African Journal of Biotechnology Vol. 9(7), pp. 1079-1085, 15 February, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB09.1639
ISSN 1684–5315 © 2010 Academic Journals

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Full Length Research Paper

Effect of *Gymnema inodorum* on postprandial peak plasma glucose levels in healthy human

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Accepted 18 January, 2010

***Gymnema inodorum* (GI), a vegetable widely used in a Northern Thai food, is known for not only its health nourishing effect, but also its hypoglycemic effect. But no scientific evidence on the hypoglycemic effect of GI has ever been reported in human. In this study, the effect of GI consumption on peak plasma glucose concentrations in healthy subjects was investigated. Either oral glucose load (75 g) or standard meal was given to the subjects with respect to the presence or absence of GI consumption and postprandial peak glucose levels were compared. When GI was consumed, 15 min after oral glucose load, the glucose concentration with GI was significantly lower (130 ± 32 vs. 145 ± 27 mg/dl, $p < 0.05$; N = 73). Doubling dose of GI showed much greater decrease in peak blood glucose concentration than that of the single dose (108 ± 15 vs. 130 ± 32 mg/dl, $p < 0.05$). When standard meal was used instead of oral glucose load, similar hypoglycemic effect was observed in GI group; 16 out of 20 subjects had a lowered peak glucose concentration (129 ± 27 vs. 147 ± 39 mg/dl, $p < 0.05$). In order to evaluate the impact of long term GI consumption on plasma glucose concentration and liver function, fasting plasma glucose and liver function test (AST, ALT, GGT and ALP) were monitored at days 0, 2, 4, 7, 14, 21 and 28. The results showed no change in both fasting plasma glucose and liver enzymes. To envisage the mechanism of this hypoglycemic effect, GI leaves were extracted with various solvents and tested for insulinotropic property in INS-1 cells as well as the determination of its inhibition on α-glucosidase activity. Neither increase in insulin level nor inhibition of α-glucosidase enzyme was observed, suggesting that the hypoglycemic effect of GI is involved with other mechanisms than the activation of beta cell or enzymatic inhibition of carbohydrate absorption.**

Key words: *Gymnema inodorum*, hypoglycemic effect, plasma glucose, human, liver function.

INTRODUCTION

The projection of diabetes mellitus worldwide is increasing in an alarming trend. Diabetes itself along with its complications causes psychosocial implications, as well as the financial burden associated with the management of the disease. Existing treatment options are costly and have limited palliative effects. In addition, many current therapies to control glycemia have harmful side effects, such as hypoglycemia, liver and kidney damage. One treatment that is emerging as a potential strategy for the management of diabetes is herbal medicine (Yeh et al., 2003).

Gymnema sylvestre (GS), the Asclepiad plant which grows in tropical forests of South and South-eastern Asia, is the well-known herb that has been used for diabetic treatment for more than 2,000 years in Ayuravedic medicine. The medicinally active parts of the plant are the leaves and the roots. Recent clinical trials conducted in

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Abbreviations: ALP, Alkaline phosphatase; **ALT,** alanine transaminase; **AST,** aspartate transaminase; **GI,** *Gymnema inodorum*; **GGT,** gamma glutamyltransferase; **GS,** *Gymnema sylvestre*; **OGTT,** oral glucose tolerance test.

India have shown that an extract of *G. sylvestre* is useful for controlling blood sugar (Shanmugasundaram and Panneerselvam, 1981).

The hypoglycemic action of GS leaves was first documented in the late 1920s. This action is attributed to members of a family of substances called gymnemic acids. GS leaves raise insulin levels, according to research in healthy volunteers. Based on animal studies, this may be due to regeneration of the cells in the pancreas that secrete insulin, or by increasing the flow of insulin from these cells. Other study using animal models show that GS can also reduce glucose absorption from the intestine, improve uptake of glucose into cells and prevent adrenal hormones from stimulating the liver to produce glucose, thereby reducing blood sugar levels (Shanmugasundaram et al., 1983, 1990; Baskaran et al., 1990).

A number of studies showed that GS extract can inhibit glucose absorption in both animal and human intestines by suppressing potassium-induced contraction of ileal longitudinal muscle. Oral administration of GS extract reduced postprandial serum glucose and improved glucose tolerance in mildly diabetic rats. The water-extracted fraction of GS leaves can return fasting blood glucose levels to normal after a long period of oral administration by revitalization of pancreatic beta cells (Murakami et al., 1996; Yoshikawa et al., 1997; Persaud et al., 1999; Sugihara et al., 2000). However, GS suppresses sweet taste on taste buds and has bitterness, therefore it may irritate the flavor of food or drink (Gent et al., 1999).

Gymnema inodorum (GI), also a member of Asclepiad strain, is found ubiquitously in South-eastern Asia including Thailand. Since GI does not have gustatory modifying action, its leaves and stems have been used as vegetables for Thai cuisine, especially in the Northern and Eastern parts of the country. GI has been shown to have an ability to inhibit glucose absorption in guinea pig intestines (Shimizu et al., 1997, 2001). Klungsupya et al. (2008) found that GI leaves, extracted with both water and ethanol, showed high antioxidant activity with polyphenols as the major antioxidant. They also found that water extraction of young GI leaves can decrease blood glucose in alloxan-induced diabetic rats. However, the study with regard to hypoglycemic effect of GI has never been done in human. For this reason, we studied the hypoglycemic effect of GI in healthy human. In addition, such possible underlying mechanisms of hypoglycemic effect of *G. inodorum* as the insulinotropic effect and the inhibition of the carbohydrate digestive enzyme of the intestine (α-glucosidase) were investigated

MATERIALS AND METHODS

Subjects

Healthy subjects of both sexes aged 18-25 years old without history of diabetes or other evident diseases voluntarily participated in this study. Absence of diabetes was confirmed by evaluating normal fasting plasma glucose concentration (70 - 100 mg/dl) and seventy-three subjects were included. This study was approved by the Ethic Committee on Human Studies, Faculty of Medicine, Chulalongkorn University and written informed consents were obtained from all participants.

Gymnema inodorum

Fresh *G. inodorum* (Lour.) Decne was obtained from Chiangmai, the Northern Province of Thailand. The sample of GI was sent to Forest Herbarium for identification and the voucher specimen is BKF 154237. The fresh leaves were used for further extraction.

GI tea bag was prepared according to the method of Jinda Rungruang manufacturer. Briefly, GI leaves collected from a single garden were oven-dried and crushed in powder and 1.5 g was packed in one tea bag. This tea prepared was subsequently utilized for oral glucose load or standard meal study. Before use, one or two bags of GI tea was immersed in 150 ml of boiling water and left for 5 min.

Study of hypoglycemic effect of GI tea in human

The study was designed as a before/after experiment and oral glucose tolerance test (OGTT) was used as a tool. The “before” or control group was the group of subjects performed OGTT without GI tea consumption (n = 73) and the “after” or treatment group was the same group of subjects that was studied for OGTT with the addition of GI tea consumption. Plasma glucose level was measured at 0, 15 and 30 min after the oral glucose load (75 g) to determine the appropriate time for GI tea consumption. Usually, GI tea prepared from one bag was used. The subjects were divided into 4 groups. Groups I, II and III subjects drank one bag of GI tea immediately, at 15 and 30 min after oral glucose load, respectively, while group IV, subjects drank two bags of GI tea at 15 min after the oral glucose load. The patterns of OGTT curves were analyzed and the peaks of glucose concentration were compared and statistically analyzed using Student’s *t*-test.

To investigate whether the GI tea has hypoglycemic effect when consumed with meal, standard meal prepared from 66 g of baguette French bread (glycemic index = 95) and 15 g of strawberry jam (1 pack of Smuckers’ strawberry jam, glycemic index = 49), total glycemic index of 88 (glycemic load = 72) and total calories of 225 kcal was used instead of glucose load. Twenty subjects were assigned to eat standard meal and drink one cup of GI tea (one bag) at 15 min after the first bite of the meal. Blood sample was drawn before taking the meal (0 min) and at 30, 60 and 120 min after the first bite of the meal. Baseline or control group was the same group of subjects that ate standard meal without GI tea. The time interval between baseline and treatment condition (wash out period) was 7 days.

To determine the peak plasma glucose concentration, plasma glucose was measured using glucose oxidase method (Human Gesellschaft fur Biochemica and Diagnostica mbH, Wiesbaden, Germany).

The effect of long term consumption of GI tea on fasting plasma glucose and liver function

Twenty healthy subjects with normal plasma glucose and liver function enzymes (aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and gamma glutamyltransferase (GGT)) were included in this study. During the period of 28 days, the subjects were assigned to have daily consumption of 1 pack of GI tea (1.5 g in 150 ml hot water) at 15

min after their regular lunch, then liver enzymes and fasting plasma glucose levels were monitored from blood obtained at days 0, 2, 4, 7, 14, 21 and 28.

Preparation of GI extract

GI leaves were extracted into four fractions according to the method of Shimizu et al. (2001). Briefly, the fresh GI leaves were dried at 60°C and crushed into 0.5 - 2 mm pieces; the dried GI powder was pretreated/ decolored with citric acid solution, pH 2.5. The acid-treated leaves were extracted with an aqueous 50% ethanol solution to obtain GI-I and concentrated in a rotary evaporator at 80°C. After evaporation, partition extractions were performed using n-butanol and water in a ratio of 1:1 to obtain n-butanol fraction (GI-II) and residue fraction was concentrated from aqueous phase. The n-butanol fraction was defatted and decolorized with petroleum ether. The defatted mixture was filtered and defatted again with ethyl acetate to obtain GI-III and purified with methanol to obtain GI-IV.

Culture of INS-1 cells

The INS-1 rat insulinoma cell line (kindly provided by Dr. S. Adisakwatana, Faculty of Allied health sciences, Chulalongkorn University, Thailand) was cultured under 5% CO_2/ 95% air at 37°C in the RPMI-1640 medium (Invitrogen, Carlsbad, CA) containing 11.2 mM glucose and 2 mmol/l L-glutamine. The medium was supplemented with 10% fetal bovine serum (FBS), 1 mmol/l pyruvate, 10 mmol/l HEPES buffer, pH 7.4, 50 µmol/l 2-mercaptoethanol, 100 units/ml penicillin and 100 µg/ml streptomycin. All experiments were performed using INS-1 cells between the 20^{th} and 30^{th} passages. The cells were passed 2 - 4 days before each experiment and plated in 24-multiwell plates at a density of 5 x 10^5 cells per well in the presence of glucose (100 mg/dl).

MTT assay

INS-1 cells (3 x 10^5 cells/150 µl/well) were cultured on 96-well microplates for 24 h. Cells were incubated with various concentration of GI extract (0, 5,10 and 15 mg in 1 ml of 0.2% DMSO) for another 24 h. The cultures were incubated with MTT (C,N-diphenyl-N'-4,5-dimethyl thiazol 2yl tetrazolium bromide) (Sigma, St Louis, MO), dissolved in Krebs-Ringer bicarbonate (KRB) buffer, pH 7.4 (2.5 mM $CaCl_2$, 15 mM $NaHCO_3$, 10 mM HEPES and 0.1% BSA) for 4 h. The assay was performed as described by Mosmann, 1983. The concentration of 5 mg/ml of GI extract (Fraction I-IV) gave the highest percentage of cell viability (data not shown).

Measurement of insulin secretion

One day before an insulin release experiment, the glucose concentration in the medium was reduced to 5 mM. Cultured medium was replaced using the medium with KRB buffer, pH 7.4, containing 3 mM glucose. After washing cells twice with Krebs-Ringer-HEPES-BSA solution, an insulin secretion assay was performed in 1 ml of the same solution with or without GI extract. 5 mg of each GI extract; GI I-IV fractions were dissolved with 1 ml of 0.2% DMSO prior to incubation with INS-1 cells for 1 h at 37°C, then insulin secretion was measured. Plates with DMSO were used as negative controls. After 1 h at 37°C, the solution was collected and centrifuged at 25°C, 2,000 rpm for 10 min. The supernatant fraction was removed and saved for insulin measurement. Insulin level in culture medium was measured using an immunoradiometric assay. The insulina IRMA kit was purchased from RADIM S.p.A., Italy. The experiment was done in duplicate and the results were calculated for mean and standard deviation.

Measurement of alpha-glucosidase inhibitory activity

Dried GI leaves were extracted with boiling deionized water and 70% methanol and the extracts were lyophilized before use for the determination of inhibitory effect on digestive enzyme, α-glucosidase. Acarbose was used as a positive control. The α-glucosidase inhibitory assay was done by the chromogenic method (Sigma, St Louis, MO). In brief, GI extract powders were dissolved in 1 M PBS and incubated for 5 min with yeast α-glucosidase enzyme, then substrate (p-nitrophenyl-α-D-glucopyranoside) was added and after 20 min of incubation at 37°C, sodium carbonate was added. The reaction tubes were recorded at 405 nm spectrophotometrically. The increase in absorbance from pre-substrate addition to post substrate reaction was obtained. Percent inhibition was calculated by (1-Absorbance test/Absorbance control) × 100 and inhibitory concentration 50% (IC50) was calculated by applying suitable regression analysis.

Statistical analysis

Results were expressed as mean ± SD throughout the study. The effects of GI extract on peak glucose concentration and insulin level were analyzed by one-way and two-way analyses of variance (ANOVA) and paired Student's t-test. All other clinical and parameters were analyzed by one-way ANOVA, followed by paired Student's t-test as appropriate. Significance was accepted when $p < 0.05$.

RESULTS

Hypoglycemic effect of *G. inodorum* tea in human (OGTT study)

Data in Table 1 showed that drinking GI tea immediately and at 15 min after oral glucose load (group I and II) could significantly reduce plasma glucose ($p = 0.035$ and 0.004 respectively). Double concentration of GI tea (group IV) could reduce plasma glucose better than one pack of GI tea drinking ($p < 0.001$). Drinking GI tea at 30 min after glucose load (group III) did not reduce plasma glucose significantly ($p = 0.662$). The average values of OGTT for all groups were shown in Figure 1.

Hypoglycemic effect of GI tea on standard meal

The result showed that 16 out of 20 of the subjects (80%) had decreased peak plasma glucose concentration. The mean peak glucose concentration in the GI group (treatment group) was significantly lower than that of the control group (147 ± 39 vs. 129 ± 27 mg/dl, $p = 0.016$) as shown in Table 2. Thus, *G. inodorum* tea has hypoglycemic effect regardless of glucose source consumed.

Effect of GI tea consumption on liver function test

Liver enzymes and fasting glucose in blood of subjects

Table 1. Hypoglycemic effect of GI tea on peak plasma glucose concentration in healthy human.

Group	n	Average glucose peak (mg/dl)
Control [a]	73	145 ± 27.17
Group I [b]	40	130 ± 31.50 *
Group II [c]	73	131 ± 27.25 **
Group III [d]	20	143 ± 41.18
Group IV [e]	19	108 ± 14.49 **

Data were expressed as mean ± SD.
[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; [c] the subjects drank 1.5 g of GI tea at 15 min after oral glucose load; [d] the subjects drank 1.5 g of GI tea at 30 min after oral glucose load; [e] the subjects drank 3.0 g of GI tea at 15 min after oral glucose load; * statistical significance ($p \leq 0.05$); ** statistical significance ($p \leq 0.01$).

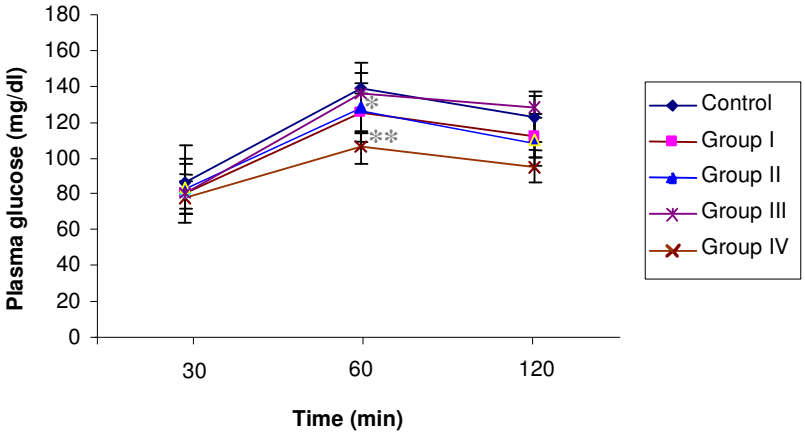

Figure 1. Effect of oral administration of GI tea (1.5 g) on plasma glucose levels. Values are means ± SD. *p < 0.05 and **p < 0.01 vs. control.

Table 2. Hypoglycemic effect of GI tea on standard meal (n=20).

Group	Peak glucose concentration (mg/dl)
Control	147 ± 39
Treatment	129 ± 27*

Data were expressed as mean ± SD; * statistical significance ($p \leq 0.05$).

who consumed one pack of GI tea daily for 28 days were monitored at days 0, 2, 4, 7, 14, 21 and 28. As shown in Table 3 and Figure 2, it was found that there was no significant difference with regards to the liver enzymes of the control group (no GI tea consumption) and the treatment group (with GI tea consumption). The fasting plasma glucose of all subjects remained within normal limit throughout the period of the study (Figure 3).

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

The result showed that none of GI fractions could stimulate insulin secretion from INS-1 cells (Figure 4).

Study on alpha-glucosidase inhibitory activity of GI extract

GI extract with both boiling water and 70% methanol did not inhibit α-glucosidase enzyme as shown in Figure 5.

DISCUSSION

The present study suggested that suitable time for GI tea to exert its hypoglycemic effect is to drink the tea immediately or at 15 min after oral glucose load or meal (groups I and II), but not at 30 min (group III). One pack of GI tea can significantly reduce peak plasma glucose

Table 3. Liver function test (AST, ALT, ALP, GGT) in healthy subjects (n = 20) after 28 days of GI tea consumption.

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

Each value is 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 for 28 days.

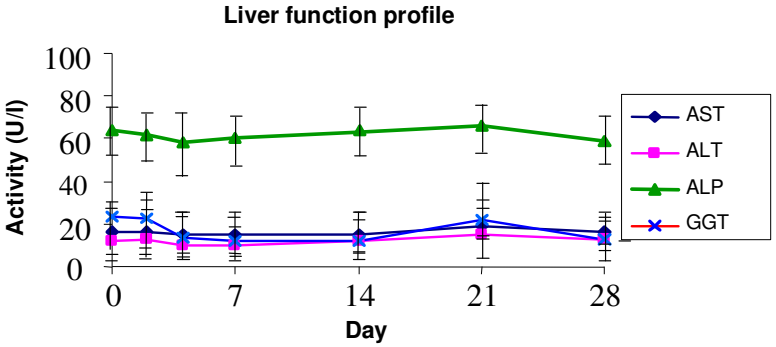

Figure 2. Average liver function profile (AST, ALT, ALP, GGT) in healthy subjects within 28 day period of GI tea consumption.

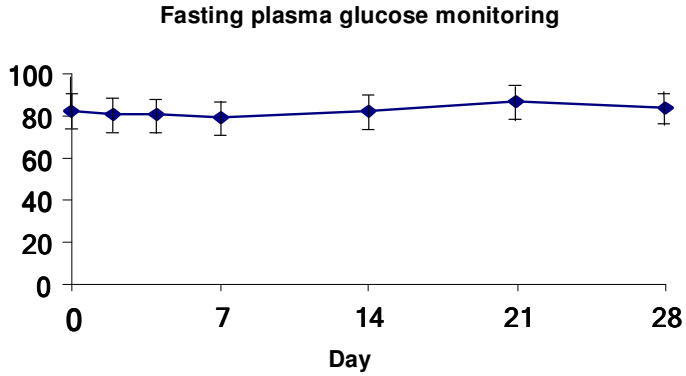

Figure 3. Fasting plasma glucose in healthy subjects within 28 days period of GI tea consumption.

10 and 11%, respectively, and more reduction (25%) was obtained with double dosage of GI (group IV). This dose-responsive finding confirms the hypoglycemic activity of GI. Natalucci et al. (2003) studied a pattern of glucose absorption from OGTT and found that the average rate of glucose appearance into plasma was highest at time 30 - 45 min after glucose load. This may explain why drinking GI tea at 30 min after glucose load cannot reduce peak plasma glucose. At 30 min, glucose was readily absorbed and transported to blood circulation thus GI cannot display its effect.

α-Glucosidase (EC 3.2.1.20) is an enzyme that hydrolyzes α-glucosides to glucose. It is usually found at the brush border of the small intestinal epithelium, where it can hydrolyze oligosaccharides, trisaccharides and disaccharides such as maltose to glucose and other saccharides for absorption. α-Glucosidase inhibitor can act as a competitive inhibitor of α-glucosidase; hence, it

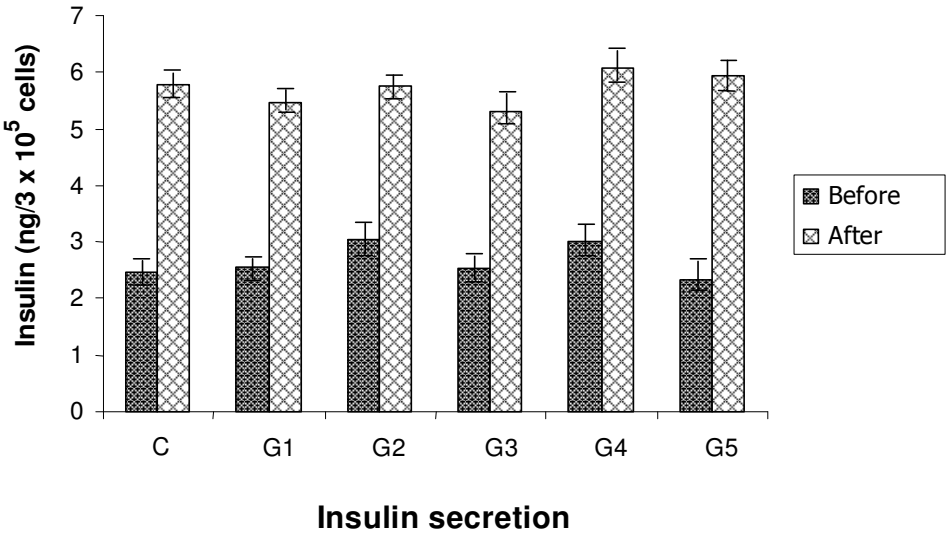

Figure 4. Effects of GI extract on insulin secretion in INS-1 cells. The insulin in each well was measured by Insulina IRMA kit (^{125}I) as before and after glucose addition. The figures are the average of the duplicate study. C = control group; measure insulin release from INS-1 cell; Group 1= INS-1 cell treated with GI extract from boiling water; Group 2 = INS-1 cell treated with GI I fraction (ethanol extract); Group 3 = INS-1 cell treated with GI II fraction (n-butanol extract); Group 4 = INS-1 cell treated with GI III fraction (ethyl acetate extract); Group 5 = INS-1 cell treated with GI IV fraction (methanol extract).

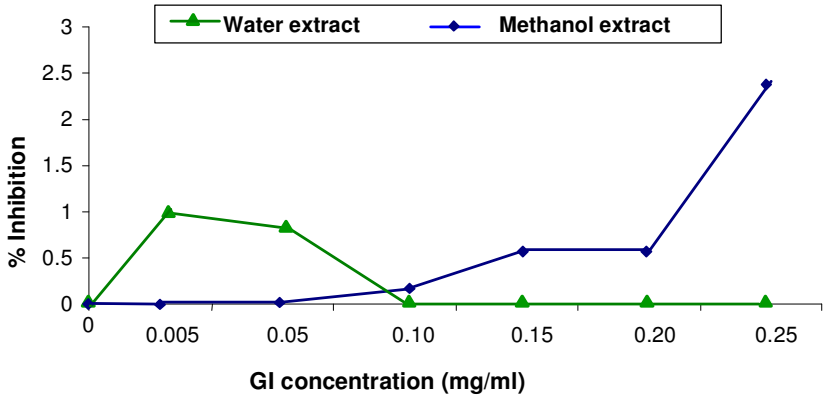

Figure 5. Effect of GI extracted with water and 70% methanol on inhibition of alpha -glucosidase activity.

reduces the impact of carbohydrates on blood sugar (Kakavanos et al., 2006).

Inhibition of α-glucosidase activity is one possible mechanism for the hypoglycemic effect of certain medicinal herbs used for diabetes therapy such as *Rosa damascene* (rose), *Rosmarinus offinalis* (rosemary), *Pistacia vera* (pistachio), *Entada rheedii* (African dream herb) and *Albizia lebbeck* (lebbeck-tree) (Gholamhoseinian et al., 2008; Tunsaringkarn et al., 2008). In this study, the α-glucosidase inhibitory property of GI leave extracts was tested both with water and methanol, but no inhibitory effect on α-glucosidase enzyme was found. The present finding is in accordance with the study of Sugihara et al. (2000) who also found that gymnemic acid IV, a compound derived from *G. sylvestre* leaves (1 mg/ml) did not inhibit α-glucosidase activity in the brush border membrane vesicles of normal rat small intestines.

Shimizu et al. (1997, 2001) showed that the crude saponin mixtures extracted from GI leaves can inhibit glucose absorption in the isolated intestinal tract and suppressed the increased blood glucose in rats. They found that triterpenoid saponin in GI extracts can suppress the high K^+-induced contraction of intestinal smooth muscle which affected Na^+/K^+ pump. When the pump was suppressed, the electrochemical potential of Na^+ inside the cell changed and this affected the Na^+-dependent co-

transport system. Thus, this experimental evidence is suggested to be the possible mechanism of the inhibitory effect of GI on glucose absorption from the intestinal tract.

The insulinotropic action of the extract of GI was further investigated using INS-1 rat insulinoma cell line and immunoradioassay. It was found that GI extracts failed to increase insulin secretion from the INS-1 cells after incubating with GI extract for 1 h. The finding that GI extract could not stimulate insulin secretion in INS-1 cells despite its hypoglycemic effect in the oral glucose tolerance test may explain that GI lowers blood glucose concentration via incretins-insulin stimulation which can occur only *in vivo*. Consumption of GI after meal may stimulate more incretins secretion from small intestine and the incretins further stimulate pancreatic β-cells to secrete insulin resulting in the decrease of blood glucose concentration (Baggio and Drucker, 2007). Since incretins have very short half-life in blood and is not possible to measure, the measurement of plasma insulin after GI consumption with meal will elucidate this hypothesis. As well, further study is needed to explore this activity on long-term use in human.

In this study, commercial GI was used and the product has been approved by Thai Food and Drug Administration (FDA) for consumption as a regular beverage. To confirm the safety of GI tea, a group of 20 healthy subjects was studied and it was found that GI tea has no toxicity to liver upon a once daily consumption for 28 days. Furthermore, GI tea did not reduce normal fasting plasma glucose level in healthy human after this period. This may implied that daily consumption of one pack of GI tea will not induce hypoglycemia in healthy human.

Conclusion

G. inodorum has hypoglycemic effect in healthy human. Consumption of GI tea with meal or 15 min after meal can significantly decrease peak plasma glucose. Double dose of GI reduce peak plasma glucose in a dose-responsive manner. Long term consumption of GI for 28 days does not cause fasting hypoglycemia or hepatotoxicity. The mechanism of this hypoglycemic effect does not relate to increase insulin secretion or inhibition of α-glucosidase enzyme.

ACKNOWLEDGEMENTS

This work was financially supported from the Chulalongkorn University Centenary Academic Development Project and the 90th Anniversary of Chulalongkorn University (Ratchadaphieksomphot Endowment) funds. We gratefully acknowledge the generous gift of the INS-1 cell line from Dr. S. Adisakwatana.

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