European Review for Medical and Pharmacological Sciences 2011; 15: 803-808

α-glucosidase inhibitory activity and lipid-lowering mechanisms of *Moringa oleifera* leaf extract

S. ADISAKWATTANA, B. CHANATHONG

Program in Nutrition and Dietetics and The Medical Food Research and Development Center, Department of Transfusion Medicine, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok (Thailand)

Abstract. – *Objectives and Materials and Methods:* **Medicinal plants constitute an important source of potential therapeutic agents for diabetes and hyperlipidemia. The purpose of present study was to investigate the effect of leaf extract of *Moringa oleifera* on inhibition of α-glucosidase and pancreatic α-amylase related to diabetes mellitus. Moreover, the study was also determined in vitro bile acid binding capacity as well as inhibition of cholesterol micellization, pancreatic lipase, and cholesterol esterase activity.**

Results: **The phytochemical analysis indicated that total phenolic, flavonoid, and condensed tannin contents in the extract were 45.21±0.96 mg gallic acid equivalents/g extract, 15.39±0.58 mg catechin equivalents/g extract, and 4.90±0.20 catechin equivalents/g extract, respectively. In addition, the extract contained a specific inhibitor of intestinal sucrase than intestinal maltase with IC_{50} value of 0.78±0.21 mg/ml, whereas it slightly inhibited pancreatic α-amylase and pancreatic cholesterol esterase. However, the extract had no inhibitory activity against pancreatic lipase. Furthermore, taurodeoxycholic acid, taurodeoxycholic acid, and glycodeoxycholic acid were bound to the extract (1 mg/ml) with a degree of 26.90±0.37%, 21.78±0.68%, and 22.59±1.02%, respectively. Finally, the extract (10 mg/ml) markedly inhibited the formation of cholesterol micelle about 40.22±2.64%.**

Conclusion: **Our results indicated that the leaf extract of *Moringa oleifera* may be used for the control of blood glucose and lipid concentration and prevention of hyperglycemia and hyperlipidemia.**

Key Words:

Moringa oleifera, Bile acid binding, Cholesterol micellization, Pancreatic cholesterol esterase, Pancreatic lipase, α-glucosidase.

Introduction

Diabetes mellitus is a group of metabolic disorders which result from defects in both regulations of insulin secretion and/or insulin action. Diabetes mellitus is associated with a reduced quality of life and increased risk of mortality and morbidity. Hyperlipidemia is a group of metabolic disorders caused by hypertriglyceride and/or hypercholesterol in blood circulation. The long-term hyperglycemia and hyperlipidemia is an important contributor to develop the progression of micro- and macro-vascular complications including microangiopathy, cardiovascular, cerebrovascular, and metabolic syndrome diseases. The prevalence of hyperlipidemic and diabetic patients has dramatically increased worldwide due to a modern lifestyle and an increase of consumption of high-carbohydrate and high-fat diets[1-3]. One of the most important strategies for prevention of postprandial hyperglycemia and hyperlipidemia is inhibition of carbohydrate digestive enzymes (α-glucosidase and α-amylase), inhibition of fat digestion and absorption[4]. World ethnobotanical information reported that a number of herbal medicines from plants and vegetables are used for the control of hyperglycemia and hyperlipidemia[5]. Many herbal medicines have potential and opportunity for the development of newer therapeutics for anti-hyperglycemic and anti-hyperlipidemic agents from natural resources[6-8].

Moringa oleifera (Ma-rum) is the most widely cultivated species of a monogeneric family, the Moringaceae, which includes 13 species of trees and shrubs distributed in India, Sri Lanka, Pakistan, Bangladesh North-eastern and South-western Africa, Arabia, and Thailand. *Moringa oleifera* has shown various beneficial pharmacological effects in prevention or treatment of a variety of diseases such as anti-diabetic[9], anti-microbial[10], anti-inflammatory[11], anti-oxidant properties[12]. It has recently reported that the leaf extract of *Moringa oleifera* decreases the blood glucose level in normal and streptozotocin (STZ)-induced diabetic rats[9]. Moreover, the ad-

Corresponding Author: Sirichai Adisakwattana, Ph.D.; e-mail: Sirichai.a@chula.ac.th

ministration of the crude leaf extract of *Moringa oleifera* decreases cholesterol levels in serum, liver, and kidney in the rat fed with a high-fat diet[13].

However, anti-diabetic and anti-hyperlipidemic effects of the leaf extract of *Moringa oleifera* through the inhibition of carbohydrate digestive enzymes and the inhibition of lipid digestion and absorption are not well known. Therefore, the aim of the present study was to investigate the effect of the leaf extract of *Moringa oleifera* on inhibition of α-glucosidase, pancreatic α-amylase, pancreatic lipase, and pancreatic cholesterol esterase activities. Moreover, inhibition of cholesterol micellization formation, and bile acid binding capacity of the extract were also determined.

Material and Methods

Chemicals

Folin-Ciocalteu, quercetin, catechin, gallic acid, rat intestinal acetone powder, porcine pancreatic α-amylase, vanillin, aminoguanidine, 3,5-dinitrosalicylic acid, glucose oxidase kits, *p*-nitrophenylbutylrate (*p*-NPB), oleic acid, phosphatidylcholine, glycodeoxycholic acid, taurodeoxycholic acid, taurocholic acid, porcine cholesterol esterase, porcine pancreatic lipase were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Cholesterol test kits were purchased from HUMAN GmbH Co. (Wiesbaden, Germany). Total bile acid kit was purchased from Bio-Quant Co. (San Diego, CA, USA). All others chemicals used were of analytical grade.

Preparation of the Extract

The dried leaves were extracted with distilled water at 90°C for 2 h. The samples were filtered through Whatman 70 mm filter paper. The solution was then centrifuged at 8,000 rpm for 10 min. The aqueous solution was concentrated before being charged in the spray-dryer.

Phytochemical Analysis

Total phenolic content was determined with the Folin-Ciocalteu's reagent[14]. Estimation of total flavonoid content was assayed spectrophotometrically according to a previous report[15]. Condensed tannin was performed by using vanillin method according to a previous literature[16].

α-Glucosidase Inhibition Assay

α-Glucosidase inhibitory activity was performed according to our previous report[8]. Briefly, rat intestinal acetone powder was homogenized in 0.9% NaCl solution. After centrifugation, 10 µl of the supernatant was incubated with 70 µl of substrate solution (37 mM maltose, 54 mM sucrose), and 20 µl of extract at various concentrations in 0.1 M phosphate buffer pH 6.9 at 37°C for 30 min (maltase assay) and 60 min (sucrase assay). The mixtures were suspended in boiling water for 10 min to stop the reaction. The concentrations of glucose released from the reaction mixtures were determined by glucose oxidase method.

Pancreatic α-Amylase Inhibition Assay

The pancreatic α-amylase inhibition assay was performed according to our previous reports[17]. The extract was added to solution containing in starch (1 g/l) and phosphate buffer (pH 6.9). The reaction was initiated by adding amylase (3 U/ml) to the mixture to a final volume of 500 µl. After 10 min the reaction was stopped by adding 500 µl dinitrosalicylic (DNS) reagent to the reaction mixture. The mixtures were heated at 100°C for 10 min and 500 µl of 40% potassium sodium tartrate solution was added to the mixtures. The absorbance was recorded at 540 nm using spectrophotometer (Biotek, Vinooski, VT, USA).

Pancreatic Lipase Inhibition

Pancreatic lipase activity was slightly modified according to previous method[18]. The extract (25 µl) was mixed with 25 µl of pancreatic lipase solution (50 U/ml) in the well of a microplate. Fifty microliters of oleate ester of fluorescent 4-methylumbelliferone (4MUO) solution (0.1 mM) dissolved in phosphate-buffered saline (PBS) was then added to initiate the enzyme reaction. After incubation at 37°C for 20 min, 100 µl of 0.1 M sodium citrate (pH 4.2) was added to stop the reaction. The amount of 4-methylumbelliferone released by lipase was measured using a fluorescence microplate reader (Biotek, Vinooski, VT, USA) at an excitation wavelength of 320 nm and an emission wavelength of 450 nm.

Pancreatic Cholesterol Esterase Inhibition

The pancreatic cholesterol esterase inhibition was performed spectrophotometrically[19] at 25°C. The extract was incubated with mixtures containing 5.16 mM taurocholic acid, 0.2 mM *p*-NPB in

100 mM sodium phosphate buffer, 100 mM NaCl, pH 7.0. The reaction was initiated by adding porcine pancreatic cholesterol esterase (1 µg/ml). After incubation for 5 min at 25ºC, the mixtures were measured the absorbance at 405 nm.

Cholesterol Micellization

Artificial micelles were prepared according to previous method with minor modifications[20]. In brief, the mixtures (2 mM cholesterol, 1 mM oleic acid, and 2.4 mM phosphatidylcholine) were dissolved in methanol and dried under nitrogen before adding 15 mM PBS containing 6.6 mM taurocholate salt, at pH 7.4. The suspension was sonicated twice for 30 min using a sonicator (Crest Ultrasonics, Ewing, NJ, USA). The micelle solution was incubated overnight at 37°C. Thereafter, the extract or equivalent PBS as control were added to the mixed micelle solution and incubated for further 2 h at 37°C and centrifuged at 16,000 rpm for 20 min. The supernatant was collected for the determination of cholesterol by using total cholesterol test kits (HUMAN GmbH Co., Wiesbaden, Germany).

Bile Acid Binding

The bile acid binding assay was slightly modified according to previous method[21]. Taurocholic acid, glycodeoxycholic acid and taurodeoxycholic acid were used as bile acid in this experiment. Briefly, the extract (1 mg/ml) was incubated with each bile acid (2 mM) containing in 0.1 M phosphate buffer-saline (pH 7) at 37°C for 90 min. The mixtures were filtered through 0.2 µm filter and frozen at –20°C until analysis was carried out. The bile acid concentration was analyzed spectrophotometrically at 540 nm by using bile-acid analysis kit (Bio-Quant Co., San Diego, CA, USA).

Statistical Analysis

The IC_{50} values were calculated from plots of log concentration of inhibitor concentration versus percentage inhibition curves by using the Sigma Plot 10.0 software (Systat Software Inc., San Jose, IL, USA).Values were expressed as mean ± standard error (SE) for $n = 3$.

Results

The amount of total phenolic compounds, flavonoids, and condensed tannins in the extract are listed in Table I. Total phenolic compound in the extract was 45.21±0.96 mg gallic acid equivalent/g extract. It was found that the content of flavonoid in the extract was 15.39±0.58 mg quercetin equivalent/g extract. Furthermore, the content of condensed tannins in the extract was 4.90±0.20 mg catechin equivalent/g extract.

As shown in Table II, the IC_{50} value of the extract was 0.98±0.21 mg/ml for intestinal sucrase, whereas the extract (5 mg/ml) inhibited intestinal maltase by 22.3± 2.8%. The findings indicated that the extract was more specific inhibitor of intestinal sucrase than intestinal maltase. In addition, the extract (5 mg/ml) was less potent inhibitor against pancreatic α-amylase (% inhibition = 5.3±0.5%). The extract inhibited pancreatic cholesterol esterase activity with the IC_{50} value of 4.34±0.25 mg/ml. However, it found that the extract had no inhibitory activity on pancreatic lipase.

The percentage inhibition of formatting cholesterol micellization of the extract (10 mg/ml) was 40.22±2.64%. The percentage bile acid binding by the extract (1 mg/mL) is shown in Figure 1. The results showed that the percentage taurodeoxycholic acid, taurodeoxycholic acid, and glycodeoxycholic acid binding were 26.90±0.37, 21.78±0.68, and 22.59±1.02, respectively.

Table I. Total phenolic, flavonoid, and condensed tannin contents of *Moringa oleifera* leaf extract.

	Phytochemical analysis		
	Total phenolics (mg/g extract)	Flavonoids (mg/g extract)	Condensed tannins (mg/g extract)
Moringa oleifera	45.21 ± 0.96	15.39 ± 0.58	4.90 ± 0.20

Results are expressed as means ± S.E.M., n = 3.

Table II. The inhibitory effect of *Moringa oleifera* leaf extract on pancreatic α-amylase, intestinal α-glucosidase (maltase and sucrase), pancreatic lipase, and pancreatic cholesterol esterase.

	IC_{50} values (mg/ml)				
	α-Amylase	Maltase	Sucrase	Pancreatic lipase	Pancreatic cholesterol esterase
Moringa oleifera	> 5.00	> 5.00	0.98 ± 0.21	N.I.	4.34 ± 0.25

Results are expressed as means ± S.E.M., n = 3, N.I. = No inhibition.

Discussion

This is the first study to investigate the effect of leaf extract of *Moringa oleifera* on inhibition of α-glucosidase, pancreatic α-amylase related to anti-hyperglycemic activity. Our findings show that the extract markedly inhibits intestinal sucrase activity. The inhibition of α-glucosidase and pancreatic α-amylase activity results in delaying carbohydrate digestion to absorbable monosaccharide, causing reduction of postprandial hyperglycemia. It has been established that reduction of postprandial hyperglycemia contributes to decrease in hemoglobin A_{1C} (HbA_{1C}) in diabetic patients, consequently, reduces the appearance of chronic vascular complications[22]. The number of patients with pre-diabetic condition is currently increasing in worldwide[23]. An intake of *Moringa oleifera* leaf may delay glucose absorption to blood circulation in pre-diabetic patients that help to prevent the development of type 2 diabetes.

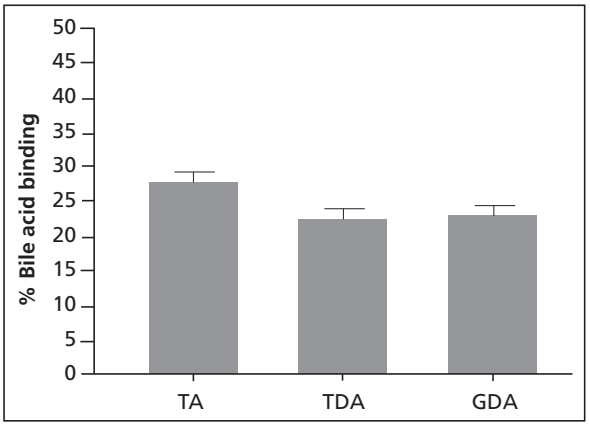

Figure 1. The effect of *Moringa oleifera* leaf extract on taurocholic acid (TA), taurodeoxycholic acid (TDA) and glycodeoxycholic acid (GDA) binding. Results are expressed as means ± S.E.M., n = 3.

Recent evidence strongly supports that aggressive delaying dietary fat digestion and absorption is one of successful strategy for treatment of hyperlipidemia[24]. In the present study, *Moringa oleifera* leaf extract was investigated anti-hyperlipidemic properties through inhibition of pancreatic lipase, cholesterol micellization formation, pancreatic cholesterol esterase, and bile acid binding. Pancreatic lipase and cholesterol esterase plays a pivotal role in hydrolyzing dietary triglyceride and cholesterol esters[25,26]. The hydrolysis of cholesterol ester is catalyzed by pancreatic cholesterol esterase which liberates free cholesterol in the small intestine. Moreover, pancreatic cholesterol esterase plays an important role in regulating the incorporation of cholesterol into mixed micelles[27]. Therefore, inhibition of cholesterol esterase activity is anticipated to limit the absorption of dietary cholesterol, resulting in delayed cholesterol absorption[28]. Consequently, the principal steps in the absorption of dietary cholesterol are involved in the formation of micellar solubilization, and absorption in the proximal jejunum[29]. The reduction of cholesterol absorption by inhibiting this mechanism is a new target site of intervention for treatment of hyperlipidemia and obesity[20]. Moreover, binding bile acids by forming insoluble complexes and increasing their fecal excretion have been hypothesized as a possible mechanism of lowering plasma cholesterol level[30]. In this study, we firstly present anti-hyperlipidemic mechanisms of *Moringa oleifera* leaf extract by inhibiting cholesterol esterase activity as well as inhibiting the formation of cholesterol micellization and bile acid binding. As previous studies mentioned above, it suggests that anti-hyperlipidemic activities of *Moringa oleifera* leaf extract may be involved in these mechanisms.

Published research suggests that there is a direct relationship between of phenolic compound,

flavonoids, and condensed tannin in the extract and the ability to inhibit α-glucosidase and α-amylase activities[8,31,32]. It has been reported that phenolic compounds show the ability to inhibit the formation of cholesterol micelles[33]. In this study, it can be hypothesized that phenolic compounds, flavonoids, and condensed tannins in *Moringa oleifera* may play important role for inhibition of α-glucosidase, pancreatic cholesterol esterase activity, as well as bile acid binding and inhibiting the formation of cholesterol micellization. Further studies are needed to clarify this important hypothesis.

In conclusion, our results have demonstrated the beneficial biological effects of *Moringa oleifera* by inhibiting α-glucosidase, cholesterol esterase, formation of cholesterol micellization, and bile acid binding. A daily intake of this plant may help to prevent hyperglycemia and hyperlipidemia.

Acknowledgements

This research was fully supported by The Chaipattana Foundation, Thailand. The Author gratefully acknowledges The Medical Food Research and Development Center, which has been financially and institutionally supported by Chulalongkorn University.

References

1) Jacobson TA, Miller M, Schaefer EJ. Hypertriglyceridemia and cardiovascular risk reduction. Clin Ther 2007; 29: 763-777.

2) Misra A, Khurana L. Obesity and the metabolic syndrome in developing countries. J Clin Endocrinol Metab 2009; 93(11 Suppl 1): S9-S30.

3) Bessesen DH, Bull S, Cornier MA. Trafficking of dietary fat and resistance to obesity. Physiol Behav 2008; 94: 681-688.

4) Raptis SA, Dimitriadis GD. Oral hypoglycemic agents: insulin secretagogues, alpha-glucosidase inhibitors and insulin sensitizers. Exp Clin Endocrinol Diabetes 2001; 109(Suppl 2): S265-287.

5) Dahanukar SA, Kulkarni RA, Rege NN. Pharmacology of medicinal plants and natural products. Indian J Pharmacol 2000; 32: S81-S118

6) Zhang J, Kang MJ, Kim MJ, Kim ME, Song JH, Lee YM, Kim JI. Pancreatic lipase inhibitory activity of taraxacum officinale in vitro and in vivo. Nutr Res Pract 2008; 2: 200-203.

7) Kurihara H, Shibata H, Fukui Y, Kiso Y, Xu JK, Yao XS, Fukami H. Evaluation of the hypolipemic property of Camellia sinensis Var. ptilophylla on postprandial hypertriglyceridemia. J Agric Food Chem 2006; 54: 4977-4981.

8) Adisakwattana S, Charoenlertkul P, Yibchok-Anun S. alpha-Glucosidase inhibitory activity of cyanidin-3-galactoside and synergistic effect with acarbose. J Enzyme Inhib Med Chem 2009; 24: 65-69.

9) Jaiswal D, Kumar Rai P, Kumar A, Mehta S, Watal G. Effect of *Moringa oleifera* Lam. leaves aqueous extract therapy on hyperglycemic rats. J Ethnopharmacol 2009; 123: 392-396.

10) Cáceres A, Cabrera O, Morales O, Mollinedo P, Mendia P. Pharmacological properties of *Moringa oleifera*. 1: Preliminary screening for antimicrobial activity. J Ethnopharmacol 1991; 33: 213-216.

11) Mahajan SG, Banerjee A, Chauhan BF, Padh H, Nivsarkar M, Mehta AA. Inhibitory effect of n-butanol fraction of *Moringa oleifera* Lam. seeds on ovalbumin-induced airway inflammation in a guinea pig model of asthma. Int J Toxicol 2009; 28: 519-527.

12) Atawodi SE, Atawodi JC, Idakwo GA, Pfundstein B, Haubner R, Wurtele G, Bartsch H, Owen RW. Evaluation of the polyphenol content and antioxidant properties of methanol extracts of the leaves, stem, and root barks of *Moringa oleifera* Lam. J Med Food 2010; 13: 710-716.

13) Ghasi S, Nwobodo E, Ofili JO. Hypocholesterolemic effects of crude extract of leaf of *Moringa oleifera* Lam in high-fat diet fed wistar rats. J Ethnopharmacol 2000; 69: 21-25.

14) Adisakwattana S, Jiphimai P, Prutanopajai P, Chanathong B, Sapwarobol S, Ariyapitipan T. Evaluation of alpha-glucosidase, alpha-amylase and protein glycation inhibitory activities of edible plants. Int J Food Sci Nutr 2010; 61: 295-305.

15) Sriplang K, Adisakwattana S, Rungsipipat A, Yibchok-anun S. Effects of Orthosiphon stamineus aqueous extract on plasma glucose concentration and lipid profile in normal and streptozotocin-induced diabetic rats. J Ethnopharmacol 2002; 109: 510-514.

16) Toda S. Antioxidative effects of polyphenols in leaves of Houttuynia cordata on protein fragmentation by copper-hydrogen peroxide in vitro. J Med Food 2005; 8: 266-268.

17) Adisakwattana S, Chantarasinlapin P, Thammarat H, Yibchok-Anun S. A series of cinnamic acid derivatives and their inhibitory activity on intestinal alpha-glucosidase. J Enzyme Inhib Med Chem 2009; 24: 1194-1200.

18) Sugiyama H, Akazome Y, Shoji T, Yamaguchi A, Yasue M, Kanda T, Ohtake Y. Oligomeric procyanidins in apple polyphenol are main active components for inhibition of pancreatic lipase and triglyceride absorption. J Agric Food Chem 2007; 55: 4604-4609.

19) Pietsch M, Gütschow M. Synthesis of tricyclic 1,3-oxazin-4-ones and kinetic analysis of cholesterol esterase and acetylcholinesterase inhibition. J Med Chem 2005; 48: 8270-8288.

20) Kirana C, Rogers PF, Bennett LE, Abeywardena MY, Patten GS. Naturally derived micelles for rapid in vitro screening of potential cholesterol-lowering bioactives. J Agric Food Chem 2005; 53: 4623-4627.

21) Yoshie-Stark Y, Wäsche A. *In vitro* binding of bile acids by lupin protein isolates and their hydrolysate. Food Chem 2004; 88: 179-184.

22) Baron AD. Postprandial hyperglycaemia and alpha-glucosidase inhibitors. Diabetes Res Clin Pract 1998; 40(Suppl): S51-55.

23) Abdul-Ghani MA, DeFronzo RA. Pathophysiology of prediabetes. Curr Diab Rep 2009; 9: 193-199.

24) Ros E. Intestinal absorption of triglyceride and cholesterol. Dietary and pharmacological inhibition to reduce cardiovascular risk. Atherosclerosis 2000; 151: 357-379.

25) Birari RB, Bhutani KK. Pancreatic lipase inhibitors from natural sources: unexplored potential Drug Discov Today 2007; 12: 879-889

26) Brodt-Eppley J, White P, Jenkins S, Hui DY. Plasma cholesterol esterase level is a determinant for an atherogenic lipoprotein profile in normolipidemic human subjects. Biochim Biophys Acta1995; 1272: 69-72.

27) Myers-Payne SC, Hui DY, Brockman HL, Schroeder F. Cholesterol esterase: a cholesterol transfer protein. Biochemistry 1995; 34: 3942-3947.

28) Heidrich JE, Contos LM, Hunsaker LA, Deck LM, Vander Jagt DL. Inhibition of pancreatic cholesterol esterase reduces cholesterol absorption in the hamster. BMC Pharmacol 2004; 4: 5.

29) Hui DY, Howles PN. Molecular mechanisms of cholesterol absorption and transport in the intestine. Semin Cell Dev Biol 2005; 16: 183-192.

30) Insull W Jr. Clinical utility of bile acid sequestrants in the treatment of dyslipidemia: a scientific review. South Med J 2006; 99: 257.

31) Mai TT, Thu NN, Tien PG, Van Chuyen N. Alpha-glucosidase inhibitory and antioxidant activities of Vietnamese edible plants and their relationships with polyphenol contents. J Nutr Sci Vitaminol 2007; 53: 267-276.

32) Lee YA, Cho EJ, Tanaka T, Yokozawa T. Inhibitory activities of proanthocyanidins from persimmon against oxidative stress and digestive enzymes related to diabetes. J Nutr Sci Vitaminol 2007; 53: 287-292.

33) Vermeer MA, Mulder TP, Molhuizen HO. Theaflavins from black tea, especially theaflavin-3-gallate, reduce the incorporation of cholesterol into mixed micelles. J Agric Food Chem 2008; 56: 12031-12036.