Antidiabetic Effects of Leaves Extracts of *Psidium guajava L.* and *Lagerstroemia speciosa L.* in STZ−induced Rats

Sang Geun Roh, Kyun Ha Kim and Won Chul Choi*

Department of Biology, College of Natural Sciences, Pusan National University

Guava (*Psidium guajava L.*) and banaba (*Lagerstroemia speciosa L.*) are well known as medicinal plants for their antidiabetic effects. These contain a great deal of polyphenol compound and work on the treatment of diabetes mellitus effectively. In this study, the extracts of guava and banaba are consumed by streptozotocin (STZ) induced diabetic rats to compare the antidiabetic effects. According to the comparison result, the glucose level of those STZ-induced diabetic rats has decreased by 19-32%, total cholesterol by 24-46%, triglyceride by 22-67% and free fatty acid by 49-71% approximately compared to the diabetic rats, while the generation of insulin and the recovery of beta cells have increased. However, the result showed that the antidiabetic effect of guava extracts was higher than that of banaba extracts. This is because the hydrophilic polyphenol compounds contained in banaba leaves were not extracted during the ethanol extraction process, and the antidiabetic activity of the extracted corosolic acid was low to surprise.

Key words : Guava (*Psidium guajava*), banaba (*Lagerstroemia speciosa*), polyphenol, anti-diabetic effects

Introduction

Diabetes mellitus (DM) causes the chronical metabolism disorder which makes approx. 4% of the world population suffer and this figure is expected to increase to 5.4% [16]. Hyperglycemia triggered by DM is usually caused due to the decrease in insulin secretion, decrease in glucose utilization of tissue and increase in the generation of glucose. Hyperglycemia's symptoms include obsessive thirst, glucosuria, polyuria, lipemia and hunger, and if left without treatment, this can result in patient's death due to critical ketoacidosis [27].

For ages, many countries have applied traditional medical plants to treat hyperglycemia and diabetes mellitus [1,6,13,26], and more than 400 plants have been reported with their glucose reduction effects [2]. Recently the interest on medical plants is getting high as oral agents used to treat DM cause various side effects [4,15] while taking traditional medical plants in a natural form of extracts show treatment effect as good as that of therapeutic agents without side effects.

In general, traditional medical plants contain a wide range of polyphenol compounds such as flavonoids, glycosides and terpenoids, which are effective in treating DM [5,7,31]. However, the amount and efficacy of polyphenol com-

pounds may differ depending on extraction methods. If the ethanol extraction method is used, polyphenol compounds can hardly extracted.

Psidium guajava L. known as guava is a representative medical plant that has antidiabetic effects and the interest on guava has been increased recently. Guava leaves contain a variety of polyphenol compounds such as polyphenol [25], terpenoids [3,23], flavonoids [20] and tannins [29]. And it was reported that guava leaves have antidiabetic effects direct and indirect through controlling glucose increase [22] and carbohydrate hydrolyzing enzyme's activity [21]. *Lagerstroemia speciosa L.* known as banaba has been used in treating DM and kidney diseases for ages. The antidiabetic effects of banaba was reported by Garcia in 1940 for the first time, and its effects in the glucose decreasing and insulin-like activity were proved through further researches [9-12]. Murakami and workers (1993) proposed that corosolic acid is the most effective substance among banaba leaves's antidiabetic effects [24] and banaba leaves were used widely to treat DM. In this vein, the ethanol-extract of banaba leaves and water-extract of guava leaves were consumed by streptozotocin (STZ) induced diabetic rats and their antidiabetic effects were compared.

Materials and Methods

Preparation of the plant extract

The banaba (*Lagerstroemia speciosa*) leaves extract was pur-

---

*Corresponding author

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

 E-mail : wcchoi@pusan.ac.kr

chased from Japan (Wellness banaba Inc, Korea) which was extracted containing 1% of corosolic acid by 90% ethanol extraction. As for guava (*Psidium guajava*) leaves extract, 150 kg of well dried guava leaves were purchased from Daegu Yangyeongsi Herb Market (Daegu, Korea) and 10 times of DW was added. This was boiled for 6 hr at 98°C to get extracts. This plant water-soluble extract was filtered with a 50 μm filter and concentrated under vacuum and freeze-dried to powder form.

Animal

Six-week-old male Wistar Hannover rats (n=40) were purchased from Samtako Experiment Animal (Samtako Bio, Kyung Gi-Do, Korea) and they were acclimatized under a controlled temperature of 20±2°C, humidity of 55±5%, 12h light and 12 hr dark cycle conditions for 1 week before starting the experiments. They had free access to standard pellets (Samtako Bio, Korea) and water.

Reagent and equipment

The glucose concentration of animals was measured using ACCU-CHEK Active (Roche, Germany) and the lipid concentration was analyzed using ADVIA 1650 (Bayer, Deerfield, IL, USA). The insulin concentration was measured with Sunrise Remote Control (TECAN, Salzburg, Austria) using Rats Insulin ELISA Kit (Shibayagi, Japan). Pancreas was cut using Rotary microtome (Leica, Wetzlar, Germany) and employed Hematoxylin-eosin. These preparations were observed under a light microscope (Carl Zeiss, Göttingen, Germany).

Causing diabetics

To cause diabetics, streptozotocin (STZ) (65 mg/kg) was solved into the 0.01M citrate buffer (pH 4.5) and then 250 μl was injected with single I.P. injection to rats who were not fed for 24 hr. Three days after injecting STZ, we measured the level of glucose using the blood from the vein in the tail. Those rats with blood glucose level of 300 mg/dl or higher were regarded as diabetic rats. For normal control rats, the same amount of 0.01M citrate buffer was injected (I.P. injection). Blood sugar levels were measured using ACCU-CHEK Active (Roche, Germany), and those rats who exceeded 600 mg/dl, which is the limit of glucose measuring range of the device were excluded in the experiments.

Grouping animals and feeding extracts

Rats with similar in weight were selected and classified as follows. NC served as normal controls and received vehicle only, DC served as diabetic controls and received vehicle. PG received the guava water extract, LS received the banaba ethanol extract. Each group was composed of 10 rats. The weight of rats per group was measured daily for 4 wk. Guava and banaba extracts were orally administered by rats twice a day based on the ratio of 1.5 ml per 1 kg of weight. In this case, the same amount of normal saline was taken by diabetic rats.

Measuring glucose, insulin and lipid concentration

The glucose concentration of the animals was measured once a week. To measure the glucose concentration, all the animals were not fed for 8 hr and then blood was sampled from the vein in the tail. The levels of insulin, total cholesterol (TC), HDL-cholesterol (HDL-C), triglyceride (TG) and free fatty acid (FFA) were measured in week 4. After leaving animals without feeding for 8 hr, they were anesthetized with ethyl ether and then the blood from the heart was sampled and analyzed.

Histological examination

After sampling blood from the animals in week 4, the pancreas was separated. Then, the pancreas was fixed in 10% neutral formalin for 1 day and processed in a routine manner to 4 μm thick paraffin sections. Hematoxylin-eosin (HE) stain was undertaken for the pancreas tissues and its histological damage was scored as follows based on the Tang et al., method [30]. 0: normal; Ⅰ: minor injury; Ⅱ: moderate injury; Ⅲ: obvious injury; Ⅳ: severe injury. Each sample was observed at 200x magnification. The degree of injuries of the islet cells was expressed as the mean of 10 different fields in each slide.

Statistical analysis

All values are expressed as means±S.D. The data were analyzed by Mann-Whitney test and Kruskal-Wallis test with SPSS WIN, ver. 12.0. The level of statistical significance in the study was $p < 0.05$.

Results

Effect of plant extracts on Langerhans islets' morphology

Fig. 1 shows the result of HE stain for the pancreas. The shape of DC's Langerhans islets is smaller and shrunken
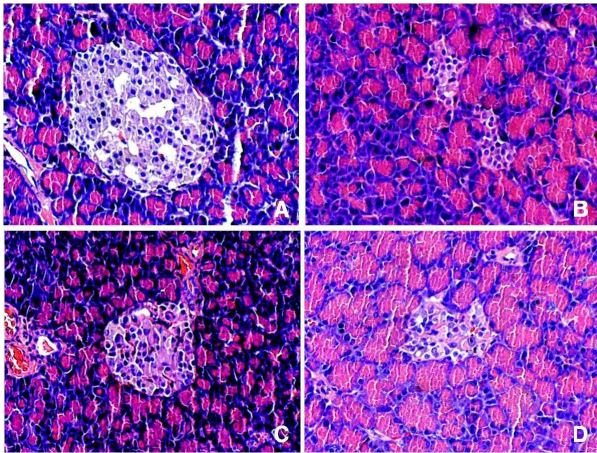
Fig. 1. Effect of guava and banaba extracts on the histological micrograph of rats Langerhans islets cell. (HE 200×). Abbreviations: A, Normal control rats; B, Diabetic control rats; C, Diabetic rats fed with guava leaves extract; D, diabetic rats fed with banaba leaves extract. A showed a normal shape of Langerhans islets cell; B have severely injured Langerhans islets cell; C, D showed recovery of Langerhans islets cell.

Table 1. Effect of guava and banaba extracts on the histopathological damage of pancreas in diabetic rats ($n=10$)

Group	Pathological grading of pancreas					P
	0	I	II	III	IV	
NC	10	0	0	0	0	-
DC	0	0	1	4	5	0.000[*]
PG	0	1	3	3	3	0.000[*]
LS	0	1	2	3	4	0.000[*]

[*]$P<0.01$ are calculated by Kruskal-Wallis' test and compared with normal control group.
Abbreviations: NC, Normal control; DC, Diabetic control; PG, Diabetic mice fed with guava leaves extract; LS, Diabetic mice fed with banaba leaves extract.

compared to that of NC. Furthermore, in the pathological grading of pancreas (Table. 1), NC's Langerhans islets were all normal while DC's were injured mostly (p<0.01). PG's and LS's Langerhans islets were recovered in terms of shape and size compared to DC's. PG's injured Langerhans islets were more recovered than LS's. In the pathological grading of pancreas, the number of injured PG's and LS's Langerhans islets was smaller than DC's, although there was no significant difference between the two groups in terms of statistics.

Effect of plant extracts on glucose, insulin and lipid concentration

Fig. 2 shows the change in the animals' glucose concentration
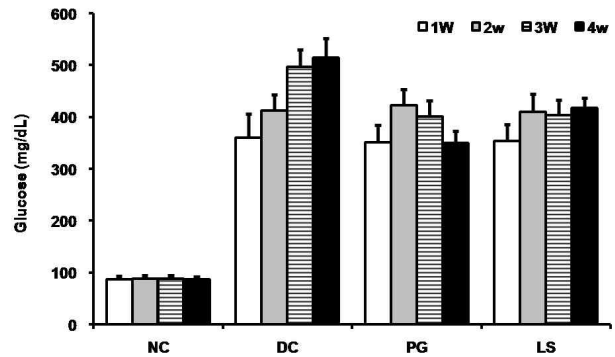

Fig. 2. Effect of guava and banaba extracts on blood glucose level in diabetic rats ($n=10$; mean±S.D.). $P<0.01$, compared with DC; $P<0.01$, PG compared with LS. Abbreviations: NC, Normal control; DC, Diabetic control; PG, Diabetic mice fed with guava leaves extract; LS, Diabetic mice fed with banaba leaves extract.

measured in week 4. NC's glucose concentration was low as 86-88 mg/dl for 4 weeks while DC's glucose concentration increased gradually up to higher than 500 mg/dl in week 4. PG's and LS's glucose concentration started decreasing from week 3 compared to DC's (p<0.01), and PG's glucose concentration was lower than LS's (p<0.01).

Fig. 3 shows the animals' insulin concentration measured in week 4. NC's insulin concentration was higher as much as 752.2 pg/ml while DC's decreased by approx. 8% of NC's (p<0.01). Both PG's and LS's insulin concentration increased more than two times compared to DC's, and PG's insulin concentration was higher than LS's (p<0.01).

Fig. 4 - Fig. 6 show the animals' lipid concentration measured in week 4. DC's total cholesterol concentration was more two times higher than NC's, and the concentration of triglyceride and free fatty acid increased by more than 4 times (p<0.01). The concentration levels of PG's and
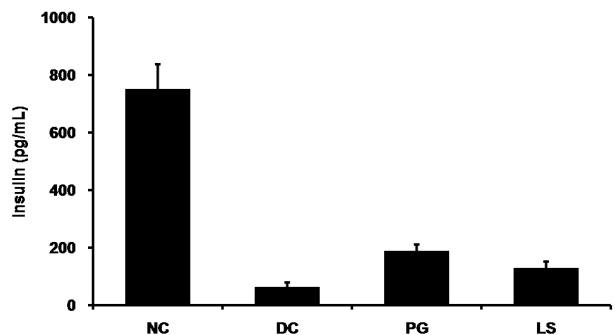

Fig. 3. Effect of guava and banaba extracts on serum insulin level in diabetic rats ($n=10$; mean±S.D.). $P<0.01$, compared with DC; $P<0.01$, PG compared with LS. NC, DC, PG, LS See Fig. 2.
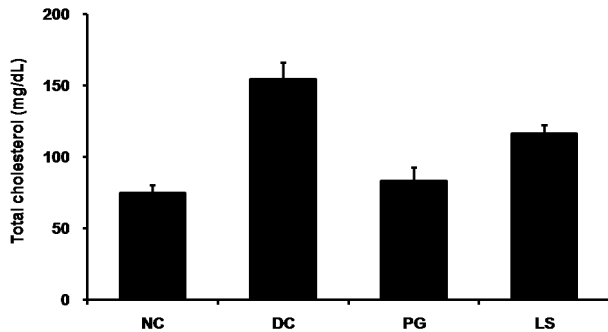
Fig. 4. Effect of guava and banaba extracts on total cholesterol concentration in diabetic rats (n=10; mean±S.D.). P<0.01, compared with DC; P<0.01, PG compared with LS. NC, DC, PG, LS See Fig. 2.
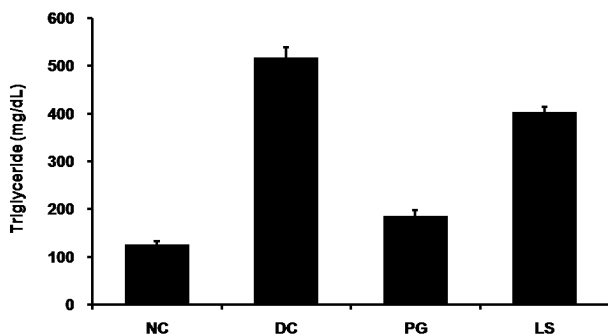

Fig. 5. Effect of guava and banaba extracts on triglyceride concentration in diabetic rats (n=10; mean±S.D.). P<0.01, compared with DC; P<0.01, PG compared with LS. NC, DC, PG, LS See Fig. 2.
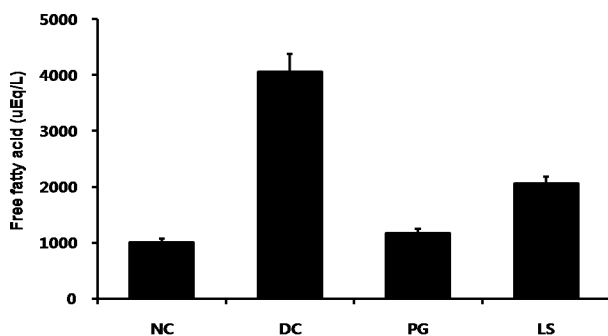

Fig. 6. Effect of guava and banaba extracts on free fatty acid concentration in diabetic rats (n=10; mean±S.D.). P<0.01, compared with DC; P<0.01, PG compared with LS. NC, DC, PG, LS See Fig. 2.

LS's total cholesterol, triglyceride and free fatty acid were lower than DC's (p<0.01), and PG's was lower than LS's when comparing just PG's and LS's (p<0.01).

Discussion

Type 1 diabetes mellitus (DM) is a disease caused due to the interaction of the genetic, environmental and immune factors, which results in the destruction of beta cell in the pancreas [27]. As the insulin secretion decreases, Type 1 DM develops hyperglycemia and hyperlipidemia, and if hyperglycemia is further developed, this can result in various complications. Thus, increasing insulin secretion and reducing hyperglycemia is very important in treating type 1 DM patients.

In this experiment, the glucose concentration of diabetic control rats was 500 mg/dl or higher, and the insulin concentration was reduced by 8% of the normal control rats. In addition, the lipid concentration of diabetic control rats increased 2 to 4 times higher than that of normal control rats, which showed the characteristics of Type 1 DM (hyperglycemia, hyperlipidemia, and hypoinsulinemia). However, consuming guava and banaba leaves extracts reduced the glucose concentration level of diabetic rats by 19% or higher, and increased the insulin secretion by more than 2 times. These extracts had antidiabetic effects by reducing the developed hyperlipidemia and recovering the damaged beta cell of lgerhans islets. However, there was difference in antidiabetic effects of the extracts used in this experiment as consuming guava extracts was more effective compared to consuming banaba extracts in increasing insulin secretion, reducing glucose level, improving hyperlipidemia and recovering the beta cell of Langerhans islets. In general, polyphenols are contained in the seeds, fruits, leaves and barks of medicinal plants [28] and protect plants from other external invaders [26]. Therefore, the antidiabetic effects of medical plants can be triggered by various flavonoids, glycosides and terpenoids [5,7,31]. The amount of polyphenol may vary depending on the extraction method as the more ethanol we use for extraction, the less hydrophilic polyphenol compounds we can extract. Actually, in the previous experiment that used different extract solvents [17], the amount of extracted polyphenol of banaba leaves extracts was as low as half of the current experiment since it used 90% ethanol only. So far, it is known that the major antidiabetic compound contained in banaba leaves is corosolic acid [8,18,24], and this substance is hydrophobic material which is hardly soluble in water. The solvents with higher ethanol ratio has been used to extract corosolic acid during the extraction process of banaba leaves, and the ethanol extracted banaba leaves that contain 1% of corosolic acid has been commercialized. However, other researchers [14,19] propose that the antidiabetic compound of these extracts is

not corosolic acid which is hydrophobic material, but another polyphenol which is hydrophilic material as the water extracted banaba extracts that rarely contain corosolic acid showed higher antidiabetic effects. On the other hand, guava water extracts are known to have antidiabetic effects such as decreasing plasma sugar level and restraining the activity of carbohydrate hydrolyzing enzyme [21,22]. Thus, the reason that the antidiabetic effects of banaba ethanol extracts were lower than those of guava water extracts is because the majority of hydrophilic polyphenol compounds contained in banaba leaves were not extracted during the extraction process with ethanol, and the extracted corosolic acid had low antidiabetic activity compared to what we expected.

In conclusion, guava water extracts showed higher antidiabetic effects on STZ induced rats compared to banaba extracts that contain 1% of corosolic acid. This is because the corosolic acid contained in banaba extracts has low antidiabetic activity and less hydrophilic polyphenol compounds which have high antidiabetic effects. Therefore, further study should be carried out to reveal what kind of hydrophilic polyphenol has high antidiabetic effects.

Acknowledgments

References

1. Ahmed, I., M. S. Lakhani, M. Gillett, A. John and H. Raza. 2001. Hypotriglyceridemic and hypocholesterolemic effects of anti-diabetic *Momordica charantia* (karela) fruit extract in streptozotocin-induced diabetic rats. *Diabetes Research and Clinical Practice* **51**, 155-161.
2. Bailey, C. J. and C. Day. 1989. Traditional plant medicines as treatments for diabetes. *Diabetes Care* **12**, 553-564.
3. 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.
4. Braunwald, E., A. S. Fauci, D. L. Kasper, S. L. Hauser, D. L. Longo and J. L. Jameson. 2001. Harrison's Principles of Internal Medicine. pp. 2109-2137, 15th eds., McGraw-Hill, New York.
5. Cetto, A. A. and H. Wiedenfiend. 2001. Hypoglycemic effect of *Cecropiaobtusifolia* on STZ diabetic rats. *Journal of Ethnopharmacology* **78**, 145-149.
6. Cignarella, A., M. Nastasi, E. Cavalli and L. Puglisi. 1996. Novel lipid-lowering properties of *Vaccinium myrtillus* L. leaves, a traditional antidiabetic treatment, in several models of rats dyslipidaemia: a comparison with ciprofibrate. *Thrombosis Research* **84**, 311-322.
7. Farkes, L. 1980. Active principles of plants of traditional medicine as models of new drugs. *Journal of Ethnopharmacology* **2**, 45-48.
8. Fukushima, M., F. Matsuyama, N. Ueda, K. Egawa, J. Takemoto, Y. Kajimoto, N. Yonaha, T. Miura, T. Kaneko, Y. Nishi, R. Mitsui, Y. Fujita, Y. Yamada and Y. Seino. 2006. Effect of corosolic acid on postchallenge plasma glucose levels. *Diabetes Research and Clinical Practice* **73**, 174-177.
9. 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.
10. Garcia, F. 1955. Plantisul compared with insulin. *Journal of the Philippine Medicinal Associations* **31**, 276-282.
11. Garcia, F. 1956, Plantisul tablets in the treatment of diabetes mellitus. *Journal of the Philippine Medicinal Associations* **31**, 216-224.
12. Garcia, F. and P. Melencio-Maglalang. 1957. Application of banabins (a plantisul preparation) and S.B. menus to diabetics. *Journal of the Philippine Medicinal Associations* **33**, 7-15.
13. Gori, M. and R. K. Campbell. 1998. Natural products and diabetes treatment. *The Diabetes Educator* **24**, 2001-2008.
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 traansport in fat cells. *Planta Medica* **68**, 173-175.
15. Holman, R. R. and R. C. Turner. 1991. Oral agents and insulin in the treatment of NIDDM. pp. 467-469 *In* Pickup J. and G. Williams (eds.), Text Book of Diabetes. Blackwell, Oxford.
16. Kim, S. H., S. H. Hyun and S. Y. Choung. 2006. Anti-diabetic effect of cinnamon extract on blood glucose in db/db mice. *Journal of Ethnopharmacology* **104**, 119-123.
17. Kim, K. H., S. G. Roh, C. R. Li, C. F. Jin, A. Kim and W. C. Choi. Anti-diabetic effect of banaba leaf extracts (lagerstroemia speciosa pers.) through solvents. 2008. *Journal of Life Science* **18**, 1305-1311.
18. Kotaro, Y., M. Hosokawa, S. Fujimoto, H. Fujiwara, Y. Fujita, N. Harada, C. Yamada, M. Fukushima, N. Ueda, T. Kaneko, F. Matsuyama, Y. Yamada, Y. Seino and N. Inagaki. 2008. Effect of corosolic acid on gluconeogenesis in rat liver. *Diabetes Research and Clinical Practice* **80**, 48-55.
19. 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.
20. 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* **25**, 11-15.
21. Mai, T. T. and N. V. Chuyen. 2007. Anti-hyperglycemic activity of an aqueous extract from flower buds of *Cleistocalyx*

operculatus (Roxb.) Merr and Perry. *Bioscience, Biotechnology, and Biochemistry* **71**, 69-76.

22. Maruyama, Y., H. Matsuda, R. Matsuda, M. Kubo, T. Hatano and T. Okuda. 1985. Study on *Psidium guajava* L. (I). Anti-diabetic effect and effective components of the leaf of *Psidium guajava* L. (Part I). *Shoyakugaku Zasshi* **39**, 261-269.

23. Meckes, M., F. Calzada, J. Tortoriello, J. L. Gonzalez and M. Martinez. 1996. Terpenoids isolated from *Psidium guajava* hexane extract with depressant activity on central nervous system. *Phytotherapy Research* **10**, 600-603.

24. 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.

25. 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.

26. Olajide, O. A., S. O. Awe, J. M. Makinde and O. Morebise. 1999. Evaluation of the anti-diabetic property of *Morinda Lucida* leaves in streptozotocin-diabetic rats. *The Journal of*

Pharmacy and Pharmacology **51**, 1321-1324.

27. 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. pp. 2275-2304, 17th eds., McGraw-Hill Companies, New York.

28. Prior, R. L. and L. Gu. 2005. Occurrence and biological significance of proanthocyanidins in the American diet. *Phytochemistry* **66**, 2264-2280.

29. 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.

30. 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.

31. Vertichevan, T. and M. Jegadeesan. 2002. Anti-diabetic activity of alcholic extracts of *Aervalanata* (L.) Juss, ex Schultes in rats. *Journal of Ethnopharmacology* **80**, 103-107.

초록 : STZ으로 당뇨를 유발한 실험쥐에 대한 *Psidium guajava* L.과 *Lagerstroemia speciosa* L. 잎 추출물의 항당뇨 효과

노상근 · 김균하 · 최원철*

(부산대학교 생물학과)

구아바(*Psidium guajava L.*)와 바나바(*Lagerstroemia speciosa L.*)는 항당뇨 효과를 갖는 대표적인 약용식물로 알려져 있다. 이들 식물은 다양한 폴리페놀(polyphenol) 성분을 함유하며 당뇨병의 치료에 효과적으로 작용한다. 따라서 본 연구는 이들 추출물을 streptozotocin (STZ)으로 당뇨를 유발한 실험쥐에 섭취시켜 항당뇨 효과를 상호 비교하였다. STZ으로 당뇨를 유발한 실험쥐에 구아바 및 바나바 잎 추출물을 섭취시킨 결과 당뇨쥐에 비해 혈당은 약 19-32%, 총콜레스테롤(total cholesterol)은 약 24-46%, 중성지방(triglyceride)은 22-67% 그리고 유리지방산(free fatty acid)은 49-71% 감소하였고 또한 인슐린 분비와 췌장에 존재하는 베타세포의 회복도 증가되었다. 한편 구아바 추출물의 섭취가 바나바 추출물의 섭취에 비해 항당뇨 효과가 더 높았는데 그 이유는 바나바 잎에 포함된 친수성 폴리페놀(polyphenol) 성분이 에탄올 추출과정에서 대부분 추출되지 않았고 또한 추출된 코로소릭산(corosolic acid)은 지금까지 알려진 것과는 달리 항당뇨 활성이 낮았기 때문이다.