

Antidiabetic Synergetic Effects of Plant Extract-Mixtures in Streptozotocin-Diabetes Rats

Sang Geun Roh, Jong Hae Kim¹ and Won Chul Choi*

Department of Biology, College of Natural Sciences, Pusan National University, Busan, 609-735, Korea

¹Division of Electronic Engineering, Sun Moon University, A-San, 336-708, Korea.

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This study investigates the effects of *Psidium guajava* L. leaf (Pg), *Lagerstroemia speciosa* L. leaf (Ls) and mixture A (Pg, Ls, *Morus indica* L. leaf extract, *Pinus densiflora* needles extract, *Acanthopanax senticosus* M. root extract) on streptozotocin (STZ)-diabetes rats. For four weeks, STZ-diabetes rats were fed crystallized extracts of Pg, Ls, and mixture A. Compared to the diabetic control group, extracts of Pg, Ls, and mixture A decreased glucose levels in rats by 20%, 14% and 24% respectively. These extracts also decreased the level of total cholesterol, triglyceride and free fatty acid, compared to the diabetic control group, while effectively increasing levels of insulin and high-density lipoprotein-cholesterol. These results showed that mixture A had greater antihyperglycemic, antihyperlipidemic, and insulin-increasing effects than the Pg and Ls extracts. Mixture A also showed better restoration of damaged beta cell function compared to Pg and Ls extracts. Therefore, it was proved that mixture A provides a beneficial synergistic effect when compared with Pg and Ls extracts used individually.

Key words : STZ-diabetes rats, antihyperglycemic effect, antihyperlipidemic effect, synergistic effect

Introduction

Diabetes mellitus (DM) is caused by abnormal changes including those to carbohydrate, lipid and lipoprotein metabolism and it causes various complications such as hyperglycemia, hyperlipidemia, hypoinsulinemia, hypertension and atherosclerosis [6]. Unlike type 2 DM, type 1 DM shows hypoinsulinemia as beta cells of Langerhans' islets in the pancreas are destroyed by the interaction of genetic, environmental and immune factors, thus causing lack of insulin secretion [31]. However, interest in traditional medical plants has increased due to the side effects of therapeutic agents such as oral hypoglycemic agent and insulin used in the treatment of diabetes [16]. More than 400 plants are reported as having blood glucose-lowering potential [5,10]. These medicinal plants contain polyphenol in their seeds, fruits, leaves, and barks [32]. The main role of polyphenol is to protect plants against external invaders [9], but it also plays an important role in human health [39]. *Lagerstroemia speciosa* L. and *Psidium guajava* L. leaves are representative traditional antidiabetic medical plants. The glucose-lowering effect of *L. speciosa* leaves was reported initially in early 1940

[12], and its antidiabetic effect has since been confirmed [17,35]. *P. guajava* leaves were also reported to have effective antihyperglycemic activity [1,2]. This study, using *in vitro* pancreas β cells, proved the insulin secretion activity of *L. speciosa* and *P. guajava* leaves. Furthermore, the insulin secretion effect of *Morus indica* L. leaves, *Pinus densiflora* needle, and *Acanthopanax senticosus* M. roots extracts, which grow wild in Korea and have been used as traditional herbs, was confirmed. The extracts of these plants are known for antidiabetic actions by way of pharmacological mechanisms including increase in insulin sensitivity [15,20,27], insulin-like activity [3,11], insulin secretion [34,38], and antioxidant activity [7,8,29]. They also restrain the activity of intestinal glycosidase [18,19,27]. The objective of the author's study was to compare the antidiabetic effects of individual *L. speciosa* and *P. guajava* leaf extracts on streptozotocin (STZ)-diabetes rats, with effects due to the combined antidiabetic mechanisms provided by mixture A. It was thought that synergism might make the mixtures superior to extracts from single plant sources.

Materials and Methods

Preparation of the plant extracts

Psidium guajava L. leaves (Pg), *Morus indica* L. leaves (Mi),

*Corresponding author

Tel : +82-51-510-2262, Fax : +82-51-510-3736

E-mail : wcchoi@pusan.ac.kr

Pinus densiflora needles (Pd), and *Acanthopanax senticosus* M. roots (As) were purchased from the Daegu Yangyeongsi herbmarket (Daegu, Korea). 15 kg of well dried herb, were added to 150 L of distilled water for each of the four (Pg, Mi, Pd, and As). Each herb was boiled for 6 hours at 98°C to get extracts. Each of the water soluble plant extracts was filtered (50 µm), concentrated under vacuum, then freeze-dried to form a powder (0.8-1% in yield). *Lagerstroemia speciosa* L. leaf (Ls) extract, which contained 1% corosolic acid powder extracted using ethanol, was purchased from the Use Techno Corporation Co., Ltd (Kyoto, Japan). Mixture A contained the same amounts of freeze-dried Pg, Ls, Mi, Pd, and As powders.

Reagents and equipment

To cause diabetes, streptozotocin (Sigma-Aldrich, USA) was used. The levels of blood glucose, HDL-cholesterol (HDL-C), total cholesterol (TC), triglyceride (TG), and free fatty acid (FFA) were measured using an automatic analyzer (ADVIA1650, Bayer, Deerfield, IL, USA). Blood insulin was measured using the Sensitive Rat RIA kit (Linco Research, USA) and automatic analyzer (Gammacounter COBRA-II, Packard, Ramsey, MN, USA). The Periodic Acid-Schiff (PAS) stain for the liver was measured with an SS002 Kit (BioGenex, USA). The mouse anti-insulin and rabbit anti-glucagon antibody (BioGenex, USA) was used as a primary antibody and a Sensitive™ polymer-HRP detection kit (BioGenex, USA) as a secondary antibody for the immunohistochemical stain of the pancreas. The entire histological examination process was done using light microscopy (AxioScope, CarlZeiss, Göttingen, Germany).

Cell culture

Hamster pancreas beta cells, HIT-T15 were purchased from the Korean Cell Line Bank (Seoul, Korea) and maintained as monolayer in RPMI 1640 (Gibco BRL Life Technology, USA) supplemented with 10% fetal bovine serum (Gibco BRL Life Technology, USA) and 1% penicillin/streptomycin (Gibco BRL Life Technology, USA) at 37°C under a humidified atmosphere containing 5% CO₂.

Animal groups and treatment

Five-week-old male Wistar Hannover rats (n=100) were purchased from the Samtako Experiment Animal Center (Korea) and acclimatized for 1 week before starting the experiments. Animals were rendered diabetic by a single I.P.

injection of streptozotocin (STZ) (65 mg/kg) prepared in 250 µl of freshly prepared 0.01 M citrate buffer (pH 4.5) after an overnight fast and normal control rats were injected with 250 µl 0.01 M citrate buffer (pH 4.5). Three days after STZ administration, their tails were nipped and blood collected to measure glucose concentration, and the rats with glucose concentration higher than 300 mg/dl were considered to have been rendered diabetic. The blood glucose concentrations were measured using Accu-Chek Active (Roche Diagnostics, Germany) and rats that exceeded 600 mg/dl, which was the maximum measure level were excluded. The rats were randomly divided into five groups with 20 animals in each group. The NC group served as normal controls and received vehicle (normal saline) only. The DC group served as diabetic controls and also received vehicle only. The PG and LS groups received the single plant extracts; Pg extract and Ls extract respectively. The MA group received mixture A. The vehicle or plant extract (100 mg/kg, w/w) was orally administered daily over the experimental period of 4 weeks. They controlled temperature of 20±2°C, humidity of 55±5%, and 12 hr light and 12 hr dark cycle for 4 week and had free access to standard pellets (Samtako Bio, Korea) and water.

Measurement of insulin level in cells

HIT T15 cells were divided into 30 wells with 5×10³ cells each, and cultured for 24 hr in a 5% CO₂ incubator in the RPMI 1640 culture solution that was deprived of glucose. After the concentration was adjusted to 50 µg/ml by adding DW to each of the plant extract powders, each extract was divided into five wells and cultured for another 24 hr. Five wells of that plant extract were used as control. After culturing for 24 hr, the insulin concentration of each well was measured using the Rats Insulin ELISA Kit (Shibayagi, Japan) and the results were read using an automatic analyzer (TECAN, Salzburg, Austria).

Measurement of blood glucose, insulin, lipoprotein and lipids in serum

Five rats from each group were selected each week of the 4 weeks, and blood from the heart was collected using a syringe by making an incision in the chest after using ethyl ether as anesthesia. The collected blood was centrifuged for 10 minutes at 4,000 rpm and 4°C, and the serum was stored at -70°C. After completion of the experiment, blood glucose, insulin, HDL-cholesterol (HDL-C), total cholesterol (TC), tri-

glyceride (TG), and free fatty acid (FFA) levels were measured by using an automatic blood chemical analyzer and the HDL-cholesterol/total cholesterol ratio (HTR) calculated.

Histological examination

After the 4th week of the experiment, the liver and pancreas of each rat were dissected and fixed in 10% neutral formalin for 1 day. Then, these were processed to produce 4 μ m thick paraffin sections (m.p. 58°C). The PAS reaction was employed for liver, and the immunohistochemical avidin-biotin complex (ABC) method was used for insulin or glucagon-detects in pancreas tissue. All the histological examination results reported were the means of measures from 10 different fields on each slide. Histological damage was scored as follows, based on the ABC stain using insulin antibodies: 0 - normal; I - minor injury; II - moderate injury; III - obvious injury; IV - severe injury. Each sample was observed at 400x magnification [37]. While the insulin or glucagon occupied proportion in Langerhans' islet cells was magnified 200x, the picture of each slide was analyzed using the Cell Image Scanner program. The Cell Image Scanner program for determining the insulin or glucagon occupied proportion was developed by Professor Jong Hae Kim from the Division of Electronic Engineering, Sun Moon University. The proposed Cell Image Scanner program on the basis of HIS (Hue, Intensity, and Saturation) color coordinates has been developed by Visual Basic Net 2005 in order to allow detailed analyses of cell images. For the various analyses of cell images, the user can adjust the ranges of hue, intensity, and saturation.

Statistical analysis

All values were expressed as means \pm S.D. The results were compared using the Kruskal-Wallis test of SPSSWIN, ver. 12.0. The level of statistical significance in the study was either $p < 0.05$ or $p < 0.01$.

Results

Effect of plant extracts on insulin secretion of cells

The average levels of insulin in cells are shown in Fig. 1. The insulin levels produced by the As and Pd extracts were higher than the control by 43.4% and 40.5% respectively ($p < 0.01$). While Pg, Ls, and Mi extract each produced higher insulin levels than the control ($p < 0.01$), the insulin levels resulting from the use of Pg, Ls, and Mi extracts

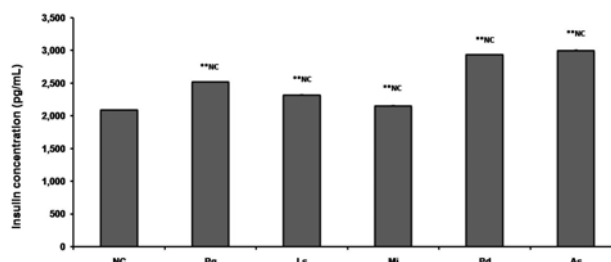


Fig. 1. Effect of plant extracts on insulin secretion (n=5; mean \pm S.D.). Probability less than 0.05 indicated by '**', and less than 0.01 indicated by '***'. Letter superscripts refer to comparison groups. NC: normal control, Pg: *Psidium guajava* L. leaves, Ls: *Lagerstroemia speciosa* L. leaves, Mi: *Morus indica* L. leaves, Pd: *Pinus densiflora* needles, As: *Acanthopanax senticosus* M. roots.

were higher than the control by 20.5%, 10.8%, and 2.9% respectively. The Pg extract produced higher insulin levels compared to Ls extract ($p < 0.01$).

Effect of plant extracts on body weight

After 4 weeks, the body weight gain of the diabetic control did not differ from when the experiment started, and showed body weight loss characteristics of type 1 diabetes (Table 1). Compared to that of the diabetic control, body weight gain for the experimental groups increased (PG 3.5 times and MA 4 times). However, though the body weight gain of LS group was higher than the diabetic control ($p < 0.01$), it did not differ from before intake.

Effect of plant extracts on blood glucose

In Table 2, the glucose level of the diabetic control increased as the experiments went on, and showed hyperglycemia of over 600 mg/dl in week 3. After 4 weeks, the glucose levels

Table 1. Effect of plant extracts on body weight gain in diabetic rats (n=5)

Group	Body Weight (mean \pm S.D.) (g)		
	Initial	Final	Gain
NC	207.5 \pm 3.0	311.0 \pm 3.3 ^{*initial}	103.5 \pm 0.4
DC	187.4 \pm 18.1	192.5 \pm 17.8	5.1 \pm 0.8
MA	198.4 \pm 17.7	221.0 \pm 17.1 ^{*initial}	22.6 \pm 2.0 ^{**DC}
PG	189.3 \pm 15.2	206.9 \pm 15.2 ^{*initial}	17.6 \pm 0.7 ^{**DC}
LS	189.8 \pm 22.6	201.0 \pm 21.7	11.3 \pm 2.9 ^{**DC}

Probability less than 0.05 indicated by '**', and less than 0.01 indicated by '***'. Letter superscripts refer to comparison groups. NC: Normal control, DC: Diabetic control, MA: Diabetic mice fed with mixture A, PG: Diabetic mice fed with Pg, LS: Diabetic mice fed with Ls extract.

Table 2. Effect of plant extracts on blood glucose level in diabetic rats (n=5)

Group	Blood glucose (mean±S.D.) (mg/dl)			
	1 Week	2 Week	3 Week	4 Week
NC	184.3±20.8	196.0±9.5	196.9±10.6	180.5±22.0
DC	575.8±13.5	590.4±13.8	635.6±21.4	634.0±29.0
MA	476.6±26.6 ^{**DC}	508.6±15.6 ^{**DC}	538.3±20.1 ^{**DC}	479.3±16.0 ^{**DC}
PG	463.2±18.0 ^{**DC}	526.1±25.4 ^{**DC}	546.3±25.1 ^{**DC}	502.3±13.4 ^{**DC}
LS	459.3±23.5 ^{**DC}	555.5±22.3 ^{**DC}	573.5±29.8 ^{**DC}	541.7±13.5 ^{**DC}

NC - LS and superscripts as in Table 1.

of the PG, LS, and MA groups decreased compared to that of the diabetic control (p<0.01). The glucose level of the MA group was lower than that of other groups (p<0.01).

Effect of plant extracts on serum insulin

In Table 3, the insulin level of the diabetic control decreased to 9.5% of the normal control since week 2 and showed hypoinsulinemia. After 4 weeks, compared to that of the diabetic control, the insulin levels of PG, LS, and MA groups increased (PG 2.7 times, LS 2.7 times, and MA 9.3 times). The insulin level of the MA group was higher than that of the other groups (p<0.01). After 4 weeks, unlike the PG and LS groups, the insulin level of the MA group increased compared to that of the week 1 (p<0.01).

Effect of plant extracts on serum TC, HDL-C and HTR

The level of serum TC, HDL-C, and HTR in each group is shown in Fig. 2. The TC level of the diabetic control increased constantly until week 4, and showed hypercholesterolemia over 2 times greater than that of the normal control (p<0.05). After 4 weeks, compared to that of diabetic control, the TC levels of PG, LS, and MA groups decreased (PG 50%, LS 36%, and MA 50%). The HDL-C level of the normal and diabetic controls increased during the 4 weeks, but there was no significant difference between normal and diabetic controls. The HDL-C level of PG and MA groups increased compared to that of the diabetic control in week 3 (p<0.01), but there was no significant difference in the 4th week. After 4 weeks, the HTR of the diabetic control decreased by 50% compared to the normal control (p<0.01), showing no complementary increase of HDL-C correspondent with the TC level. The HTR of PG, LS, and MA groups increased compared to that of the diabetic control (p<0.01), and the HTR of PG and MA groups showed no

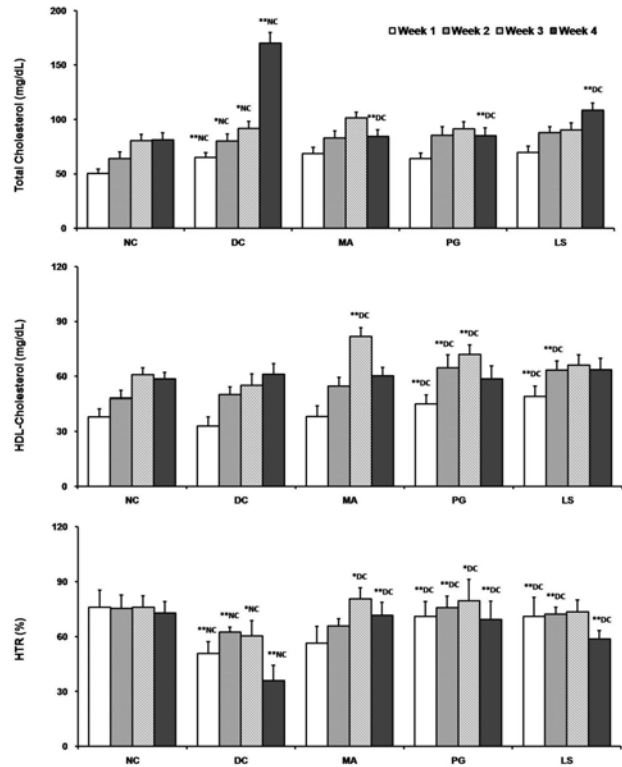


Fig. 2. Effect of plant extracts on serum total cholesterol, HDL-cholesterol and HTR level in diabetic rats (n=5; mean±S.D.). HTR: HDL-cholesterol/total cholesterol ratio, NC: Normal control, DC: Diabetic control, MA: Diabetic mice fed with mixture A, PG: Diabetic mice fed with Pg, LS: Diabetic mice fed with Ls extract. Superscripts as in Fig. 1.

significant difference compared to the normal control.

Effect of plant extracts on serum TG and FFA

The level of serum TG and FFA in each group is shown in Fig. 3. The TG level of the diabetic control constantly increased until week 4 and showed an abnormal lipid profile of more than 5 times higher than the normal control (p<0.01). After 4 weeks, compared to that of the diabetic control, the TG level of the PG, LS, and MA groups decreased (PG 3 times, LS 1.5 times, and MA 5 times). The FFA level of the diabetic control increased until week 4 and showed an abnormal lipid profile over 4 times higher than normal rats (p<0.01). After 4 weeks, compared to that of the diabetic control, the FFA level of PG, LS, and MA groups decreased (PG 4 times, LS 1.8 times, and 4 times).

Effect of plant extracts on glycogen accumulation in liver

Changes of glycogen accumulation are displayed in Fig.

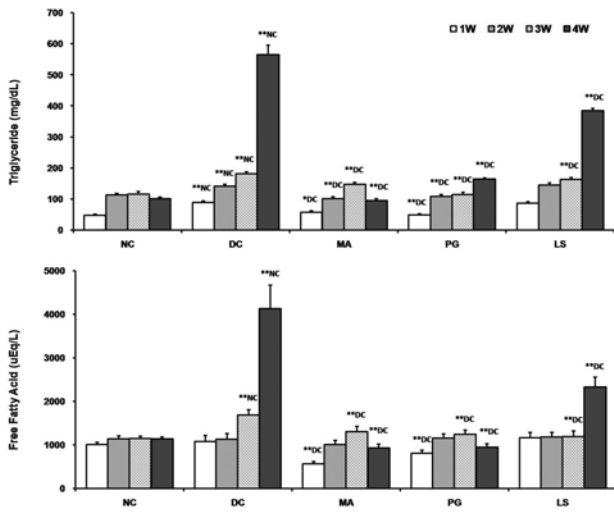


Fig. 3. Effect of plant extracts on serum triglyceride and free fatty acid level in diabetic rats (n=5; mean±S.D.). NC - LS and superscripts as in Fig. 2.

Table 3. Effect of plant extracts on serum insulin level in diabetic rats (n=5)

Group	Serum insulin (mean±S.D.) (ng/dl)			
	1 Week	2 Week	3 Week	4 Week
NC	0.84±0.13	0.82±0.10	0.83±0.13	0.84±0.15
DC	0.12±0.04	0.07±0.03	0.08±0.03	0.07±0.02 ^{*1W}
MA	0.10±0.03	0.18±0.08	0.22±0.07 ^{*DC,*1W}	0.65±0.10 ^{**DC,**1W}
PG	0.11±0.06	0.17±0.06	0.19±0.06 ^{*DC}	0.20±0.05 ^{*DC}
LS	0.12±0.04	0.14±0.04	0.17±0.05 ^{*DC}	0.19±0.06 ^{**DC}

NC - LS and superscripts as in Table 1.

4. Based on PAS-stained tissue sections, the portions stained red color show glycogen accumulation. The normal control showed a low level of glycogen accumulation in the liver but the diabetic control showed a high level. The MA group showed levels of glycogen accumulation that were similar

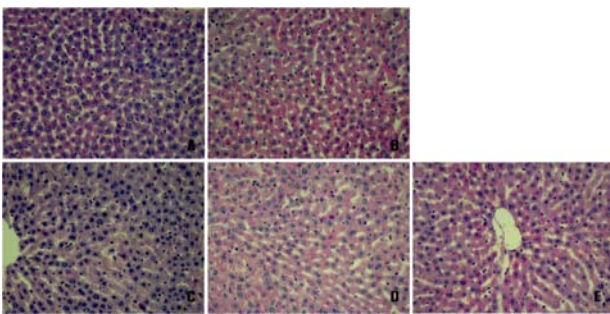


Fig. 4. Effect of plant extracts on the histological micrograph of rats liver (PAS 200×). A: Normal control, B: Diabetic control, C: Diabetic mice fed with mixture A, D: Diabetic mice fed with Pg, E: Diabetic mice fed with Ls extract.

to that of the normal control. The PG group showed a lower level of glycogen accumulation compared to that of the diabetic control, but the LS group showed a level of glycogen accumulation that was similar to that of the diabetic control.

Effect of plant extracts on β-cell recovery in Langerhans' islet

Histopathological changes of Langerhans' islets are displayed in Table 4 and Figs 5, 6. In Table 4, the pathological grading score of MA group was significantly higher compared to that of the diabetic control (p<0.01). But the scores of PG and LS groups were not significantly different compared to that of the diabetic control. The insulin occupied proportion of the Langerhans' islets of the diabetic control, decreased to 6 times less than that of the normal control (p<0.01) and the insulin occupied proportion of the Langerhans' islets of the PG, LS, and MA groups were significantly higher compared to that of the diabetic control (p<0.01). As for the MA group, the insulin occupied proportion of the Langerhans' islets and pathological grading of pancreas tissue was higher compared to those of the PG and LS groups (p<0.01). The glucagon occupied proportion

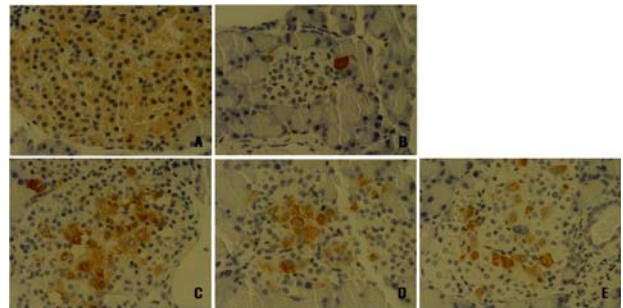


Fig. 5. Effect of plant extracts on the histological micrograph of rats Langerhans' islets cell and insulin secretion (ABC 400×). A-E as in Fig. 4.

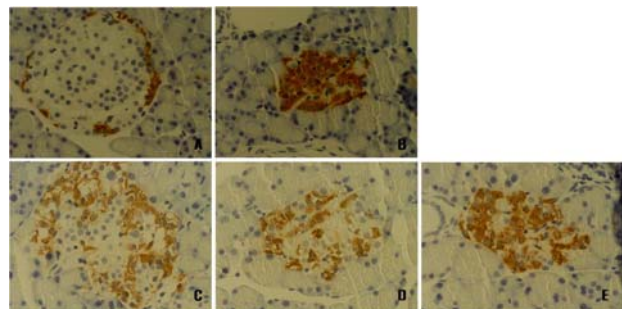


Fig. 6. Effect of plant extracts on the histological micrograph of rats Langerhans' islets cell and glucagon secretion (ABC 400×). A-E as in Fig. 4.

Table 4. Effect of plant extracts on the histopathological damage of pancreas in diabetic rats (n=10)

Group	Pathological grading of pancreas					P	Occupy proportion (mean±S.D.) (%)	
	0	I	II	III	IV		Insulin	Glucagon
NC	10	0	0	0	0	-	67.5±5.6	9.3±3.4
DC	0	0	2	4	4	0.000 ^{**NC}	10.8±3.3	72.9±7.1
MA	1	5	3	1	0	0.007 ^{**NC,**DC}	44.6±5.1 ^{**DC}	24.6±4.6 ^{**DC}
PG	0	1	3	3	3	0.000 ^{**NC}	33.2±4.8 ^{**DC,**MA}	36.6±5.5 ^{**DC,**MA}
LS	0	1	2	4	3	0.000 ^{**NC}	26.2±5.3 ^{**DC,**MA}	48.4±5.2 ^{**DC,**MA}

NC - LS and superscripts as in Table 1.

of the Langerhans' islets of the diabetic control was 7 times higher than that of the normal control ($p < 0.01$). The glucagon occupied proportion of the Langerhans' islets of the PG, LS, and MA groups was lower than that of the diabetic control ($p < 0.01$). The glucagon occupied proportion of the Langerhans' islets of MA group was lower than those of the PG and LS groups ($p < 0.01$).

In Fig. 5, Based on ABC-stained tissue sections using insulin antibodies, the normal control showed a normal shape of Langerhans' islets cell and insulin secretion but the diabetic control showed severely injured Langerhans' islet cell and little insulin secretion. The PG, LS, and MA groups showed recovery of Langerhans' islet cell and insulin secretion. In Fig. 6, based on ABC-stained tissue sections using glucagon antibodies, the Langerhans' islets of PG, LS, and MA groups showed glucagon secretion in only some parts, unlike the diabetic control, and in particular, glucagon secretion was observed only in the periphery in MA showing that beta cells in the center eventually recovered.

Discussion

The main purpose of dietary therapy in type 1 diabetes patients is to maintain a normal blood glucose level which includes controlling the increased postprandial blood glucose. Stabilizing blood glucose in diabetes patients is very important in the prevention of complications and hyperglycemia related to diabetes. According to the result of this study, effects of both the Pg and Ls extracts included increases in insulin secretion and lowering of glucose. However, the Pg extract had a higher recovery effect from a hyperglycemic state, because the Pg extract had more polyphenolic compounds that show antidiabetic activity. Pg extracts contain a variety of polyphenolic compounds such as polyphenol [28], terpenoids [2], flavonoids [22], and tannins [36]. The Ls extract used in this study was an ethanol extract including 1% corosolic acid. The hydrophilic polyphenol

compounds were removed during the extraction process, so the Ls extract contained less hydrophilic polyphenol compounds than the Pg extracts. In 1993, Murakami and coworkers [26] observed that the corosolic acid in their methanol extracts was the most effective antidiabetic compound. But through a study using water-soluble Ls extracts, Hayashi [14] and Liu [21] claimed that Lagerstroemin, which is a polyphenol component and tannic acid, and not the corosolic acid is responsible for the antidiabetic effect of Ls extracts. Therefore, the reason Pg extract's antidiabetic effect is greater than that of the Ls extracts is because the Pg extract contains more hydrophilic polyphenol compounds. This study showed that mixture A showed a higher level of insulin-secretion and glucose-lowering activity compared to that of the Pg and Ls extracts. This is because each extract included antidiabetic activities that were complimentary. Mixture A is composed of a mixture of Pg, Ls, Mi, Pd, and As extracts. The Mi extract's main components fagomine and 1-deoxynojirimycin, stimulate insulin release [13,38] and block intestinal glucose uptake by inhibiting the carbohydrate hydrolysing enzyme in the small intestine [19]. Also, the As extract includes components such as acanthoside, eleutheroside, daucosterine, β -sitosterol, sesamine, and savi-nine [30], for which Liu and coworkers [20] proved their ability to increase insulin-sensitivity. Meanwhile, the Pd extract has inhibition activity on the carbohydrate hydrolysing enzyme [18], and the pinitol included in this extract is converted into D-chiro-inositol *in vivo*, giving it insulin-like activity [11]. Therefore, mixture A can accomplish antidiabetic effects through antidiabetic pharmacological mechanisms such as: insulin-secretion increase activity by Pg, Ls, and Mi extracts; intestinal glucose-uptake blocking activity by Pg, Mi, and Pd extracts; insulin-sensitivity activity by the Pg, Ls and As extracts; and insulin-like activity by the Pd extract. Meanwhile, the insulin-secretion effect of mixture A was higher than Pg and Ls extracts in this study, which is due to the cell recovery capability of the included extracts. While

in type 1 DM, the Langerhans' islets of beta cells are destroyed and insulin production is reduced, the insulin-secretion increasing activities of plant extracts increase insulin secretion by stimulating those beta cells which are left and not destroyed [24]. Hence, the Pg and Ls extracts stimulated insulin release by stimulating residual beta cells which had not been destroyed. Unlike the rest of the extracts, the mixture A significantly enabled recovery of beta cells in Langerhans' islets, and increased the blood insulin level close to normal. Also, the glucose-lowering effect of mixture A in this study proved to be greater than the Pg and Ls extracts, because each extract included in the mixture A have enabled synergetic activities.

Under normal circumstances, insulin increases TG synthesis by increasing fatty acid uptake in adipose cells and furthermore, inhibits lipolysis [33]. However, when insulin is depleted, lipolysis is not inhibited and the increased lipolysis eventually causes hyperlipidemia. Therefore, the decrease of the plasma lipid level through dietary or drug therapy in type 1 DM patients reduces the risk of vascular disease and related complications [4], and moreover, the decrease of total cholesterol and increase of HDL-C is a desirable biochemical state in the prevention of atherosclerosis and coronary artery disease [23]. The reason the serum FFA level increases in insulin-deficient diabetes is that the balance of the FFA esterification-triglyceride lipolysis cycle shifts toward lipolysis, which in turn causes FFA outflow from fat depots. In this study, the diabetic control showed an abnormality of the lipid profile. Hyperlipidemia observed in type 1 diabetes may be reversed through an increase of the depleted insulin. In addition to insulin recovery, the increase of insulin-like activity and insulin sensitivity also contributes to the improvement of hyperlipidemia, and the activities of nutritional antioxidants also decrease cholesterol absorption, cholesterologenes, and fatty acid synthesis. The results of this study showed that the Pg and Ls extracts reverse the abnormality of the lipid profile, and the reason the Pg extracts had greater effects was because the Pg extracts displayed more antidiabetic pharmacological mechanisms than the Ls extracts. While Pg extracts have antioxidant activity [7], insulin-secretion increase, insulin-sensitivity increase, and intestinal glucose-uptake inhibit activities, Ls extracts only have insulin-secretion and sensitivity increasing activity. This leads to less improvement of hyperlipidemia. Mixture A improved hyperlipidemia, and showed more effectiveness than Pg and Ls

extracts. This was due to the fact that the effects of mixture A extract for releasing insulin and lowering glucose were greater than that of other extracts and mixture A contained many extracts that have antioxidant activities [7,8,29].

Gluconeogenesis is caused when a sufficient amount carbohydrate is not obtained by diet or from glycogen reserves. In type 1 diabetes, hepatic gluconeogenesis is dramatically increased due to insulin depletion and amino acid is used as an energy source, causing body weight loss [31]. The improvement of body weight loss is used as an important index that indicates the recovery from type 1 diabetes. In this study, the diabetic control showed more body weight loss than the normal control, indicating weight loss from excessive breakdown of tissue proteins. Meanwhile, the liver is the major gluconeogenic tissue [25]. The diabetic control had excessive accumulation of glycogen in the liver, but normal utilization of carbohydrates through glycogen reserves had failed; leading to a rapid increase of gluconeogenesis resulting in body weight loss. In this study, Pg extract and mixture A reversed the muscle wasting condition, and this was due to the improvements of hyperglycemia and hyperlipidemia caused by the plant extracts. In particular, the mixture A caused recovery of the abnormal glycogen accumulation within the liver close to normal, and the amount of body weight increase was also higher than that observed with other extracts. Therefore, mixture A was not only effective in recovering from hyperglycemia, hypoinsulinemia, and hyperlipidemia, but also in recovering abnormal glycogen accumulation in liver and the muscle-wasting condition.

In conclusion, this study showed that Pg extract showed higher antihyperglycemic, antihypoinsulinemic, and antihyperlipidemic effect compared to the Ls extract. This is because the corosolic acid contained in Pg extracts has lower antidiabetic activity and less of the hydrophilic polyphenol compounds that have high antidiabetic effects. Mixture A showed greater antihyperglycemic, antihyperlipidemic, and insulin increase effects. It also reduced the effects from the functional damage of beta cells compared to the Pg and Ls extracts. Therefore, it was proved that mixture A ensured a more beneficial synergistic antidiabetic effect compared to the effect of either the Pg or Ls extract used alone.

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References

1. Andallu, B. and N. C. Varadacharyulu. 2003. Antioxidant role of mulberry (*Morus indica* L. cv. Anantha) leaves in streptozotocin-diabetic rats. *Clinica Chimica Acta* **338**, 3-10.
2. Begum, S., S. I. Hassan, B. S. Siddiqui, F. Shaheen, M. N. Ghayur, and A. H. Gilani. 2002. Triterpenoids from the leaves of *Psidium Guajava* *Phytochemistry* **61**, 399-403.
3. Broadhurst, C. L., M. M. Polansky, and R. A. Anderson. 2000. Insulin-like biological activity of culinary and medical plant aqueous extracts in vitro. *Journal of Agricultural and Food Chemistry* **48**, 849-852.
4. Brown, B. G., XQ. Zhao, D. E. Sacco, and J. J. Albers. 1993. Lipid lowering and plaque regression. New insights into prevention of plaque disruption and clinical events in coronary disease. *Circulation* **87**, 1781-1791.
5. Cha, J. Y., B. S. Jun, C. H. Lee, K. S. Yooi, J. C. Moon, and Y. S. Cho. 2005. Hypoglycemic and antioxidative effects of fermented Chaga Mushroom (*Inonotus obliquus*) on streptozotocin-induced diabetic rats. *Journal of Life Science* **15**, 809-818.
6. Chait, A. and J. D. Brunzell. 1996. Diabetes, lipids, and atherosclerosis. In LeRoith, D., S. I. Taylor and J. M. Olefsky (eds.), *Diabetes Mellitus*. pp. 467-469, Lippincott-Raven Publishers, Philadelphia.
7. Chen, H. Y. and G. C. Yen. 2007. Antioxidant activity and free radical-scavenging capacity of extracts from guava (*Psidium guajava* L.) leaves. *Food Chemistry* **101**, 686-694.
8. Devaraj, S., N. Kaul, F. Schönlaue, P. Rohdewald, and I. Jialal. 2002. Pycnogenol[®] supplementation increases antioxidant capacity and has a favourable effect on the lipid profile in humans. *Lipids* **37**, 931-934.
9. Dixon, R. A., D. Y. Xie, and C. B. Sharma. 2005. Proanthocyanidins-a final frontier in flavonoid research. *The New Phytologist* **165**, 9-28.
10. Ernst, E., 1997. Plants with hypoglycemic activity in human. *Phytomedicine* **4**, 73-78.
11. Fonteles, M. C., L. C. Huang, and J. Larner. 1996. Infusion of pH 2.0 D-chiro-inositol glycan insulin putative mediator normalizes plasma glucose in streptozotocin diabetic rats at a dose equivalent to insulin without inducing hypoglycemia. *Diabetologia* **39**, 731-734.
12. Garcia, F. 1940. On the hypoglycemic effect of decoction of *Lagerstroemia speciosa* leaves (banaba) administered orally. *Journal of the Philippine Medicinal Associations* **20**, 395-402.
13. Gerich, J. E. 2001. Matching treatment to pathophysiology in type 2 diabetes. *Clinical Therapeutics* **23**, 646-659.
14. Hayashi, T., H. Maruyama, R. Kasai, K. Hattori, S. Takasuga, O. Hazeki, K. Yamasaki, and T. Tanaka. 2002. Ellagitannins from *Lagerstroemia speciosa* as Activators of Glucose Transport in Fat Cells. *Planta Medica* **68**, 173-175.
15. Hong, J. H., M. S. Lee, E. Y. Bae, Y. H. Kim, H. Oh, W. K. Oh, B. Y. Kim, and J. S. Ahn. 2004. Screening for the inhibitory activity of medicinal plants against protein tyrosine phosphatase 1B. *Korean Journal of Pharmacognosy* **35**, 16-21.
16. Kar, A., B. K. Choudhary and N. G. Bandyopadhyay. 2003. Comparative evaluation of hypoglycaemic activity of some Indian medical plants in alloxan diabetic rats. *Journal of Ethnopharmacology* **84**, 105-108.
17. Kim, K. H., S. G. Roh, C. R. Li, C. F. Jin, A. Kim, and W. C. Choi. 2008. Anti-diabetic effects of banaba leaf extracts (*Lagerstroemia speciosa* Pers.) through solvents. *Journal of Life Science* **18**, 1305-1311.
18. Kim, Y. H., Y. K. Jeong, M. H. Wang, W. Y. Lee, and H. I. Rhee. 2005. Inhibitory effect of pine extract on alpha-glucosidase activity and postprandial hyperglycemia. *Nutrition* **21**, 756-761.
19. Kimura, T., K. Nakagawa, H. Kubota, Y. Kojima, Y. Goto, K. Yamagishi, S. Oita, S. Oikawa, and T. Miyazawa. 2007. Food-grade mulberry powder enriched with 1-deoxynojirimycin suppresses the elevation of postprandial blood glucose in human. *Journal of Agricultural and Food Chemistry* **55**, 5869-5874.
20. Liu, T. P., C. S. Lee, S. S. Liou, I. M. Liu, and J. T. Cheng. 2005. Improvement of insulin resistance by *Acanthopanax senticosus* root in fructose-rich chow-fed rats. *Clinical and Experimental Pharmacology and Physiology* **32**, 649-654.
21. Liu, X., J. K. Kim, Y. Li, J. Li, F. Liu, and X. Chen. 2005. Tannic acid stimulates glucose transport and inhibits adipocyte differentiation in 3T3-L1 cells. *The Journal of Nutrition* **135**, 165-171.
22. Lozoya, X., M. Meckes, M. Abou-Zaid, J. Tortoriello, C. Nozzolillo, and J. T. Arnason. 1994. Quercetin glycosides in *Psidium guajava* L. leaves and determination of a spasmolytic principle. *Archives of Medical Research Arch* **25**, 11-15.
23. Luc, G. and J. C. Fruchart. 1991. Oxidation of lipoproteins and atherosclerosis. *The American Journal of Clinical Nutrition* **53**, 206S-209S.
24. Marles, R. J. and N. R. Farnsworth. 1995. Antidiabetic plants and their active constituents. *Phytomedicine* **2**, 137-189.
25. Mayes, P. A. and D. A. Bender. 2003. Gluconeogenesis & control of the blood glucose. In Murray, R. K., D. K. Granner, and V. W. Rodwell (eds.), *Harper's Illustrated Biochemistry*. 26th eds. pp. 145-162, McGraw-Hill Companies, New York.
26. Murakami, C., K. Myoga, R. Kasai, K. Ohtani, T. Kurokawa, S. Ishibashi, F. Dayrit, W. G. Padolina, and K. Yamasaki. 1993. Screening of plant constituents for effect on glucose transport activity in Ehrlich Ascites tumor cells. *Chemical and Pharmaceutical Bulletin* **41**, 2129-2131.
27. Oh, W. K., C. H. Lee, M. S. Lee, E. Y. Bae, C. B. Sohn, H. Oh, B. Y. Kim, and J. S. Ahn. 2005. Antidiabetic effects of extracts from *Psidium guajava*. *Journal of Ethnopharmacology* **96**, 411-415.
28. Okuda, T., T. Yoshida, T. Hatano, K. Yazaki, Y. Ikegami, and T. Shingu. 1987. Guavins A, C and D, complex tannins from *Psidium Guajava*. *Chemical and Pharmaceutical Bulletin* **35**, 443-446.
29. Onogi, A., K. Osawa, H. Yasuda, A. Sakai, H. Morita, and H. Itokawa. 1993. Flavonol glycosides from the leaves of *Morus alba* L. *Nature Medicine* **47**, 423-425.

30. Park, S. Y., S. Y. Chang, C. S. Yook, and T. Nohara. 2000. New 3,4-seco-lupane-type triterpene glycosides from *Acanthopanax senticosus forma inermis*. *Journal of Natural Products* **63**, 1630-1633.
31. Powers, A. C. 2008. Diabetes mellitus. In Fauci, A.S., E. Braunwald, D. L. Kasper, S. L. Hauser, D. L. Longo, J. L. Jameson, and J. Loscalzo (eds.), *Harrison's Principles of Internal Medicine*. 17th ed. pp. 2275-2304, McGraw-Hill Companies, New York.
32. Prior, R. L. and L. Gu. 2005. Occurrence and biological significance of proanthocyanidins in the American diet. *Phytochemistry* **66**, 2264-2280.
33. Shirwaikar, A., K. Rajendran, C. D. Kumar, and R. Bodla. 2004. Antidiabetic activity of aqueous leaf extract of *Annona squamosa* in streptozotocin-nicotinamide type 2 diabetic rats. *Journal of Ethnopharmacology* **91**, 171-175.
34. Sunagawa, M., S. Shimada, Z. Zhang, A. Oonishi, M. Nakamura, and T. Kosugi. 2004. Plasma insulin concentration was increased by long-term ingestion of guava juice in spontaneous non-insulin-dependent diabetes mellitus (NIDDM) rats. *Journal of Health Science* **50**, 674-678.
35. Suzuki, Y., T. Unno, M. Ushitani, K. Hayashi, and T. Kakuda. 1999. Antiobesity activity of extracts from *Lagerstroemia speciosa* L. leaves on female KK-Ay mice. *Journal of Nutritional Science and Vitaminology* **45**, 791-795.
36. Tanaka, T., N. Ishida, M. Ishimatsu, G. Nonaka, and I. Nishioka. 1992. Tannins and related compounds. CXVI. Six new complex tannins, guajavins, psidinins and psiguavin from the bark of *Psidium guajava* L. *Chemical and Pharmaceutical Bulletin* **40**, 2092-2098.
37. Tang, L. Q., W. Wei, L. M. Chen, and S. Liu. 2006. Effects of berberine on diabetes induced by alloxan and a high-fat/high-cholesterol diet in rats. *Journal of Ethnopharmacology* **108**, 109-115.
38. Taniguchi, S., N. Asano, F. Tomino, and I. Miwa. 1998. Potentiation of glucose-induced insulin secretion by fagomine, a pseudo-sugar isolated from mulberry leaves. *Hormone and Metabolic Research* **30**, 679-683.
39. Yang, C. S., J. M. Landau, M. T. Huang, and H. L. Newmark. 2001. Inhibition of carcinogenesis by dietary polyphenolic compound. *Annual Review of Nutrition* **21**, 381-406.

초록 : STZ으로 유발된 당뇨쥐에 대한 식물추출 혼합물의 항당뇨 상승효과

노상근 · 김중해¹ · 최원철*

(부산대학교 생물학과, ¹선문대학교 전자공학과)

본 연구는 streptozotocin (STZ)으로 당뇨를 유발한 실험쥐에 대한 *Psidium guajava* L. 잎(Pg), *Lagerstroemia speciosa* L. 잎(Ls) 추출물 그리고 혼합물 A (Pg, Ls, *Morus indica* L. 잎 추출물, *Pinus densiflora* needles 추출물, *Acanthopanax senticosus* M. roots 추출물)의 효과를 조사하였다. 4주간 streptozotocin (STZ)으로 당뇨를 유발한 실험쥐에 이들 추출물을 섭취시킨 결과 Pg, Ls 그리고 혼합물 A의 섭취는 당뇨 대조군에 비해 혈당을 각각 20%, 14% 그리고 24% 감소시켰다. 또한 이들 추출물의 섭취는 총 콜레스테롤, 중성지방, 유리지방산을 감소시켰고 인슐린과 HDL-콜레스테롤을 효과적으로 증가시켰다. 결론적으로 혼합물 A는 Pg와 Ls 추출물에 비해 혈당 및 지질 감소, 인슐린 증가 효과 그리고 기능적으로 손상된 베타세포의 회복이 더 높은 것으로 나타났다. 따라서 혼합물 A는 개별적으로 사용한 Pg와 Ls 추출물에 비해 더욱 유의한 상승효과를 발휘하는 것으로 증명되었다.