B will belong to intervention group A. On all 3 days, blood samples will be taken from participants before, as well as 60, 120 and 240 minutes after consuming the meals. The results of the analyses will be statistically processed to present data regarding postprandial levels of selected biomarkers and plasma antioxidant status.

✓ **Theoretical background and study objectives**

2.1. Argument, Research evidence, Significance of study

According to the scientific background to date, indices of postprandial lipemia, glycemia and oxidative stress appear to be associated with various chronic diseases, especially cardiovascular diseases (Koutelidakis and Dimou, 2016). A diet rich in highly processed, calorie-dense foods, which are also devoid of nutrients, often leads to excessive postprandial spikes in blood glucose and lipids. This condition, called postprandial dysmetabolism, causes immediate oxidative stress, which increases directly in proportion to the increases in glucose and triglycerides after a meal. The transient increase in free radicals triggers acute atherogenic changes, such as inflammation, endothelial dysfunction, blood hypercoagulability and sympathetic hyperactivity. Postprandial dysmetabolism is an independent predictor of future cardiovascular events even in non-diabetic individuals (O'Keefe et al., 2008). In response to this fact, studies have been conducted to investigate the postprandial effect of functional ingredients and foods on biochemical markers and markers of inflammation.

Taking olive leaf extract, rich in antioxidants and polyphenols, may be able to attenuate the increase in glucose, triglycerides and inflammation after a meal, positively affecting postprandial dysmetabolism. Olive leaves, being rich in antioxidants, without adding additional calories, when added to a high glycemic index meal, may reduce the postprandial glucose passage into the blood, partly by slowing gastric emptying. Clinical studies that study the effect of olive leaf tea on human health are mostly long-term, as a result of which there is no scientific data available on the effect of its administration at a post-prandial level. Considering the above, this clinical study aims to investigate a possible post-prandial effect of products prepared with dried olive leaves, on selected biomarkers and on the antioxidant status of plasma, in healthy volunteers.

Study Objectives / Research Questions

Main Objective

The aim of the study is to investigate the postprandial effect of products (biscuits and tea) with dried olive leaves, on indicators of postprandial lipemia, glycemia and oxidative stress in the blood (plasma antioxidant capacity, cholesterol, LDL, HDL, triglycerides, glucose, insulin, CRP, etc.).

Research Questions

The research questions of the present clinical study are the following:

- ✓ What is the postprandial effect of consuming tea with dried olive leaves on the aforementioned blood biomarkers?
- ✓ Does the postprandial effect of tea with olive leaves differ from the postprandial effect of the biscuit enriched with olive leaf powder, with regard to the biomarkers under examination?
- ✓ Can the consumption of olive leaf tea or an olive leaf cookie, before a meal rich in fat and carbohydrates (cookies with flour, butter and sugar), statistically significantly affect postprandial glycemia, lipemia, inflammatory response?
- ✓ Could the inclusion of olive leaf powder, in a cookie rich in carbohydrates and fats, be a practice to reduce spikes in glycemia, lipemia and oxidative stress?

✓ **Summary presentation / study design**

Summary

A nutritional intervention - clinical study of cross-over design (cross over design: acute study) will take place in a sample of healthy volunteers. This specific research will be carried out in the specially designed area of the Human Nutrition Unit of the University of the Aegean in collaboration with an external physician who will undertake the work of blood sampling.

Study design

In a total sample of approximately 35 healthy volunteers, the number of which has been determined through a special statistical program (G-power, see 4.1.) for calculating the required sample of the research to draw safe and reliable conclusions and in which a possible dropout of 10-15% has been taken into account, a nutritional intervention - clinical study of crossover design will be carried out. The study for each participant will last 15 days.

Each participant on the first day in a 12-hour fasting state will be invited to visit between 08:30 am and 13:00 pm, the specially designed blood collection room of the Human Nutrition Unit, Department of Food Science and Nutrition, housed in the building on Dimokratias Avenue of the University of the Aegean. In this specially designed space, questionnaires will be completed (24-hour recall), blood pressure will be measured for each volunteer (3 times in total on the right arm) and blood will be taken by catheterization (baseline) by a doctor. Afterwards, a meal will be served that will include 2-3 pieces of biscuits (with or without olive leaves) and 1 cold (~4°C) tea that will have been prepared with or without olive leaves. The consumption of each meal will take place within a period of 15 minutes and in the next 10 minutes, the tea must also be completed by all participants. Finally, the following

blood and blood pressure samples will be taken for the volunteers, which will be carried out 60, 120 and 240 minutes (min) after the completion of the meal.

After a seven-day washout period, on the eighth day, the same participants will be asked to return to the specially designed area of the University of the Aegean, at the previously determined hours (08:30am-13:00pm) to repeat the nutritional intervention and blood sampling process. Finally, on the fifteenth day, the same volunteers will return to the specially designed area of the University of the Aegean, at the previously determined hours (08:30am-13:00pm) to repeat the same process as on the first day. That is, a discussion will take place with the volunteer, questionnaires will be completed, their blood pressure will be measured and blood will be taken by catheterization (baseline) by a doctor. The meals that will be served to the volunteers are determined by the control or intervention group to which they belong. After each solid meal (biscuits) is consumed within 15 minutes and the liquid meal (tea) follows in the next ten minutes, the next blood pressure measurements and blood sampling will be performed for the participants 60, 120 and 240 minutes (min) after completing the meal.

- ✓ Time point t0 = 0 minutes (min): Blood pressure measurement and taking a 10 ml blood sample in a fasting state and before taking any food, by venipuncture (Baseline).

- ✓ Time point t1 = 25 minutes (min) / Meal consumption:

Control group: 2-3 pieces of biscuits with caramel coloring (total 120 gr.) and a glass (250 mL) of tea (natural mineral water with caramel coloring) for the control group or

Intervention Group A: 2-3 pieces of biscuits with 1.25 g. dried olive leaf powder (total 120 g.) and a glass (250 mL) of tea (natural mineral water with caramel coloring) for intervention group A and

Intervention Group B: 2-3 pieces of biscuits with caramel coloring (total 120 g.) and a glass (250 mL) of tea (natural mineral water with 1.25 g. dried olive leaves) for intervention group B. Solid meals (biscuits) should be consumed within 15 minutes (min) and tea intake should be completed within the next 10 minutes.

- ✓ Time point t2 = 60 minutes (min), after the end of the meal: Blood pressure measurement and taking a 10 ml blood sample 60 minutes (min) after consuming the meal.
- ✓ Time point t3 = 120 minutes (min), after the end of the meal: Blood pressure measurement and taking a 10 ml blood sample 120 minutes (min) after consuming the meal.

✓ Time point t4 = 240 minutes (min), after the end of the meal: Blood pressure measurement and taking a 10 ml blood sample 240 minutes (min) after consuming the meal.

Participant registration and case of study withdrawal

The goal is to gather an initial sample of individuals (at least 40-50) from Greece in order to collect the required number of cases for the study. The research team will then proceed to examine the inclusion/exclusion criteria and select the participants to whom the meals will be provided, on the three different days of the study.

Sample size calculations

The sample size was calculated using G*power 3.1 (University of Düsseldorf, Germany). The sample size calculation revealed that 30 subjects were sufficient to detect a statistically significant group-time interaction, with an effect size of 0.25, at the 5% significance level with a 95% probability. Taking into account a dropout rate of 15%, the final sample size reached 35 subjects

Inclusion criteria

The criteria that those interested in conducting the study must meet are the following:

- Healthy, without imminent diseases (e.g. diabetes, cardiovascular, hypertension, hypercholesterolemia, hypertriglyceridemia, morbid obesity with BMI> 40 kg/m2)
- Aged 18-65 years at the start of the study
- Signed written consent to participate in the study and access by doctors to their medical history (diseases, medication)

Exclusion criteria

Participants who meet the following exclusion criteria at the initial stage will be excluded from the study for reasons of safety, as well as for the effectiveness of the study:

- Individuals with a BMI >40

- Professional athletes
- Individuals taking contraceptives in the last 3 months
- Individuals with a history of food allergy with hypersensitivity to any of the components of the administered product (e.g. milk protein or allergy-hypersensitivity to plants of the Oleaceae family)
- Individuals taking supplements (e.g. antioxidants) or medications that affect bowel function (e.g. antibiotics) in the last 3 months or medication (hypoglycemic drugs or antihypertensive drugs or diuretics) that may interact with the administered product
- Individuals taking any medication or supplement that may affect metabolism (e.g. GLP-1 analogues and receptor agonists, caffeine supplement, weight loss/gain supplements)
- Pregnant, lactating or menopausal women
- Individuals with substance abuse or chronic alcoholism or total daily alcohol intake > 50 g/d
- Individuals with a chronic condition (history of cardiovascular disease, cancer, diabetes, hypertension, hyperlipidemia, neurological and neuropsychological disorders, active liver disease, severe renal dysfunction, severe stroke and conditions associated with increased risk of bleeding)
- Individuals with any other serious medical condition that may affect the individual's ability to participate in a nutritional intervention study.
- Individuals whose dietary preferences do not allow them to participate (e.g. vegetarians, vegans)
- Individuals who are not willing to follow the study protocol and the fasting and eating periods
- Individuals who are considered unreliable by the researcher or have a shorter life expectancy than the expected duration of the study, due to an illness or if they are in any condition that in the opinion of the researcher does not allow their safe participation in the study (drug addiction, alcohol abuse)

Strategy for the inclusion and retention of participants

- The study participants will be recruited by the University and the wider local community of Lemnos. The volunteers will be either students or members of the teaching staff of the University of the Aegean or residents of the island. One of the members of the research coordinators will undertake the briefing of each healthy volunteer, which will take place in a special area of the University. Those who meet all the study criteria to be accepted and at the same time agree in writing to participate in it, can participate in the study. On the days they come to this special area, they will have the opportunity at any time to ask for help either from the principal investigator or from any other representative of the research to record any difficulties or questions they may have. The members of the research team will be responsible for promptly informing the participants, in writing or by telephone, about the visits and the biological samples that need to be collected.
- The scientific director of the study or his/her representatives will be responsible for the safekeeping of the data and results. The anonymity of the participants will be maintained and the safe storage of the history of the healthy subjects will be ensured, to which only the personnel who will be employed in this research will have access.

Participant Withdrawal Criteria

Participants are free to withdraw from the study at any time they wish. Accordingly, the investigator will be able to ask the participant to leave the study if:

o Clinical adverse events, laboratory abnormalities, or other medical conditions or conditions are identified that would compromise the participant's continued participation in the study

o Participants meet one of the aforementioned exclusion criteria (whether newly developed or previously unrecognized) that precludes further participation in the study

o Participants appear to be inadequately adhering to the protocol requirements

Suspension or early termination of the study

This study may be suspended or terminated prematurely only if there is a sufficient reason. A written notice documenting the reason for the interruption or suspension of the study will be provided by the person responsible for this decision (research team, funding agency, hospital).

Study Intervention

- ✓ **Intervention Description**

The meals will be given to the volunteers at the Nutrition and Public Health laboratory of the Human Nutrition Unit, University of the Aegean.

For olive leaves, there does not seem to be any data suggesting that at these doses it may have a potential risk or negative impact on health, as on the one hand there is no confirmed adverse effect of its individual components on health based on the scientific literature and on the other hand, according to corresponding research on similar foods, their possible beneficial role in promoting health emerges.

- ✓ **Assessment of participant compliance for the administration**

The assessment of the compliance of the study participants with regard to the administration of meals and compliance with the required study criteria during blood sampling, of the

volunteers who will come to the University premises to consume all the meals in the correct order and at the correct time, will be achieved after continuous supervision by a member of the research team.

- ✓ **Study Procedures**

- ✓ **Questionnaires**

A specially designed questionnaire, based on bibliographic data, will be used to collect important data regarding the study participants.

The questionnaire will concern the clinical status and habits (especially dietary) of the volunteers of the clinical study and will include the following parts:

Part 1

General descriptive data of the study population (gender, age, anthropometric indicators)

Part 2

Clinical status of each participant (medication, nutritional supplements, etc.)

Part 3

Dietary and other habits before the start of the intervention

In addition, study participants will be given a 24-hour questionnaire, each day they attend for blood draws.

- ✓ **Biological samples**

The collection of blood samples, by a competent doctor, from healthy volunteers of the clinical study will be used to determine indicators of postprandial lipemia, glycemia and antioxidants in the blood (antioxidant capacity of the plasma, lipid profile and specifically triglycerides, HDL & LDL cholesterol, total cholesterol, insulin, glucose, hs-CRP, etc.) which will be analyzed using a special biochemical and immunological analyzer as well as in vitro laboratory methods.

- ✓ **Statistical analysis**

After the above analyses are carried out using a special biochemical and immunological analyzer, the use of the statistical package SPSS (Statistical package for social sciences: SPSS) will follow in order to record, process and analyze the results of the study.

- ✓ **Retention of study records**

The study records will remain registered for a maximum of 3 years from the date of their registration in special areas of the Human Nutrition Unit, University of the Aegean. Study records should be kept for a maximum of 2 years after the last marketing authorization or for longer if required by local regulations.

- ✓ **Ethical issues, Consent and Privacy Statement**

Ethical standards

The members of the research team agree to provide their professional expertise and knowledge for the conduct of the study in accordance with the requirements of the protocol and in accordance with the provisions of the Declaration of Helsinki and the provisions of the General Data Protection Regulation.

Research ethics and conduct rules

During the conduct of this specific clinical study, all fundamental rules of ethical, scientific and research ethics will be observed. Therefore, an application for approval of the clinical study will be made by the Bioethics and Research Ethics Committee of the University of the Aegean, with the research volunteers also being informed of its purpose, the confidentiality of the data and the voluntary nature of their participation. Participants will take part in the study, after signing a declaration of consent to participate in it.

Formal consent process

Formal consent in the clinical study is a process that will begin before the registration of the interested parties in the study and will continue throughout their participation in it. Interested parties will be provided with a detailed discussion of the benefits and potential risks of the study, as well as a relevant consent form where the necessary procedures will be presented. Each interested party must read and review it or in special cases (e.g. blind) be informed by third parties. Interested parties will be provided with a copy. The investigator or representative will explain the study and its objectives to each participant and will resolve any questions they may have.

The rights and care of interested parties will be protected by the fact that the quality of their clinical care will not be affected in any way if they refuse to participate in this clinical trial.