

JOURNAL OF APPLIED SCIENCES RESEARCH

JOURNAL home page: <http://www.aensiweb.com/jasr.html>

2013 Special, 9(12) pages: 6185-6188

Published Online :15 January 2014

Research Article

Antidiabetic Activity of The Methanolic Extract from *Betula alnoides* Buch-Ham. ex G. Don¹Yutana Pongpiriyadacha, ²Patcharin Nuansrithong and ³Dhiravit Chantip¹Department of Thai Traditional Medicine,²Department of Science, Faculty of Science and Technology, Rajamangala University of Technology Srivijaya, Thungsong, Nakhon Si Thammarat, Thailand³Department of Veterinary Science, Faculty of Veterinary Science, Rajamangala University of Technology Srivijaya, Thungyai, Nakhon Si Thammarat, Thailand

Received: 12 November 2013; Revised: 14 December, 2013; Accepted: 20 December 2013.

© 2014 AENSI PUBLISHER All rights reserved

ABSTRACT

Antidiabetic activity of 80% methanolic extract from *Betula alnoides* were examined. The aqueous methanolic extract exhibited the inhibition of α -glucosidase activity, sucrase and maltase activities, with IC₅₀ values of 26.5 and 5.4 μ g/ml, respectively. A standard α -glucosidase inhibitor, acarbose also showed very potent inhibition of an enzymes, sucrase and maltase with IC₅₀ values of 1.0 and 1.1 μ g/ml, respectively. Furthermore, the extract (100-400 mg/kg, *p.o.*) significantly inhibited the increase of serum glucose levels in rats after oral administration of 20% sucrose at 30 and 60 min. Similarity, a standard acarbose (5-10 mg/kg, *p.o.*) also significantly inhibited the increase of blood glucose levels in sucrose-loaded rats at 30 and 60 min. These results indicate that *Betula alnoides* may benefit in the treatment of diabetes mellitus as α -glucosidase inhibitory activity and lowering of the blood glucose levels.

Key words: *Betula alnoides*, α -glucosidase inhibitor, antidiabetic, glucose tolerance

INTRODUCTION

Diabetes mellitus is the most serious of metabolic disorder disease and leading causes of morbidity and mortality in the world and still challenging health problem in the 21st century. According to prevalence estimates of the International Diabetes Federation (IDF), 366 million people have diabetes in 2011, and by the year 2030, about 552 million people will have the disease [3]. The Institute of Medical Research and Technology Assessment (IMRTA) reported that diabetes mellitus is reported to be the most prevalent endocrine disorder and rapidly increase about 4 times from years 2000-2011 and caused of dead about 7,019 persons per year [4]. One therapeutic approach to treat diabetes is to retard the absorption of glucose via inhibition of enzymes, such as α -glucosidase which catalyzes the liberation of α -glucose from the non reducing end of the substrate oligosaccharides. The management of diabetes mellitus is mostly use hypoglycemic drugs or exogenous insulin which sometimes must pay a high cost and have various side effects. In developing country, Thailand, the couple of modern drugs and folklore medicine maintain the good way for the treatment of diabetes mellitus. During the past few decades, the search for

more effective and safer antidiabetic agents has continued to be an interesting area, especially from medicinal plants. It has been reported that many Thai medicinal plants useful to manage diabetes mellitus such as *Salacia chinensis*, *Borassus flabellifer* and *Cotylelobium melanoxylon* [20,21,10].

The Betulaceae plant *Betula alnoides* is known as “Khamlang suea khrong” or “Khamlang phaya suea khrong” in Thai and its stem bark has been used as a traditional medicinal plant in many purposes, such as tonic, longevity, stomachic, appetite, carminative and aphrodisiac [12]. Recent research has reported that some chemical constituents, such as dammarane glycosides, were predominant from *Betula alnoides* [14] and it has been reported that the ethanolic extract of *Betula alnoides* exhibited the inhibition of phosphodiesterase activity [18].

In our studied of antidiabetic activity from the extract of tonic and longevity plants, we found that *Salacia chinensis* and *Erycibe expansa* showed very high potential for inhibition of intestinal α -glucosidase activity and lowering of serum glucose levels [9]. However, the antidiabetic effects of several tonic and longevity medicinal plants, including *Betula alnoides* have not been clarified. In the present study, the effects of aqueous methanolic extract from *Betula alnoides* on α -glucosidase

Corresponding Author: Yutana Pongpiriyadacha, Department of Thai Traditional Medicine, Department of Science, Faculty of Science and Technology, Rajamangala University of Technology Srivijaya, Thungsong, Nakhon Si Thammarat, Thailand
E-mail: yutanap@hotmail.com

activity and increase of blood glucose level were examined.

Materials and Methods

Plant Material:

The stem bark of *Betula alnoides* was collected in April 2012 from Nakhon Si Thammarat province, Thailand, and was identified by one of authors (Rajamangala University of Technology Srivijaya, Pongpiriyadacha Y.). Voucher specimens (TL013) have been deposited in our laboratory, Medicinal Plant Research and Development Centre (MPRDC), Faculty of Science and Technology, Rajamangala University of Technology Srivijaya, Thungyai, Nakhon Si Thammarat, Thailand.

Extract Preparation:

The dried stem bark of *Betula alnoides* was pulverized to rough powder and extracted three times with 80% methanol under reflux at 80 °C for 3 hours. Subsequently, it was filtered over Whatman No.1 paper. Finally, the filtrate was evaporated under reduced pressure provided the aqueous methanolic extract which use for test sample.

Animals:

Male Wistar rats weighing about 130-160 g were purchased from National Laboratory Animal Centre, Mahidol University, Thailand. The animals were housed at a constant temperature of 25±2 °C and were fed a standard laboratory chow. The animal were fasted for 24-26 hours prior to the beginning of the experiment in individual cages with raised mesh bottom, but were allowed free access to tap water. All of experiments were performed with conscious rats unless otherwise noted. The conduct of experiments and the procedure of sacrifice were approved by the Experimental Animal Research Committee of Rajamangala University of Technology Srivijaya.

Effects on Rat Intestinal α -Glucosidase Activity:

The inhibitory activity of the extract on rat small intestinal α -glucosidase was performed according to the method described previously (Nishioka et al., 1998, Yoshikawa et al., 2002, 2003) with slight modifications. The rat small intestinal brush border membrane fraction was prepared and its suspension in 0.1 M maleate buffer (pH 6.0) was used to determine the small intestinal α -glucosidase enzyme activity of maltase and sucrase. The enzyme suspension was diluted to hydrolyze maltose and sucrose to produce about 0.30 and 0.20 μ mol/tube of D-glucose, respectively. In the total assay mixture

100 μ l containing a substrate (sucrose 74 mM, maltose 74 mM) 50 μ l, methanolic extract 25 μ l and start reaction by added the enzyme 25 μ l, incubated at 37° C, 30 min. Then, 0.4 ml of water was added to the test tube, and the tube was immediately immersed in boiling water for 2 min to stop the reaction and then cooled with water. The glucose concentration determined by enzymatic method using glucose C-II test Wako (Wako Pure Chemical Co., Ltd.) and measured absorbance at 500/655 nm with microplate reader. The intestinal α -glucosidase inhibitor acarbose was used as a reference compound.

Effects on increase of blood glucose level:

The effect of methanolic extract on increase of blood glucose level in sucrose-loaded rats was performed according to our method described previously [20,21]. Briefly, male Wistar rats (130-160 g) were fasted for 20-24 h and the test compound (suspended in 5% acacia solution) were orally administered (5 ml/kg). After sample administration 30 min, 20 % sucrose was administered (5 ml/kg, *p.o.*), except in normal rat group, water was given orally instead of sucrose solution. Blood samples (*ca.* 0.4 ml) were collected from the infraorbital venous plexus under ether anesthesia 0.5, 1 and 2 h after the oral administration of sucrose. The blood was centrifuged and serum glucose levels were determined by the glucose oxidase method (Glucose C-II test Wako).

Statistics:

Values were expressed as means±S.E.M. One-way analysis of variance (ANOVA) followed by Dunnett's test was used for statistical analysis.

Results and Discussion

As shown in Table 1, the methanolic extract of *Betula alnoides* showed inhibitory effects on the enzyme α -glucosidase activity, sucrase and maltase in a concentration-related manner. The inhibitory effects on maltase revealed more effectiveness than sucrase at the same dose. The extract (25 μ g/ml) exhibited the inhibition of rat intestinal sucrase and maltase about 48% and 95% with the IC₅₀ values of 26.5 and 5.4 μ g/ml, respectively. The standard compound, acarbose also exhibited the inhibition of α -glucosidase, sucrase and maltase with IC₅₀ values of 1.0 and 1.1 μ g/ml, respectively. Furthermore, the extract (100-400 mg/kg, *p.o.*) significantly inhibited the increase of blood glucose levels after orally administered 20% sucrose (5ml/kg, *p.o.*) at 30 and 60 min, but did not any effects at 120 min. Similarity, a commercial diabetic medicine, acarbose (5 mg/kg, *p.o.*) also show strongly reduced elevation of blood glucose levels (*p*<0.01) at 30 min, while administration of acarbose at dose 10 mg/kg, (*p.o.*)

significantly inhibited the blood glucose elevation both 30 and 60 min.

It is generally recognized that α -glucosidase plays an important role in carbohydrate metabolism by hydrolyzing the non-reducing end of saccharide polymers to release α -glucose. The inhibition of this enzyme leads to impaired glucose absorption and decrease in postprandial blood glucose levels [6,7]. Many medicinal plant species have an α -glucosidase inhibitory activity [17] and composed of various active components such as alkaloid, stilbenoids, triterpene, flavonoids, flavanone glucosides, and anthrones [5,1]. In addition, It has been reported that some phenolic compounds, possess antioxidant activity, have also been found to inhibit α -glucosidase and it has a positive correlation between α -glucosidase inhibition and antioxidant activity

[11,8]. Pongpiriyadacha et al. [15] reported that the 80% methanolic extract of *Betula alnoides* showed strong antioxidant activity. Therefore, the inhibitory activity of this methanolic extract might be, at least in part, mediated via antioxidant properties. α -Glucosidase inhibitor, acarbose, is shown to control postprandial blood glucose level and useful for the treatment of hyperglycemia, because it is a high degree of safety with regard to severe side effects and complications, and effects on body weight [16]. Ghadyale et al. [2] reported that the effective control of postprandial glucose level through the inhibition of intestinal α -glucosidase. The methanolic extract of *Betula alnoides* showed potent inhibition of the elevation of blood glucose level. Hence, this action, might be, associated with the inhibitory activity of the enzyme, α -glucosidase, both sucrose and maltase.

Table 1: Inhibitory Effects of 80% Methanolic Extract from *Betula alnoides* on Rat Intestinal α -Glucosidase Activity

Treatment	Sample conc. (μ g/ml)	Inhibition (%)		IC ₅₀ (μ g/ml)	
		Sucrase ^{a)}	Maltase ^{b)}	Sucrase	Maltase
80% MeOH extract	3.125	15.5	34.0	26.5	5.4
	6.25	22.0	65.2		
	12.50	37.2	79.9		
	25	48.6	95.2		
	50	70.4	–		
	100	84.5	–		
Acarbose	0.5	22.3	19.8	1.0	1.1
	1.0	48.5	46.7		
	2.0	91.2	88.3		

α -Glucosidase inhibitory activities were monitored as described in the method. a) 74 mM sucrose as substrate, b) 74 mM maltose as substrate

Table 2: Inhibitory Effects of 80% Methanolic Extract from *Betula alnoides* on Increase of Blood Glucose Levels in Sucrose-Loaded Rats

Treatment	Dose (mg/kg, p.o.)	N	Blood glucose levels (mg/dl)		
			30 min	60 min	120 min
Normal	–	7	77.8 \pm 4.1**	90.5 \pm 5.0**	95.5 \pm 3.4**
Control	–	7	183.4 \pm 2.6	166.7 \pm 3.5	128.6 \pm 2.8
80% MeOH extract	100	6	141.4 \pm 4.2**	135.5 \pm 6.1	127.2 \pm 2.4
	200	6	129.2 \pm 3.1**	131.4 \pm 3.2**	122.7 \pm 5.3
	400	6	107.8 \pm 7.5**	118.3 \pm 3.3**	118.8 \pm 7.3
Acarbose	5	6	134.2 \pm 1.2**	148.4 \pm 2.3	119.7 \pm 3.8
	10	6	107.4 \pm 5.1**	120.7 \pm 2.6**	108.2 \pm 5.5

Mean \pm S.E.M., Significantly different from the control group, ** p <0.01. For statistical analysis, one-way analysis of variance followed by Dunnett's test was used.

Conclusion:

The results of this study have shown that the methanolic extract of *Betula alnoides* possess significant inhibition of intestinal α -glucosidase and lowering of blood glucose levels in sucrose-loaded rats. In our best knowledge, this is the first report of its activity which different from traditional use and support for the Thai medicinal plants used for tonic and longevity tend to be benefited for the treatment of diabetes mellitus. It is suggested that *Betula alnoides* can be considered as a good source of natural products and promising potential for the management of type II diabetes mellitus. In addition, the pharmacologically active compounds and the possible mechanism of action are needed to clarify.

Acknowledgement

The authors wish to express our sincere gratitude to the Pharmacognosy Laboratory, Kyoto Pharmaceutical University, Japan, for bioassay guidelines and animals experimental training and grateful to The National Research Council of Thailand (NRCT) for financial support.

Reference

1. Benalla, W., S. Bellahcen and M. Bnouham, 2010. Antidiabetic medicinal plants as a source of alpha glucosidase. Curr. Diabetes Rev., 6: 247-254.

2. Ghadyale, V., S. Takalihar, V. Haldavnekar and A. Arvindekar, 2012. Effective control of postprandial glucose level through inhibition of intestinal alpha glucosidase by *Cymbopogon martini* (Roxb.). Evid-Based. Complement. Alternat. Med. Pubmed. doi:10.1155/2012/372909.
3. International Diabetes Federation. IDF Diabetes Atlas. 5th ed. Brussel, Belgium: International Diabetes Federation. 2011. [Access June 16, 2013] Available from: <http://www.idf.org/diabetesatlas>.
4. Institute of Medical Research and Technology Assessment (IMRTA). Fact sheet: diabetes, hypertension, and their complications. 2013.[Accessed July 1, 2013]. Available from:http://www.dms.moph.go.th/imrta/images/data/doc_dm_ht.pdf
5. Jung, M., M. Park, H.C. Lee, Y.H. Kang, E.S.Kang and S.K. Kim. 2006. Antidiabetic agents from medicinal plants. *Curr. Med. Chem*, 13: 1203-1218.
6. Kim, K.Y., K.A. Nam, H. Kurihara and S.M. Kim, 2008. Potent α -glucosidase inhibitors purified from the red alga *Grateloupia elliptica*. *Phytochem.*, 69: 2820-2825.
7. Lebovitz, H.E., 1997. α -Glucosidase inhibitors. *Endocrinol. Metab. Clin. North.*, 26: 539-551.
8. Mai, T.T., N.N. Thu, P.G. Tien and N.V. Chuyen, 2007. Alpha-glucosidase inhibitory and antioxidant activities of Vietnamese edible plants and their relationships with polyphenol contents. *J. Nutri. Sci. Vitaminol.*, 53: 267-276.
9. Matsuda, H., T. Morikawa, F. Xu, K. Ninomiya and M. Yoshikawa, 2004. New isoflavones and Pterocarpane with hepatoprotective activity from the stems of *Erycibe expansa*. *Planta Med.*, 70: 1201-1209.
10. Matsuda, H., Y. Asao, S. Nakamura, M. Hamao, S. Sugimoto, M. Hongo, Y. Pongpiriyadacha, M. Yoshikawa 2009. Antidiabetogenic constituents from the Thai traditional medicine *Cotylelobium melanoxylon*. *Chem Pharm. Bull.*, 57: 487-494.
11. Matsumoto, M., F. Ishigaki, A. ishigaki, H. Iwashina and Y. Hara, 1993. Reduction of blood glucose levels by tea catechin, *Biosci. Biotech. Biochem.*, 57: 525-527.
12. Nanthawan, B. and C. Oranuch. 1996. Samunprai Maipunban 1. Prachachon Publishing, Bangkok.
13. Nishioka, T., J. Kowabata and Y. Aoyama, 1998. Baicalein, an alpha-glucosidase inhibitor from *Scutellaria baicalensis*. *J. Nat. Prod.*, 61: 1413-1415.
14. Phan, M.G., T.T. Chinh Truong, T.S. Phan, K. Matsunami and H. Otsuka, 2011. Three new dammarane glycosides from *Betula alnoides*. *Phytochem. Lett.*, 4: 179-182.
15. Pongpiriyadacha, Y., P. Nuansrithong and T. Chantip, 2013. Free radical scavenging potential and xanthine oxidase inhibitor of water extract from tonic and longevity medicinal plants. *Proceeding of the International Conference, Burapha University*, pp: 903-914.
16. Rosak, C. and G. Mertes, 2012. Critical evaluation of the role of acarbose in the treatment of diabetes: patient considerations. *Diabetes Metab. Syndr. Obes.*, 5: 357-367.
17. Shai, L.J., P. Masoko, M.P.Mokgato, S.R. Magano, A.M. Mogale, N. Baoduo and J.N. Eloff., 2010. Yeast alpha glucosidase inhibitory and antioxidant activities of six medicinal plants collected in Phalaborwa, South Africa. *South African Journal of Botany*, 76: 465-470.
18. Temkitthawon, P., J. Viyoch, N. Limpeanchob, W. Ponggamornkul, C. Sirikul, A. Kumpila, K. Suwanborirux and K. Ingkaninan, 2008. Screening for phosphodiesterase inhibitory activity of Thai medicinal plants. *J Ethnopharmacol.*, 119: 214-217.
19. Yoshikawa, M., T. Morikawa, H. Matsuda, G. Tanabe and O. Muraoka, 2002. Absolute stereostructure of potent α -glucosidase inhibitor, salacinol, with unique thiosugar sulfonium sulfate inner salt structure from *Salacia reticulata*. *Bioorg. Med. Chem*, 10: 1547-1554.
20. Yoshikawa, M., Y. Pongpiriyadacha, A. Kishi, T. Kageura, T. Wang, T. Morikawa and H. Matsuda, 2003. Biological activities of *Salacia chinensis* originating in Thailand: The quality evaluation guided by α -glucosidase inhibitory activity., *Yakugaku Zasshi.*, 123: 871-880.
21. Yoshikawa, M., F. Xu, T. Morikawa, Y. Pongpiriyadacha, S. Nakamura, Y. Asao, A. Kumahara and H. Matsuda, 2007. Medicinal flowers. XII. New spirostane-type steroid saponins with antidiabetogenic activity from *Borassus flabellifer.*, *Chem. Pharm. Bull.*, 55: 308-316.