

## CHAPTER V

### RESULTS

#### Subject recruitment

The researcher interviewed and examined all patients with newly-diagnosed diabetes mellitus attending the Nutrition Clinic and the Outpatient Medical Clinic and Preventive Medicine Clinic at the Chulalongkorn Hospital from April to August 1995. Those patients who met the eligible criteria got instructions on dietary control and exercise. They came back for further evaluation every month until their fasting plasma glucose and dietary and exercise habits did not change over 2 consecutive months. For this run-in period, fasting plasma glucose levels for each subject must not have rise or lower more than 15% from the previous months.

Sixty-two patients with newly-diagnosed diabetes mellitus attended the Clinics during the recruitment period. There were 27 male patients and 35 female patients. Of these 62 patients only 16 patients participated in this study, the other 46 patients did not participate for various reasons as shown in Table 9.

Table 9 Reasons that caused the exclusion of patients from the study

Reason	No. of patients
1. Patients wanted or needed treatment for diabetes without delay	12
2. Patients successfully normalized plasma glucose by diet and exercise	12
3. Patients took other herbal medicine	1
4. Patients took other drugs e.g. steroids	5
5. Patients who the researchers could not contact	12
6. Patient took alcohol	1
7. Previous treatment of diabetes mellitus	2
8. Symptomatic Acquired Immune Deficiency Syndrome (AIDS)	1

Twelve patients did not participate in the study because they needed diabetic treatment without delay for various reasons. The majority of this group wanted to take hypoglycemic agents and continued treatment in the provinces. Two needed to bring the blood glucose level down to normal or near normal level before surgical procedures.

It is remarkable that twelve patients successfully controlled their plasma glucose level in the normal range after dietary control and exercise. The successful glycemic control applied both in patients without classic diabetic symptoms and also in patients with severe classic diabetic symptoms.

Transportation, communication problems led to exclusion of another twelve patients from the study. Most of them did not come back for follow-up and the researcher could not reach them through ordinary ways of contacting patients. The researchers considered them as having high risk of dropping out of the study if recruited.

#### Patient characteristics

The characteristics of patients who participated in the study is shown in Table 10. Patients were randomized into two groups according to the randomization schedule (Table 8). Group 1 consisted of 8 patients. Patients in Group 1 took Placebo juice for the first treatment sequence period and took Aloe vera juice for the second treatment sequence period. Group 2 also consisted of 8 patients. Patients in Group 2 took Aloe vera juice for the first treatment sequence period then took Placebo juice in the second treatment sequence period.

Subjects in the two groups did not differ significantly in variables that may affect the outcome variables. The average age in both groups did not differ significantly. The average body mass index suggested that most of the subjects were near the upper limit of normal range for body mass index. Male subjects participated almost as many as female.

Table 10 Characteristics of participating patients of the two treatment-sequence groups.

	Group 1, n = 8 (Placebo-Aloe vera) $\bar{X} \pm S.D.$	Group 2, n = 8 (Aloe vera-placebo) $\bar{X} \pm S.D.$	p Value
Age (years)*	56.5 $\pm$ 5.6	58.1 $\pm$ 10.1	0.70
Body weight* (kilograms)	57.5 $\pm$ 8.8	63.5 $\pm$ 13.7	0.32
Body Mass Index* (Kilograms/meter)	24.2 $\pm$ 2.8	24.5 $\pm$ 3.1	0.84
Sex			
Male	5	4	
Female	3	4	

\* No significant differences between the two groups by student t-test.

Biochemical indices of the two treatment-sequence groups

Table 11 shows biochemical indices comparing the two treatment-sequence groups at the end of run-in period.

Table 11 Biochemical indices of participating patients of the two treatment-sequence groups.

Biochemical indices	Group 1, n = 8 (Placebo-Aloe vera) $\bar{X} \pm S.D.$	Group 2, n = 8 (Aloe vera-Placebo) $\bar{X} \pm S.D.$	p Value
HbA1 (%) *	10.6 $\pm$ 1.7	10.4 $\pm$ 0.6	0.75
Fructosamine (mM/L)	456.1 $\pm$ 116.6	413.8 $\pm$ 80.7	0.41
Total cholesterol (mg/dl)	231.6 $\pm$ 27.4	215.5 $\pm$ 35.9	0.33
Total triglyceride (mg/dl)	198.0 $\pm$ 91.5	191.5 $\pm$ 99.6	0.89
HDL (mg/dl)	45.3 $\pm$ 7.1	47.6 $\pm$ 9.8	0.59

The indices for glycemic control, glycosylated hemoglobin and fructosamine, and the lipid profiles of the two treatment groups were comparable before treatment.

#### Changes of outcome variables during the study

Table 12 shows changes of outcome variables during the period of the study.

Table 12 Outcome variables during the run-in period and at the end of trial

Outcome variables	End of run-in $\bar{X} \pm S.D.$	End of trial $\bar{X} \pm S.D.$	p Value
Body weight (kg)	60.5 $\pm$ 11.6	60.4 $\pm$ 11.3	0.99
HbA <sub>1</sub> (%)	10.5 $\pm$ 1.3	10.2 $\pm$ 1.5	0.5
Fructosamine (mM/L)	434.9 $\pm$ 99.3	424.6 $\pm$ 85.3	0.75
Total cholesterol (mg/dl)	223.6 $\pm$ 31.9	222.9 $\pm$ 29.7	0.95
Triglyceride (mg/dl)	194.8 $\pm$ 92.5	184.6 $\pm$ 109.0	0.78
HDL cholesterol (mg/dl)	46.4 $\pm$ 8.4	44.6 $\pm$ 9.1	0.56
BUN (mg/dl)	12.4 $\pm$ 1.9	11.9 $\pm$ 1.6	0.48
Cr (mg/dl)	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1	0.67
AST (U/l)	22.1 $\pm$ 7.1	23.5 $\pm$ 8.2	0.6
ALT (U/l)	26.9 $\pm$ 9.0	24.8 $\pm$ 8.0	0.5

The subjects maintained body weight within 2 kilograms from the first visit. This is in accord with the exclusion criteria which excluded patients who were symptomatic or having weight loss, polyuria, polydipsia, and/or polyphagia. However, the subjects have a wide range of body weight.

Two indices for glycemic control, hemoglobin A<sub>1</sub> and fructosamine, did not change significantly from the start to the end of the study. The higher value of standard deviation for fructosamine

reflects the rapid changes which plasma glucose levels might affect the level of fructosamine.

The lipid profiles, total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDLc), did not change significantly. The levels of total cholesterol and HDLc changed slightly and non-significantly. The level of triglyceride varied widely among subjects and within subjects and did not change significantly by the treatments.

The livers and kidneys did not deteriorate by the treatments as reflected by the liver function tests (AST, ALT) and the kidney function tests (BUN, Cr).

The effect of treatments on the level of fasting plasma glucose

Table 13 Fasting plasma glucose (FPG) levels in milligrams per deciliter (mg/dl) measured

Sequence*	FPG (mg/dl)			
	Subject	Placebo**	Aloe vera**	Crossover difference
Placebo/Aloe vera	1	309	270	39
	2	156	233	-77
	5	404	384	20
	7	224	258	-34
	9	222	220	2
	12	226	225	1
	13	256	247	9
	15	215	210	5
	Aloe vera/Placebo	3	268	272
4		168	154	14
6		281	319	-38
8		291	246	45
10		201	176	25
11		226	218	8
14		228	204	24
	16	249	240	9

\*Placebo/Aloe vera = Placebo followed by Aloe vera

\*Aloe vera/Placebo = Aloe vera followed by Placebo

\*\*Placebo = placebo period \*\*Aloe = Aloe vera period

Table 13 shows the data on fasting plasma glucose levels at the end of periods which subjects received placebo or aloe vera juices. The data shows changes on fasting plasma glucose levels in each subject's response over two treatment periods.

Data from Table 13 were plotted in Figure 3 to show the subject profiles for fasting plasma glucose levels. For each subject  $k$ , in group I, the pairs of points  $y_{i1k}$  and  $y_{i2k}$  are plotted against the period labels and joined up.

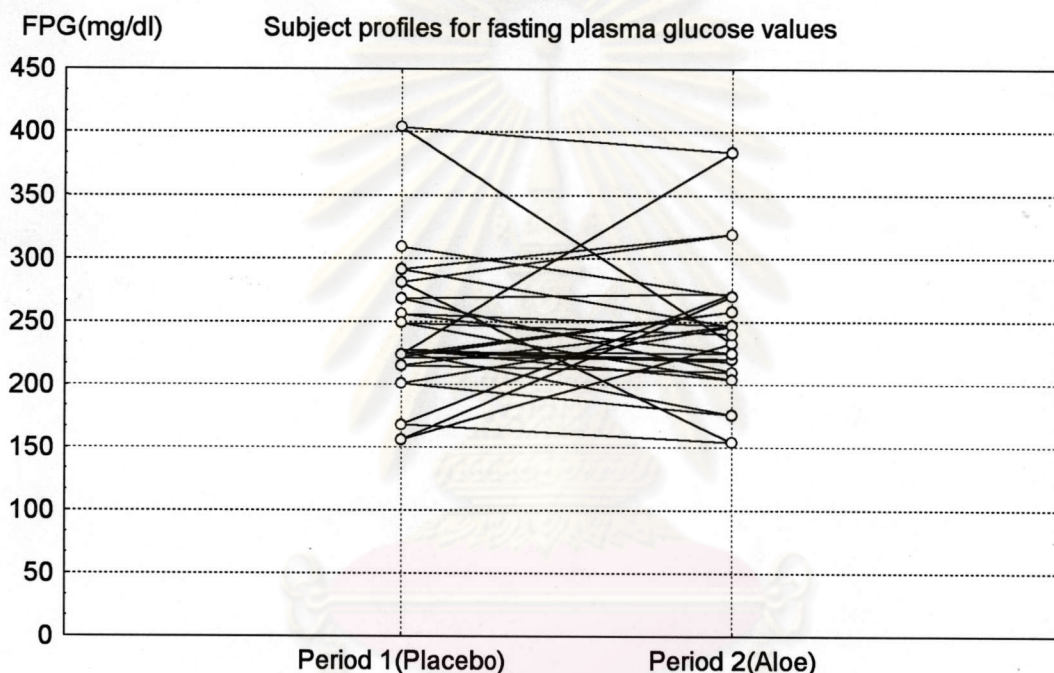


Fig. 3 Subject profiles for fasting plasma glucose values

It is clear from the subject profiles plot above (Fig. 3) that there is no tendency for the fasting plasma glucose to be lower on treatment with Aloe vera juice. Moreover, some subjects have markedly higher values on Aloe vera juice. Also, two subjects exhibit large drop in value between the two periods.

The analysis on fasting plasma glucose levels followed the statistical model proposed by Grizzle (1965). Table 14 - 16 are constructed to provide values used for further analysis.

Table 14 Fasting plasma glucose values from Group 1 (receiving  
Placebo - Aloe vera sequence)

Group 1 (Placebo-Aloe vera)				
Subject	Period 1 y1	Period 2 y2	Total y1 + y2	Difference y1 - y2
1	309	270	579	39
2	156	233	389	-77
5	404	384	788	20
7	224	258	482	-34
9	222	220	442	2
12	226	225	451	1
13	256	247	503	9
15	215	210	425	5

Table 15 Fasting plasma glucose values from Group 2 (receiving Aloe  
vera - Placebo sequence)

Group 2 (Aloe vera - Placebo)				
Subject	Period 1 y1	Period 2 y2	Total y1 + y2	Difference y1 - y2
3	272	268	540	4
4	154	168	322	-14
6	319	281	600	38
8	246	291	537	-45
10	176	201	377	-25
11	218	226	444	-8
14	204	228	432	-24
16	240	249	489	-9

Table 16 The group-by-period means for fasting plasma glucose values

Group	Period 1	Period 2	Mean
1 (Placebo-Aloe vera) = 8	$\bar{y}_{11} = 251.5$	$\bar{y}_{12} = 255.9$	$\bar{y}_{1..} = 253.7$
2 (Aloe vera-Placebo) = 8	$\bar{y}_{21} = 228.6$	$\bar{y}_{22} = 239.0$	$\bar{y}_{2..} = 233.8$
Mean	$\bar{y}_{.1} = 240.1$	$\bar{y}_{.2} = 247.4$	$\bar{y}_{...} = 243.8$

The analysis of data of fasting plasma glucose using t-tests

Testing  $\lambda_1 = \lambda_2$ ,

This test is the first test to evaluate data from an AB-BA crossover trial. The test is a two-sample t-test to test the equality of the carry-over effect.

To derive a test of the null hypothesis that  $\lambda_1 = \lambda_2$ , we note that the subject totals

$$t_{1k} = y_{11k} + y_{12k} \text{ for the } k\text{th subject in group 1}$$

and

$$t_{2k} = y_{21k} + y_{22k} \text{ for the } k\text{th subject in group 2}$$

have expectations

$$E[t_{1k}] = 2\mu + \pi_1 + \pi_2 + \tau_1 + \tau_2 + \lambda_1$$

and

$$E[t_{2k}] = 2\mu + \pi_1 + \pi_2 + \tau_1 + \tau_2 + \lambda_2$$

If  $\lambda_1 = \lambda_2$  these two expectations are equal. Consequently, to test if  $\lambda_1 = \lambda_2$ , we apply the two-sample t-test to the subject totals.

$$\text{Let } \lambda_d = \lambda_1 - \lambda_2 \text{ and } \hat{\lambda}_d = \bar{t}_1 - \bar{t}_2.$$

$$\text{Let } E[\hat{\lambda}_d] = \lambda_d \text{ and } V[\hat{\lambda}_d] = 2(\sigma_s^2 + \sigma^2)\left(\frac{1}{n_1} + \frac{1}{n_2}\right) = \sigma_T^2 m$$

where,

$$\sigma_T^2 = \sigma(2\sigma_s^2 + \sigma^2) \text{ and } m = \frac{n_1 + n_2}{n_1 n_2}$$



We estimate  $\sigma_T^2$  by using the formula

$$\hat{\sigma}_T^2 = \frac{\sum_{i=1}^2 \sum_{k=1}^{n_i} (t_{ik} - \bar{t}_i)^2}{(n_1 + n_2 - 2)}$$

the pooled sample variance which has  $(n_1 + n_2 - 2)$  degrees of freedom(d.f.)

On the null hypothesis that  $\lambda_1 = \lambda_2$  the statistic

$$T_\lambda = \frac{\hat{\lambda}_d}{(\hat{\sigma}_T^2 m)^{1/2}}$$

has Student's t-distribution on  $(n_1 + n_2 - 2)$  d.f.

Using data in Table we obtain the following results:

$$\bar{t}_1 = 251.500 + 255.875 = 507.375$$

$$\bar{t}_2 = 228.625 + 239.000 = 467.625$$

$$\hat{\lambda}_d = 39.75$$

$$\sum_{k=1}^8 ((t_{1k} - \bar{t}_1)^2) = 112793.875$$

$$\sum_{k=1}^8 (t_{2k} - \bar{t}_2)^2 = 59277.875$$

$$\hat{\sigma}_T^2 = (112793.875 + 59277.875) / 14 = 12290.839$$

$$T_\lambda = \frac{39.75}{\left(\frac{16}{64} \times 12290.839\right)^{1/2}} = 0.717094 \text{ on } 14 \text{ d.f.}$$

According to Grizzle's advice(1965) to test the null hypothesis at the 10% two-sided level prior to testing for a treatment difference, the t-statistic calculated above gave insufficient evidence to reject the null hypothesis. It indicates that the carry over effect of aloe vera juice may be similar to the carry over effect of placebo juice. As the test shows that  $\lambda_1 = \lambda_2$ , we can proceed to test the null hypothesis of equal direct effects.

Testing  $\tau_1 = \tau_2$  (assuming  $\lambda_1 = \lambda_2$ )

If  $\lambda_1 = \lambda_2$ , then the period differences

$$d_{1k} = y_{11k} - y_{12k} \text{ for the } k\text{th subject in group 1}$$

and

$$d_{2k} = y_{21k} - y_{22k} \text{ for the } k\text{th subject in group 2}$$

have expectations

$$E[d_{1k}] = \pi_1 - \pi_2 + \tau_1 - \tau_2$$

and

$$E[d_{2k}] = \pi_1 - \pi_2 + \tau_2 - \tau_1$$

On the null hypothesis that  $\tau_1 = \tau_2$  these two expectations are equal and so we can test the null hypothesis by applying the two-sample t-test to the period differences.

Let  $\tau_d = \tau_1 - \tau_2$  then  $\hat{\tau}_d = \frac{1}{2}[\bar{d}_1 - \bar{d}_2]$  is such that

$$E[\hat{\tau}_d] = \tau_d$$

and

$$\begin{aligned} V[\hat{\tau}_d] &= 2\sigma^2/4(1/n_1 + 1/n_2) \\ &= \sigma_D^2/4, \text{ say,} \end{aligned}$$

where,

$$\sigma_D^2 = 2\sigma^2.$$

The pooled estimate of  $\sigma_D^2$  is

$$\hat{\sigma}_D^2 = \sum_{i=1}^2 \sum_{k=1}^{n_i} \frac{(\bar{d}_{ik} - \bar{d}_i)^2}{(n_1 + n_2 - 2)}$$

On the null hypothesis that  $\tau_1 = \tau_2$  the statistic

$$T_\tau = \frac{\hat{\tau}_d}{(\hat{\sigma}_D^2 m / 4)^{1/2}}$$

has Student's t-distribution on  $(n_1 + n_2 - 2)$  d.f.

Using data in Table we obtain the following results:

$$\bar{d}_1 = -4.375$$

$$\bar{d}_2 = -10.375$$

$$\hat{d} = 1/2[-4.375 - (-10.375)] = 3$$

$$\sum_{k=1}^8 (d_{1k} - \bar{d}_1)^2 = 8963.875$$

$$\sum_{k=1}^8 (d_{2k} - \bar{d}_2)^2 = 4165.875$$

$$\hat{\sigma}_D^2 = (8963.875 + 4165.875) / 14 = 937.8393$$

$$T_\tau = \frac{3}{\left(\frac{16}{64} \times \frac{937.8393}{4}\right)^{1/2}} = 0.391847 \text{ on } 14 \text{ d.f.}$$

The t-statistic calculated also gave insufficient evidence to reject the null hypothesis at the 5% two-sided significance level. In this case the direct treatment effect of aloe vera juice may have similar hypoglycemic effect as placebo juice. This result implies that aloe vera juice may not have hypoglycemic effect.

Testing  $\pi_1 = \pi_2$  (assuming  $\lambda_1 = \lambda_2$ )

In order to test the null hypothesis  $\pi_1 = \pi_2$  we use the "cross-over" differences

$$c_{1k} = d_{1k} = Y_{11k} - Y_{12k} \quad \text{for the } k\text{th subject in group 1}$$

and

$$c_{2k} = -d_{2k} = Y_{22k} - Y_{21k} \quad \text{for the } k\text{th subject in group 2}$$

If  $\lambda_1 = \lambda_2$  then

$$E[c_{1k}] = \pi_1 - \pi_2 + \tau_1 - \tau_2$$

and

$$E[c_{2k}] = \pi_2 - \pi_1 + \tau_1 - \tau_2$$

If  $\pi_1 = \pi_2$  these expectations are equal and we can apply the two-sample t-test to the cross-over differences.

Let  $\pi_d = \pi_1 - \pi_2$  then  $\hat{\pi}_d = \frac{1}{2}[\bar{c}_1 - \bar{c}_2]$  is such that

$$E[\hat{\pi}_d] = \pi_d$$

and

$$\begin{aligned} V[\hat{\pi}_d] &= 2\sigma^2/4(1/n_1 + 1/n_2) \\ &= \sigma_D^2 m/4, \text{ say,} \end{aligned}$$

where,

$$\sigma_D^2 = 2\sigma^2.$$

The pooled estimate of  $\sigma_D^2$  is  $\hat{\sigma}_D^2 = \frac{\sum_{i=1}^2 \sum_{k=1}^{n_i} (\bar{d}_{ik} - \bar{d}_i)^2}{(n_1 + n_2 - 2)}$

On the null hypothesis that  $\pi_1 = \pi_2$  the statistic  $T_\pi = \frac{\hat{\pi}_d}{(\hat{\sigma}_D^2 m / 4)^{1/2}}$

has Student's t-distribution on  $(n_1 + n_2 - 2)$  d.f.

Using data in Table we obtain the following results:

$$\bar{c}_1 = -4.375$$

$$\bar{c}_2 = 10.375$$

$$\hat{\pi}_d = \frac{1}{2}(-4.375 - 10.375) = -7.375$$

$$T_\pi = \frac{-7.375}{\left(\frac{16}{64} \times \frac{937.8393}{4}\right)^{1/2}} = -0.96329 \text{ on } 14 \text{ d.f.}$$

The t-statistic calculated also gave insufficient evidence to reject the null hypothesis at the 5% two-sided significance level. The result suggests that there is no difference in period effect between the period the subjects took placebo juice and the period the subjects took aloe vera juice.

#### Subject compliance

All subjects attended the Nutrition Clinic at Chulalongkorn Hospital within one week of appointment. The researcher visited, collected blood, and dispensed Aloe vera juice or Placebo juice for two subjects at home and workplace regularly due to subjects' difficulties to attend the Clinic.

Subjects adhered to dietary habits and exercise well. However, since evaluation of dietary control and exercise depended on subjective information voluntarily told by subjects, it was possible that some subjects might have deviated from their usual dietary habits and exercise at some points. This deviation may be responsible for the cross-over differences in some cases.

#### Drug compliance

Each subject brought to the Clinic all containers of treatment juices. The researcher counted and examined the content of containers.

All subjects took Aloe vera juice and Placebo juice as directed and on schedule except for three irregularities involving two subjects. One subject missed two-day dosage of Placebo juice once and another two-day dosage of Aloe vera juice. The other subject did not take Aloe vera juice for two days. However, these irregularities in omitting treatments occurred in the middle of the periods. So they were unlikely to affect fasting plasma glucose levels.

### Drug tolerability and side-effects

Both Aloe vera and Placebo juices were well-tolerated. Subjects had no difficulty in taking the juices. They did not experience any gastrointestinal side effects.

One subject in Group 1 (Placebo-Aloe vera) experienced generalized erythematous maculopapular rashes over her body, arms and legs. The itchy rashes started after one and half-month of taking Aloe vera juice in the second period. She continued to take Aloe vera juice for the last two weeks before she attended the Clinic at the end of the trial. A dermatologist examined the rash and described the lesions as pityriasis-like. Side effect to drug (Aloe vera) or other constituents (coloring or flavoring agents, preservatives) is a high possibility in the differential diagnosis of the rashes.

Subjects did not report other systemic side effects. The liver function tests and kidney function tests were within normal limits throughout the study in all subjects.



ศูนย์วิทยทรัพยากร  
จุฬาลงกรณ์มหาวิทยาลัย