RESEARCH METHODOLOGY

Research design

This study is a crossover, double-blinded, randomized , controlled trial comparing Aloe vera juice against placebo. (Fig.1)
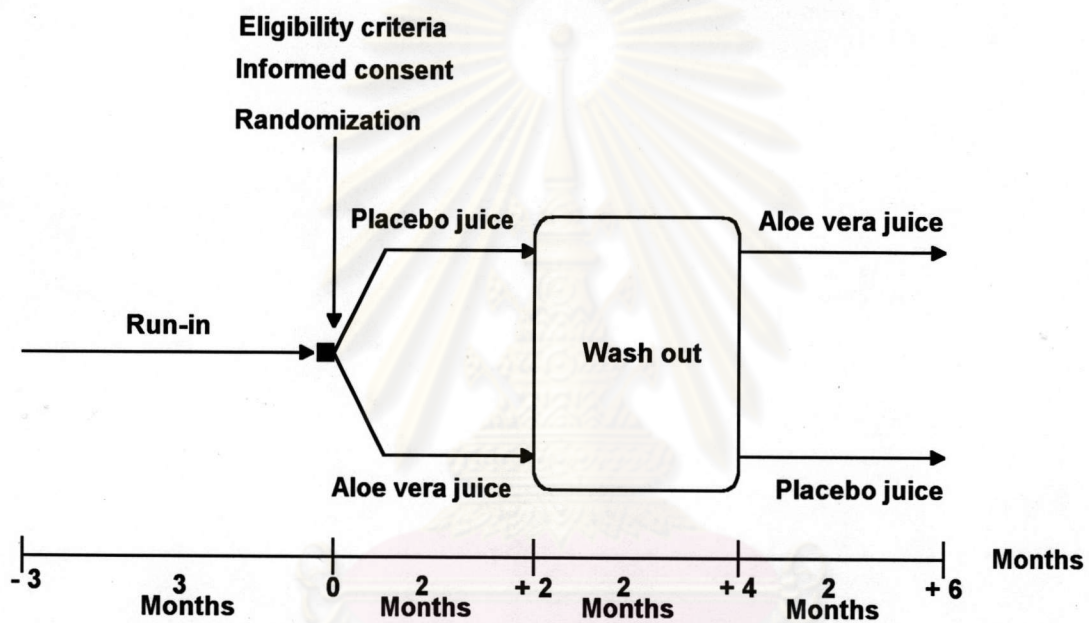

Fig. 1 Research design : crossover, double-blinded, randomized, controlled clinical trial.

Justification of the research design

The design is a two-period crossover design which is efficient in two ways. First, it requires only half the patients needed in a parallel-groups design for the same precision. So the design allows a single center to recruit adequate number of patients. With fewer patients in the study, the study costs the investigators less. Second, the design provides statistical efficiency. It compares the patients with themselves and in this way gives more precise estimate of the treatment difference.

Overall, the design offers efficiency and can be completed in the one-year time frame and within the budget constraint.

In order to use a crossover design, the investigator should ascertain that certain requirements are met(Table 4). If some requirements are not met, the investigator should have the means to measure the effects of the unmet requirements on the results.

Table 4 Requirements and desirable attributes for use of crossover
clinical trial designs(Spilker, 1991)

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1. Adequate number of patients enrolled to detect clinically
   significant within-patient difference
2. Objective measurements are possible to assess
3. Relatively stable chronic disease is being evaluated, or
   volunteers are enrolled
4. Patient groups start at the same baseline values of major
   parameters
5. Patient groups return to their baseline values between
   treatments
6. No period effect[i.e., patients respond better (or worse) to
   the first treatment received] observed
7. No physical or psychological carryover effects are present in
   the second treatment period that cannot be readily and
   convincingly eliminated
8. Appropriate statistical analyses are conducted
9. Each period of the crossover is of sufficient duration to
   provide convincing data
10. Patients are willing to enter a clinical trial that is twice
    as long as needed to demonstrate efficacy

The present design meets most of the requirements listed in Table 4. Sample size calculation(see below) gives the number of patients that is needed to detect clinically significant within-patient difference. The main outcome variables, fasting plasma glucose is objectively measured. The scientists World Health Organization run the laboratory tests do not know the identities of the patients and the treatments the patients received. They do not have any bias relating to the nature of the research in running tests and in reporting test results. Diabetes mellitus is a chronic disease that is relatively stable for individual patient. During the three-month run-in period, the patients will learn how to control their diet and how to exercise according to the guidelines by the American Diabetes Association (American Diabetes Association, 1993a, American Diabetes Association, 1993b). Adherence to the guidelines is evaluated by simple questioning on their dietary control and exercise. The two-month wash-out period is long enough for the effects of the aloe vera juice, which last shorter than 4 weeks after drug discontinuation in a preliminary clinical study, and disappear before the second period of treatment. We cannot be perfectly sure that there are no period effect and no physical or psychological carryover effects. But appropriate statistical analyses as described in Data Analysis section will help estimate these effects.

Population and Sample

Population

The target populations are non-insulin-dependent diabetic (NIDDM) patients who do not have classical symptoms related to hyperglycemia, for examples, polyuria, polydipsia, polyphagia, and weight loss. It is not unethical to withhold hypoglycemic agents in NIDDM patients World Health Organization do not have symptoms. In fact, the standard management of non-insulin-dependent diabetes mellitus is to start with dietary therapy, weight control and

exercise. Only patients with newly-diagnosed non-insulin-dependent diabetes mellitus, World Health Organization never took any antidiabetic agent before, participated in this study.

The studied populations are NIDDM patients World Health Organization attend Nutrition Clinic and Medical Outpatient Clinic at Chulalongkorn Hospital.

Sample

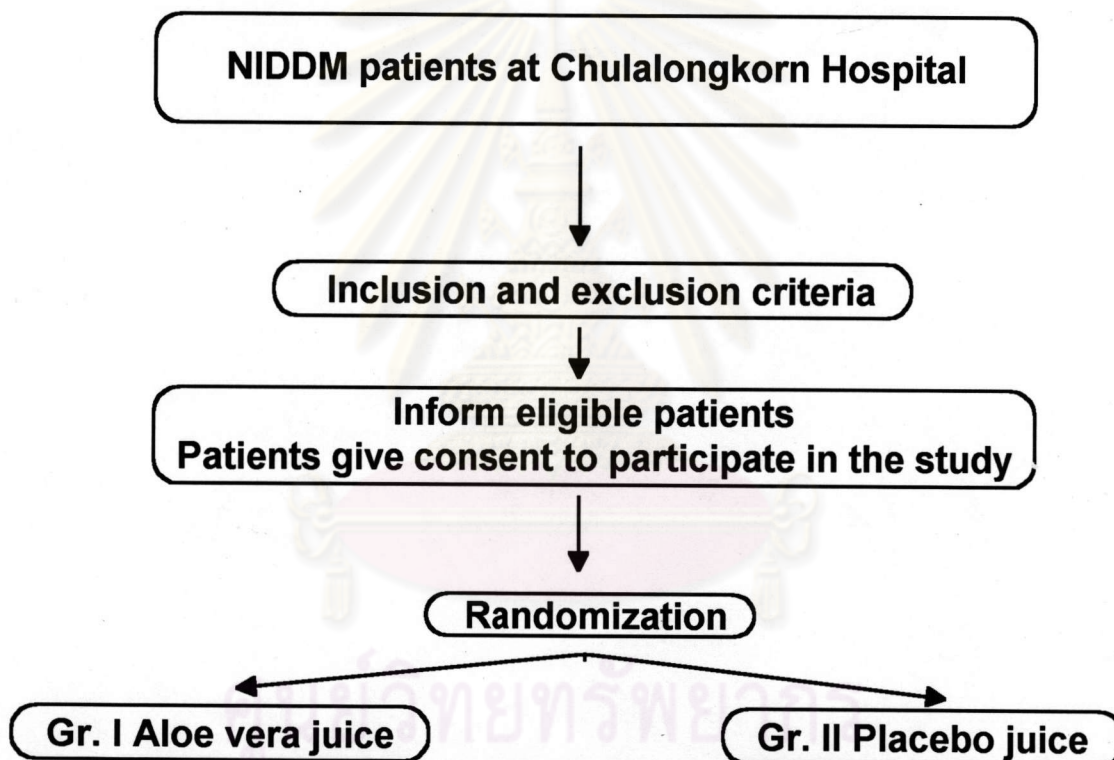The recruitment and sampling processes is shown in Fig. 2.

NIDDM patients at Chulalongkorn Hospital

Inclusion and exclusion criteria

Inform eligible patients
Patients give consent to participate in the study

Randomization

Gr. I Aloe vera juice   **Gr. II Placebo juice**

Fig.2 The recruitment and sampling processes

Samples are selected according to the inclusion and exclusion criteria below.

Inclusion criteria:

1. Non-insulin-dependent diabetes mellitus (NIDDM) patients as defined by WORLD HEALTH ORGANIZATION criteria(WORLD HEALTH ORGANIZATION Expert Committee on Diabetes Mellitus, 1980)(Table 5).

2. Age between 25 - 65 years.

3. No history of receiving hypoglycemic agents.

4. Freely consent to participate in the study.

Table 5 Diagnostic values for oral glucose tolerance test under standard conditions.

	Glucose concentration, mmol/litre(mg/dl)			
	Blood		**Plasma**	
	Venous	**Capillary**	**Venous**	**Capillary**
Diabetes mellitus				
Fasting value	≥ 6.7 (≥ 120)	≥ 6.7 (≥ 120)	≥ 7.8 (≥ 140)	≥ 7.8 (≥ 140)
2 hrs after glucose load	≥ 10.0 (≥ 180)	≥ 11.1 (≥ 200)	≥ 11.1 (≥ 200)	≥ 12.2 (≥ 200)
Impaired glucose tolerance				
Fasting value	< 6.7 (< 120)	< 6.7 (< 120)	< 7.8 (<140)	< 7.8 (<140)
2 hrs after glucose load	6.7-10.0	7.8-11.1	7.8-11.1	8.9-12.2
	(120 - 180)	(140-200)	(140-200)	(160-220)

Observation and Measurement

Measurements

The following measurements will be recorded:

a. Administrative variables

1. I.D. No.

2. Hospital number

3. Sex: Male/Female

4. Age

  b. Baseline variables.

    1. Body weight

    2. Height

    3. Body mass index

    4. Use of tobacco, ingestion of caffeine and/or alcohol

    5. Hypersensitivity to a medicine or test

    6. Kidney function tests: blood urea nitrogen, creatinine

    7. Liver function tests : serum transaminases (serum glutamic oxaloacetic transaminase SGOT, serum glutamic pyruvic transaminase SGPT)

Outcome variables

A. Main outcome variable

    1. Fasting plasma glucose

B. Other outcome variable

    1. Serum fructosamine level for evaluating glycemic control

    2. Serum glycosylated hemoglobin (HbA_1)

    3. Lipid profiles: serum total cholesterol, total triglyceride, high-density lipoprotein cholesterol(HDLc).

    4. Kidney function tests: blood urea nitrogen, creatinine

    5. Liver function tests: serum transaminases(serum glutamic oxaloacetic transaminase SGOT, serum glutamic pyruvic transaminase SGPT).

Fasting plasma glucose and serum fructosamine level are done monthly until the end of the study. Lipid profiles, HbA_1, kidney and liver function tests are done every two months. Only values at the beginning and at the end of each period are analyzed.

Intervention

1. Aloe vera juice

Aloe vera leaves from the same source of plantation are peeled and the gel is washed with tap water. The gel is chopped, centrifuged

and filtered to get aloe juice. The juice is adjusted to 80% concentration of the original aloe juice(out of the centrifuge). Flavor and color are then added to give the juice palatability. Quality control based on electrophoresis is done on all preparations of Aloe vera juices at the Medicinal Plant Information Unit, Faculty of Pharmacy, Mahidol University, Bangkok. The Aloe vera juice has a preservative which does not interfere with the active ingredients. With the preservative, the Aloe vera juice retains its glycoprotein mass in the refrigerator at a temperature of 4°C for at least 2 years and at room temperature for up to 2 weeks. The Medicinal Plan Information Unit at Mahidol University produces enough supply of Aloe vera and placebo juices for a trial with 200 participants in a single batch. The quantity is enough to cover for accidental losses and for patients might drop out of the study. The investigator stores the juices in two separate sites to ensure the continuity of supply.

2. Placebo juice

Placebo juice is prepared from water with the addition of the same preservative used in the Aloe vera juice. It is identical in color, consistency, flavor and taste to Aloe vera juice. In a preliminary study patients could not distinguish placebo juice from Aloe vera juice. Both juices are acceptable to patients.

3. Dosage

Patients takes Aloe vera juice or placebo juice 15 ml twice a day after breakfast and dinner according to protocols. The dosage has induced the hypoglycemic effect of aloe in a preliminary study.

Data collection

Each patient visit has its own data form according to the type of visit which determines the type of data needed. Baseline visits need data to establish patient eligibility, to characterize patients and to establish a baseline for assessment of outcome variables. Follow-up visits need data to assess changes in outcome variables, to characterize the nature of the treatment and its effects, and to characterize patient compliance and adverse effect. The investigator will design and test forms to address these needs.

Data analysis

Statistical analyses :

Crossover designs are used to compare treatments that are administered to an experimental units(e.g., a patient) in a sequence. That is, each experimental unit is subjected to each treatment in a predetermined sequence. The objective of crossover designs is to eliminate variation in comparing treatments by observing all treatments on the same experimental unit.

Although crossover designs eliminate between-experimental-unit variation from treatment comparisons, other problems arise in the form of carry-over or residual effects. Carry-over effects occur when treatment A is given first and its effect has not worn off by the time treatment B is applied. If this lingering effect of treatment A interferes with the response of the subject to treatment B(either positively or negatively), then there is a residual effect of treatment A on the response of treatment B.

The crossover design model contains a sequence effect, a time effect, a treatment effect, carry-over effects, an experimental unit error term, and a time interval error term.

The model for analysis of data is the model proposed by Grizzle

(1965) for two sequences of treatments, A:B and B:A, as follows:

Table 6 Model for a two-period crossover design

Period	Treatment sequence	Subjects $S_{11}..S_{1n_1}$	Mean	Treatment sequence	Subjects $S_{21}..S_{2n_2}$	Mean
1	A	$y_{111}..y_{1n_{11}}$	$y_{1.1}$	B	$y_{211}..y_{2n_{21}}$	$y_{2.1}$
2	B	$y_{112}..y_{1n_{12}}$	$y_{1.2}$	A	$y_{212}..y_{2n_{22}}$	$y_{2.2}$

Grizzle(1965) proposed a model to describe the response of an

observation in a crossover design. The model is:

$$Y_{ijk} = \mu + x_{ij} + \pi_k + \tau_l + \lambda_l + e_{ijk}$$

$$j = 1, ...n_i; \; i = 1, 2; \; k = 1, 2, \; l = 1, 2;$$

where

μ = general mean,

x_{ij} = the effect of the j-th patient within the i-th

sequence, which we must assume to be a normally distributed random

variable with mean 0, and variance σ^2_s,

π_k = the effect of the k-th period,

τ_l = the direct effect of the l-th drug,

λ_l = the residual or carry-over effect of the l-th drug,

and

e_{ijk} = the random fluctuation which is normally distributed

with mean 0 and variance σ^2_e, and is independent of the x_{ij}.

The variance of an observation is $\sigma^2_e + \sigma^2_s$.

The analysis examines three important effects in the model.

First the carry-over effects(λ) in the two treatment sequences are

tested to find inequality. If the carry-over effects in the two treatment sequences are not equal, the study need to be analyzed as a parallel study. If the carry-over effects are equal, it is possible to examine whether the direct effects(τ) of the two treatments are equal. If the direct effects of the two treatments are equal, it means that Aloe vera and Placebo juices have the same hypoglycemic effect. It is also possible to examine whether the period effects(π) of the two treatments are equal. If the period effects are equal, the observed response differences may not be due to the period effects.

In the settings which Aloe vera juice exerts hypoglycemic effect, the expected analytic results are: the carry-over effects are equal, the direct effects are not equal and the period effects are equal.

Sample size

Table 7 shows the sample size required for a study power of 0.95 with the significance level of 0.05(two-tailed) according to Grizzle(1965). Calculation using the limited data from a preliminary study by Ghannam(1985) gives $\delta/\sigma = 60/51.43 = 1.17$. The data in the study did not allow calculation of δ but should not be less than 0 for the crossover design. From Table 9 the sample size required for each treatment is between 6 for r = 0.75 and 12 for r = 0.25. The total sample size is between 12 and 24. To get the exact sample size we need to conduct a pilot study.

Table 7 Sample size required for each treatment to achieve a power of
0.95 when the significance level is 0.05(two-tailed) for
different designs.

Type of design	δ/σ				
	0.4	0.6	0.8	1.0	2.0
Completely randomized	86	38	22	15	6
Crossover with unequal residual effects	>100	78	43	28	9
Crossover with equal residual effects $\rho = -0.50$	> 100	56	33	21	8
$\rho = -0.25$	> 100	47	27	18	6
$\rho = 0$	86	38	22	15	6
$\rho = 0.25$	66	31	17	12	5
$\rho = 0.50$	43	20	12	9	4
$\rho = 0.75$	22	11	7	6	3

Randomization

Construction of the blocked randomization schedule followed the
Moses-Oakford algorithm and used a table of random numbers. The
construction followed the following steps:

a. Specification of the number of treatment group: 2

b. Specification of treatment allocation ratio: 1:1

c. Specification of block size: 2

d. Specification of treatment codes: C = placebo, T = Aloe vera

e. Arbitrarily setting the starting point in a table of random
numbers. In this case The first 25 lines of page 17 of the Rand
Corporation's 1 million random digits was used.

f. Reading single integer from the starting point from left to right
to the end of row, then down, row by row.

g. Establishment of correspondence between numbers selected and
treatment assignments.

The resulting randomization schedule is shown in Table 8.

Table 8 The randomization schedule

Case No.	Treatment assignment
1	PLACEBO
2	PLACEBO
3	ALOE VERA
4	ALOE VERA
5	PLACEBO
6	ALOE VERA
7	PLACEBO
8	ALOE VERA
9	PLACEBO
10	ALOE VERA
11	ALOE VERA
12	PLACEBO
13	PLACEBO
14	ALOE VERA
15	PLACEBO
16	ALOE VERA

Allocation of treatments for patients

A pharmacologist prepared the placebo and Aloe vera juices and put 30 milliliters(ml) of either juice in a small plastic bottle. Each bottle represented a dose of treatment and had a identification code on it. The identification code corresponded to the randomization schedule as in Table and helped identify what treatment a patient had taken. The researcher and the patients did not know what kind of treatment they were receiving. Thus, this scheme achieved the randomization process and guaranteed complete masking for the researcher and the patients.