The Effect of Daily Lemon Balm Tea Consumption on LDL Levels in a Washington Heights Population with Mild to Moderate Hypercholesterolemia: A Randomized , Double Blind Crossover Interventional Study

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A. Study purpose and rationale

The consumption of tea rich in antioxidant flavenoids has been associated with decreased cardiovascular mortality in observational studies [1,2]. In animal models, flavenoids have been found to inhibit the development of atherosclerosis by 3 separate mechanisms: (1) reduction of serum LDL levels (2) antioxidant activity with inhibition of LDL cholesterol oxidation (3) fibrinolytic mechanisms [3]. Although the exact mechanism of LDL reduction is not known, potential mechanisms which have been observed in several animal studies include reduced micellar solubility and intestinal absorption of cholesterol[4], increased fecal excretion of fat and cholesterol[5], reduced hepatic cholesterol concentration, with up-regulation of the LDL receptor in liver cells[6].

Lemon balm (*Melissa officinalis*), widely consumed within the Latino community (known as *toronjil*) as an herbal infusion and perceived to possess qualities that are cardio-protective, has been found to contain a high antioxidant content (102.4mmol/100g)[7,8]. with 6 different flavenoids, especially rich in luteolin[9], found in previous observational studies to be inversely related to coronary heart disease mortality[1,2]. The aim of this study is to determine whether the regular consumption of lemon balm tea, which has not been previously studied, is associated with improved lipid profiles in patients with mild to moderately high cholesterol.

B. Study Design and Statistical Analysis

This is a randomized, double-blind, single center, interventional study using a crossover design. Each subject will be randomized to consume lemon balm tea or placebo for a period of 8 weeks then switched to the alternative regimen after a wash out period of 1 week. Patients will be given a supply of lemon balm tea or placebo made from inert ingredients, identical to the tea in odor, appearance and taste. Each patient will be given instructions on infusion preparation and their performance in this task will be observed. The preparation is to be consumed 4 times per day at the end of each meal and before bedtime. . The tea will be prepared by infusion of a single tea-bag steeped with 8oz boiling water for 2 minutes, then tea bag removed without squeezing. Subjects will receive a questionnaire at the initiation of the trial which details their dietary intake and physical activity which will then be reviewed by Dr. Wahida Karmally who will counsel all patients on maintaining a stable, low fat diet and stable level of physical activity throughout the intervention.

The primary endpoint measured will be the change in LDL-cholesterol levels as determined by the average of 3 measurements on weeks 6,7,8 and weeks 15, 16,17 of the cross-over, 17 week study. Secondary outcomes will include total cholesterol, HDL cholesterol, and triglyceride levels. All specimens will be collected and processed according to standardized protocol and aliquots will be stored at –80c until the end of the study, when all specimens will be analyzed. Serum concentrations of total and HDL cholesterol and triglycerides will be determined by enzymatic assays. LDL cholesterol levels will be calculated [LDL cholesterol=total cholesterol-(HDL cholesterol + triglyceride/5)].

This study will include 9 visits in total. Each subject will also receive a phone call on week 3 and week 11 to assess for adverse events and compliance. Each visit to the study center will consist of the following:

- Visit 1, day, Day –7 (week –1): Inclusion/exclusion criteria assessed
  - Medical history and physical exam including height, weight, BP
  - Serum HDL, LDL
  - Dietary and physical activity questionnaire and computerized dietary analysis

- Visit 2, Day 1, week 1: final enrollment
  - Informed consent
  - Randomization
  - Study tea/placebo distributed w/ demonstration and observation of prep by study personnel
  - Dietary and exercise counseling

- Visit 3, Day 42, week 6:
  - Assess for adverse events and compliance
  - Weight
  - Serum LDL, HDL, triglyceride and total cholesterol blood levels drawn
  - Dietary and physical activity questionnaire

- Visit 4, Day 49, week 7
  - Adverse events, compliance
  - Serum lipid levels as above

- Visit 5, Day 56, week 8
  - Adverse events, compliance
  - Serum lipid levels

- Visit 6, Day 63, week 9 (after one-week washout period)
  - Adverse events, compliance
  - Study tea or placebo will be distributed w/ demonstration and observation of prep by study staff

- Visit 7, Day 105, week 15
  - Adverse events, compliance
  - Weight
  - Serum lipid levels
  - Dietary and physical activity questionnaire

- Visit 8, Day 112, week 16
  - Adverse events, compliance
  - Serum lipid levels

- Visit 9, Day 119, week 17
  - Adverse events, compliance
  - Serum lipid levels

a. Statistical analysis

The differences in average lipid concentrations in subjects for each treatment group (active to placebo, treatment group 1, and placebo to active, treatment group 2 will be analyzed utilizing the paired T-test. 34 subjects per group will be required to achieve 90% power to detect a 10% difference in LDL at

the end of the study, assuming a common SD of 17mg/dL as found in a recent randomized controlled trial of green tea extract[10] and assuming a 10% average dropout rate. To detect a smaller difference of 5%, a much larger sample size: 122 per group for 90% power would be required. Assuming 10% drop out rate, the sample size is increased to 135 per group.

C. Study Procedure

A total of 7 blood samples will be drawn on all subjects during weeks –1, 6,7,8 and again on weeks 15,16,17 (the last 3 weeks of the trial). Phlebotomies will all occur according to institutional protocol at the ICCR study site. Standard clinical care would not require that these measurements be performed with this same frequency. The duration of the entire study will be 17 weeks after enrollment completion.

D. Study Drugs

Lemon balm tea (*melissa officionalis*) manufactured by Tazo tea has no known side effects and is used for consumption as an herbal tea infusion. The study tea will originate from a single source of lemon balm and prepared into identical tea bags with rigorous quality control. This tea contains the following flavenoids as determined by spectral data[9]:

- luteolin 7-O-beta-D-glucopyranoside
- apigenin 7-O-beta-D-glucopyranoside
- luteolin 7-O-beta-D-glycuronopyranoside
- luteolin 3'-O-beta-D-glucuronopyranoside and luteolin 7-O-beta-D-glucopyranoside-3'-O-beta-D-glucuronopyranoside.

E. Medical device

N/A

F. Study questionnaire

A questionnaire in both Spanish and English will be developed detailing each subjects dietary habits and level of physical activity which will be used at week –1 to identify individuals already on a low fat diet (<30% total calories from fat) and weeks 3 and 7.

G. Study subjects

Patients eligible for enrollment will include adults > 18 years of age who are Spanish or English speaking with mild-moderate hypercholesterolemia (LDL 130-190), who do not regularly consume tea and who are already on a low fat diet (<30% total calories from fat) as determined by a dietary questionnaire and computerized dietary analysis. Patients will be excluded if they are known to have cardiovascular or hepatic disease, are known diabetics, have high blood pressure (>160/90), a +family history of early CAD (male <55, female<65), or HDL levels<40, Triglyceride levels >400. They will also be excluded if they are currently on anti-lipid therapy (statin, fibrates, niacin, or bile acid binding resins) or other drugs that might interfere with lipid metabolism including anti-thyroid agents, OCPs, dietary supplements such as fish oils and anti-oxidants.

H. Recruitment of subjects

Patients will be recruited from the surrounding community through flyers written in both Spanish and English and local newspaper ads in both languages over an 8-12-week period.

I. Confidentiality of study data

To insure confidentiality of all trial participants, all study data will be coded using a unique code number. Data will be stored in a secure location, accessible only to investigators.

J. Potential conflicts of interest

There is no known conflict of interest in this study.

K. Location of the study

This study will be taking place at Columbia Presbyterian ICCR center where all visits and phlebotomies will take place.

L. Potential risks

There are no known adverse risks associated with the consumption of lemon balm tea. The risk of phlebotomy is that of standard venipuncture by trained professionals according to protocol.

M. Potential benefits

Lemon balm tea may improve lipid profiles and thus potentially reduce cardiovascular mortality.

N. Alternative therapies

Altenative therapies exist for hypercholesterolemia such as lifestyle changes and medical interventions such as the use of statins which have proven benefit. However, in our study population, patients will not meet the ATPIII guidelines for initiating statin therapy.

O. Compensation to subjects

Subjects will be fully reimbursed for all travel to the study center at the time of each visit.

P. Cost to subjects

None after travel reimbursement

Q. Minors as research subjects

N/A

R. Radiation or radioactive substances

N/A

S. References

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