CHAPTER III

MATERIALS AND METHODS

1. Subjects

Male or female subjects aged between 20-70 years were recruited to participate in this study. All subjects had plasma cholesterol concentrations higher than 200 mg/dl and they were not currently on lipid lowering medications. They were free from diabetes mellitus, cardiovascular diseases, endocrine or gastrointestinal disease or other conditions that might be expected to influence the study findings. They were all nonsmokers. Thirty four subjects were recruited into this study.

The experimental protocol was approved by the committee on Human Study of Makarak Hospital. The written informed consent was obtained from each subject after the experimental protocol had been explained.

2. Experimental design

The subjects participated in a 12-week study with two consecutive periods: a 6-week nutrition counseling period (week 1-6), followed by a 6-week nutrition counseling with *O. canum* seed supplemented period (week 7-12). Dried *O. canum* seeds (15 g/day) were given to each subject in 3 divided doses (5 g/pack) during the second period of the study. At the beginning of week 7 the subjects were instructed to take 5 g of the seeds after breakfast, lunch and dinner, and they continued consuming the seeds until the end of week 12. They were advised to soak the seeds into approximate 200 ml of water for 10-15 minutes and stir thoroughly before consuming.

Venous blood was drawn after a 12 hour fast to determine lipid profile at week 0, 6 and12. Three-day dietary records and dietary assessment questionnaires modified from ATP III were completed by the subjects. Appropriate dietary advice was re-iterated. Weight and height were also recorded. The subject's compliance with the dosing regimen was assessed by interviewing and counting the remaining packs of the seeds. The experimental diagram of the study is presented in Figure 1.

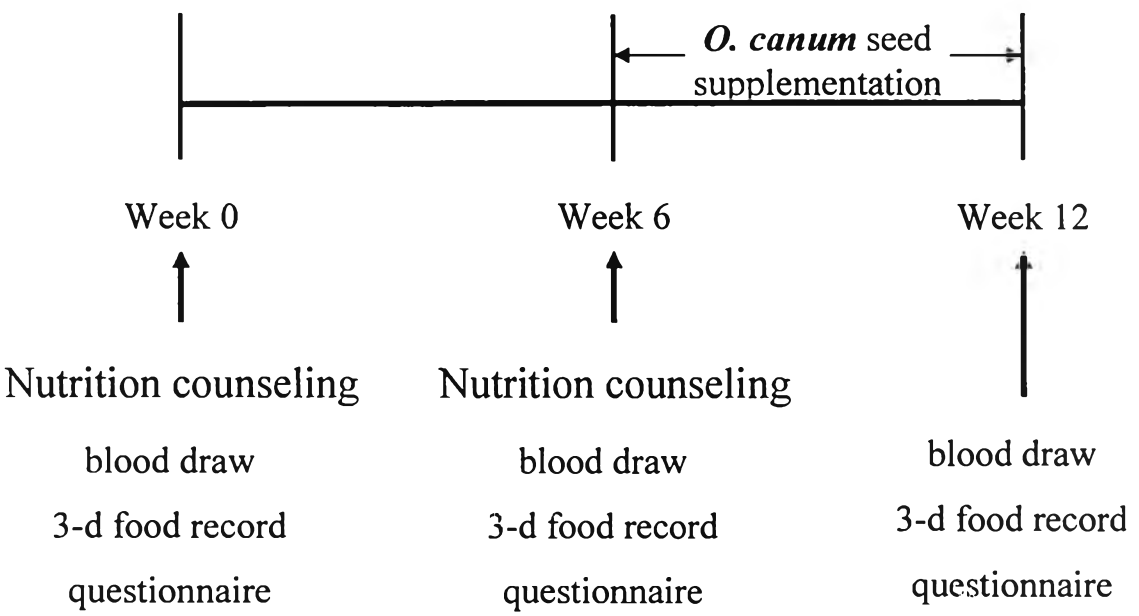

Figure 1 Experimental diagram

3. Research instruments

1. A 3-day food record

This food record involved documenting dietary intake. The record was used for assessment of food consumption and nutrient intake. The subjects should record

the amounts of all foods and liquids consumed during a set times (1 weekend day and 2 weekdays) prior to their visit.

2. Dietary assessment questionnaire modified from ATP III

This questionnaire was done by the subjects prior to their visit. It was a semi-quantitative food frequency questionnaire modified from ATP III (NCEP, 2001). Dietary assessment was evaluated by a score of questionnaire. The score of questionnaires was classified into 3 levels; score > 70: need to make some dietary change, score 40-70: achieve heart healthy diet, and score < 40: achieve TLC diet.

4. Nutrition counseling

Dietary advice involved a consideration of energy intake and the proportion of protein, fat, carbohydrate and other nutrients to ensure that optimal nutrition was maintained. On the first day of the study, each subject received dietary guideline to control lipid levels and the booklet with the details of dietary recommendation. They were instructed to plan and prepare their diets by food exchange lists for selection and calculation for dietary consumption. Each subject was informed about the principle and the importance of the diets.

5. Dietary assessment

A 3-day food record was done by the subjects prior to their visit. They were instructed how to record a 3-day dietary intake. The example of dietary record was also given to each subject. All items and portions of food consumed including name and method of preparation and cooking were asked to record. The subjects estimated food portion size by using standard household measuring cups and spoons.

Portion size measures were converted into grams of foods. The food records were analyzed for total energy intake and its distribution from protein, fat, and carbohydrate. The contents of dietary fiber, cholesterol, SFA, PUFA and MUFA were also calculated from food records. The nutrient consumed was analyzed by the computerized program "Nutrisurvey" modified for Thai food by Associate Professor Rungsunn Tungtrongchitr, Faculty of Tropical Medicine, Mahidol University.

Dietary assessment questionnaire modified from ATP III were self-administered. The subjects were instructed to complete the questionnaire. They completed these dietary assessment instruments on their own at home and returned them during their visit. The scores of questionnaire were calculated following ATP III (NCEP, 2001). It was designed for hypercholesterolemic patients and used to study eating habits.

6. Anthropometric measurement

Weight was measured by balance-beam scale (Supreme Products, Bangkok). Height was measured by height meter (Agsornpunkanchanaburi, Kanchanaburi). Body Mass Index (BMI) was calculated from weight and height as follow:

$$\text{BMI (kg/m}^2\text{)} = \frac{\text{weight (kg)}}{[\text{height (m)}]^2}$$

7. Determination of serum lipids

At baseline, week 6 and week 12, 5 ml of venous blood was obtained from each subject after a 12 hour fast. Whole blood, serum or plasma was appropriately prepared for biochemical determination. Serum total cholesterol, triglyceride, and HDL cholesterol were measured by enzymatic-colorimetric methods using automatic

analyzer (Meditop, India) at Makarak Hospital. LDL cholesterol level was calculated from Friedewald's formula (Friedewald, 1972) as follow:

LDL cholesterol = Total cholesterol – (Triglycerides / 5) –HDL cholesterol

This formula is applicable when triglyceride levels are less than 400 mg/dl

8. Compliance and adverse effects during *O. canum* seed supplementation

At the end of the study, the subjects returned uneaten packs. The returned packs were counted and calculated for % of compliance as follow:

$$\% \text{ of compliance} = \frac{\text{no. of eaten packs}}{126} \times 100$$

During 6-week of *O. canum* seed supplementation, adverse effects were assessed by direct questions.

9. Statistical analysis

Statistical analysis was performed by SPSS version 13 for windows program (SPSS Inc., Chicago). The descriptive values were presented as mean ± SD. The differences among means of individual parameters were tested by repeated measure analysis of variance (repeated measure ANOVA). When the significant differences between experimental groups were detected, a Bonferroni test was performed to test the differences of all pairwise comparisons for significance. The differences were considered statistical significant at $p<0.05$.