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EFFECT OF THE AQUEOUS GREEN LEAF EXTRACT OF *PSIDIUM GUAJAVA* ON GLUCOSE LEVEL IN RAT

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ABSTRACT

Psidium guajava belongs to the family of Myrtaceae, is widely grown in Sistan and Balochistan provinces in the South of Iran. *P. guajava* is known in folk medicine as a medicinal plant that used as hypotensive and anti-diabetic. In the present studies aqueous green leaf extract of *P. guajava* (400 mg/kg) showed a strong glucose lowering effect after oral administration in rats. The decrease of glycemia has reached to 30% of the control value 2 h after glucose loading. The amount of glucose absorbed in a segment jejunum in situ was 9.32±0.32 mg in the presence of tea extract as compared to control (14.55 ±045 mg) during 2 h ($P<0.05$). The results indicate that aqueous extract of tea has a significant anti hyperglycemic effect that may be caused in part by the reduction of intestinal glucose absorption.

Keywords: *Psidium guajava*, green leaf, anti-hyperglycemia, Iran

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease, which can be classified into type 1 diabetes (insulin-dependent diabetes mellitus or IDDM) and type 2 diabetes (non-insulin dependent diabetes mellitus or NIDDM). The prevalence of diabetes is rapidly increasing in industrialized countries and type 2 diabetes accounts for 90% of the disease. In type 2 diabetes, insulin-resistance is a characteristic feature and several drugs to increase the insulin sensitivity are currently being used in clinic. However, currently available drugs for type 2 diabetes have a number of limitations, such as adverse effects and high rates of secondary failure (Ebadi *et al*., 2005; Aguilar *et al*., 1994; Kim *et al*., 2001; Joy and Kuttan, 1999).

Many indigenous Iranian medicinal plants have been found to be useful to successfully manage diabetes (Shokrzadeh *et al*., 2005; Ebadi *et al*., 2005). Despite the introduction of hypoglycemic agents from natural and synthetic sources diabetes and its complications continue to be a major problem in the world population (Loew and Kaszkin, 2002**)**.

In the course of this investigation, an aqueous extract of *Psidium guajava* L. (Myrtaceae) exhibited promising inhibitory activity. Psidium guajava is commonly known as guava, guyava and kuawa or in false Zeytun in Sistan and Balochistan province in the south of Iran (Ebadi *et al*., 2005; Begum *et al*., 2002) . The place of origin of the guava is uncertain but needs a tropical location and full sun. This small evergreen tree belongs to the Myrtaceae family and has been claimed to be useful in the treatment of diarrhea, dysentery and acute gastrointestinal inflammation (Aguilar *et al*., 1994; Lozoya, 1999). An alcohol extract of guava leaves exhibited spasmolytic effects on isolated rat and guinea-pig ileum (Lozoya *et al*., 1990) and effects of the extract from guava leaves on acute diarrheic disease has been described (Lozoya *et al*., 2002). Leaves of this plant have been reported to contain several compounds such as various terpenoids (Meckes *et al*., 1996; Begum *et al*., 2002), flavonoids (Lozoya *et al*., 1994) and tannins (Tanaka *et al*., 1992). In particular, quercetin, the best-known flavonoid from guava leaves, exerted spasmolytic action through a calcium-mediated mechanism (Morales *et al*., 1994). Maruyama *et al*. (1985) reported that the butanolsoluble fraction prepared from 50% ethanol extract from *P. guajava* leaves inhibited the increase of the plasma sugar level in alloxan-induced diabetic rats and decreased plasma glucose level in the glucose tolerance test. In addition, the butanol- and water-soluble fractions were all found to suppress adrenalin-induced lipolysis in fat cells from rat epididymal adipose tissues (Maruyama *et al*., 1985). Hypoglycemic effect in alloxan-treated mice and diabetic volunteers exerted by the fruit extract from *P. guajava* has also been reported (Cheng and Yang, 1983).

The purpose of this study is to study the hypoglycemic effect of aqueous leaf extract of *P. guajava* grown in Sistan and Balochistan province on hyperglycemia induced by oral glucose tolerance test (OGTT) and on alloxan-induced diabetic rats. The intestinal glucose absorption was measured *in situ* in a perfused jejunum segment in order to determine one of the probable mechanism of antihyperglycemic effect of *P. guajava*.

MATERIALS AND METHODS

All procedures in this study was carried out at December 2003, in Central laboratory of Mazandaran medical canter (Sari – Iran). Samples of *P. guajava* was collected from some areas of Zahedan city in Sistan and Balochistan provinces in the south of Iran and prepared for experiment based on Zhang *et al*., (1999). Male wistar rats weighing 250–350 g prepared from Pasteur institute and were kept in a room maintained at a temperature of 23°C .Animals were fasted for 16 h before the OGTT. Glucose (1 g kg^{-1}) was administered by gavages 30 min after oral administration of 400 mg kg^{-1} leaf water extract of *P.guajava*. Glibenclamide at dose of 2 mg kg^{-1} was used as a reference drug. Blood glucose level was measured each hour after glucose loading in rats under light ether anesthesia (Bergmeyer and Bernt, 1980). Rats were treated intraperitoneally with 150 mg kg^{-1} day^{-1} of alloxan for 3 Consecutive days. Three days after the last alloxan injection, only rats with fasting glycemia more than 1.5 g l^{-1} were used. They were divided into two groups and After 16 h of fasting, treated orally with 400 mg kg^{-1} of plant extract or distilled Water (control), respectively. Blood samples were obtained 30 min before and 60,120, 180min after treatment .The effect of the water leaf extract obtained from *P. guajava* was tested in a perfused Jejunum segment (6 cm) in fasted rats for 36 h and anaesthetized with sodium Pentobarbital (50 mg kg^{-1}, i.m.). The bilberry infusion was added to a 3 final concentration 400 mg kg^{-1}. The system was set at constant temperature of 37°C, and the perfusion rate was 0.53 ml min^{-1} for 2 h. The controls were perfused with the perfusing solution without plant extract. Student's t-test was used for statistical analysis and $P<0.05$ was considered to be significant (Bergmeyer and Bernt, 1980).

RESULTS AND DISCUSION

A strong antihyperglycemic effect of *P. guajava* (400 mg kg^{-1}) was observed at the first Hour after glucose loading in rats under OGTT (Table 1). In alloxan-induced diabetic rats, the oral administration of the aqueous extract of *P. guajava* did not modify the blood glucose level. On the contrary, a strong antihyperglycemic effect of *P. guajava* (400 mg kg^{-1}) was observed. The fall of glycemia was approximately 30% vs. control. This effect was still present 120 min after the oral administration of glucose.
Glibenclamide at dose of 2 mg kg^{-1} resulted more active at all the time tested. The intestinal glucose absorption in situ on jejunum segment showed that leaf water extract significantly reduced the absorption of glucose. The amount of glucose absorbed in controls during 2 h was (14.55 ±045mg) vs. (9.32±0.32mg) in the presence of bilberry extract ($P<0.05$).

Table. 1. Effect of oral treatment of 400 mg kg^{-1} of *P. guajava* aqueous leaf extract on plasma glucose level of rats.

Time (Min)	Glucose level (g l^{-1})		
	Controls	Leaf Extract	Glibenclamide
-30	1.23	1.21	1.13
0	1.22	1.23	1.15
30	1.45	0.89	1.12
60	1.55	0.90	1.15
90	1.23	0.82	0.79
120	1.45	0.81	0.82
150	1.21	0.86	0.85
180	1.33	0.87	0.82

We have not observed any sign of intestinal irritation due to the presence of plant aqueous extract. *P. guajava* traditionally has been used for a number of ailments including diabetes. In the present study we demonstrated an antihyperglycemic effect of the aqueous leaf extract of *P. guajava* grown in Sistan and Balochistan province. The lack of hypoglycemic effect of *P. guajava* aqueous leaf extract in alloxan-induced diabetic rats which is a model of diabetes with hypoinsulinemia demonstrates that this extract may act on glucose homeostasis by extra pancreatic way. It indicates that the presence of insulin is required for the hypoglycemic activity of *P. guajava*. We have shown that *P. guajava* inhibits significantly glucose absorption in small intestine in rats under anesthesia. This can be considered as one of the mechanisms by which this extract can regulate the glucose homeostasis in glucose loaded rats. However, this result does not exclude the other mechanisms regulating the peripheral glucose homeostasis (Tonks, 2003; Dadke and Chernoff, 2003)

The results indicate that aqueous extract of *P. guajava* grown in Sistan and Balochistan province had a significant antihyperglycemic effect which may be caused in part by the reduction of intestinal glucose absorption. The integration of a menu with *P. guajava* may be possible and recommendable for the management of diabetes. Other experiments are necessary to determine the other mechanisms of antihyperglycemic action of *P. guajava* and the active fractions involved in this effect. However, in some studies of laboratory cultures and animals, an extract of *P. guajava* leaves has shown the opposite effect but in many cases, researchers noted that animals treated with *P. guajava* water leaf extract produced increased amounts of insulin. In one of the studies, decreased blood sugar occurred in animals with both high and normal blood sugar levels (Nagarajan *et al*, 1987; Perfumi and Tacconi, 1996 ; Ponnachan *et al*, 1993 ; Rajasekharan and Tuli, 1976). We observed that P. guajava improved oral glucose tolerance in rats. It is therefore likely that *P. guajava* is prophylactic against diabetes and ameliorates diabetic hyperglycemia. *P. guajava* consumption at moderate doses may be associated with a reduced risk of type 2 diabetes in apparently healthy individuals by controlling postprandial hyperglycemia. Much more research is needed to prove or disprove the possible effects of *P. guajava* 's aerial parts on blood sugar (Cheng and Yang, 1983 ; Shokrzadeh *et al*., 2005; Hamaguchi *et al*., 1995; Hong *et al*., 2004 ; Illing *et al*.,1951; Saltiel and Kahn, 2001; van Huijsduijnen *et al*., 2002).

REFERENCES

Aguilar, A., A. Argueta and L. Cano (1994). Flora medicinal ind´ýgena de Mexico. *Treinta y cinco monograf´ýas del Atlas de las Plantas de la Medicina Tradicional Mexicana*, vol. 2. INI, Mexico, p. 45.

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.

Bergmeyer, H.W. and E. Bernt (1980). In: *Practical Clinical Biochemistry* (Varley, H., Gowenlock, A.H.,Bell, M. eds.). William Heinemann Medical Books Ltd, London, p: 741.

Burke, T.R., B. Ye, X. Yan, S. Wang, Z. Jia, L. Chen, Z.Y. Zhang and D. Barford (1996). Small molecule interactions with protein-tyrosine phosphatase PTP1B and their use in inhibitor design. *Biochemistry* 35: 15989–15996.

Cheng, J.T. and R.S. Yang, (1983). Hypoglycemic effect of guava juice in mice and human subjects. *American J. Chinese Medicine* 11: 74–76.

Cheung, A., J. Kusari, D. Jansen, D. Bandyopadhyay, A. Kusari and M. Bryer-Ash (1999). Marked impairment of protein tyrosine phosphatase 1B activity in adipose tissue of obese subjects with and without type 2 diabetes mellitus. *J. Lab. and Clinical Medicine*, 134: 115–123.

Dadke, S. and J. Chernoff (2003). Protein-tyrosine phosphatase 1b as a potential drug target for obesity. Current drug targets. *Immune, endocrine and metabolic disorders*, 3: 299–304.

Ebadi, A.G., M. Babaee and S. Zare (2005). The effect of *Salvia* officinalis Linn for reducing blood glucose in normal and alloxan diabetic rats. *IVth Intl. con. of Ethnobotany*, 21-26 August , Istanbul , Turkey.

Hamaguchi, T., T. Sudo and H. Osada (1995). RK-682, a potent inhibitor of tyrosine phosphatase, arrested the mammalian cell cycle progression at G1 phase. *FEBS Letters*, 372: 54–58.

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 J. Pharmacognosy*, 35: 16–21.

Illing, E.K.B., C.H. Gray and R.D.Lawrence (1951). Blood glutathione and non-glucose reducing substances in diabetes. *Journa. Biochemistry*, 48: 637-640.

Johnson, T.O., J. Ermolieff and M.R. Jirousek (2002). Protein tyrosine phosphatase 1b inhibitors for diabetes. *Nature Rev. Drug Discovery*, 1: 696–709.

Kim, J.K., J.J. Fillmore, Y. Chen, C. Yu, I.K. Moore, M. Pypaert, E.P. Lutz, Y. Kako, W. Velez-Carrasco, I.J. Goldberg, J.I. Breslow, G.I. Shulman (2001). Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. Proc. of the *Nat. Acad. of Sciences of the U. S. A.* 98: 7522–7577.

Joy, K.L., and R. Kuttan, (1999). Anti-diabetic activity of *Picorrhiza kurroa* extract. *J. Ethnopharmacol*, 167: 143-148.

Klaman, L.D., O. Boss, O.D. Peroni, J.K Kim, J.L. Martino, J.M. Zabolotny, N. Moghal, M. Lubkin, Y.B. Kim, A.H. Sharpe, A. Stricker-Krongrad, G.I. Shulman, B.G. Neel, B.B. Kahn (2000). Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. *Mol. and Cellular Bio.*, 20: 5479–5489.

Loew, D., and M. Kaszkin (2002). Approaching the problem of bioequivalence of herbal medicinal products. *Phytother. Res*, 16: 705-711.

Lozoya, X., M.N. About-Zaid, C. Nozzilillo, J.T. Arnason (1990). Spasmolytic effect of the methanolic extract of *Psidium guajava. Planta Medica*, 56: 686–689.

Lozoya, X., M. Meckes, M. Abou-Zaid, J. Tortoriello, C. Nozzolillo, J.T. Arnason (1994). Quercetin glycosides in *Psidium guajava* L. leaves and determination of a spasmolytic principle. *Archives of Med. Res.* 25: 11–15.

Lozoya, X., (1999) . Xiuhpatli. In: *Herba Officinalis. SSA/UNAM,* Mexico, p. 127.

Lozoya, X., H. Reyes-Morales, M.A. Chavez-Soto, C. Martinez-Garcia Mdel, Y. Soto-Gonzalez, S.V. Doubova (2002). Intestinal antispasmodic effect of a phytodrug of *Psidium guajava* folia in the treatment of acute diarrheic disease. J. of Ethnopharmacology , 83: 19–24.

Maruyama, Y., H. Matsuda, R. Matsuda, M. Kubo, T. Hatano, 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.

Meckes, M., F. Calzada, J. Tortoriello, J.L. Gonzalez, M. Martinez (1996). Terpenoids isolated from *Psidium guajava* hexane extract with depressant activity on central nervous system. . *R*es., 10: 600–603.

Morales, M.A., J. Tortoriello, M. Meckes, D. Paz, X. Lozoya (1994). Calcium-antagonist effect of quercetin and its relation with spasmolytic properties of *Psidium guajava* L. *Archives of Medical Res.*, 25: 17–21.

Nagarajan, S., H.C. Jain and G.S. Aulakh, (1987). *Indigenous Plants used in the Control of Diabetes*. Publication and information directorate CSIR, New Delhi, p. 586.

Perfumi, M. and R. Tacconi (1996). Anti hyperglycemic effect of fresh *Opuntia dillenii* fruit from Tenerife (Canry islands). *Intl.. J. .Pharmacology*, 34: 40- 41.

Ponnachan, P.T.C., C.S. Paulose and K.R. Panikkar (1993). Effect of leaf extract of *Aegle marmelos* in diabetic rats. Indian. *J. .Experl. Biol.* 31: 345-347.

Rajasekharan, S. and S.N. Tuli (1976). Vijaysar' (*Pterocarpus marsupium*) in the treatment of 'Madhumeha' (diabetes mellitus) a clinical trial. *J. Res. .Indian .Medicine.Yoga. Homeopathy*, 9: 76-78.

Saltiel, A.R. and C.R. Kahn (2001). Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* 414: 799–806.

Shokrzadeh, M., A.G. Ebadi, and M. Babaee (2005). Ethnobotanical and ethnopharmacology survey of medicinal plants used in the treatment of diabetes in Mazandaran province (Iran), *IVth Intl.l cong. of Ethnobotany* , 21-26 August , Istanbul , Turkey.

Tanaka, T., N. Ishida, M. Ishimatsu, G. Nonaka, I. Nishioka (1992). Tannins and related compounds. CXVI. Six new complex tannins, guajavins, psidinins and psiguavin from the bark of *Psidium guajava* L. *Chem. and Pharm. Bull*., 40: 2092–2098.

Tonks, N.K. (2003). PTP1B: From the sidelines to the front lines! *FEBS Letters*, 546: 140–148.

van Huijsduijnen, R.H., A. Bombrun, D. Swinnen (2002). Selecting protein tyrosine phosphatases as drug targets. *Drug Discovery Today*, 7: 1013–1019.

Zhang, B., G. Salituro, D. Szalkowski, Z. Li, Y. Zhang, I. Royo, D. Vilella, M.T. Diez, F. Pelaez, C. Ruby, R.L. Kendall, X. Mao, P. Griffin, J. Calaycay, J.R. Zierath, J.V. Heck, R.G. Smith, E.D. Moller (1999). Discovery of small molecule insulin mimetic with antidiabetic activity in mice. *Science*, 284: 974–977.

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