CHAPTER IV

RESULTS

Hypoglycemic effect of *G. inodorum* tea in human

In order to determine the hypoglycemic effect of *Gymnema inodorum* tea on healthy subjects, we divided the subjects into 5 groups: group one or a control group, the standard OGTT was performed without GI tea consumption after 75 g glucose load; group 2 the treatment I group, the subjects were asked to drink one pack of GI tea (1.5 g in 150 ml hot water) immediately after oral glucose load; group 3 the treatment II group, the subjects were asked to drink 1 pack of GI tea 15 minutes after oral glucose load; group 4 the treatment III group, the subjects were asked to drink 1 pack of GI tea 30 minutes after oral glucose load; group 5 the treatment IV group, the subjects were asked to drink 2 packs of GI tea (3.0 g in 150 ml hot water) 15 minutes after 75 g oral glucose load.

The data in table 4 showed that drinking GI tea immediately and 15 minutes after oral glucose load (treatment I and II) can significantly reduced plasma glucose at p-values equal 0.035 and 0.004 respectively. Double concentration of GI tea (treatment IV) can even reduced plasma glucose better than one pack of GI tea drinking ($p = 0.000$). Drinking GI tea 30 minutes after glucose load (treatment III) cannot reduce plasma glucose ($p=0.662$). The average values of standard OGTT for all 5 groups were shown in figure 5.

Table 4 Hypoglycemic effects of GI tea on plasma glucose in healthy humans.

	Control[a]	Treatment I[b]	Treatment II[c]	Treatment III[d]	Treatment IV[e]
N	73	40	73	20	19
Average Peak glucose (mg/dL)	145±27.17	130±31.50	131±27.25	143±41.18	108±14.49
p-value (compared with control group)	-	0.035*	0.004*	0.662	0.000*

a The subjects performed standard OGTT without GI tea consumption

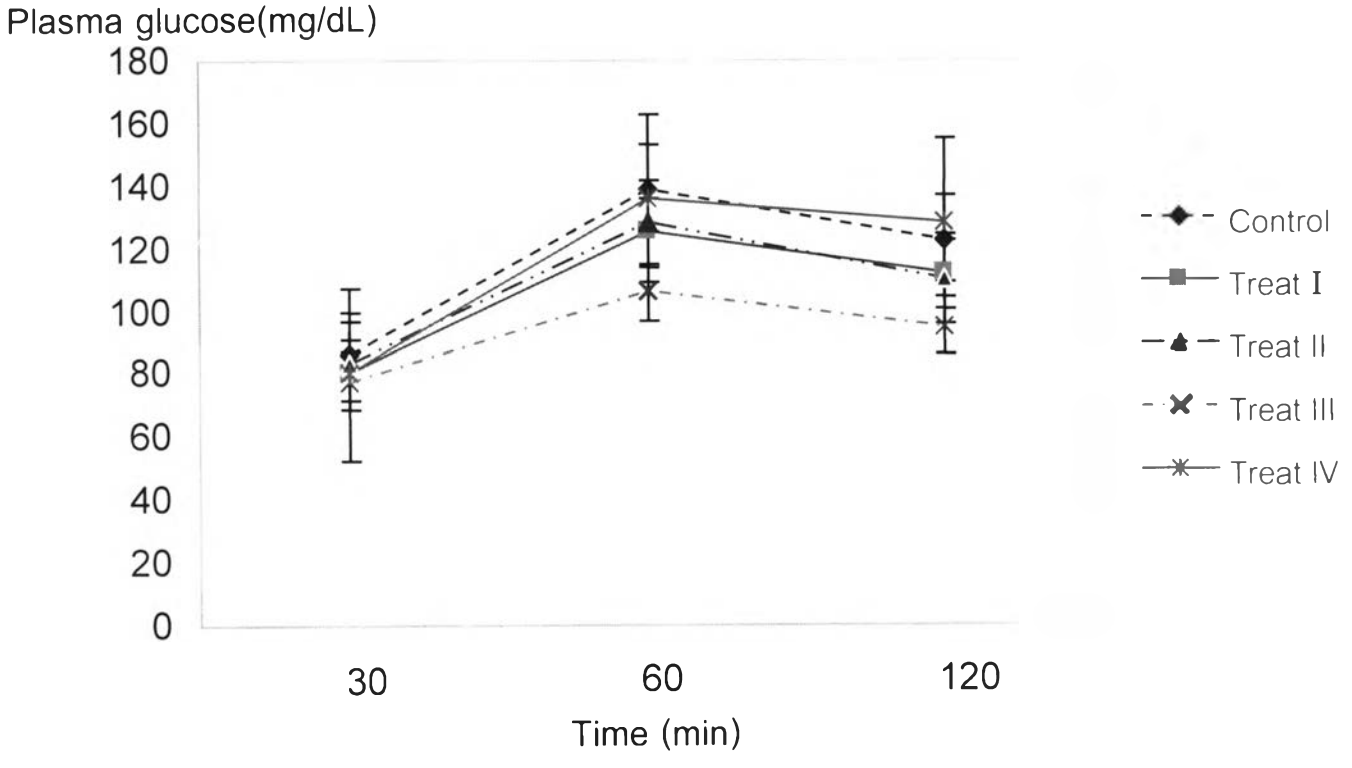
b The subjects drank 1.5 g of GI tea immediately after oral glucose load (treatment I).

c The subjects drank 1.5 g of GI tea 15 minutes after oral glucose load (treatment II).

d The subjects drank 1.5 g of GI tea 30 minutes after oral glucose load (treatment III).

e The subjects drank 3.0 g of GI tea in 150 ml boiling water (double concentration of GI tea) 15 minutes after oral glucose load (treatment IV)

* Statistic significant ($p \leq 0.05$)



Data were expressed as mean ± SD

Figure 5 Hypoglycemic effects of GI tea on plasma glucose (OGTT) in healthy human

Hypoglycemic effect of GI tea on standard meal

The standard meal was prepared as described in chapter III, B. Treatment: The result showed that 16 out of 20 (80 %) of the subjects had decreased peak of glucose concentration. The mean peak glucose concentration in the GI group (treatment group) is significantly lower than the before group (147 ± 39 VS 129 ± 27 mg/dL: $p = 0.016$) as shown in table 5. The results indicated that *Gymnema inodorum* tea has hypoglycemic effect and can decrease blood glucose of the test subjects.

Table 5 Hypoglycemic effect of GI tea on standard meal

	N	Peak glucose concentration (mg/dL)	P=value
Control	20	147 ± 39	0.016*
Treatment	20	129 ± 27	

* Statistical significant ($p \leq 0.05$)

Effect of GI tea consumption on liver function test

Twenty healthy subjects with normal fasting plasma glucose and normal liver enzyme level (aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and gamma glutamyl transferase (GGT)) were included in this study. During the period of 28 days of 1 pack of GI tea (1.5 g of GI in 150 ml hot water) daily consumption, the subjects were asked to monitor liver function enzyme and fasting plasma glucose in blood at day 0, 2, 4, 7, 14, 21, and 28. Table 6 and Figure 6 showed that there was no significant difference between the liver enzymes of the control baseline group (no GI tea consumption) and the treatment group (GI tea consumption). The fasting plasma glucose of all subjects remained within normal limit throughout the period of the study (Table 7 and Figure 7).

Table 6 Liver function test (AST,ALT, ALP, GGT) in healthy subjects after 28 days of GI tea consumption.

Liver enzyme	N	Treat		p-value
		Baseline[a]	Treatment[b]	
AST	20	17.2±6.4	18.3±3.3	0.872
ALT	20	12.3±4.5	13.1±3.3	0.475
ALP	20	72.4±7.4	73.8±8.0	0.100
GGT	20	17.8±4.5	18.4±5.0	0.759

Data was shown as mean ± SD

a 'Baseline' is the liver enzyme level measured at day 0 before the treatment

b 'Treatment' is the liver enzyme level measured after GI tea consumption on day 28

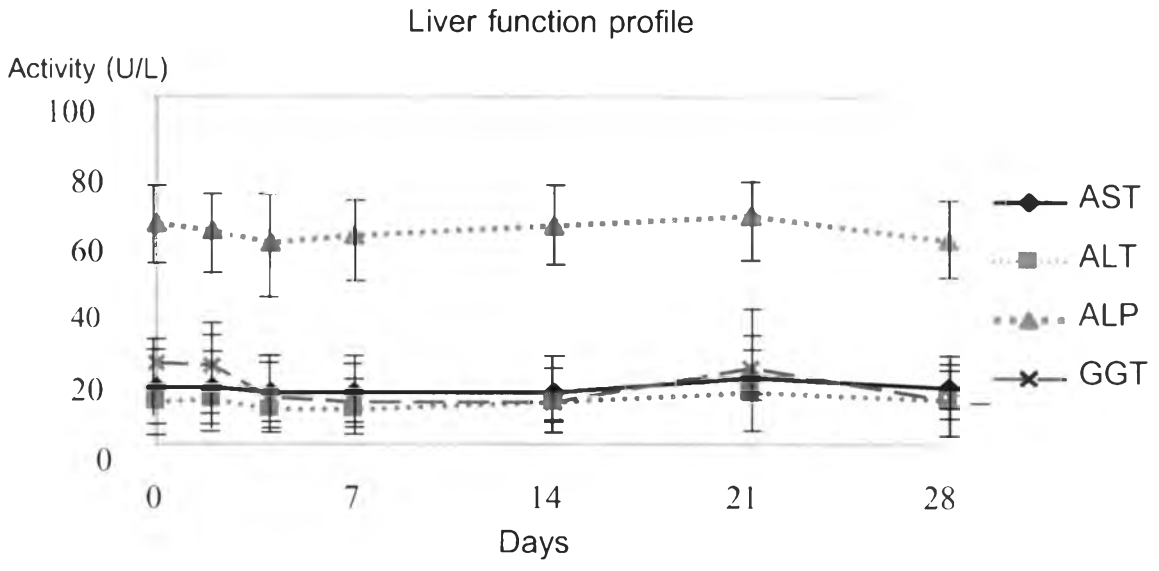


Figure 6 Average liver function profile (AST,ALT, ALP, GGT) in healthy subjects within 28 day period of GI tea consumption.

Table 7 Plasma glucose monitoring of healthy subjects during 28 days of GI tea consumption.

Subj No.	Blood glucose (mg/dL)						
	day 0	day 2	day 4	day 7	day 14	day 21	day 28
1	86	69	77	81	73	86	60
2	76	74	76	70	85	82	79
3	83	86	85	77	81	87	87
4	78	90	82	75	73	74	83
5	82	81	81	80	82	87	84
6	82	77	76	78	84	90	89
7	90	89	79	83	88	90	84
8	77	75	83	84	85	90	59
9	86	76	87	79	79	80	82
10	94	81	80	82	89	81	75
11	72	77	73	75	87	78	81
12	67	53	78	65	70	84	66
13	89	85	83	88	92	100	87
14	68	73	69	67	62	76	80
15	83	83	86	81	87	91	88
16	79	78	76	77	84	87	79
17	74	77	82	69	77	95	82
18	82	68	72	69	83	77	70
19	85	84	87	87	89	86	85
20	87	86	90	84	85	82	83
Average	81	78.1	80.1	77.55	81.75	85.15	79.15
SD	7.2	8.5	5.5	6.7	7.5	6.6	8.8

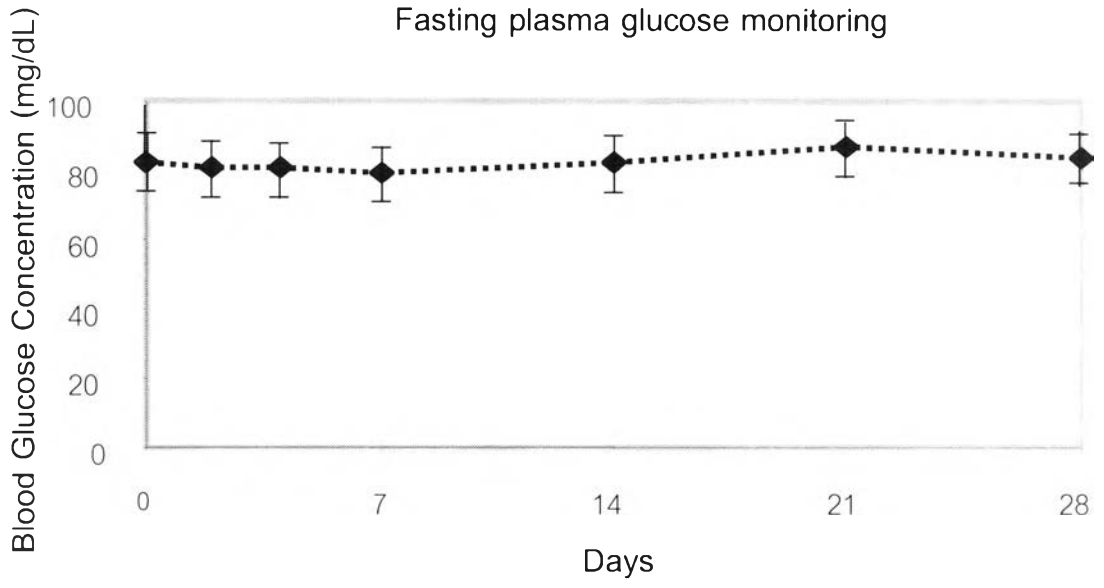


Figure 7 Fasting plasma glucose monitoring in healthy subjects within 28 day period of GI tea consumption.

Effect of GI extract on insulin secretion of INS-1 cells

INS-1 cells is a rat insulinoma beta cell line that can secrete insulin. In control group, after 1 hour of glucose exposed, the level of insulin was measured using radioimmuno assay kit (Insulina IRMA kit). In the treatment group, GI extract, fraction I-IV (as described in chapter III) were added together with glucose. We found that, all GI fractions cannot stimulate insulin secretion from INS-1 cell as shown in table 8 and figure 8. Insulin level in all s are not significantly different ($p>0.05$).

Table 8 Effects of GI extract on insulin secretion in INS-1 cells.

	Insulin secretion (ng/3 x 10^5 cell)					
	Control	GI water	GI I	GI II	GI III	GI IV
Before	2.45	2.56	3.05	2.54	3.00	2.34
After	5.79	5.48	5.74	5.31	6.08	5.95
Difference	3.34	2.92	2.63	2.77	3.09	3.61
p-value		0.262	0.184	0.211	0.306	0.745

The insulin in each well was measured by Insulina IRMA kit (^{125}I) as before and after glucose addition. Although the insulin level increased in all treatment groups when compared with the control group, but it is not significantly different..

Control : treat INS-1 cell with glucose for 1 h

GI water : treat INS-*1* cell with glucose for 1 h and GI extract from boiling water

GI I : treat INS-1 cell with glucose for 1 h and GI I fraction (ethanol extract)

GI II : treat INS-1 cell with glucose for 1 h and GI II fraction (n-butanol extract)

GI III : treat INS-1 cell with glucose for 1 h and GI III fraction (ethyl acetate extract)

GI IV : treat INS-1 cell with glucose for 1 h and GI IV fraction (methanol extract)

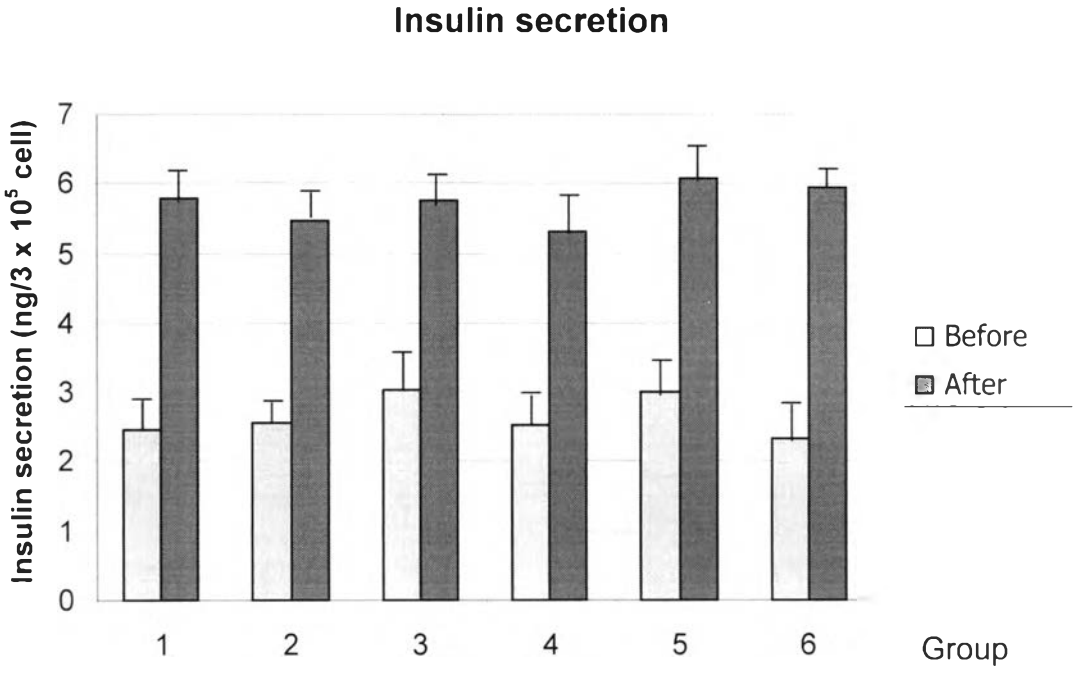


Figure 8 Effects of GI extract on insulin secretion in INS-1 cells. The insulin in each well was measured by Insulina IRMA kit (^{125}I) as before and after glucose addition.

Group 1: control group; measure insulin release from INS-1 cell

Group 2: treat INS-1 cell with GI extract from boiling water

Group3: treat INS-1 cell with GI I fraction (ethanol extract)

Group4: treat INS-1 cell with GI II fraction (n-butanol extract)

Group5: treat INS-1 cell with GI III fraction (ethyl acetate extract)

Group6: treat INS-1 cell with GI IV fraction (methanol extract)

Alpha glucosidase inhibitor determination

Dried GI leaves were extracted with boiling water and 70% methanol and then determined for alpha-glucosidase inhibitor activity. We found that GI extract with both boiling water and 70% methanol did not inhibit alpha-glucosidase enzyme as shown in table 9, figure 9, table 10 and figure 10. However, GI extract with 70% methanol showed slight inhibition on alpha-glucosidase enzyme at higher concentration.

Table 9 Effect of GI extracted with boiling water on inhibition of alpha glucosidase activity

GI concentration (mg/mL)	Average alpha-glucosidase activity (unit/mL)	% inhibition
0	0.709 ± .014	0
0.005	0.703 ± .009	0.9
0.050	0.704 ± .008	0.75
0.100	0.713 ± .021	0
0.150	0.714 ± .018	0
0.200	0.720 ± .026	0
0.250	0.722 ± .010	0
0.005 mg/ml Acarbose (control)	0.353 ± .053	51.1

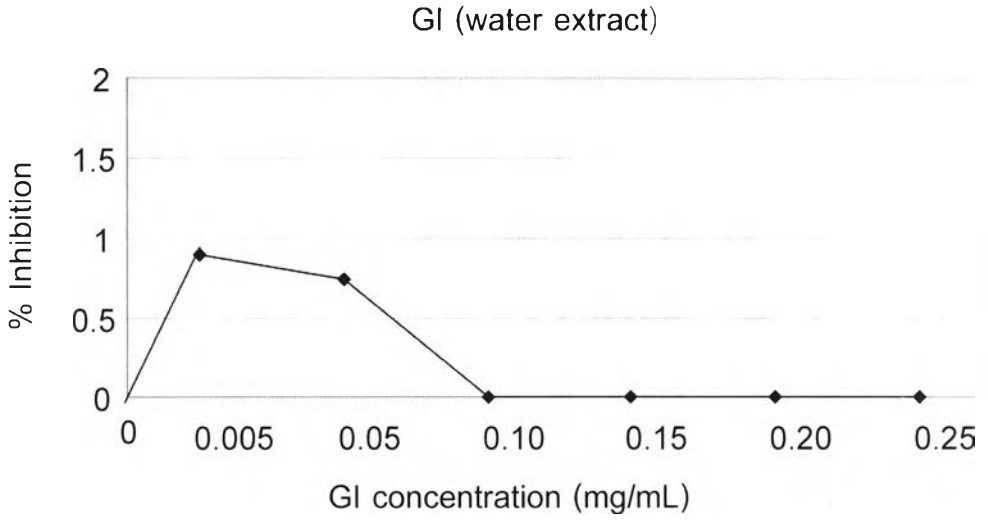


Figure 9 Effect of GI extracted with boiling water on inhibition of alpha-glucosidase activity.

Table 10 Effect of GI extracted with 70% methanol on inhibition of alpha glucosidase activity.

GI concentration (mg/mL)	Average alpha-glucosidase activity (unit/mL)	% inhibition
0	0.708 ± .028	0
0.005	0.713 ± .015	0
0.050	0.708 ± .009	0.14
0.100	0.707 ± .033	0.15
0.150	0.704 ± .008	0.57
0.200	0.704 ± .016	0.57
0.250	0.691 ± .032	2.4
0.005 mg/ml Acarbose (control)	0.353 ± .053	51.1

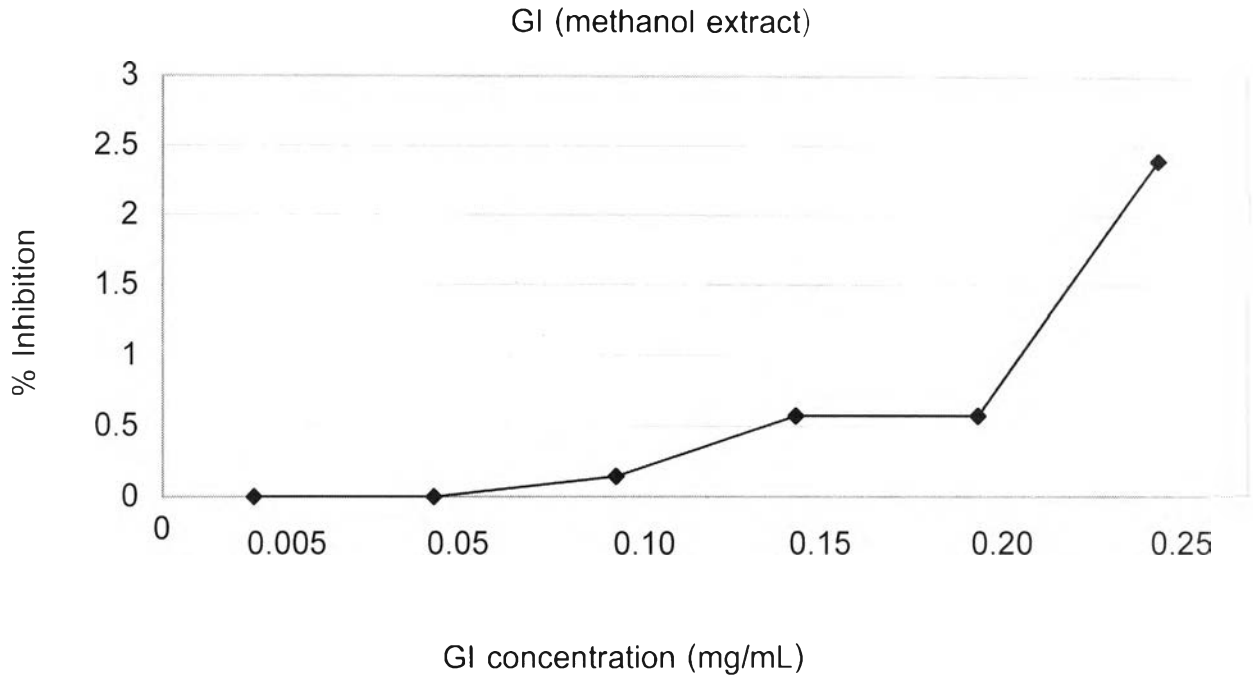


Figure 10 Effect of GI extracted with 70% methanol on inhibition of alpha - glucosidase activity.

Polymorphism of RAGE gene and complication of diabetes

Genotype distribution and allele frequency of -429 T/C RAGE gene polymorphism was shown in table 11. The PCR products of -429T/C RAGE gene were cut with restriction enzyme Alu I before separation on 3% agarose gel electrophoresis. The RFLP product of wild-type (TT) is one piece of DNA band at 344 bp. The TC (mutant) had been cut into 3 pieces at 161, 183, and 344 bp. The CC (mutant) had been cut at 161, and 183 bp. [123-124] The genotype distribution of healthy control (n=160) are TT: 71.88% (n=115), TC:23.75% (n=38), and CC:4.37% (n=7). The allele frequencies are 83.75% for allele T and 16.25% for allele C. In diabetic patients (n=190), the genotypic distribution were TT:75.79% (n=144), TC:19.47%(n=37), and CC:4.74%(n=9). The allele frequencies were 85.53% for allele T and 14.47% for allele C. There was no statistical significant difference in allele frequencies between the two groups ($p>0.05$), suggesting that the association between the -374 T/A polymorphism and diabetic neuropathy did not exist (Table 11).

Table 12 showed that there was no statistic difference in allele frequencies between healthy control and diabetic patients, with and without complication ($p> 0.05$). The association between -374 T/A polymorphism and diabetic complication did not exist.

When classified diabetic complication into individual group depended on pathogenesis; nephropathy, retinopathy, neuropathy, coronary artery disease (CAD), cardiovascular disease (CVD), and skin disease, the association between each complication group and -374T/A polymorphism did not exist ($p > 0.05$, table 13-18)

Table11 Gene and allele frequency of -429T/C RAGE gene polymorphism in healthy subjects (control) and diabetic patients (DM) groups

	Control (n = 160)	DM (n = 190)
Genotype distribution		
T/T	115 (71.88%)	144 (75.79%)
T/C	38 (23.75%)	37 (19.47%)
C/C	7 (4.37%)	9 (4.74%)
Allele frequency		
T	268 (83.75%)	325 (85.53%) [a]
C	52 (16.25%)	55 (14.47%)

[a] $P = 0.5856$

Table 12 Genotype distribution and allele frequency of -429T/C RAGE gene polymorphism in healthy subjects (control) and diabetic patients (DM) with and without complication

	Control (n = 160)	DM (n = 190)	
		With complication (n = 132)	Without complication (n = 58)
Genotype distribution			
T/T	115 (71.88%)	101 (76.52%)	43 (74.14%)
T/C	38 (23.75%)	26 (19.70%)	11 (18.97%)
C/C	7 (4.37%)	5 (3.78%)	4 (6.89%)
Allele frequency			
T	268 (83.75%) [a]	228 (86.36%)	97 (83.62%) [b]
C	52 (16.25%)	36 (13.64%)	19 (16.38%)

[a] $P = 0.4457$, [b] $P = 0.5881$

Table 13 Genotype distribution and allele frequency of -429T/C RAGE gene polymorphism in healthy subjects (control) and diabetic patients (DM) with and without diabetic nephropathy

	Control (n = 160)	DM (n = 190)	
		With nephropathy (n = 82)	Without nephropathy (n = 108)
Genotype distribution			
T/T	115 (71.88%)	64 (78.05%)	80 (74.07%)
T/C	38 (23.75%)	15 (18.29%)	22 (20.37%)
C/C	7 (4.37%)	3 (3.66%)	6 (5.56%)
Allele frequency			
T	268 (83.75%) [a]	143 (87.20%)	182 (84.26%) [b]
C	52 (16.25%)	21 (12.80%)	34 (15.74%)

[a] $P = 0.3853$, [b] $P = 0.51$

Table 14 Genotype distribution and allele frequency of -429T/C RAGE gene polymorphism in healthy subjects (control) and diabetic patients (DM) with and without diabetic retinopathy

	Control (n = 160)	DM (n = 190)	
		With retinopathy (n = 45)	Without retinopathy (n = 145)
Genotype distribution			
T/T	115 (71.88%)	37 (82.22%)	107 (73.79%)
T/C	38 (23.75%)	7 (15.56%)	30 (20.69%)
C/C	7 (4.37%)	1 (2.22%)	8 (5.52%)
Allele frequency			
T	268 (83.75%) [a]	81 (90.00%)	244 (84.14%) [b]
C	52 (16.25%)	9 (10.00%)	46 (15.86%)

[a] $P = 0.4997$, [b] $P = 0.2265$

Table 15 Genotype distribution and allele frequency of -429T/C RAGE gene polymorphism in healthy subjects (control) and diabetic patients (DM) with and without diabetic neuropathy

	Control (n = 160)	DM (n = 190)	
		With neuropathy (n = 78)	Without neuropathy (n = 112)
Genotype distribution			
T/T	115 (71.88%)	61 (78.21%)	83 (74.11%)
T/C	38 (23.75%)	14 (17.95%)	23 (20.54%)
C/C	7 (4.37%)	3 (3.84%)	6 (5.35%)
Allele frequency			
T	268 (83.75%) [a]	136 (87.18%)	189 (84.38%) [b]
C	52 (16.25%)	20 (12.82%)	35 (15.62%)

[a] $P = 0.3987$, [b] $P = 0.5378$

Table 16 Genotype distribution and allele frequency of -429T/C RAGE gene polymorphism in healthy subjects (control) and diabetic patients (DM) with and without CAD

	Control (n = 160)	DM (n = 190)	
		With CAD (n = 23)	Without CAD (n = 167)
Genotype distribution			
T/T	115 (71.88%)	20 (86.96%)	124 (74.25%)
T/C	38 (23.75%)	3 (13.04%)	34 (20.36%)
C/C	7 (4.37%)	0 (0.00%)	9 (5.39%)
Allele frequency			
T	268 (83.75%) [a]	43 (93.48%)	282 (84.43%) [b]
C	52 (16.25%)	3 (6.52%)	52 (15.57%)

[a] $P = 0.1321$, [b] $P = 0.1581$

Table 17 Genotype distribution and allele frequency of -429T/C RAGE gene polymorphism in healthy subjects (control) and diabetic patients (DM) with and without CVD

	Control (n = 160)	DM (n = 190)	
		With CVD (n = 15)	Without CVD (n = 175)
Genotype distribution			
T/T	115 (71.88%)	9 (60.00%)	135 (77.14%)
T/C	38 (23.75%)	6 (40.00%)	31 (17.72%)
C/C	7 (4.37%)	0 (0.00%)	9 (5.14%)
Allele frequency			
T	268 (83.75%) [a]	24 (80.00%)	301 (86.00%) [b]
C	52 (16.25%)	6 (20.00%)	49 (14.00%)

[a] $P = 0.6081$, [b] $P = 0.4141$

Table 18 Genotype distribution and allele frequency of -429T/C RAGE gene polymorphism in healthy subjects (control) and diabetic patients (DM) with and without skin disease

	Control (n = 160)	DM (n = 190)	
		With skin disease (n = 11)	Without skin disease (n = 179)
Genotype distribution			
T/T	115 (71.88%)	8 (72.73%)	136 (75.98%)
T/C	38 (23.75%)	3 (27.27%)	34 (18.99%)
C/C	7 (4.37%)	0 (0.00%)	9 (5.03%)
Allele frequency			
T	268 (83.75%) [a]	19 (86.36%)	306 (85.74%) [b]
C	52 (16.25%)	3 (13.64%)	52 (14.53%)

[a] $P = 1.000$, [b] $P = 1.000$

Note

- [a] *P* compared between healthy control group and DM patient with complication
- [b] *P* compared between DM patients with and without complication

Genotype distribution and allele frequency of -374 T/A RAGE polymorphism was showed in table 19.The PCR products were cut with restriction enzyme Tsp 5091 before separated on 3% agarose gel electrophoresis. The RFLP products of wild-type (TT) had been cut into 4 pieces at 29 bp, 70bp, 110 bp, and 130 bp. The TA (mutant) had been cut into 5 pieces at 29 bp, 70bp, 110 bp,130 bp, and 240 bp. The AA (mutant) had been cut into 3 pieces at 29 bp, 70bp, and 240 bp. [123] The genotype distribution of healthy control (n=161) are TT: 78.26%(n=126), TA:20.50% (n=33), and AA:1.24% (n=2). The allele frequencies are 87.44% for allele T and 12.56% for allele C. In diabetic patients (n=203), the genotypic distribution were TT:78.33% (n=159), TA:18.23%(n=37), and AA:3.45%(n=7). The allele frequencies were 88.51% for allele T and 11.49% for allele A. There was no statistical significant difference in allele frequencies between the two groups ($P > 0.05$), suggesting that the association between the -374 T/A polymorphism and diabetic complication did not exist (Table 19).

Table 20 showed that there was no statistic difference in allele frequencies between healthy control and diabetic patients, with and without complication ($p > 0.05$). The association between -374 T/A polymorphism and diabetic complication did not exist.

When classified diabetic complication into individual group depended on pathogenesis; retinopathy, neuropathy, coronary artery disease (CAD), and cardiovascular disease (CVD), the association between each complication group and -374T/A polymorphism did not exist ($p > 0.05$, table 21-24)

Table 19 Genotype distribution and allele frequency of -374 T/A RAGE gene polymorphism in healthy subjects (control) and diabetic patients (DM) groups

	Control (n = 161)	DM (n = 203)
Genotype distribution		
T/T	126 (78.26%)	159 (78.33%)
T/A	33 (20.50%)	37 (18.23%)
A/A	2 (1.24%)	7 (3.45%)
Allele frequency		
T	285 (87.44%)	196 (88.51%)[a]
A	37 (12.56%)	44 (11.49%)

[a] $P = 0.744$

Table 20 Genotype distribution and allele frequency of -374T/A RAGE gene polymorphism in healthy subjects (control) and diabetic patients (DM) with and without complication

	Control (n = 161)	DM (n = 203)	
		With complication (n = 115)	Without complication (n = 88)
Genotype distribution			
T/T	126 (78.26%)	95(82.61%)	64 (72.73%)
T/A	33 (20.50%)	17(14.78%)	20 (27.73%)
A/A	2 (1.24%)	3 (2.61%)	4 (4.55%)
Allele frequency			
T	285 (88.51%)	207 (90.00%)	148 (84.09%)
A	37 (11.49%) [a]	23 (10.00%) [b]	28 (15.91%)

[a] $P = 0.207$, [b] $P = 0.103$

Table 21 Genotype distribution and allele frequency of -429T/C RAGE gene polymorphism in healthy subjects (control) and diabetic patients (DM) with and without diabetic retinopathy

	Control (n = 161)	DM (n = 203)	
		With retinopathy (n = 49)	Without retinopathy (n = 154)
Genotype distribution			
T/T	126 (78.26%)	39 (79.60%)	120 (77.92%)
T/A	33 (20.50%)	7 (14.29%)	30 (19.48%)
A/A	2 (1.24%)	3 (6.12%)	4 (2.60%)
Allele frequency			
T	285 (88.51%)	85 (86.37%)	270 (87.66%)
A	37 (11.49%) [a]	13 (13.27%)	38 12.34%)

[a] $P = 0.904$

Table 22 Genotype distribution and allele frequency of -374T/A RAGE gene polymorphism in healthy subjects (control) and diabetic patients (DM) with and without diabetic neuropathy

	Control (n = 161)	DM (n = 203)	
		With neuropathy (n = 87)	Without neuropathy (n = 116)
Genotype distribution			
T/T	126 (78.26%)	72 (78.21%)	87 (75.00%)
T/A	33 (20.50%)	10 (17.95%)	27 (23.28%)
A/A	2 (1.24%)	5 (3.84%)	2 (1.72%)
Allele frequency			
T	285 (88.51%)	154 (88.51%)	201 (86.64%)
A	37 (11.49%) [a]	20 (11.49%)	31 (13.36%)

[a] $P = 0.681$

Table 23 Genotype distribution and allele frequency of -429T/C RAGE gene polymorphism in healthy subjects (control) and diabetic patients (DM) with and without CAD

	Control (n = 161)	DM (n = 203)	
		With CAD (n = 26)	Without CAD (n = 177)
Genotype distribution			
T/T	126 (78.26%)	23 (88.46%)	136 (76.84%)
T/A	33 (20.50%)	2 (7.69%)	35 (19.77%)
A/A	2 (1.24%)	1 (3.85%)	6 (3.39%)
Allele frequency			
T	285 (88.51%)	48 (92.31%)	307(86.72%)
A	37 (11.49%) [a]	4 (7.69%)	47 (13.28%)

[a] $P = 0.361$

Table 24 Genotype distribution and allele frequency of -374T/A RAGE gene polymorphism in healthy subjects (control) and diabetic patients (DM) with and without CVD

	Control (n = 161)	DM (n = 203)	
		With CVD (n = 16)	Without CVD (n = 187)
Genotype distribution			
T/T	126 (78.26%)	15 (93.75%)	144 (77.01%)
T/A	33 (20.50%)	1 (6.25%)	36 (19.25%)
A/A	2 (1.24%)	0 (0.00%)	7 (3.76%)
Allele frequency			
T	285 (88.51%)	31 (13.37%)	324 (86.63%)
A	37 (11.49%) [a]	1 (3.13%)	50 (13.37%)

[a] $P = 0.158$

Note

- [a] *P* compared between healthy control group and DM patient with complication
- [b] *P* compared between DM patients with and without complication