ฤทธิ์ลดระดับน้ำตาลของสารสกัดมาตรฐานบัวบก อีซีเอ 233 ในการศึกษาแบบนอกกายและแบบในกายหนอนไหมไทย

นางสาวปิยนุช วรฉัตร

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาเภสัชวิทยา ภาควิชาเภสัชวิทยาและสรีรวิทยา
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2555

GLUCOSE LOWERING EFFECT OF STANDARDIZED EXTRACT OF *CENTELLA ASIATICA* ECa 233 *IN VITRO* AND *IN VIVO* THAI SILKWORM MODEL

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A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science in Pharmacy Program in Pharmacology

Department of Pharmacology and Physiology

Faculty of Pharmaceutical Sciences

Chulalongkorn University

Academic Year 2012

Thesis Title	GLUCOSE LOWERING EFFECT OF STANDARDIZED EXTRACT OF *CENTELLA ASIATICA* ECa 233 *IN VITRO* AND *IN VIVO* THAI SILKWORM MODEL
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Field of Study	Pharmacology
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ปิยนุช วรฉัตร : ฤทธิ์ลดระดับน้ำตาลของสารสกัดมาตรฐานบัวบก อีซีเอ 233 ในการศึกษาแบบนอกกายและแบบในกายหนอนไหมไทย (GLUCOSE LOWERING EFFECT OF STANDARDIZED EXTRACT OF *CENTELLA ASIATICA* ECa 233 *IN VITRO* AND *IN VIVO* THAI SILKWORM MODEL) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : อ.ดร.สันทัด จันทร์ประภาพ, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม : อ.ดร.ชนิดา พลานุเวช, 93 หน้า.

การศึกษานี้มีวัตถุประสงค์เพื่อทดสอบฤทธิ์ลดระดับน้ำตาลของสารสกัดมาตรฐานบัวบก อีซีเอ 233 (ECa 233), asiaticoside และ madecassoside ผ่านกลไกการยับยั้งเอนไซม์แอลฟากลูโคซิเดส ทั้งแบบนอกกายและแบบในกายสัตว์ทดลองโดยใช้แบบจำลองหนอนไหมไทย การศึกษาแบบนอกกายใช้เอนไซม์แอลฟากลูโคซิเดสจากยีสต์และลำไส้หนูเพื่อประเมินการยับยั้ง เอนไซม์ดังกล่าว ของสารทดสอบทั้ง 3 ชนิดและหาค่า IC_{50} เปรียบเทียบกับ acarbose ส่วนการศึกษาแบบในกายใช้หนอนไหมไทยเพื่อศึกษาความเป็นพิษและหาค่า LD_{50} หลังจากนั้นทำการทดสอบฤทธิ์ลดระดับน้ำตาลโดยการเหนี่ยวนำให้เกิดภาวะน้ำตาลในเลือดสูงด้วยการให้อาหารสังเคราะห์ที่ผสมน้ำตาลกลูโคส 10% หรือซูโครส 10% หรือมอลโตส 10% เปรียบเทียบ กับอินซูลิน ขนาด 3.5 มก./มล.โดยฉีดเข้ากระแสเลือด , acarbose ขนาด 20 มก./มล. และสารทดสอบทั้ง 3 ชนิดโดยฉีดเข้า midgut หรือฉีดเข้ากระแสเลือด ผลการศึกษาพบ ว่าค่า IC_{50} ในการยับยั้งเอนไซม์แอลฟากลูโคซิเดสจากยีสต์ของ acarbose, ECa 233, asiaticoside และ madecassoside เท่ากับ 0.0008, >500, 92.42 และ 68.89 มก./มล. ตามลำดับ และค่า IC_{50} ในการยับยั้งเอนไซม์แอลฟากลูโคซิเดสจากลำไส้หนูเท่ากับ 0.005, 205.61, 21.81 และ 50.52 มก./มล. ตามลำดับ ค่า LD_{50} ของ ECa 233, asiaticoside และ madecassoside จากการฉีดเข้า midgut เท่ากับ 178.51, >125 และ >250 มก./มล. ตามลำดับและค่า LD_{50} จากการฉีดเข้ากระแสเลือดเท่ากับ 46.92, >125 และ 174.58 มก./มล.ตามลำดับ การทดสอบฤทธิ์ลดระดับน้ำตาลในเลือดหนอนไหมจากการให้อาหารที่ผสมกลูโคส 10%, ซูโครส 10% และมอลโตส 10% พบว่าอินซูลินสามารถลดระดับน้ำตาลได้ทุกชนิดอย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับกลุ่มควบคุม ($P< 0.05$) ส่วน acarbose สามารถลดระดับน้ำตาลได้เฉพาะอาหารที่ผสมซูโครส 10% และมอลโตส 10% จากการให้สารทดสอบฉีดเข้า midgut ในกลุ่มที่ได้รับอาหารผสมซูโครส 10% พบว่า asiaticoside ขนาด 50 มก./มล. และ madecassoside ขนาด 5, 50 มก./มล. สามารถลดระดับน้ำตาลได้อย่างมีนัยสำคัญทางสถิติ ($P< 0.05$) ส่วนในกลุ่มที่ได้รับอาหารผสมมอลโตส 10% พบว่า asiaticoside ขนาด 0.5, 5, 50 มก./มล. และ madecassoside ขนาด 0.05, 0.5, 5, 50 มก./มล. สามารถลดระดับน้ำตาลได้อย่างมีนัยสำคัญทางสถิติ ($P< 0.05$) จากการให้สารทดสอบฉีดเข้ากระแสเลือดในกลุ่มที่ได้รับอาหารผสมกลูโคส 10% พบว่า ECa 233 ขนาด 0.05, 0.5, 5 มก./มล., asiaticoside ขนาด 5, 50 มก./มล. และ madecassoside ขนาด 50 มก./มล. สามารถลดระดับน้ำตาลได้อย่างมีนัยสำคัญทางสถิติ ($P< 0.05$) ในกลุ่มที่ได้รับอาหารผสมซูโครส 10% พบว่า ECa 233 ขนาด 5 มก./มล., asiaticoside ขนาด 50 มก./มล. และ madecassoside ขนาด 50 มก./มล. สามารถลดระดับน้ำตาลได้อย่างมีนัยสำคัญทางสถิติ ในกลุ่มที่ได้รับอาหารผสมมอลโตส 10% พบว่า ECa 233 ขนาด 0.5, 5 มก./มล., asiaticoside ขนาด 0.05, 0.5, 5, 50 มก./มล. และ madecassoside ขนาด 0.05, 0.5, 5, 50 มก./มล. สามารถลดระดับน้ำตาลได้อย่างมีนัยสำคัญทางสถิติ ($P< 0.05$)

ภาควิชา เภสัชวิทยาและสรีรวิทยา ลายมือชื่อนิสิต .....

สาขาวิชา เภสัชวิทยา ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

ปีการศึกษา 2555 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

5376587333 : MAJOR PHARMACOLOGY

KEYWORDS : α-GLUCOSIDASE/ SILKWORM/ HEMOLYMPH/ GLUCOSE LOWERING

PIYANUCH WORACHAT : GLUCOSE LOWERING EFFECT OF STANDARDIZED EXTRACT OF *CENTELLA ASIATICA* ECa 233 *IN VITRO* AND *IN VIVO* THAI SILKWORM MODEL. ADVISOR: SANTAD CHANPRAPAPH, Ph.D., CO-ADVISOR: CHANIDA PALANUVEJ, Ph.D., 93 pp.

This study aims to test the effect of ECa 233 and some compounds (asiaticoside and madecassoside) in lowering glucose by the mechanism of inhibition of α-glucosidase both *in vitro* and *in vivo* using Thai silkworm model. *In vitro* study was utilized to evaluate the inhibition of yeast and intestinal α-glucosidase from rat of the test compounds and determine the IC_{50} value. Acarbose, ECa 233, asiaticoside and madecassoside showed the inhibition of yeast α-glucosidase with IC_{50} value 0.0008, >500, 92.42 and 68.89 mg/ml, respectively and inhibition of intestinal α-glucosidase from rat with IC_{50} value 0.005, 205.61, 21.81 and 50.52 mg/ml, respectively. *In vivo* study in Thai silkworm model was used to test the toxicity and determine the LD_{50}, the results showed that the LD_{50} value of ECa 233, asiaticoside and madecassoside injected via intra-midgut in Thai silkworm was 178.51, >125 and >250 mg/ml, respectively and the LD_{50} value of ECa 233, asiaticoside and madecassoside injected via intra-hemolymph in Thai silkworm was 46.92, >125 and 174.58 mg/ml, respectively. Then the hypoglycemic effect of the test compounds was determined using hyperglycemic Thai silkworm model induced by feeding diet mixed with 10% glucose or 10% sucrose or 10% maltose. It was found that sugar level in Thai silkworm hemolymph fed with 10% glucose, 10% sucrose and 10% maltose diet lowered with statistical significance using insulin comparing with the control group ($P< 0.05$). Acarbose also lowered the sugar level in diet mixed with 10% sucrose and 10% maltose diet with statistical significance comparing with the control group ($P< 0.05$). For intra-midgut administration, asiaticoside (50 mg/ml) and madecassoside (5, 50 mg/ml) lowered the sugar level in diet mixed with 10% sucrose whereas asiaticoside (0.05, 0.5, 5, 50 mg/ml) and madecassoside (0.05, 0.5, 5, 50 mg/ml) lowered the sugar level in diet mixed with 10% maltose with statistical significance comparing with the control group ($P< 0.05$). For intra-hemolymph administration, ECa 233 (0.05, 0.5, 5 mg/ml), asiaticoside (5, 50 mg/ml) and madecassoside (50 mg/ml) lowered the sugar level in diet mixed with 10% glucose whereas ECa 233 (5 mg/ml), asiaticoside (50 mg/ml) and madecassoside (50 mg/ml) lowered the sugar level in diet mixed with 10% sucrose. Moreover ECa 233 (0.5, 5 mg/ml), asiaticoside (0.05, 0.5, 5, 50 mg/ml) and madecassoside at (0.05, 0.5, 5, 50 mg/ml) lowered the sugar level in diet mixed with 10% maltose with statistical significance comparing with the control group ($P< 0.05$).

Department : Pharmacology and Physiology	Student's Signature .....
Field of Study : Pharmacology	Advisor's Signature .....
Academic Year : 2012	Co-advisor's Signature .....

ACKNOWLEDGEMENTS

My sincere and heartfelt thanks to my advisor, Santad Chanprapaph, Ph.D. for his great understandings, excellent advices, encouragements, guidance and supporting, which have given me throughout this study. Without his kindness and understanding, this work could not be accomplished.

The author wishes to express her deepest gratitude to her thesis co-advisor, Dr. Chanida Palanuvej, College of Public Health Sciences, Chulalongkorn University, for her guidance, generous advice, kindness and support throughout the course of this study.

My graduation would not be achieved without best wishes from the members and staffs of Department of Pharmacology and Physiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, especially for my respected teacher's Assoc.Prof.Pol.Lt.Col.Somsong Lawanprasert, Ph.D., who always gives me the greatest advice and encouragement.

This work was supported by a research grant from CU.GRADUATE SCHOOL THESIS GRANT, Chulalongkorn University. Thanks are also due to the Research Instrument Center of Faculty of Pharmaceutical Sciences, Chulalongkorn University, for providing research facilities.

Eventually, the extremely gratitude is expressed to my family understanding, helping, supporting and encouraging with care, which enable me to conduct this research successfully.

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LIST OF ABBREVIATIONS

%	= percent
^{o}C	= degree Celcius
µg	= microgram
µl	= microlitter
ECa 233	= standardized Extract of *Centella asiatica* ECa 233
et al.	= et alii (and other)
g	= gram
gr	= group
g/group	= gram per group
g/kg	= gram per kilogram
hr	= hour
hrs	= hours
IC_{50}	= median inhibitory concentration
larva/gr	= larva per group
LD_{50}	= median lethal dose
mg	= milligram
ml	= milliliter
mg/dL	= milligram per deciliter
mg/kg	= milligram per kilogram
mg/ml	= milligram per milliliter
min	= minute
mm	= millimeter
mmol/L	= millimolar per liter
MW	= molecular weight
T1DM	= type 1 diabetes mellitus
T2DM	= type 2 diabetes mellitus
GDM	= gestational diabetes mellitus
IFG	= impaired fasting glucose
IGT	= impaired glucose tolerance

FPG	= fasting plasma glucose
OGTT	= oral glucose tolerance test
GIP	= gastric inhibitory polypeptide
GLP-1	= glucogon like peptide-1

CHAPTER I

INTRODUCTION

Diabetes is a chronic disease that has increased every year and adversely affects the quality of life of patients such as physical, mental, emotional and social. Although there are advance in technology and science about diabetes, the incidences of disease still increase. Statistics of the number of diabetics worldwide in 2000 were more than 171 million people, and it is predicted to rise to 366 million in 2030 (Amos *et al*., 1997; Wild *et al*., 2004). The diagnosis of diabetes is often too late especially patients with the condition of high blood sugar level, dyslipidemia and metabolic syndrome who do not receive treatment, later the complications have occur. Studies have shown that about 1 in 5 of the diabetic who are newly diagnosed have complications of eye, nerve or kidney and more than 75 percent of the patients died of cardiovascular disease, which is majority complication and happen previously diagnosed pre-diabetes (Davis *et al*., 1997; Aekplakorn *et al*., 2003). Therefore, the development of new drugs is needed to help control this disease. The research and development of new drugs mostly test the toxicity and activity in animal, which is one step in the process to ensure that test substances are effective and safe enough and lead to further studies on humans in a clinical setting. Current experimental pharmacology and toxicology often use animals as mammals for example rabbits, mice and rat but now have the problems such as price or ethical. Later to reduce these problems, the researchers use invertebrate animals such as *Drosophila melanogaster* (Bernal and Kimbrell, 2000), *Caenorhabditis elegans* (Lemaitre *et al*., 1996). In addition, the researchers from the University of Tokyo, Japan has also proposed silkworm (*Bombyx mori*) as experimental animal model to test for bacterial infection (Kaito *et al*., 2005). Then studied the rate of transport of substances and found that substances with molecular weight greater than 400 Da cannot diffuse through the membrane of the midgut (Hamamoto *et al*., 2005). Recently Yasuhiko Matsumoto and his colleagues have proposed the hyperglycemic silkworm as a model for study the glucose lowering effect in hemolymph (Matsumoto *et al*., 2011). This study found that hyperglycemic silkworms induced by feeding a diet containing glucose showed an increase of amount of sugar increased in the fat body. Glucose is clearly absorbed by passive diffusion and changes into

trehalose (α-D-glucopyranosyl-α-D-glucopyranoside) which is an important sugar in insect hemolymph and accumulated in fat bodies (Treherne, 1967; Thompson, 2003).

Thai silkworm model has also investigated in the same aspect. It was found that drugs used in clinical setting for human such as insulin, glibenclamide and acarbose show glucose lowering effects in Thai silkworm model (Chaingsom, 2011). Furthermore, Standardized Extract of *Centella asiatica* ECa 233 (ECa 233) also showed such an activity using Thai silkworm model. In this study, we investigated further on the hypoglycemic effect of ECa 233 and its constituents (asiaticoside and madecassoside) toword the mechanism of inhibition of α-glucosidase both in *in vitro* and *in vivo* using hyperglycemic Thai silkworm model induced by feeding diet mixed with 10% glucose or 10% sucrose or 10% maltose. Insulin 3.5 mg/ml injected via intra-hemolymph and acarbose 20 mg/ml injected via intra-midgut were utilized as positive control and 0.9% NaCl injected via intra-hemolymph as negative control.

Research hypothesis

ECa 233 inhibits α-glucosidase *in vitro* and has hypoglycemic effect in the *in vivo* study using Thai silkworm model.

Objectives

1. To evaluate the inhibition effect of ECa 233 on α-glucosidase both *in vitro* and *in vivo* studies
2. To evaluate the inhibition effect of asiaticoside and madecassoside on α-glucosidase both *in vitro* and *in vivo* studies

Research design

Experimental research

Scope of the study

This study investigated the inhibition effects of ECa 233, asiaticoside and madecassoside on α-glucosidase both *in vitro* and *in vivo* using human insulin and acarbose as positive control.

Thai silkworm induced hyperglycemia by feeding the diet mixed with glucose, sucrose or maltose was used for the screening of glucose lowering effect.

Benefits and application

This research provides the information of ECa 233, asiaticoside and madecassoside on the inhibition of α-glucosidase *in vitro* and *in vivo* using the hyperglycemic Thai silkworm model.

CHAPTER II

LITERATURE REVIEWS

Diabetes mellitus (DM) is silent in the early stages of the disease as well as other chronic disease that patients almost did not know its beginning and the progression continue to causes severe complications and death. Because the disease is asymptomatic, most patients will feel strong like normal people but do not know that they have diabetes which could potentially undermine the body down slowly in small increments and lead to the dangerous complications. Coronary heart disease is major complication and the cause of sudden death in patients with diabetes (Horton, 1995; Ceriello *et al*., 2006; Yamagishi, 2011). Also stroke, memory loss when these conditions occurs permanently even then patient can control blood sugar level later, it does not help to restore to normal. Based on World Health Organization, the fact that diabetes has statistically the current population of at least 180 million people worldwide and the number of people with diabetes are more likely to 360 million by 2030 and around the world have died from complications of diabetes than 4 million people every year (Rathmann and Giani, 2004; Horton, 1995). Researchers from the International Collaborative Study of Cardiovascular Disease in Asia (InterASIA) found that diabetes has prevalence of 9.6 percent of the age more than 35 years (Aekplakorn *et al*., 2003). Approximately half of people with diabetes lose the opportunity to know that they are at risk and require prevention or treatment; furthermore one in five of newly diagnosed cases already had the complication of eye or nerve or kidney and 75 percent of patients with diabetes will die of cardiovascular diseases. As mentioned above, diabetes has no symptoms in its early stages; therefore, aware of the patient and diagnosed by a physician are often too slow.

Before progress to disease, patients have been impaired glucose tolerance (IGT) that call pre-diabetes (Hussain *et al*., 2007). Pre-diabetes is definitely about people detected the blood glucose levels that are higher than normal, but are not high enough to be categorized as diabetes. Nowadays, there is not recommended to screening for IGT group because it is not worth the expense, the complication has already occurred before the diagnosis of diabetes (Ceriello *et al*., 2006; Hanefeld *et al*., 2012). So if there is screening for pre-diabetic, it may help prevent the development of disease and complications (Ceriello *et al*., 2006). Diabetes is considered usually the blood glucose level in the fasting for 8 hours higher 126 mg/dl. Physicians suggest that

glycemic control should between 70-130 mg/dl to be safe. Patients with glucose value between ranging 100-200 mg/dl have no symptom until glucose values more than 200 mg/dl, the symptoms frequent urination and thirsty. Sugar benefits for the body to provide energy especially glucose, which play an important role in providing energy to the brain and stimulates the secretion of chemicals in the brain. If the body has too much glucose into the body cells to used, this excess energy is stored in the liver and muscles as glycogen for energy supply to be use in times of need. In addition, the excess sugar is converted into fatty acid as well and cause the inflammation both large and small arteries throughout the body and lead to the degeneration of various organs such as macular degeneration, chronic kidney failure, neuropathy. According diabetes is a chronic disease and often lifelong, if patient neglect or lack of properly cares so it may cause low or high blood sugar level and lead to the complications that may occur gradually. It is a serious and ongoing treatment to control the disease by targeting desirable (Yamagishi and Imaizumi, 2005; American Diabetes Association, 2008; Ceriello 2005).

Criteria for diagnosing diabetes

The basis for new diagnostic criteria have been adapted from the national diabetes data group (NDDG) and the World Health Organization (WHO) (National Diabetes Data Group, 1979; Alberti and Zimmet, 1998).

a. The symptoms of diabetes with the value of blood sugar level at any time are greater than or equal to 200 mg/dl.

b. The fasting blood glucose sugar level (or fasting plasma glucose: FPG) for 8 h are greater than or equal to 126 mg/dl. Interpretation of the glucose level in the FPG is divided into three levels.

- FPG value less than 100 mg/dl is considered normal.
- FPG value ranging 100-125 mg/dl is considered as impaired fasting glucose (IFG).
- FPG value greater than or equal to 126 mg/dl is considered diabetes.

c. Oral glucose tolerance test (OGTT) levels at 2 hours are greater than or equal to 200 mg/dl. Interpretation of OGTT is divided into three levels.

-The 2-hour plasma glucose value less than 140 mg/dl is considered normal glucose tolerance.

-The 2-hour plasma glucose value ranging from 140-199 mg/dl is impaired glucose tolerance (IGT).

-The 2-hour plasma glucose greater than or equal to 200 mg/dl is considered diabetes.

Using OGTT as 140 mg/dl have detected people with the disorder than the FPG at 100 mg/dl, so it is important to report which testing to detect abnormalities of glucose metabolism. The criteria for the diagnosis of diabetes using FPG 126 mg/dl and OGTT at 200 mg/dl since the beginning of the complication can cause such as micro vascular, diabetic retinopathy, and these is condition characterized by a specific disease of diabetes. IFG and IGT are importance of the condition increases the risk of diabetes in the future and also found that levels of these two conditions can increase the risk of atherosclerosis which leads to coronary heart disease or ischemic stroke. In addition, OGTT can detect diabetes that can cause complications and high mortality (American Diabetes Association, 2008).

Classification of diabetes (American Diabetes Association, 2008)

a. Type 1 Diabetes mellitus (T1DM) is caused by destruction of pancreatic beta cell, most likely due to unknown cause's autoimmune. This type of diabetic ketoacidosis was likely to occur. It often found in young age and under 20 years old, patients with this type of disorder of the pancreas that does not produce insulin.

b. Type 2 Diabetes mellitus (T2DM) is the most common of abnormalities insulin resistance and insulin secretion from pancreatic diabetes. Patients with type 2 diabetes often do not have to get insulin for survival like T1DM, but the latter may require insulin to control glucose levels. There is not clear cause of this type, patients are often overweight and obesity can cause resistance to insulin.

c. Other specific types of diabetes know other causes that the result is the destruction of pancreatic β-cell such injury (trauma), cancer, cystic fibrosis.

d. Gestational diabetes mellitus (GDM) that occurs during pregnancy which the body cannot control blood sugar levels.

Glucoregulation

Normally the bodies maintain blood glucose levels are ranging 70-150 mg/dl either before or after the meal. Because blood sugar levels are crucial to the functioning of various organs in the body, especially the brain have metabolic process of glucose in the body. The control of glucose level can be divided into the following two stages (DeFronzo, 2004: Triplitt, 2012).

a. Fed stage or postprandial stage: This stage that occurs after eating, which the absorption of glucose into the bloodstream. Gastrointestinal hormones: glucagon like peptide-1 (GLP-1) and/or gastric inhibitory polypeptide (GIP) that stimulate the secretion of insulin from beta cells of the pancreas. Higher levels of insulin to stimulate the body change glucose to use as energy, and stored in the form of a glycogen is also stored in the liver tissue, peripheral fat and muscle. At this stage showed that suppress hepatic glucose production and lipolysis. Normally the blood glucose levels in adults are between 70 to 99 mg/dl and may rise to 140 mg/dl after eating of a high-carbohydrate. T2DM, this stage still produce glucose from liver whereas insulin secretion.

b. Fasting stage or post absorptive stage: This stage occurs after eating about 5-6 hours and maintains blood sugar level within normal including glycolysis, which can keep blood sugar level within normal range only 6-8 minutes and produces the sugar from hepatic gluconeogenesis. This process can maintain glucose levels within normal range at least 72 hours. Higher levels of insulin, it reduces the glucose by peripheral glucose utilization and break down fats and proteins from the tissues. If the body is malnutrition over time by 24-48 hours, it will process gluconeogenesis and mostly by the breakdown of muscle, protein, fat or produce ketone compound to be used as a source of energy instead of glucose.

Diabetic complications

Diabetic complications have been found in patient with diabetes cannot control hypertension, dyslipidemia, obesity and hyperglycemia. In diabetes, increased oxidative stress and depleted antioxidant defenses have been found. The source of reactive oxygen species (ROS) relate to hyperglycemia and diabetic complications (Figure 1, 2) (Ceriello, 2005; Unger *et al*., 2008).

a. Diabetic neuropathy found the degeneration of the nerves and axon membrane due to the destruction of the nerve and result in the retention of sorbitol and fructose caused nerve degeneration (Nosadini and Tonolo, 2004)

b. Diabetic retinopathy due to changes in retinal blood vessels induced blindness in adult patients, in some cases can lead to blurred vision, glaucoma.

c. Cardiovascular system found diabetic is a risk for coronary heart disease than the two times and increased viscosity of blood with platelet function (Best *et al*. 2004).

d. Diabetic nephropathy showed a decline in renal arteries, the reduced blood flow to the kidney filtration decreased the leakage of protein into the urine.

Medical treatment of diabetes

Treatment of diabetes may start the lifestyle modification such as controlling diet fat, carbohydrate and exercise. If the glycemic control is still not on target, patient starts pharmacological treatment. Currently available hypoglycemic agents treatment for diabetes are sulfunylurea, non-sulfunylurea (stimulating the insulin secretion from the pancreas), biguanide (reduce insulin resistance and hepatic production), thiazolidinedione (improve insulin sensitivity in adipose tissue, skeletal muscle and decrease hepatic gluconeogenesis), α-glucosidase inhibiter (reduce the absorption of glucose of dietary starch), DPP-4 inhibitor (enhance the activity of incretin), insulin, and GLP-1 analog (mimic GLP-1 and promote insulin secretion) (Triplitt *et al*., 2007; Lawal, 2008, Bedekar *et al*., 2010; Mazzola, 2012).

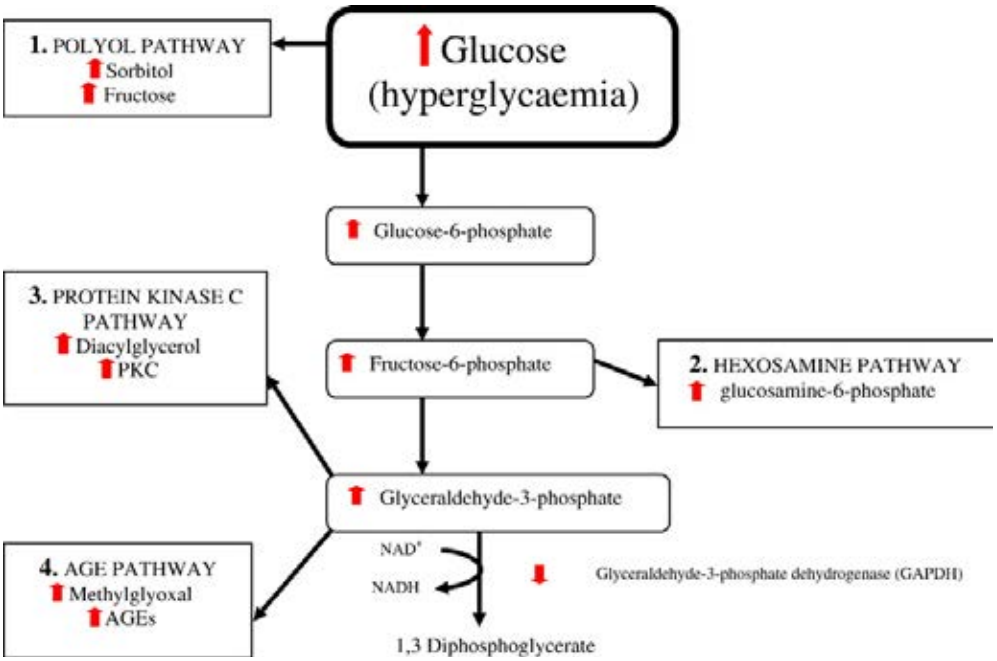


Figure 1 Proposed link between hyperglycemia and four mechanisms that lead to tissue damage (Choi *et al.*, 2008)

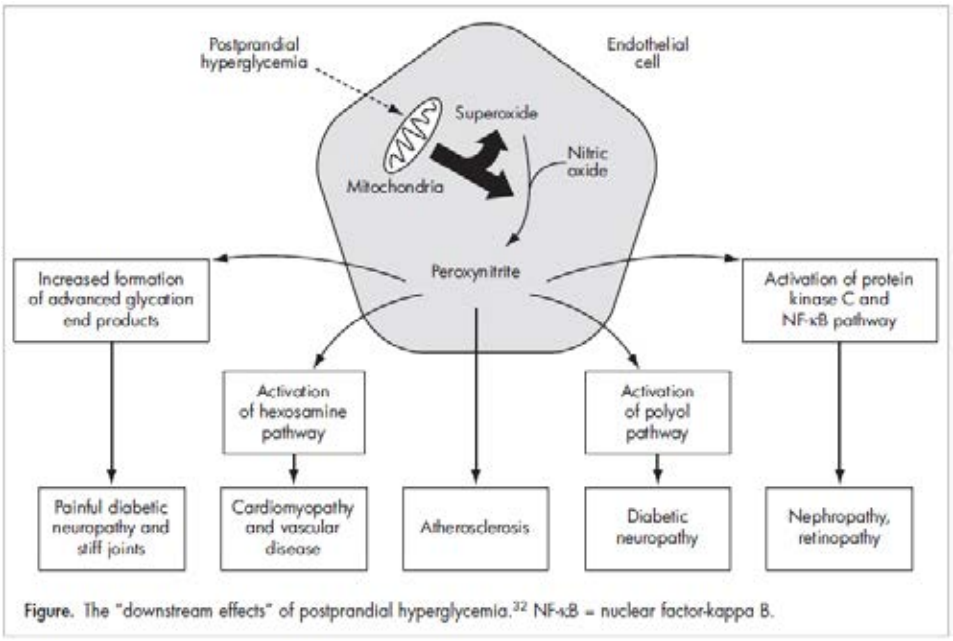


Figure 2 The downstream effects of postprandial hyperglycemia. NF-kB = nuclear factor-kappa B. (Unger *et al*, 2008)

Carbohydrate and α-glucosidase

The main structure of carbohydrate composes of carbon molecules and the hydrate. Carbohydrates are the most important source of human energy, if the malfunction the energy of the body leads to cause diabetes. The major sources of carbohydrates are from the plant such as underground stems or roots (taro, cassava and potatoes), fruit (pumpkin) or grains (rice, beans). Carbohydrate and sugar are required to process to monosaccharide molecule which can be absorbed through the intestine into the bloodstream and distribute to organs or serve as a source of energy or stored for further use. Carbohydrate is an important and continuing role of human life. It can be divided into sugar molecules that are complementary to each other (Gray, 1975; 1992; Pallardy, 2008).

a. Monosaccharide including glucose fructose and galactose, monosaccharide plays a role in human life. Monosaccharide is a type of sugar that the body can only be absorbed through the intestine and the glucose is converted into energy. Since moving into sugar in the blood circulatory system of the body, it is the source of energy in cells. Measurement of glucose in the blood sugar level as a value that indicates whether the patient is diabetic or not. Monosaccharide can be subdivided into three-dimensional structure by hydroxyl group at carbon position of a structure that is below or above the plane of the main structure are called α or β, respectively.

b. Disaccharide including sucrose lactose and maltose, as a sweetener in many foods such as sugar, milk, malt, theirs molecules are the same glucosidic bond as the monosaccharide but there are two molecules such as maltose containing glucose two molecules with a three-dimensional structure of the α-glucose linked by glucosidic bonds. The three-dimensional structure is that the α-glucosidic bond or a disaccharide α-glucosidic linked only the body can digest.

c. Complex carbohydrate can be subdivided into two types of oligosaccharide and polysaccharide. By the oligosaccharide contains 3-10 molecules of monosaccharide such as dextrin. The polysaccharide connects from the 10 molecule of monosaccharide. Complex carbohydrate such as starch is connected by α-glucosidic bonds as well as the disaccharide whereas the cell walls of plants such as cellulose, it is β-glucosidic bond that the body cannot digest.

Digestion of dietary carbohydrate is a process to change carbohydrate molecule to the simple sugar (monosaccharide) then it is absorbed and converted into energy in the body. This process occurs in the mouth through the small intestine. Initiate when food is chewed in the mouth, it stimulates the salivary glands secrete saliva enzyme called amylase, which acts as a polysaccharide and oligosaccharide to a smaller size. However, this stages only a short time because the diet move through the esophagus into the stomach and then the enzyme salivary amylase stop working due to the acidity in the stomach. Digestion occurs again when the food is through the stomach into the duodenum, which is appropriate for enzyme secreted from the pancreas called pancreatic amylase. Carbohydrate is digested and shortened to di-/tri-saccharide and limit dextrins by salivary amylase and pancreatic amylase. The end of the digestive carbohydrate occurs at the brush border, the inner wall of the small intestine contacting with diet. The brush border secrete an enzyme group known as the α-glucosidase, which cleaves di-/tri-saccharide and limit dextrins into monosaccharide (glucose) and absorbed through the cell at the brush border before entering the circulatory system.

The enzyme amylase from the salivary glands and pancreas, as well as the brush border intestinal α-glucosidase, act as α-glycosidic bond of carbohydrate. If there is the inhibition of the enzyme to digest the carbohydrate, they cannot function as usual, so glucose is absorbed slowly by.

α-glucosiadase inhibitor

A. The mechanism of action

The α-glucosiadase inhibitor including acarbose and voglibose inhibit α-glucosiadase at the brush border of the small intestine, so they delay the rate of digestion of α-linkage polysaccharides such as starch and significantly lowering postprandial glucose levels without causing hypoglycemia. Disaccharase is one of group of α-glucosiadase, for example sucrase lactase and maltase act to break the α-glucosidic bonds of sucrose, lactose and maltose, respectively (figure 3). When these enzymes are inhibited, the final step of carbohydrate breakdown to glucose is delayed resulting in lowering blood sugar level, especially postprandial blood sugar. When blood glucose level is lower, there is decreasing oxidative stress in the artery wall, resulting in reduced vascular complication in patients with diabetes. The pancreas also

stimulate insulin secretion was to reduce blood sugar levels after meals and post meal (or postprandial). Acarbose is oligosaccharide analog that inhibits α-glucosidase pancreatic amylase and also it exhibits a reversible competitive inhibition. Acarbose and voglibose are very little absorbed into the body, it excrete rapidly in the urine (Bischoff, 1994, 1995; Ceriello, 2005; Chiasson, 2006; Hussain *et al.*, 2007; Choi *et al*., 2008; Bedekar *et al*., 2010).

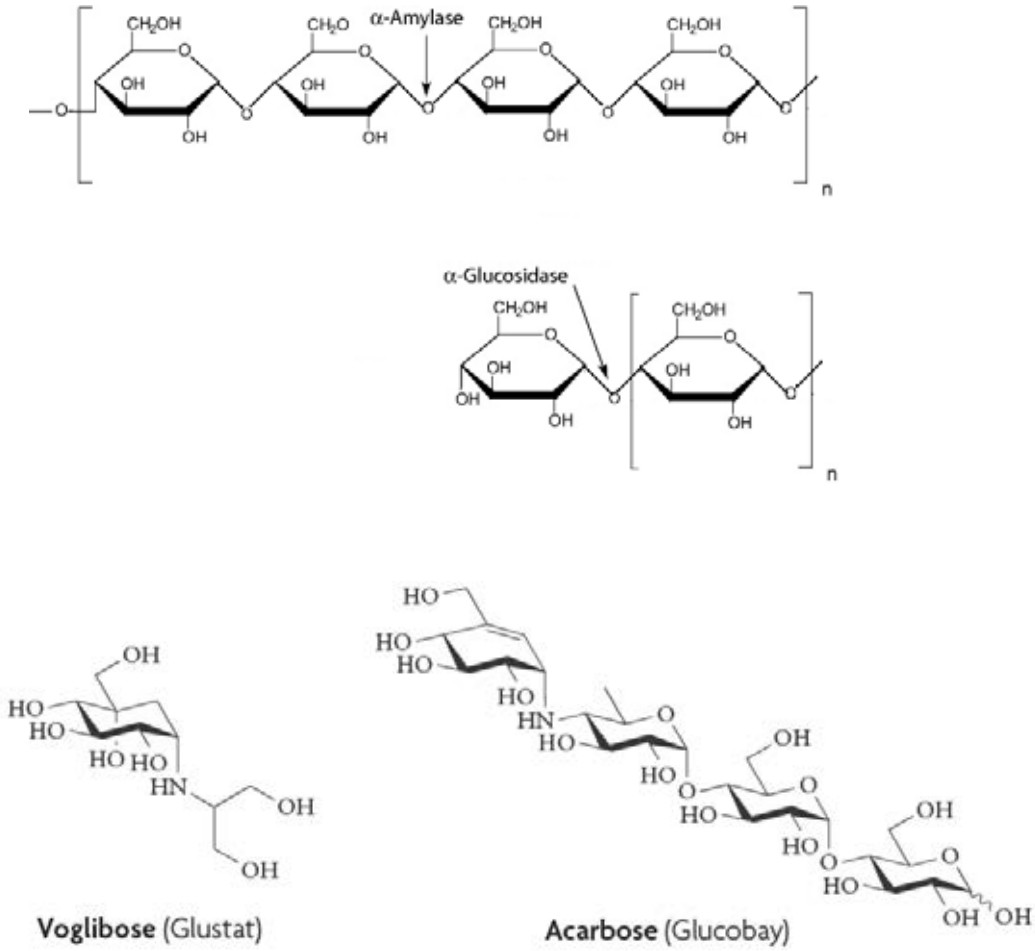


Figure 3 α-glucosidase that hydrolyze polysaccharide (upper), the α-glucosidase inhibitors that prevent the digestion of carbohydrates for the treatment of diabetes (Bedekar *et al*., 2010)

B. Effectiveness of the drug

The mechanism of inhibition of these drugs is reversible competitive inhibition. In general, all foods composed of carbohydrate are not different but low carbohydrate eating is enhancing the effectiveness. The practical problem of the poor performance drugs used caused by eating foods that contain high carbohydrate and α-glucosiadase cannot be inhibited by α-glucosiadase inhibitor intake as a substrate (Yagihashi and Imaizumi, 2005; Ceriello *et al*., 2005; Vichayanrat *et al*., 2002; Van de Laar *et al.* 2005).

C. Medical indication

-Patients with T2DM

Both acarbose and voglibose are used in patients with T2DM, or who called NIDDM (non-insulin dependent diabetes mellitus). It is effective in lowering blood sugar levels after meals (post prandial blood glucose), but HbA1C (hemoglobin A1C) and FPG (fasting plasma glucose) is not much effective when compared with other oral hypoglycemia drug. It is used in combination with other groups to control the blood level (Van Dijk *et al*. 2011; Bedekar *et al.*, 2010).

-Prevention of diabetes

The criteria those who are tend to be diabetes were based on measuring FPG which is higher than normal but still not as high as the diagnosis of diabetes (pre-diabetes, IFG, IGT). In addition to diet and exercise controlling blood sugar levels, α-glucosidase inhibitors can help control blood sugar levels then slowing the risk of diabetes (Hanefeld *et al*., 2005; Chiasson *et al*., 2002; Hussain *et al*., 2007; Waugh *et al*. 2007).

- Patients with T1DM

Using some oral hypoglycemia drugs to control blood sugar levels are not commonly used in patients with Type 1 diabetes. Metformin and α-glucosidase inhibitors can be used in conjunction with insulin to control blood sugar levels better. Both acarbose and voglibose have effect on decreasing or slowing down the absorption of sugar loading, so they are also beneficial for insulin dependent DM (Alberti and Zimmet 1998; Chiasson *et al.*, 1998).

D. Regiment indication

Mechanism of action of drugs is that drugs can have effects on the enzyme to digest the food only. The effectiveness of acarbose may be improved by chewing before swallowing the drug. Unlikely voglibose FDT (fast disintegration tablet), it can be broken down quickly by placing the tablet on the tongue. When the tablet is exposed to saliva in the mouth and swallow it, drugs will be faster action because it takes time to break down in the stomach. Dosage of acarbose is 50 mg three times daily with food and can be used with the 100 mg. Dosage of voglibose 0.2 mg three times daily with food may increase the dose of 0.3 mg (Bedekar *et al*., 2010; Mazzola, 2012).

E. The side effects and contraindications

Side effects of the α-glucosidase inhibitors are mild but common. Usually, carbohydrate molecules are broken down into smaller sugar molecules that the body can absorb. When patient takes drug, carbohydrate molecule will move through the small intestine into colon, and then they act as energy source of bacteria. As a result, the digestion of carbohydrate by the bacteria is causing substances including gases such as methane and hydrogen and various fatty acids. That these substances can cause bloating, abdominal pain, diarrhea, anorexia, nausea and vomiting which are common side effects in patients who have to start using drugs in low dose. Although effective of voglibose is higher than acarbose, but causing side effects are higher from the inhibition of carbohydrate digestion and reported that causing the hypoglycemia. Hypoglycemia found mostly when used in combination with the other groups, especially the drug increases the secretion of insulin from the pancreas such as sulfunyureas and meglitinide, then management of hypoglycemia are eating sweetened such as glucose whereas sucrose is not possible because the enzymes used to digest sucrose into glucose. The side effects often are also reported to cause liver function abnormalities jaundice and the pneumatics cystoides intestinalis but found very little. These causes the bacteria in the gut growth, so in people with a history of diseases associated with abnormalities in the digestive tract such as inflammatory bowel disease (IBD) or irritable bowel syndrome (IBS) should be use this group with caution (Vichayanra *et al*., 2002; Nakamura, 2005; Sakamoto and Tajima, 2005; Tsujimoto *et al.*, 2008).

In summary α-glucosiadase inhibitor has effect on postprandial blood glucose level but the glycemic control in HbA1c and FPG are not good. Furthermore it is used in combination with other groups including insulin. Side effects are not seriously and commonly found. However, α-glucosidase inhibitor is used in patients with T1DM, T2DM and can be used to control blood sugar levels in pre-diabetes to prevent the disease in the future.

Digestive systems of insect

The digestive system of insect is usually a long tube or coiled in a circle and will stretch the body. Starting from the mouth to the anus is the only system that is floating in the space of the body. The digestive system of insect is divided into three parts: foregut or stomodeum, midgut or mesenteron and hindgut or proctodeum (figure 4). Between intestinal and central the tongue called stomodeal or cardiac valve, and between the intestine and central part of the latter tongue barrier as well as proctodeal or pyrolic valve (Klowden, 2008).

a. Foregut: From the mouth is at the base of the hypopharynx of the preoral cavity (cibarium) and directly to the pharynx, which cibarium or pharynx or both may be adapted to help pump food with muscle. In insects such as butterfly mosquito and fly, the oesophagus which extends to the stomach, the next crop is proventriculus and stomodeum valve that acts as a valve blocking to move food through the intestine into the midgut so they cannot back up in the foregut again. Although the foregut intestine will not be an important part in digestion, it is the partial digestion in the crop by the activity of enzymes from the saliva. In Orthoptera, the intestinal tissue to be lined with a thick, called the intima, which does not allow liquid permeability, but it does not allow for fat absorption. The end of foregut is not involved with intestinal absorption of various nutrients into the bloodstream.

b. Midgut: From the stomodeal valve of this part of the body like fingers, called gastric caeca, production of digestive juices and enzymes to digest different types of caeca was varied by species of insects and gastric caeca may found another intestine. The following is a portion of the mesenteron or ventriculus, which extends into a large bag and acts as a stomach of insects. Digestion and absorption of food to use cardial epithelium and central ventricular epithelium or gut or both to create walls that contain chitin and the peritrophic membrane wall prevent friction with the epithelial membrane, which may cause ulcers or inflammation.

c. Hindgut: It starts the malpighian tubules which are structured as a long line. Theirs function excrete wastes. The valve is called the pyloric and blocks the food through the intestines after it does not go back up in the midgut. Part of the malpighian tubules is a long tube is divided into two parts, the upper part called the ileum and the lower part of the colon. Digestion may come up a little or do not occur in the hindgut, but this will serve to move the food from the gut, and excretion from the insect gut wall. Properties of liquids with small molecules can be absorbed through the hindgut. The latter is responsible for the hindgut absorption of salt and amino acid which is absorbed from the blood by the intestine and malpighian tubules, so it retains salt and water balance in the body of the insect.

Glucosidase of insect

Glucosidases secreted in the midgut hydrolyze the glucosidic bonds between the sugar residues, and their specificity depends on the type of bond that linkage α or β. Insect, α-glucosidase hydrolyzes α –glucosides of sugar molecule such as sucrose, maltose, trehalose, and melezitose whereas β-glucosidase breaks down the β-glucosides of cellobiose and gentiobiose and α-galactosidase act on α- galactosides of melibiose and raffinose. Amylases act on the α-glucosidic linkages in starch and glycogen. One of the most carbohydrases in insects is trehalase, which hydrolyzes trehalose into two glucose molecule then taken up by the fat body cells that surround the gut (Wyatt and Kale, 1957; Terra and Ferreira 1994, 2012). Ingestion of food as polysaccharides and disaccharides are broken down to monosaccharides and absorb through the gut wall by passive diffusion. The sugars that pass into the hemolymph are then taken up by the fat body cells that surround the gut. To maintain this gradient, absorbed glucose is rapidly converted to the disaccharide trehalose in the hemolymph (Thompson, 2003). Study found glucose as signaling for stimulating the releasing bombyxin from brain into hemolymph and lowering the concentration of trehalose in the midgut and muscle (Masumura *et al*., 2000; Iwami, 2000).

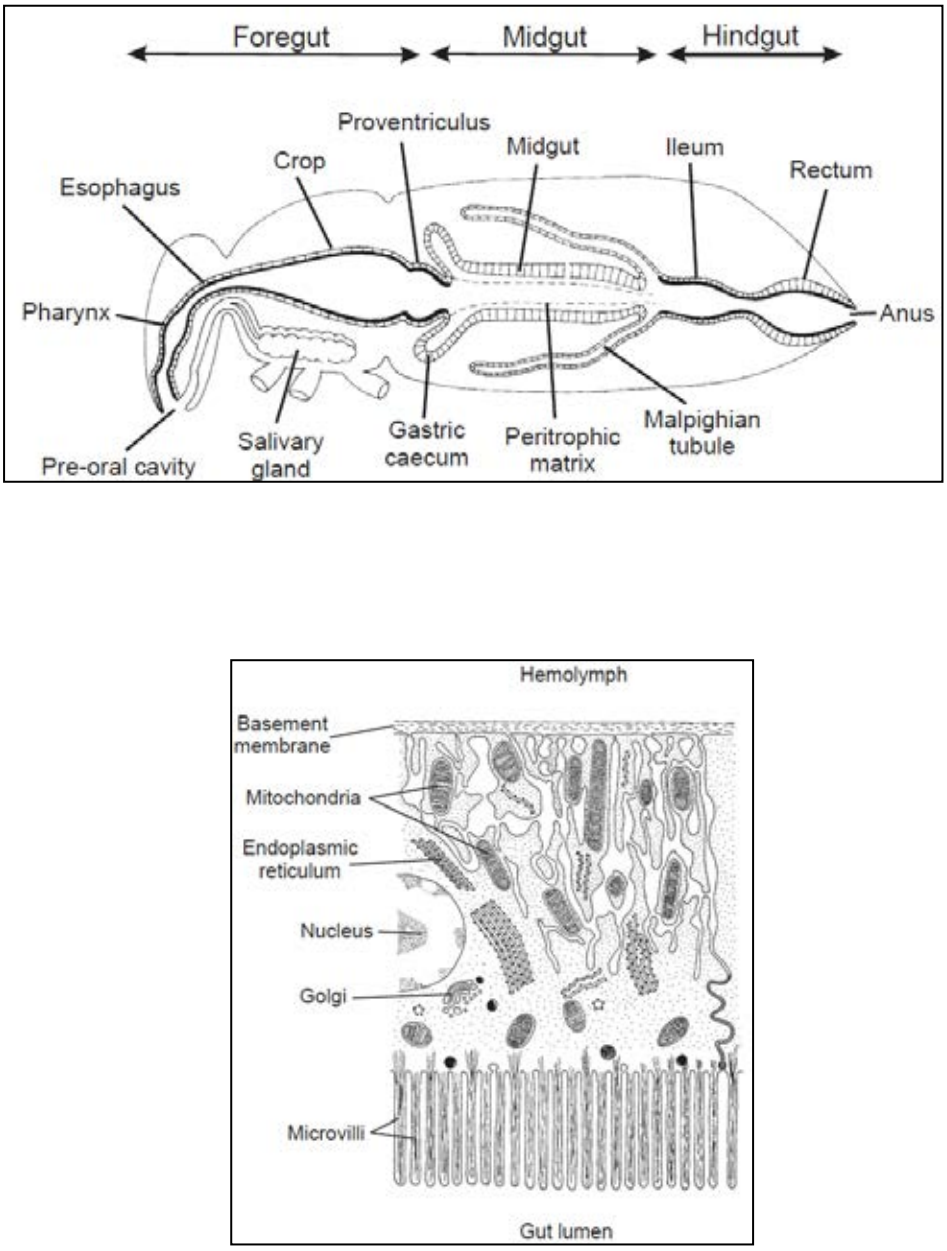


Figure 4 The three major divisions of the insect digestive tract - foregut, midgut, and hindgut - and their components (upper), and a typical columnar midgut cell (lower) (Klowden, 2008)

Centella asiatica

Study found that the extract of *Centella asiatica* and asiaticoside can decrease significantly blood glucose levels in mice after day 14 of the test substance and study the glucose tolerance test showed that blood glucose levels of the mice were decreased significantly in receiving asiaticoside group (Shigeru *et al*., 2007). Like Truong Tuyet Mai and his colleagues found that the compounds of polyphenol effect on inhibiting α-glucosidase and (Mai *et al*., 2007). Prinya Wongsa and his colleagues found that the extract of *Centella asiatica* using different solvents and at different concentrations have activity of α-amylase and α -glucosidase inhibition (Wongsa *et al*., 2012). The former study did not controlled the amount of ingredients so the researchers from Faculty of Pharmacy University has produced the standardized extract of *Centella asiatica* ECa 233, which controls the amount of triterpenoid glycosides are present in quantities not less than 80 percent and the ratio between madecassoside / asiaticoside at 1.5 ± 0.5 and can be stored for not less than two years.

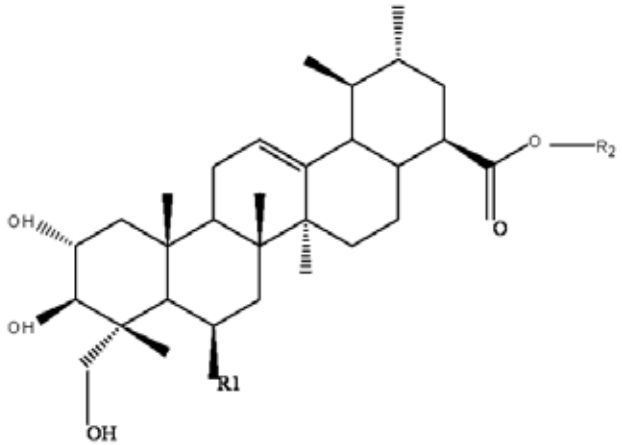


Figure 5 Structure of triterpenes of Centella asiatica (Glu: glucose, Rha: rhamnose) (Rafamantanana *et al*., 2009)

CHAPTER III

MATERIALS AND METHODS

Thai silkworm (Nang-lai) 5th instar larva weighing 0.8-1.2 g were supplied from The Queen Sirikit Department of Sericulture, Ministry of Agriculture and Cooperatives. The silkworms were kept under laboratory conditions of temperature (27 ± 2 $^{\circ}$ C) for at least 24 - 48 hrs prior to start the experiments, Silkmate 2S, an artificial diet (Nihon. Nosan, Japan) was used for feeding and mixing with either glucose or sucrose or maltose in the experiment.

Chemicals

1. Acarbose (Sigma,WGK, Germany)
2. D-Glucose MW 180.16 (Univar, Australia)
3. Dibasic sodium phosphate (Sigma, St. Louis, MO, USA)
4. Distilled water
5. 99.5% DMSO (Sigma, St. Louis, MO, USA)
6. Human insulin (Wako, USA)
7. 0.9% Normal saline
8. Maltose (Univar, Australia)
9. Monobasic sodium phosphate (Sigma, St. Louis, MO, USA)
10. 70% Perchloric acid MW 100.46 (Univar, Australia)
11. 98.5% Phenol (Lipton, England)
12. *p*-nitrophenyl-α-D-glucopyranoside (Sigma, Singapore)
13. Standardized Extract of *Centella asiatica* (ECa 233), asisticoside and madecassoside obtained from Assist. Prof. Chamnan Patarapanich, Ph.D. Department of Food and Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.
14. Sucrose (Univar, Australia)
15. Sulfuric acid MW 98.08 (J.T. Baker, Thailand)
16. α-glucosidase from *Saccharomyces cerevisiae* (Sigma,WGK, Germany)
17. α-glucosidase from rat intestinal acetone powder (Sigma,WGK, Germany)

Instruments

1. Micropipet size 20, 200 and 1,000 microliter (Gilson, France)
2. Microcentrifuge tubes size 1.5 microliter (Biologix, US)
3. Pipette tip size 0.1-10 microliter and 100-1,000 microliter (Corning incorporated, Mexico)
4. 96 Well microtiter plate (Corning incorporated, US)
5. Disposable syringe size 1 microliter (Nipro, Thailand)
6. Hypodermic needle 27G x 1" (Nipro, Thailand)
7. Centrifuge model EBA 20 (Hettich, Germany)
8. Microplate reader model Perkin Elmer (MTX Lab system, Inc., USA)

Methods

***In vitro* study**

Determination the IC_{50} values of acarbose, ECa 233, asisticoside and madecassoside

α-glucosidase from yeast prepared at concentration of 1 U/ml was used in each experiment (Palanuvej *et al*, 2009). Intestinal-rat acetone powder (200 mg) was dissolved in 4 ml of 0.9% NaCl and sonicated for 15 minutes after vigorous vortexing for 20 minutes, the suspension was centrifuged (10,000 g, 30 minutes) and the supernatant were use (Mohamed Sham Shihabudeen *et al*., 2011). The assay using 0.1M phosphate buffer at pH 6.9 and 1 mM *p*-nitrophenyl-α-glucopyranoside (PNP-G) was used as a substrate. Ten microliter of α-glucosidase was incubated in the absence or presence of various concentrations of acarbose, ECa 233, asisticoside and madecassoside (20 µl) at 37ºC. The pre-incubation time was specified at 10 min and PNP-G solution (20 µl) was added to the mixture. The reaction was carried out at 37ºC for 20 min, and then fifty microliter of 1 M Na_2CO_3 was added to terminate the reaction. Enzymatic activity was quantified by measuring the absorbance at 405 nm in a microplate reader model Perkin Elmer. Acarbose was used as a positive control and 0.9% normal saline as negative control. % Inhibition = {[(AC - AS)/AC] ×100}, where AC is the absorbance of the negative control and AS is the absorbance of the tested sample. The concentration of an inhibitor required to inhibit 50% of enzyme activity defined as the IC_{50} value was estimated using SigmaPlot version 12. The IC_{50} values were expressed as mean ± S.D. of three-independent experiments.

***In vivo* study**

Toxicity study of ECa 233, asisticoside and madecassoside

Toxicity of ECa 233, asisticoside and madecassoside was studied using 0.8-1.2 g/larva of the 5th instar larva Thai silkworms and divided into various groups (10 larvae/group). The negative control group was injected via intra-midgut or intra-hemolymph into silkworm with 50 µl of 10 % v/v DMSO in 0.9% NaCl and the treatments group were also injected via intra-midgut or intra-hemolymph into silkworm with 50 µl of ECa 233, asisticoside and madecassoside containing various concentrations. After the treatments, all of silkworms were kept at 27 ± 2 ^{o}C, the mortality of Thai silkworm were observed at 24 and 48 hrs. Median lethal dose (LD_{50}) of ECa 233, asisticoside and madecassoside in Thai silkworm was determined by the regression Probit analysis (SPSS version 16) between Probit unit of lethality of Thai silkworm versus log concentrations of ECa 233, asisticoside and madecassoside (mg/ml).

Hypoglycemic effect of ECa 233, asisticoside and madecassoside in Thai silkworm hemolymph by sugar quatification by phenol sulfuric acid (PSA) method

The optimum concentration of ECa 233, asisticoside and madecassoside that could lower the sugar levels was determined in hemolymph. The first day 5th instar larva silkworms weighing between 0.8-1.2 grams were divided into various groups of 10 larvae/group. Hyperglycemic Thai silkworm larva induced by fed 10% glucose diet or 10% sucrose diet or 10% maltose diet for 1 hour. Then each group of silkworm larva was injected via intra-midgut or intra-hemolymph with test compounds in various concentrations, insulin 3.5 mg/ml and acarbose 20 mg/ml as a positive control and 0.9% NaCl as negative control and kept for another 5 hours. Silkworm hemolymph was taken from the leg of silkworm larva. The phenol-sulfuric acid reaction was performed and the color intensity was measured by microplate reader model Perkin Elmer at wavelength 490 nm and the sugar levels were calculated from the standard curve of the serially diluted glucose solution.

Statistical analysis

In vitro study

Data were showed as means ± S.D. of three-independent experiments.

In vivo study

All results were presented as mean ± S.E.M., each experiment was performed in duplicate. Statistical analysis was performed by SPSS version 16. Differences among means were analyzed using one way analysis of variance (ANOVA) followed by turkey test for multiple comparisons. The data were considered significant, if *P*- value was less than 0.05.

CHAPTER IV

RESULTS

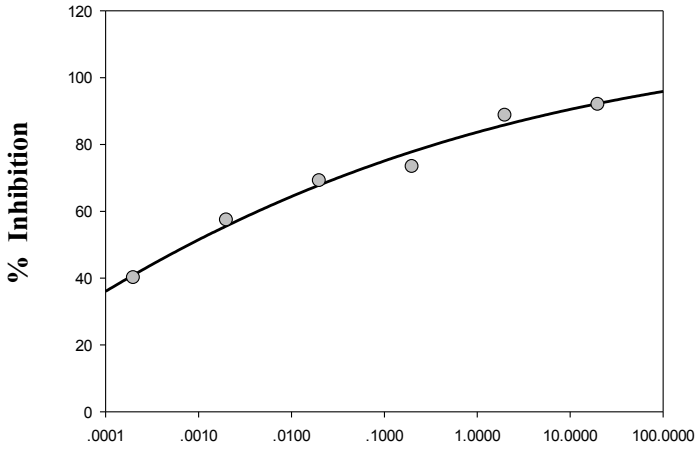
***In vitro* study**

1. *In vitro* inhibition of α-glucosidase

1.1 Inhibition of yeast α-glucosidase

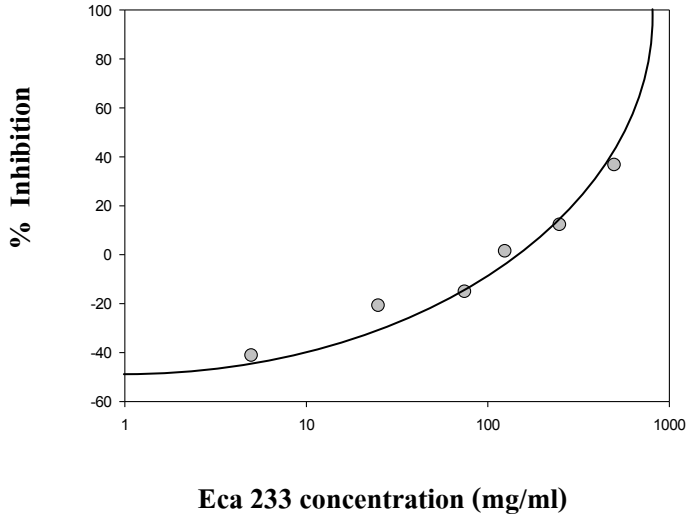
Yeast α-glucosidase inhibitory activity of the acarbose, ECa 233, asiaticoside and madecassoside were measured (Figure 6). Asiaticoside showed inhibitory potential with % inhibition ranging from 14.09-77.57 for concentrations ranging from 15–150 mg/ml. Similarly, madecassoside showed inhibitory potential with % inhibition ranging from 8.49–99.16 for concentrations ranging from 15–125 mg/ml. ECa 233, at the highest concentration of 500 mg/ml showed % inhibition of about 31.31. Acarbose, the positive control group showed a maximum percentage inhibition of 91.93 at 20 mg/ml. The IC_{50} values for acarbose, ECa 233, asiaticoside and madecassoside are 0.0008, >500.00, 92.42 and 68.89 mg/ml, respectively. However, among the compounds tested, asiaticoside and madecassoside had α-glucosidase inhibitory activity more than ECa 233.

(6a)



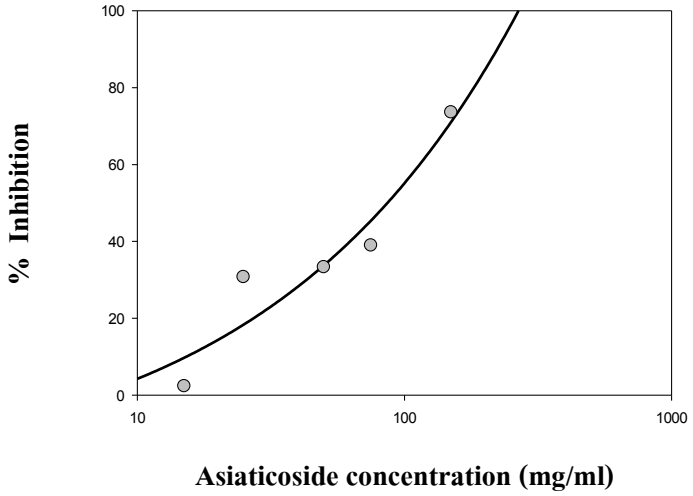
IC_{50} = 0.0008 mg/ml

(6b)



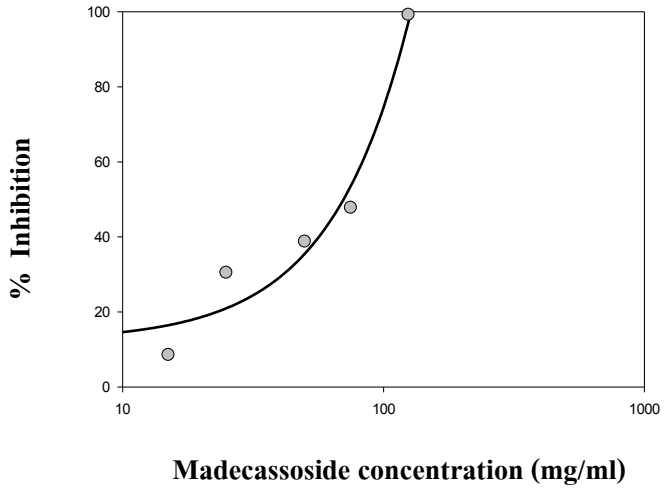
IC_{50} >500 mg/ml

(6c)



IC_{50} = 92.42 mg/ml

(6d)



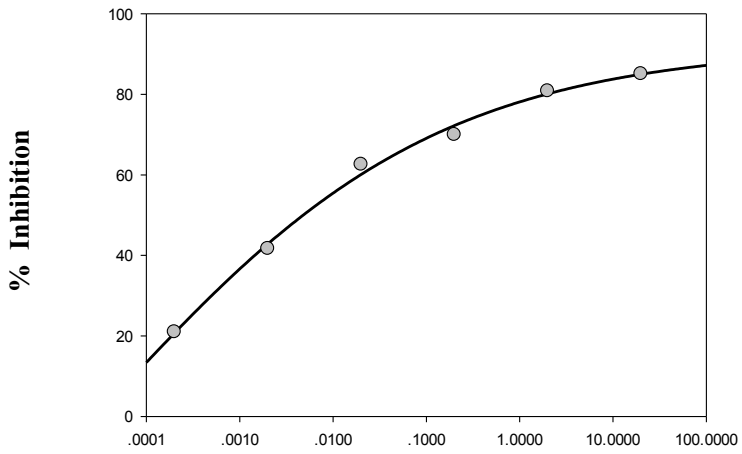
IC_{50} = 68.89 mg/ml

Figure 6 Inhibition of yeast α-glucosidase of acarbose (6a), ECa 233 (6b), asiaticoside (6c), and madecassoside (6d)

1.2 Inhibition of intestinal α-glucosidase from rat

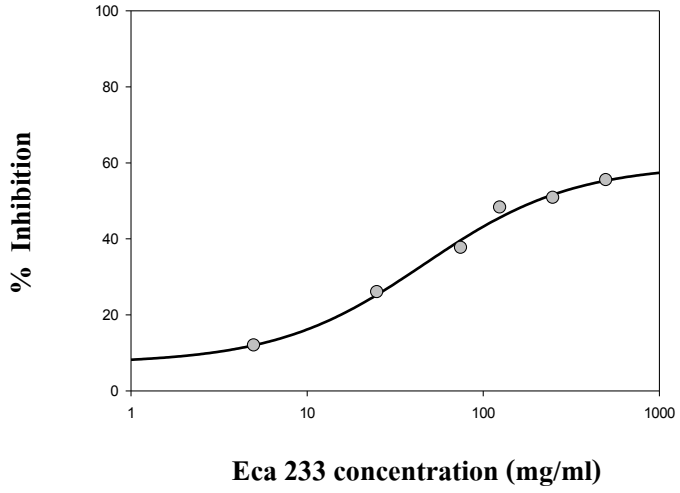
Intestinal α-glucosidase from rat inhibitory activity of acarbose, ECa 233, asiaticoside and madecassoside were measured (Figure 7). Asiaticoside showed inhibitory potential with % inhibition ranging from 31.69-88.10 for concentrations ranging from 15–150 mg/ml. Similarly, madecassoside showed inhibitory potential with % inhibition ranging from 8.36-94.68 for concentrations ranging from 15–125 mg/ml. ECa 233, at the hightest concentration of 500 mg/ml showed % inhibition of about 57.26. Acarbose as positive control group showed a maximum percentage inhibition of 85.08 at 20 mg/ml. The IC_{50} values for acarbose, ECa 233, asiaticoside and madecassoside are 0.005, 205.62, 21.82 and 50.52 mg/ml, respectively. However, among the compounds, tested asiaticoside and madecassoside had α-glucosidase inhibitory activity more than ECa 233.

(7a)



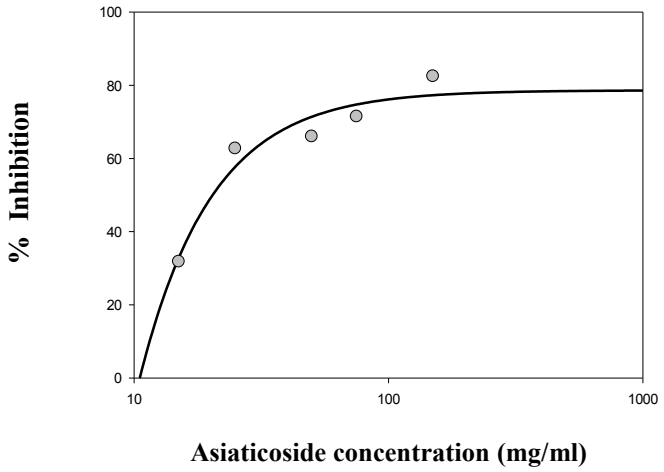
IC_{50} = 0.005 mg/ml

(7b)



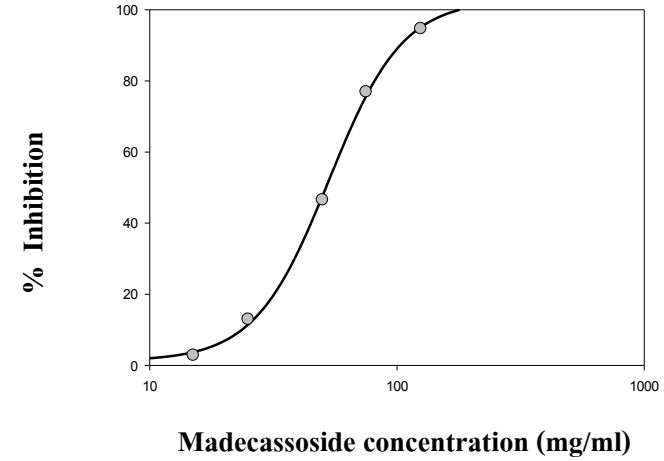
IC_{50} = 205.61 mg/ml

(7c)



IC_{50} = 21.81 mg/ml

(7d)



IC_{50} = 50.52 mg/ml

Figure 7 Inhibition of intestinal α-glucosidase from rat of acarbose (7a), ECa 233 (7b), asiaticoside (7c), and madecassoside (7d)

***In vivo* study**

2. Toxicity of test compounds injected via intra-midgut

After the 5th instar larva Thai silkworm were injected with 50 µl of test compounds via intra-midgut (ECa 233 concentration at 5, 25, 50, 100, 200, 400 mg/ml, asiaticoside concentration at 2.5, 12.5, 25, 75, 125 mg/ml, madecassoside concentration at 25, 50, 75, 125, 250 mg/ml and 0.9% NaCl as a negative control), the mortality and survival of test compounds in Thai silkworm were observed at 27 ± 2 ^{o}C after 24 and 48 hrs. Median lethal dose (LD_{50}) of test compounds in Thai silkworm was determined by the regression probit analysis (SPSS version 16). The result showed that LD_{50} of ECa 233, asiaticoside and madecassoside was 178.51 mg/ml or 8.93 mg/g larva, >125 mg/ml or 6.25 mg/g larva and >250 mg/ml or 12.5 mg/g larva, respectively. Asiaticoside up to 125 mg/ml and madecassoside up to 250 mg/ml did not have the toxic effect to the Thai silkworm.

3. Toxicity of test compounds injected via intra-hemolymph

After the 5th instar larva Thai silkworm were injected with 50 µl of test compounds via intra-hemolymph (ECa 233 concentration at 5, 25, 50, 75, 100 mg/ml, asiaticoside concentration at 2.5, 12.5, 25, 75, 125 mg/ml, madecassoside concentration at 5, 50, 100, 125, 150, 250 mg/ml and 0.9% NaCl as a negative control), then the mortality and survival of Thai silkworm were observed at 27 ± 2 ^{o}C after 24 and 48 hrs. LD_{50} of test compounds in Thai silkworm was determined by the regression probit analysis (SPSS version 16). The result showed that LD_{50} of ECa 233, asiaticoside and madecassoside was 46.92 mg/ml or 2.35 mg/g larva, >125 mg/ml or 6.25 mg/g larva and 174.58 mg/ml or 8.73 mg/g larva, respectively. Asiaticoside up to 125 mg/ml did not have the toxic effect to the Thai silkworm.

4. Time profile of sugar levels in Thai silkworm hemolymph after receiving various sugar-containing diets

4.1 Normal diet

After feeding with artificial diet 5 g/group for 1 hr, the diet then was taken out. Each group of silkworms was kept for different times. Silkworm hemolymph was collected and sugar levels in silkworm hemolymph were analyzed. The result showed that sugar levels in hemolymph of Thai silkworm reached the peak at 5 hr with the concentration of 2.93 ± 0.05 mg/ml (Figure 8).

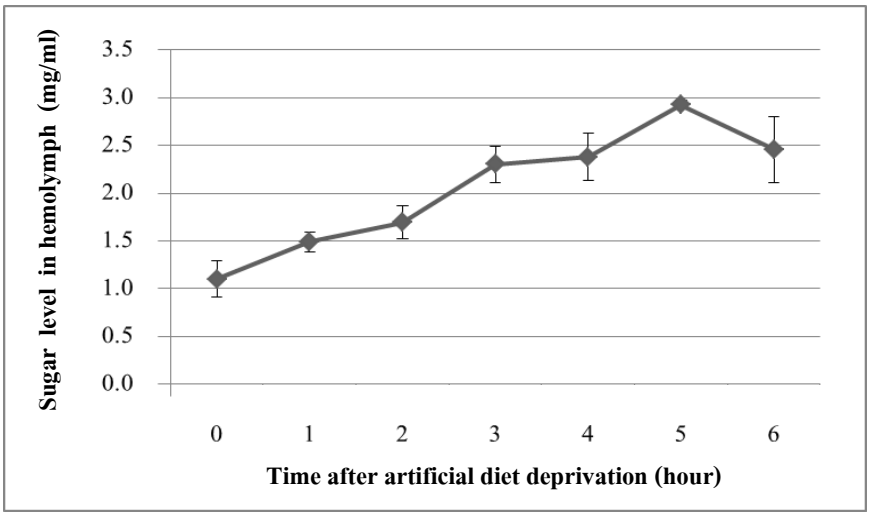


Figure 8 Time profile of sugar levels in Thai silkworm hemolymph after receiving normal diet, each value represents the mean ± S.E.M. (N=10)**.**

4.2 Glucose Diet

After feeding with 10% glucose diet 5 g/group for 1 hr, the diet then was taken out. Each group of silkworms was kept for different times. Silkworm hemolymph was collected and sugar levels in silkworm hemolymph were analyzed. The result showed that sugar levels in hemolymph of Thai silkworm reached the peak at 5 hr with the concentration of 7.02 ± 0.21 mg/ml (Figure 9).

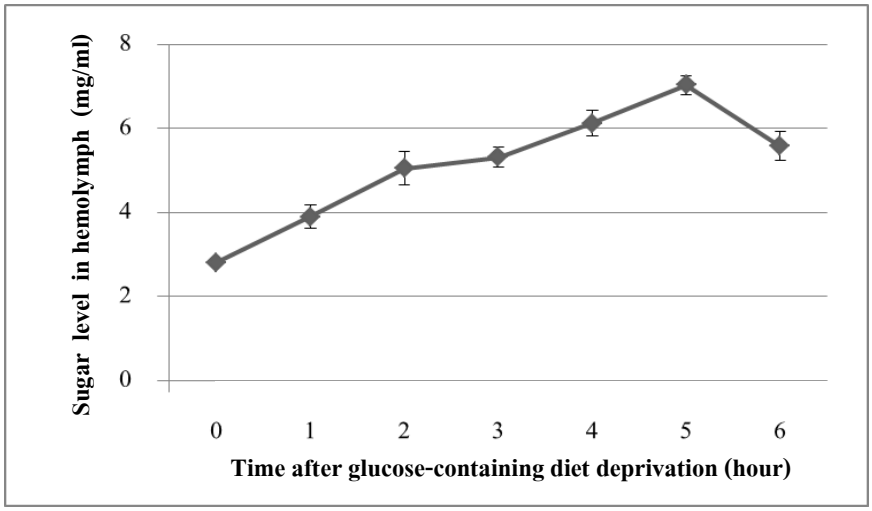


Figure 9 Time profile of sugar levels in Thai silkworm hemolymph after receiving 10% glucose diet, each value represents the mean ± S.E.M. (N=10).

4.3 Sucrose diet

After feeding with 10% sucrose diet 5 g/group for 1 hr, the diet then was taken out. Each group of silkworms was kept for different times. Silkworm hemolymph was collected and sugar levels in silkworm hemolymph were analyzed. The result showed that sugar levels in hemolymph of Thai silkworm reached the peak at 4 hr with the concentration of 8.60 ± 0.35 mg/ml (Figure 10).

Figure 10 Time profile of sugar levels in Thai silkworm hemolymph after receiving 10% sucrose diet, each value represents the mean ± S.E.M. (N=10).

4.4 Maltose diet

After feeding with 10% maltose diet 5 g/group for 1 hr, the diet then was taken out. Each group of silkworms was kept for different times. Silkworm hemolymph was collected and sugar levels in silkworm hemolymph were analyzed. The result showed that sugar levels in hemolymph of Thai silkworm reached the peak at 5 hr with the concentration of 9.52 ± 0.64 mg/ml (Figure 11).

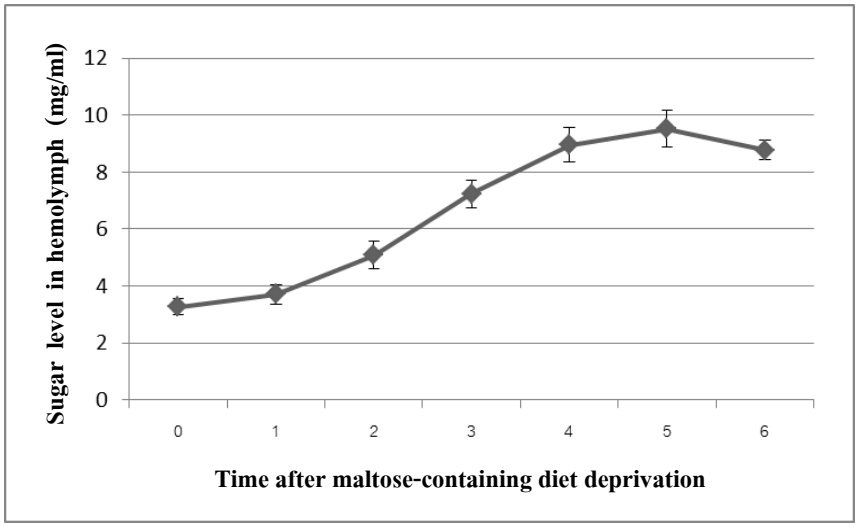


Figure 11 Time profile of sugar levels in Thai silkworm hemolymph after receiving 10% maltose diet, each value represents the mean ± S.E.M. (N=10).

5. Sugar concentration profile after receiving high sugar diet in silkworms

5.1 Glucose concentration profile

To determine the optimum concentration of glucose diet that could to induce hyperglycemia, 5%, 10% and 20% glucose diet 5 g/group was fed to silkworm for 1 hr and then diet was taken out, each group of silkworm was kept for 5 hr. Silkworm hemolymph was collected and sugar levels in silkworm hemolymph were analyzed. The result showed that sugar levels in hemolymph was significantly induced the hyperglycemia after fed 5% glucose diet group, 10% glucose diet group and 20% glucose diet group (Figure 12).

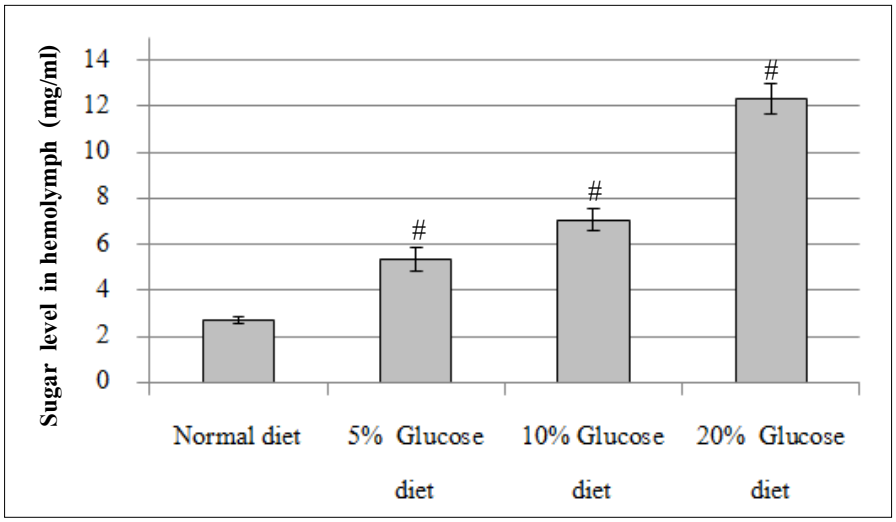


Figure 12 Glucose concentration profile in Thai silkworm hemolymph after receiving the different glucose concentrations, each bar represents the mean ± S.E.M. (N=10), # $P<$ 0.05 compared to normal diet group**.**

5.2 Sucrose diet

To determine the optimum concentration of sucrose diet that could to induce hyperglycemia, 5%, 10% and 20% sucrose diet 5 g/group was fed to silkworm for 1 hr and then diet was taken out, each group of silkworm was kept for 5 hr. Silkworm hemolymph was collected and sugar levels in silkworm hemolymph were analyzed. The result showed that sugar levels in hemolymph was significantly induced the hyperglycemia after fed 5% sucrose diet group, 10% sucrose diet group and 20% sucrose diet group (Figure 13).

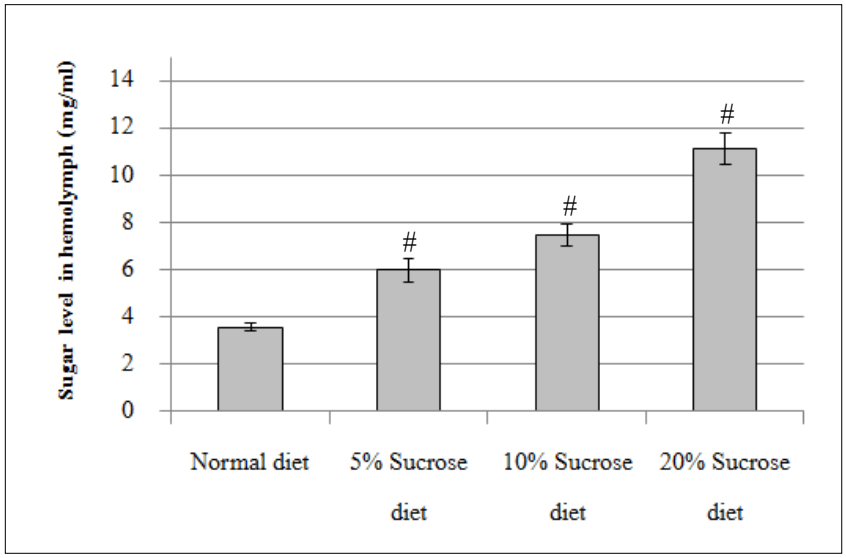


Figure 13 Sucrose concentration profile in Thai silkworm hemolymph after receiving the different sucrose concentrations, each bar represents the mean ± S.E.M. (N=10), # $P< 0.05$ compared to normal diet group**.**

5.3 Maltose diet

To determine the optimum concentration of sucrose diet that could to induce hyperglycemia, 5%, 10% and 20% maltose diet 5 g/group was fed to silkworm for 1 hr and then diet was taken out, each group of silkworm was kept for 5 hr. Silkworm hemolymph was collected and sugar levels in silkworm hemolymph were analyzed. The result showed that sugar levels in hemolymph was significantly induced the hyperglycemia after fed 5% maltose diet group, 10% maltose diet group and 20% maltose diet group (Figure 14).

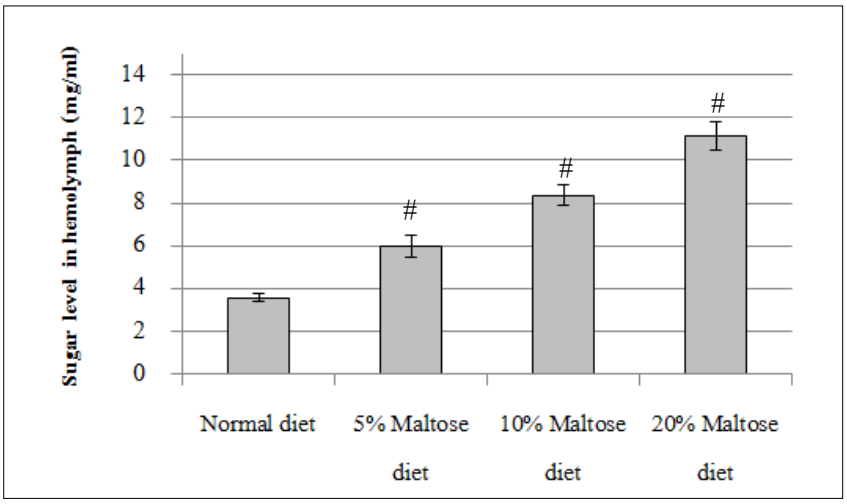


Figure 14 Maltose concentration profile in Thai silkworm hemolymph after receiving the different maltose concentrations, each bar represents the mean ± S.E.M. (N=10), # $P<$ 0.05 compared to normal diet group**.**

6. Hypoglycemic effect of ECa 233, asiaticoside and madecassoside in Thai silkworm injected via intra-midgut

6.1 ECa 233

a) Glucose diet

After feeding the 5th instar larva silkworm with 10% glucose diet 5 g/group for 1 hr, then diet was taken out and ECa 233 solution in several concentrations (0.02, 0.2, 2, 20 mg/ml and 0.9% NaCl as a negative control) volume 50 µl were injected via intra-midgut. Silkworm were kept at 27 ± 2 °C for 5 hrs, then hemolymph was collected and sugar levels were analyzed. The result showed that ECa 233 including acarbose had not hypoglycemic effect in Thai silkworm hemolymph with statistical significance ($P< 0.05$).

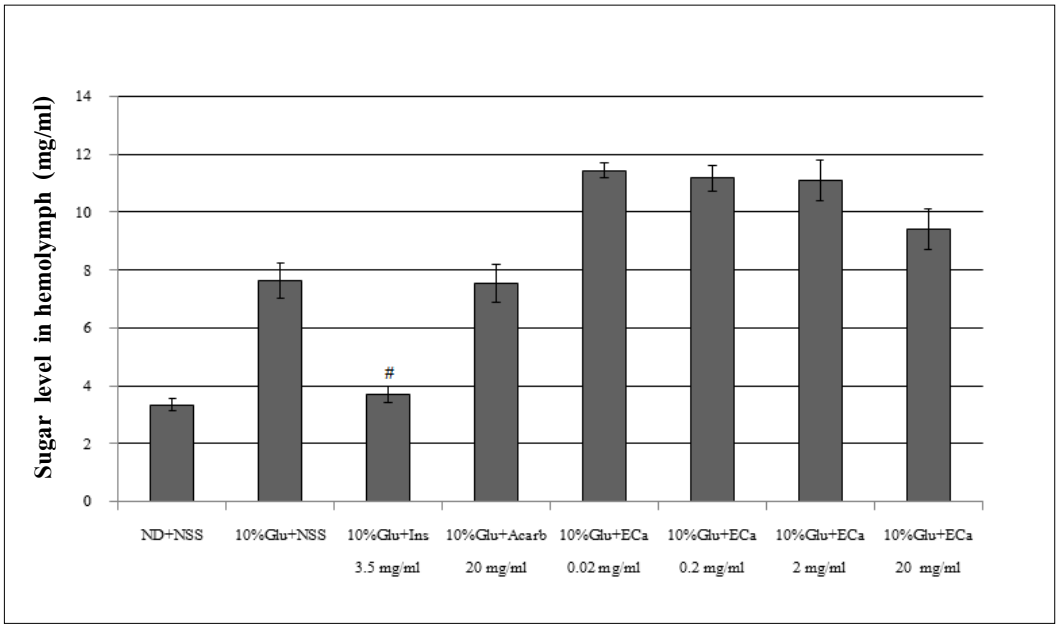


Figure 15 Hypoglycemic effect of ECa 233 in Thai silkworm hemolymph after fed 10% glucose diet, each bar represents the mean ± S.E.M. (N=10), # $P< 0.05$ compared to control group**.**

b) Sucrose diet

After feeding the 5th instar larva silkworm with 10% sucrose diet 5 g/group for 1 hr, then diet was taken out and the ECa 233 solution in several concentrations (0.02, 0.2, 2, 20 mg/ml and 0.9% NaCl as a negative control) volume 50 µl were injected via intra-midgut. Silkworm were kept at 27 ± 2 °C for 5 hrs, then hemolymph was collected and sugar levels were analyzed. The result showed that ECa 233 had not hypoglycemic effect in Thai silkworm hemolymph whereas the positive control, acarbose concentration at 20 mg/ml can decrease sugar with statistical significance ($P< 0.05$).

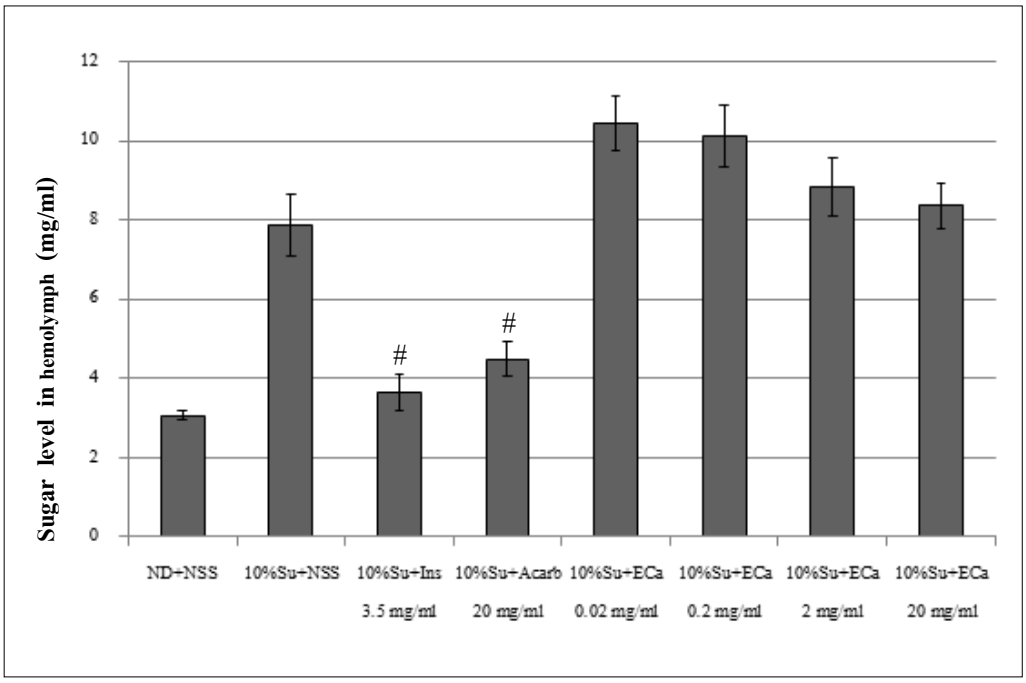


Figure 16 Hypoglycemic effect of ECa 233 in Thai silkworm hemolymph after fed 10% sucrose diet, each bar represents the mean ± S.E.M. (N=10), # $P< 0.05$ compared to control group**.**

c) Maltose diet

After feeding the 5th instar larva silkworm with 10% maltose diet 5 g/group for 1 hr, then diet was taken out and the ECa 233 solution in several concentrations (0.02, 0.2, 2, 20 mg/ml and 0.9% NaCl as a negative control) volume 50 µl were injected via intra-midgut. Silkworm were kept at 27 ± 2 °C for 5 hrs, then hemolymph was collected and sugar levels were analyzed. The result showed that ECa 233 had not hypoglycemic effect in Thai silkworm hemolymph with statistical significance ($P< 0.05$).

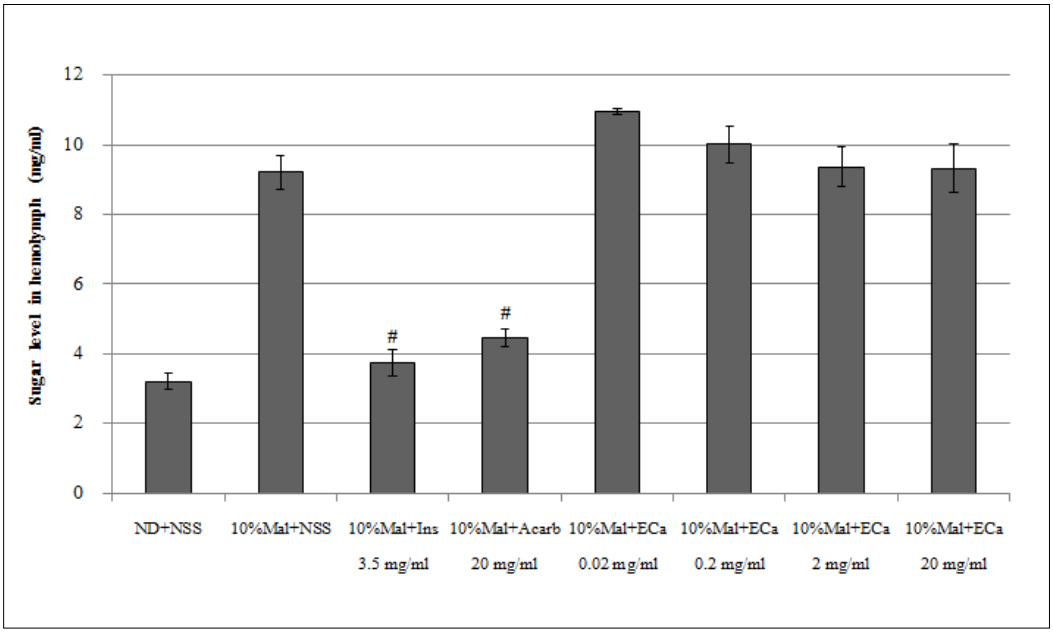


Figure 17 Hypoglycemic effect of ECa 233 in Thai silkworm hemolymph after fed 10% maltose diet, each bar represents the mean ± S.E.M. (N=10), # $P< 0.05$ compared to control group**.**

6.2 Asiaticoside

a) Glucose diet

After feeding the 5th instar larva silkworm with 10% glucose diet 5 g/group for 1 hr, then diet was taken out and asiaticoside solution in several concentrations (0.05, 0.5, 5, 50 mg/ml and 0.9% NaCl as a negative control) volume 50 µl were injected via intra-midgut. Silkworm were kept at 27 ± 2 °C for 5 hrs, then hemolymph was collected and sugar levels were analyzed. The result showed that asiaticoside and acarbose had not hypoglycemic effect in Thai silkworm hemolymph with statistical significance ($P< 0.05$).

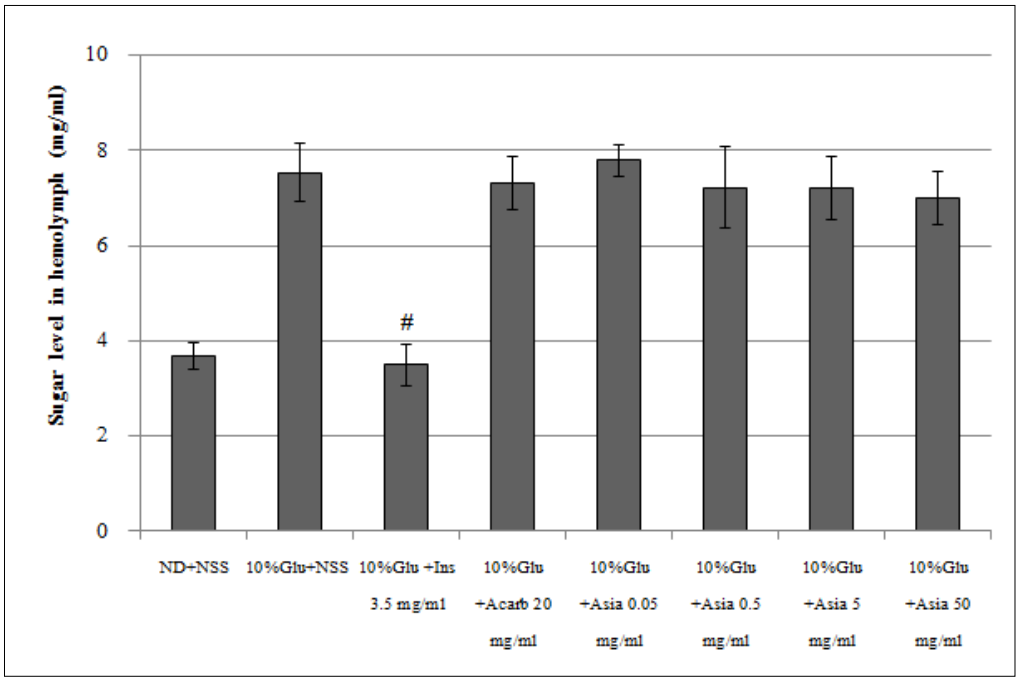


Figure 18 Hypoglycemic effect of asiaticoside in Thai silkworm hemolymph after fed 10% glucose diet, each bar represents the mean ± S.E.M. (N=10), # $P< 0.05$ compared to control group**.**

b) Sucrose diet

After feeding the 5th instar larva silkworm with 10% sucrose diet 5 g/group for 1 hr, then diet was taken out and asiaticoside solution in several concentrations (0.05, 0.5, 5, 50 mg/ml and 0.9% NaCl as a negative control) volume 50 µl were injected via intra-midgut. Silkworm were kept at 27 ± 2 °C for 5 hrs, then hemolymph was collected and sugar levels were analyzed. The result showed that asiaticoside concentration at 50 mg/ml and acarbose had hypoglycemic effect in Thai silkworm hemolymph with statistical significance ($P< 0.05$).

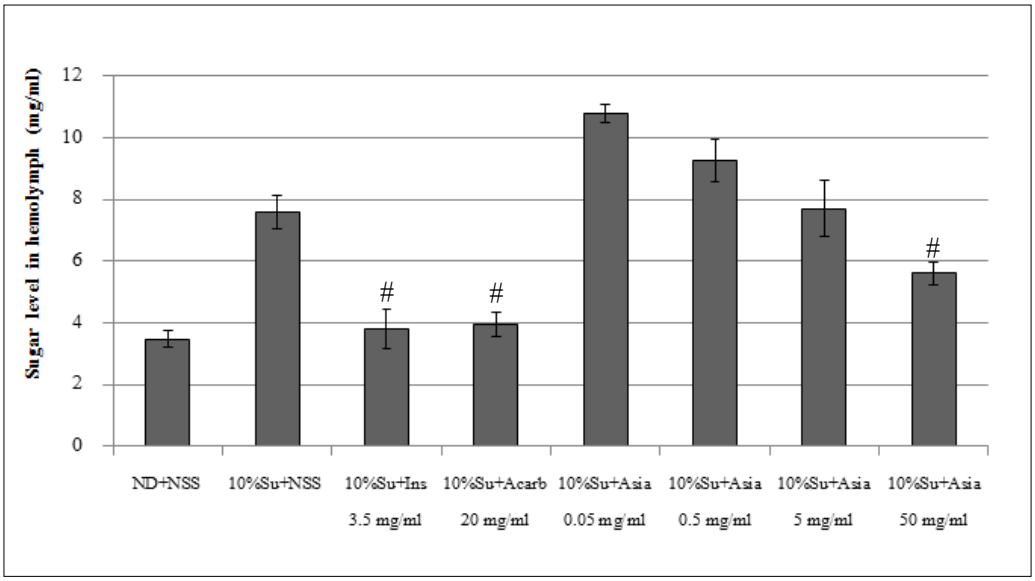


Figure 19 Hypoglycemic effect of asiaticoside in Thai silkworm hemolymph after fed 10% sucrose diet, each bar represents the mean ± S.E.M. (N=10), # $P< 0.05$ compared to control group**.**

c) Maltose diet

After feeding the 5th instar larva silkworm with 10% maltose diet 5 g/group for 1 hr, then diet was taken out and asiaticoside solution in several concentrations (0.05, 0.5, 5, 50 mg/ml and 0.9% NaCl as a negative control) volume 50 µl were injected via intra-midgut. Silkworm were kept at 27 ± 2 °C for 5 hrs, then hemolymph was collected and sugar levels were analyzed. The result showed that asiaticoside concentration at 0.5, 5, 50 mg/ml and acarbose had hypoglycemic effect in Thai silkworm hemolymph with statistical significance ($P< 0.05$).

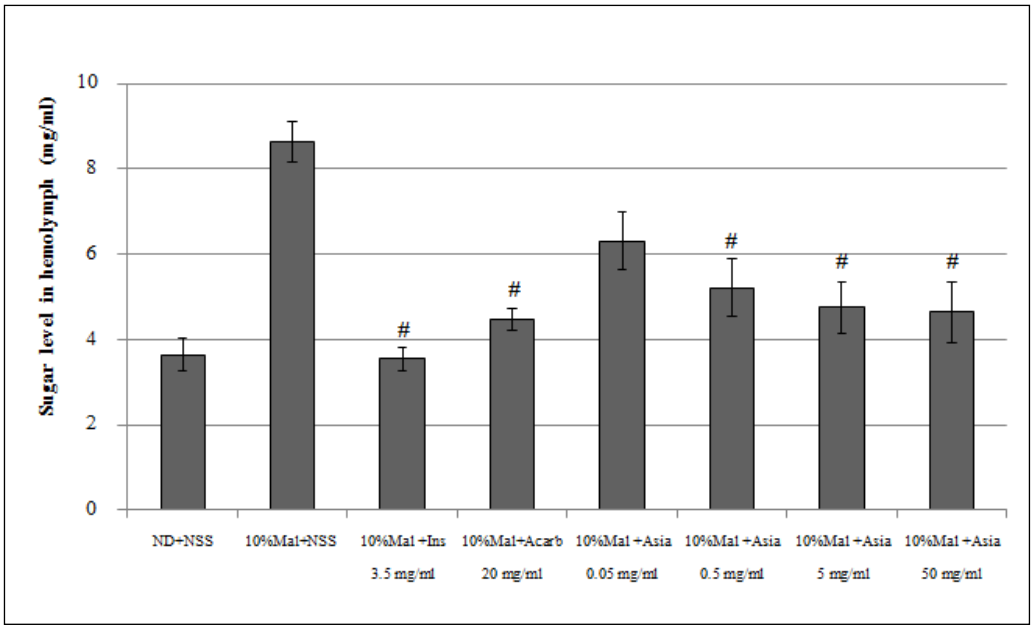


Figure 20 Hypoglycemic effect of asiaticoside in Thai silkworm hemolymph after fed 10% maltose diet, each bar represents the mean ± S.E.M. (N=10), # $P< 0.05$ compared to control group**.**

6.3 Madecassoside

a) Glucose diet

After feeding the 5th instar larva silkworm with 10% glucose diet 5 g/group for 1 hr, then diet was taken out and madecassoside solution in several concentrations (0.05, 0.5, 5, 50 mg/ml and 0.9% NaCl as a negative control) volume 50 µl were injected via intra-midgut. Silkworm were kept at 27 ± 2 °C for 5 hrs, then hemolymph was collected and sugar levels were analyzed. The result showed that madecassoside and acarbose had not hypoglycemic effect in Thai silkworm hemolymph with statistical significance ($P< 0.05$).

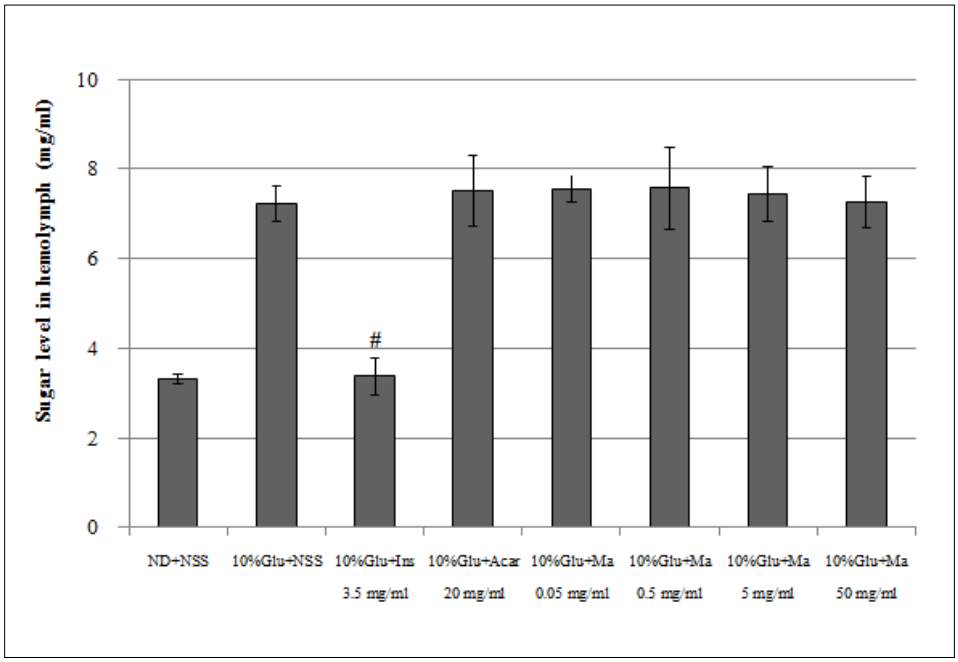


Figure 21 Hypoglycemic effect of madecassoside in Thai silkworm hemolymph after fed 10% glucose diet, each bar represents the mean ± S.E.M. (N=10), # $P< 0.05$ compared to control group**.**

b) Sucrose diet

After feeding the 5th instar larva silkworm with 10% sucrose diet 5 g/group for 1 hr, then diet was taken out and madecassoside solution in several concentrations (0.05, 0.5, 5, 50 mg/ml and 0.9% NaCl as a negative control) volume 50 µl were injected via intra-midgut. Silkworm were kept at 27 ± 2 °C for 5 hrs, then hemolymph was collected and sugar levels were analyzed. The result showed that madecassoside concentration at 5, 50 mg/ml and acarbose had hypoglycemic effect in Thai silkworm hemolymph with statistical significance ($P< 0.05$).

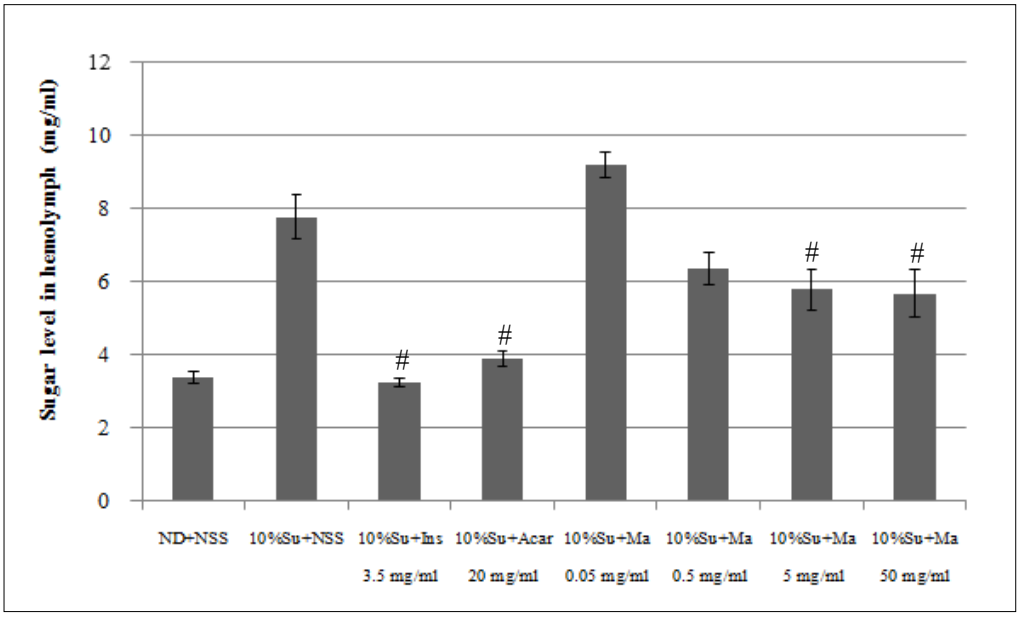


Figure 22 Hypoglycemic effect of madecassoside in Thai silkworm hemolymph after fed 10% sucrose diet, each bar represents the mean ± S.E.M. (N=10), # $P< 0.05$ compared to control group.

c) Maltose diet

After feeding the 5th instar larva silkworm with 10% maltose diet 5 g/group for 1 hr, then diet was taken out and madecassoside solution in several concentrations (0.05, 0.5, 5, 50 mg/ml and 0.9% NaCl as a negative control) volume 50 µl were injected via intra-midgut. Silkworm were kept at 27 ± 2 °C for 5 hrs, then hemolymph was collected and sugar levels were analyzed. The result showed that all concentrations of madecassoside and acarbose had hypoglycemic effect in Thai silkworm hemolymph with statistical significance ($P< 0.05$).

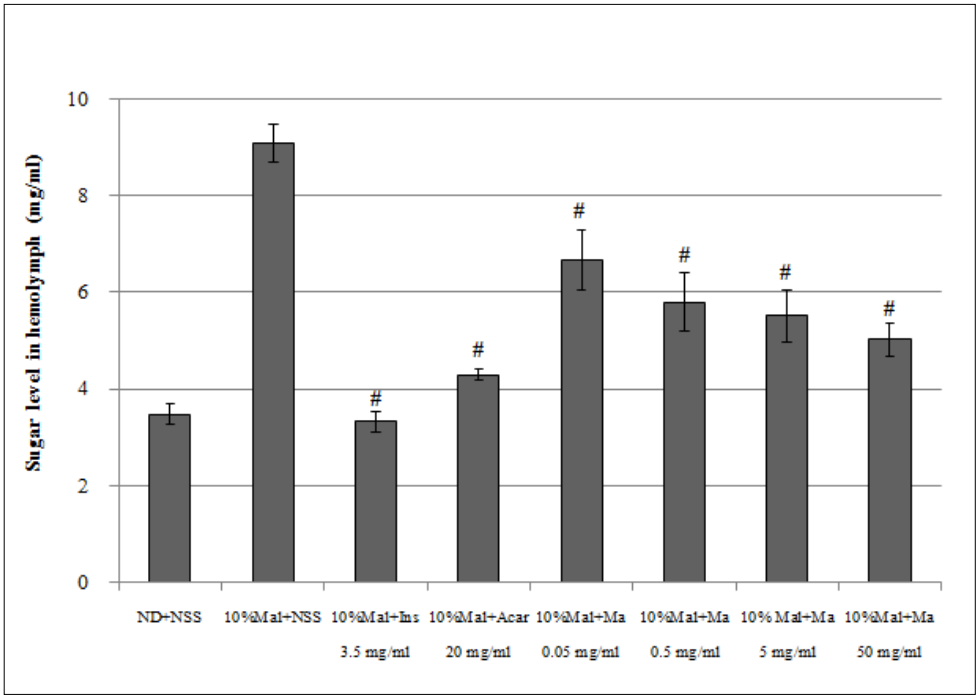


Figure 23 Hypoglycemic effect of madecassoside in Thai silkworm hemolymph after fed 10% maltose diet, each bar represents the mean ± S.E.M. (N=10), # $P< 0.05$ compared to control group**.**

7. Hypoglycemic effect of ECa 233, asiaticoside and madecassoside in Thai silkworm injected via intra-hemolymph

7.1 ECa 233

a) Glucose diet

After feeding the 5th instar larva silkworm with 10% glucose diet 5 g/group for 1 hr, then diet was taken out and ECa 233 solution in several concentrations (0.005, 0.05, 0.5, 5 mg/ml and 0.9% NaCl as a negative control) volume 50 µl were injected via intra-hemolymph. Silkworm were kept at 27 ± 2 °C for 5 hrs, then hemolymph was collected and sugar levels were analyzed. The result showed that ECa 233 concentration at 0.05, 0.5, 5 mg/ml had hypoglycemic effect in Thai silkworm hemolymph with statistical significance ($P< 0.05$).

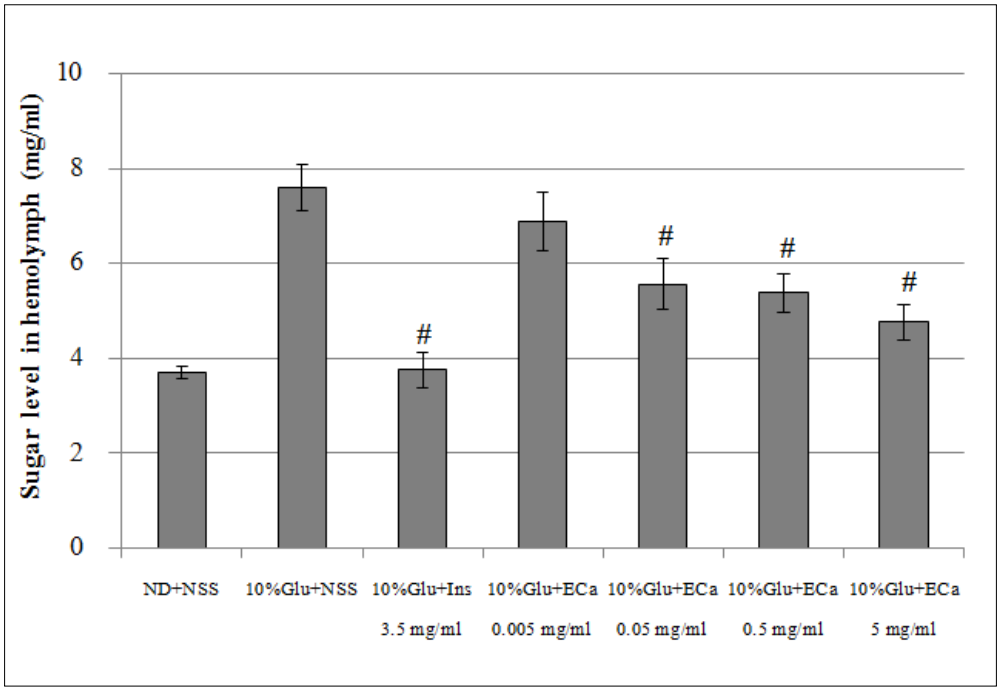


Figure 24 Hypoglycemic effect of ECa 233 in Thai silkworm hemolymph after fed 10% glucose diet, each bar represents the mean ± S.E.M. (N=10), # $P< 0.05$ compared to control group**.**

b) Sucrose diet

After feeding the 5th instar larva silkworm with 10% sucrose diet 5 g/group for 1 hr, then diet was taken out and ECa 233 solution in several concentrations (0.005, 0.05, 0.5, 5 mg/ml and 0.9% NaCl as a negative control) volume 50 µl were injected via intra-hemolymph. Silkworm were kept at 27 ± 2 °C for 5 hrs, then hemolymph was collected and sugar levels were analyzed. The result showed that only ECa 233 concentration at 5 mg/ml had hypoglycemic effect in Thai silkworm hemolymph with statistical significance (P <0.05).

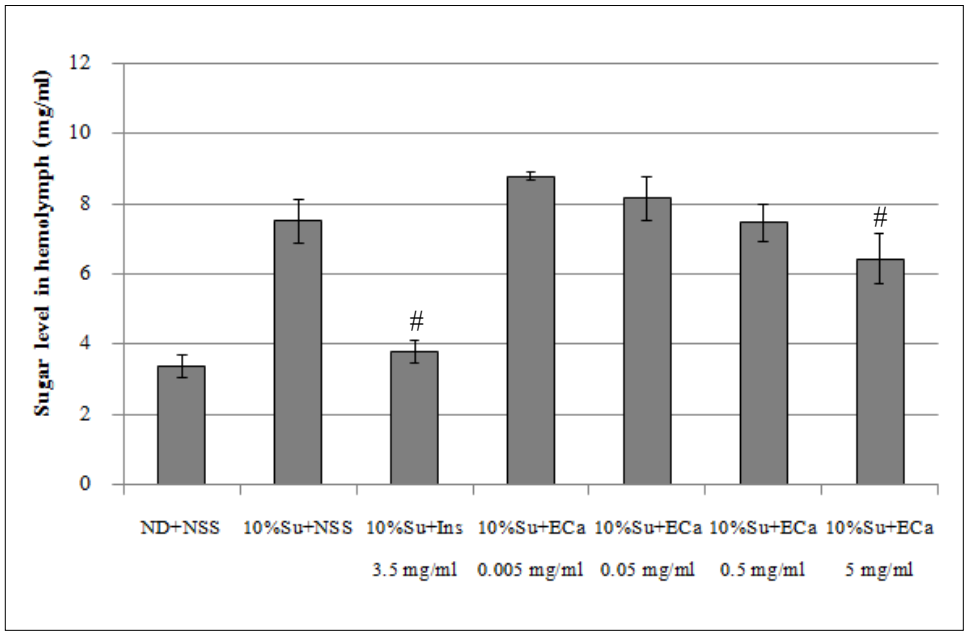


Figure 25 Hypoglycemic effect of ECa 233 in Thai silkworm hemolymph after fed 10% sucrose diet, each bar represents the mean ± S.E.M. (N=10), # P< 0.05 compared to control group**.**

c) Maltose diet

After feeding the 5th instar larva silkworm with 10% maltose diet 5 g/group for 1 hr, then diet was taken out and ECa 233 solution in several concentrations (0.005, 0.05, 0.5, 5 mg/ml and 0.9% NaCl as a negative control) volume 50 µl were injected via intra-hemolymph. Silkworm were kept at 27 ± 2 °C for 5 hrs, then hemolymph was collected and sugar levels were analyzed. The result showed that ECa 233 concentration at 0.5, 5 mg/ml had hypoglycemic effect in Thai silkworm hemolymph with statistical significance ($P< 0.05$).

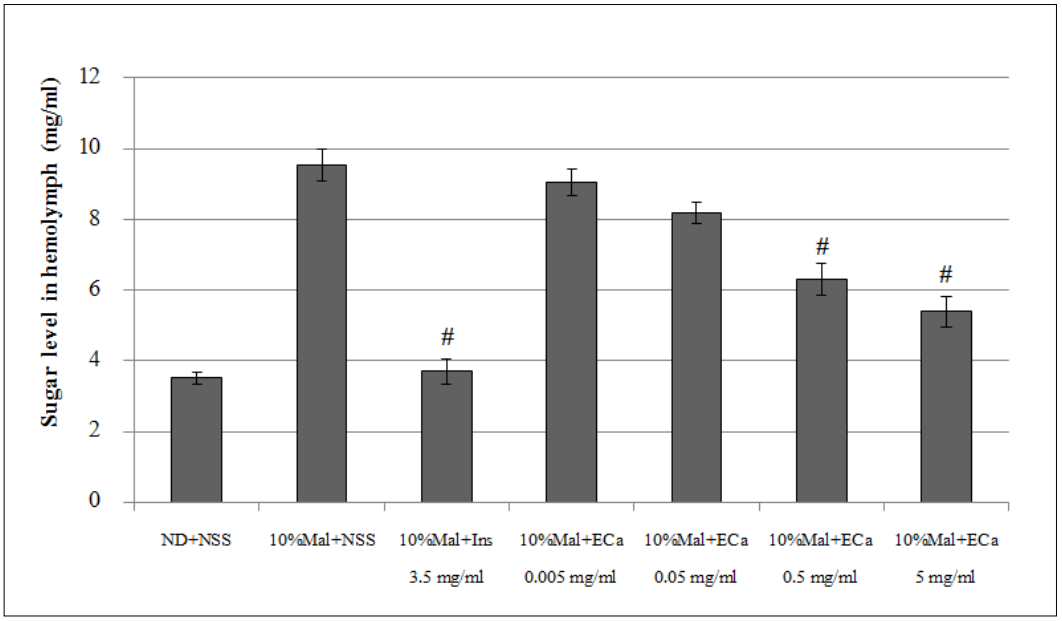


Figure 26 Hypoglycemic effect of ECa 233 in Thai silkworm hemolymph after fed 10% maltose diet, each bar represents the mean ± S.E.M. (N=10), # $P< 0.05$ compared to control group.

7.2 Asiaticoside

a) Glucose diet

After feeding the 5th instar larva silkworm with 10% glucose diet 5 g/group for 1 hr, then diet was taken out and asiaticoside solution in several concentrations (0.05, 0.5, 5, 50 mg/ml and 0.9% NaCl as a negative control) volume 50 µl were injected via intra-hemolymph. Silkworm were kept at 27 ± 2 °C for 5 hrs, then hemolymph was collected and sugar levels were analyzed. The result showed that asiaticoside concentration at 5, 50 mg/ml had hypoglycemic effect in Thai silkworm hemolymph with statistical significance ($P< 0.05$).

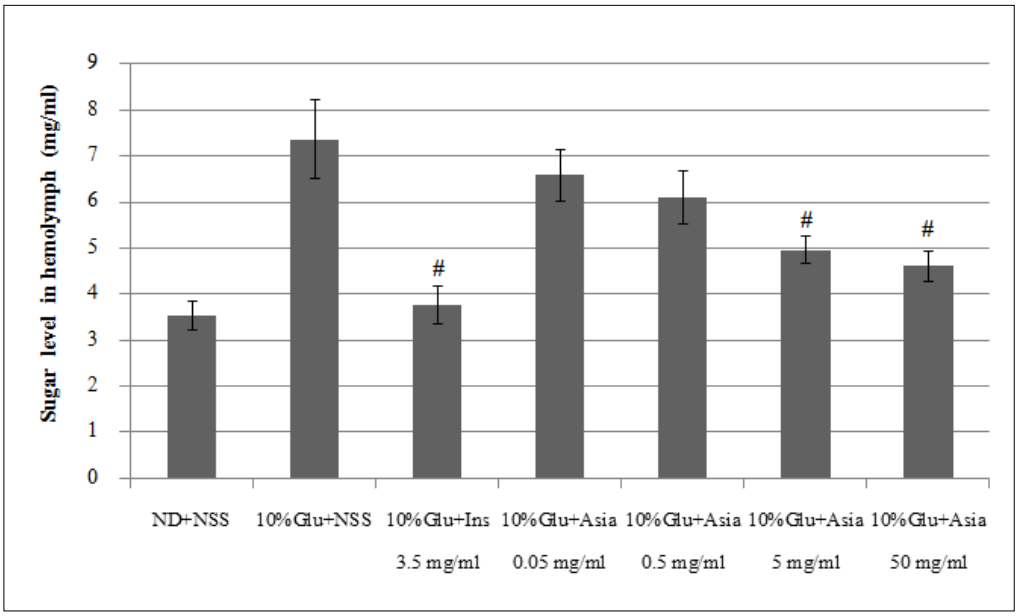


Figure 27 Hypoglycemic effect of asiaticoside in Thai silkworm hemolymph after fed 10% glucose diet, each bar represents the mean ± S.E.M. (N=10), # $P< 0.05$ compared to control group.

b) Sucrose diet

After feeding the 5th instar larva silkworm with 10% sucrose diet 5 g/group for 1 hr, then diet was taken out and asiaticoside solution in several concentrations (0.05, 0.5, 5, 50 mg/ml and 0.9% NaCl as a negative control) volume 50 µl were injected via intra-hemolymph. Silkworm were kept at 27 ± 2 °C for 5 hrs, then hemolymph was collected and sugar levels were analyzed. The result showed that asiaticoside concentration at 50 mg/ml had hypoglycemic effect in Thai silkworm hemolymph with statistical significance ($P< 0.05$).

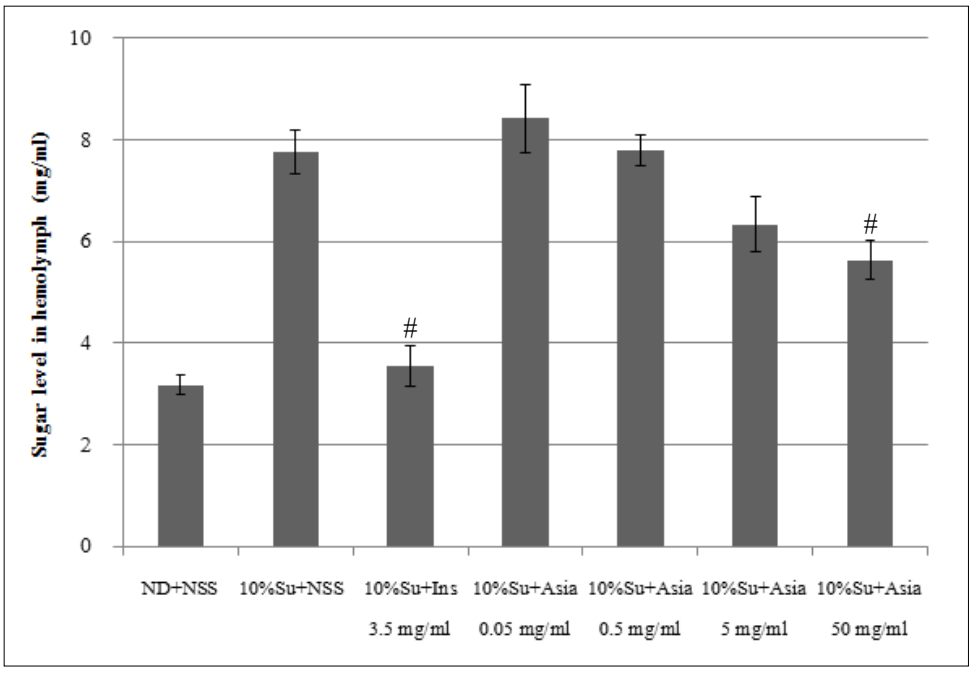


Figure 28 Hypoglycemic effect of asiaticoside in Thai silkworm hemolymph after fed 10% sucrose diet, each bar represents the mean ± S.E.M. (N=10), # $P< 0.05$ compared to control group**.**

c) Maltose diet

After feeding the 5th instar larva silkworm with 10% maltose diet 5 g/group for 1 hr, then diet was taken out and asiaticoside solution in several concentrations (0.05, 0.5, 5, 50 mg/ml and 0.9% NaCl as a negative control) volume 50 µl were injected via intra-hemolymph. Silkworm were kept at 27 ± 2 °C for 5 hrs, then hemolymph was collected and sugar levels were analyzed. The result showed that all concentrations of asiaticoside had hypoglycemic effect in Thai silkworm hemolymph with statistical significance ($P< 0.05$).

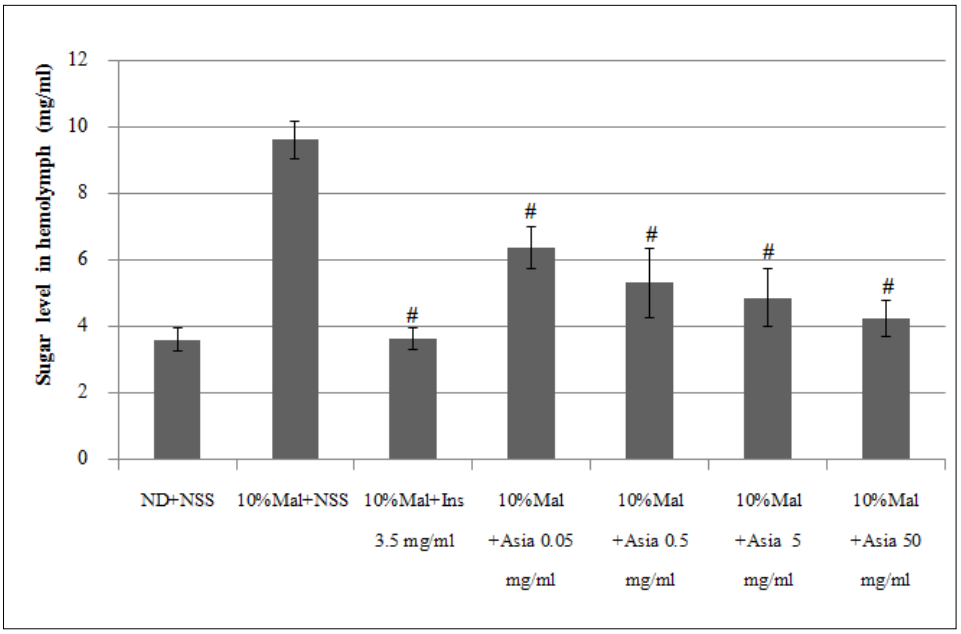


Figure 29 Hypoglycemic effect of asiaticoside in Thai silkworm hemolymph after fed 10% maltose diet, each bar represents the mean ± S.E.M. (N=10), # $P< 0.05$ compared to control group**.**

7.3 Madecassoside

a) Glucose diet

After feeding the 5th instar larva silkworm with 10% glucose diet 5 g/group for 1 hr, then diet was taken out and madecassoside solution in several concentrations (0.05, 0.5, 5, 50 mg/ml and 0.9% NaCl as a negative control) volume 50 µl were injected via intra-hemolymph. Silkworm were kept at 27 ± 2 °C for 5 hrs, then hemolymph was collected and sugar levels were analyzed. The result showed that madecassoside concentration at 50 mg/ml had hypoglycemic effect in Thai silkworm hemolymph with statistical significance ($P< 0.05$).

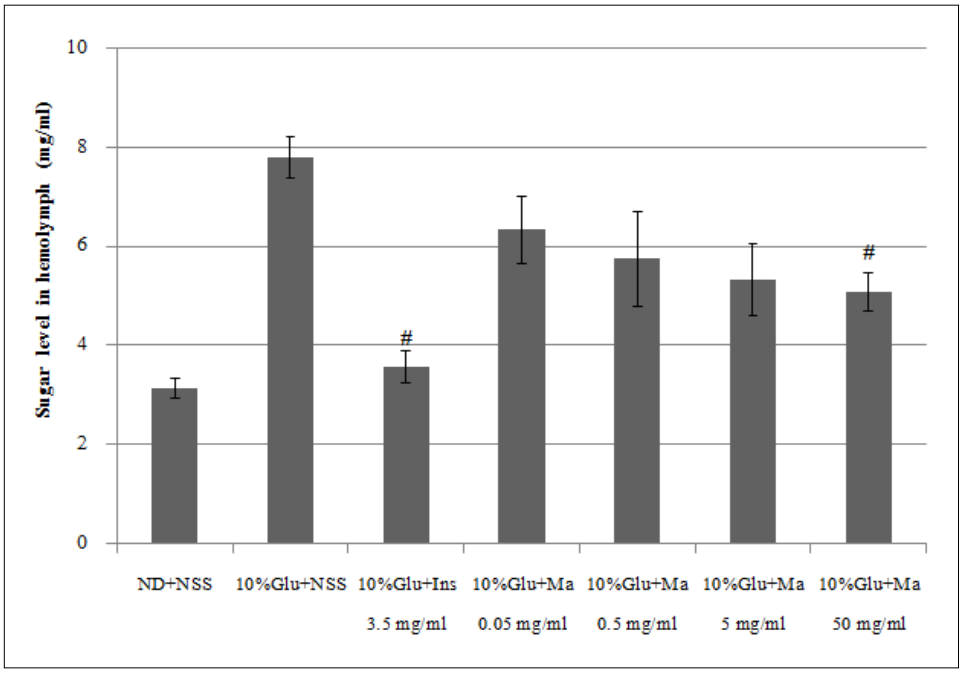


Figure 30 Hypoglycemic effect of madecassoside in Thai silkworm hemolymph after fed 10% glucose diet, each bar represents the mean ± S.E.M. (N=10), # $P< 0.05$ compared to control group.

b) Sucrose diet

After feeding the 5th instar larva silkworm with 10% sucrose diet 5 g/group for 1 hr, then diet was taken out and madecassoside solution in several concentrations (0.05, 0.5, 5, 50 mg/ml and 0.9% NaCl as a negative control) volume 50 µl were injected via intra-hemolymph. Silkworm were kept at 27 ± 2 °C for 5 hrs, then hemolymph was collected and sugar levels were analyzed. The result showed that madecassoside concentration at 50 mg/ml had hypoglycemic effect in Thai silkworm hemolymph with statistical significance ($P< 0.05$).

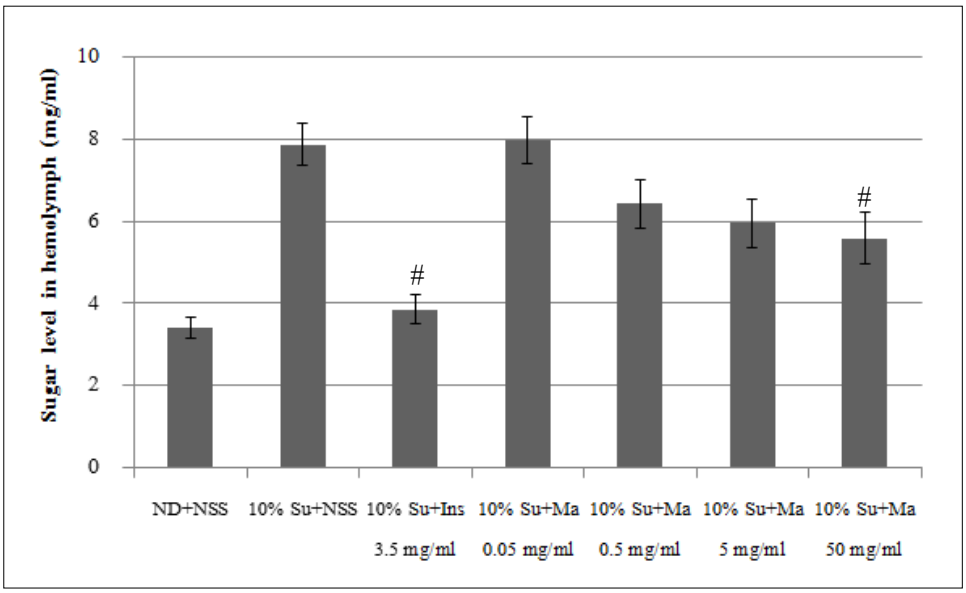


Figure 31 Hypoglycemic effect of madecassoside in Thai silkworm hemolymph after fed 10% sucrose diet, each bar represents the mean ± S.E.M. (N=10), # $P< 0.05$ compared to control group.

c) Maltose diet

After feeding the 5th instar larva silkworm with 10% maltose diet 5 g/group for 1 hr, then diet was taken out and madecassoside solution in several concentrations (0.05, 0.5, 5, 50 mg/ml and 0.9% NaCl as a negative control) volume 50 µl were injected via intra-hemolymph. Silkworm were kept at 27 ± 2 °C for 5 hrs, then hemolymph was collected and sugar levels were analyzed. The result showed that all concentrations of madecassoside had hypoglycemic effect in Thai silkworm hemolymph with statistical significance ($P< 0.05$).

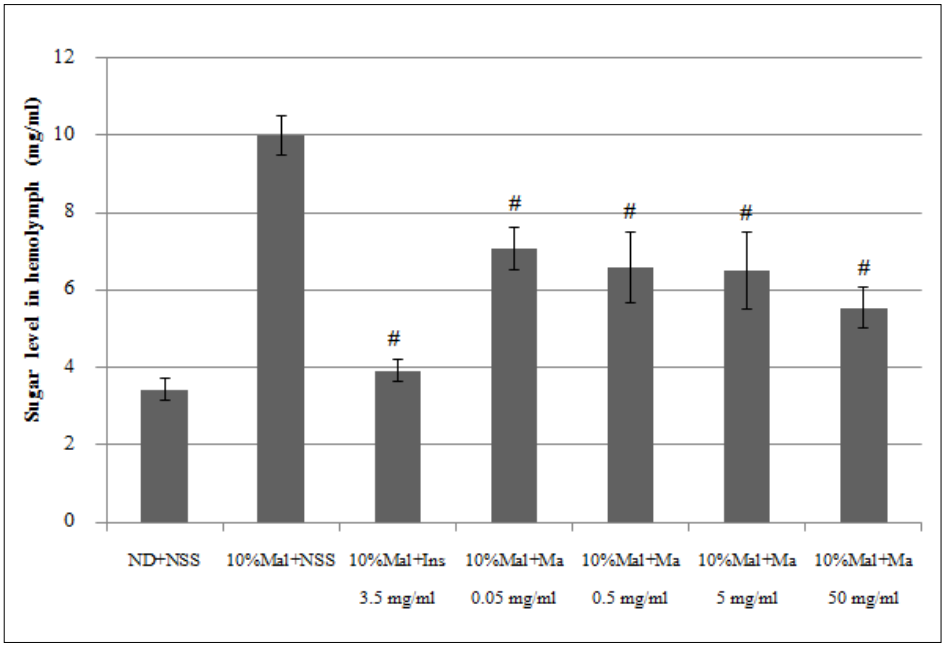


Figure 32 Hypoglycemic effect of madecassoside in Thai silkworm hemolymph after fed 10% maltose diet, each bar represents the mean ± S.E.M. (N=10), # $P< 0.05$ compared to control group**.**

8. Glucose lowering effect of ECa 233, asiaticoside and madecassoside in Thai silkworm model

8.1 Injection via intra-midgut

a) Glucose diet

After feeding the 5th instar larva silkworm with 10% glucose diet 5 g/group for 1 hr, then diet was taken out. The negative control groups (1st and 2nd group) were injected via intra-hemolymph with 50 µl of 0.9% NaCl and the treatment groups were also injected via intra-hemolymph with 50 µl of human insulin (3.5 mg/ml) and injected via intra-midgut with 50 µl of acarbose (20 mg/ml), ECa 233 (20 mg/ml), asiaticoside (50 mg/ml) and madecassoside (50 mg/ml), respectively. Silkworm larvae were kept at 27 ± 2 °C for 5 hrs, then hemolymph was collected and sugar levels were analyzed. The result showed that human insulin had hypoglycemic effect in Thai silkworm hemolymph with statistical significance ($P< 0.05$) comparing with 0.9% NaCl whereas acarbose, asiaticoside and madecassoside showed glucose lowering effect but not with statistical significance comparing with 0.9% NaCl.

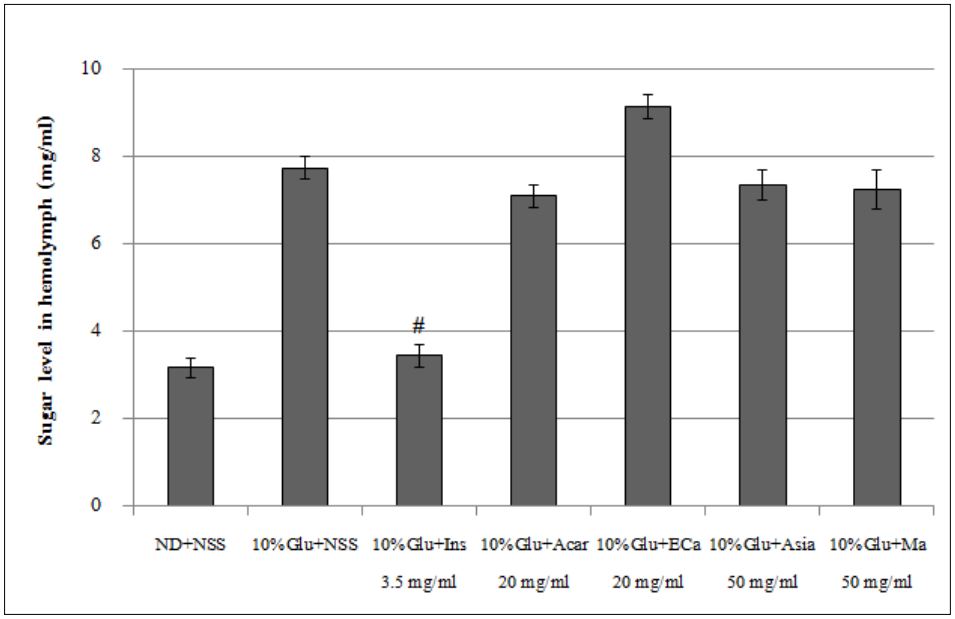


Figure 33 Hypoglycemic effect of ECa 233, asiaticoside and madecassoside in Thai silkworm by feeding 10% glucose diet and each bar represents the mean ± S.E.M. (N=10), # $P< 0.05$ compared to 0.9% NaCl.

b) Sucrose diet

After feeding the 5th instar larva silkworm with 10% sucrose diet 5 g/group for 1 hr, then diet was taken out. The negative control groups (1st and 2nd group) were injected via intra-hemolymph with 50 µl of 0.9% NaCl and the treatment groups were also injected via intra-hemolymph with 50 µl of human insulin (3.5 mg/ml) and injected via intra-midgut with 50 µl of acarbose (20 mg/ml), ECa 233 (20 mg/ml), asiaticoside (50 mg/ml) and madecassoside (50 mg/ml), respectively. Silkworm larvae were kept at 27 ± 2 °C for 5 hrs, then hemolymph was collected and sugar levels were analyzed. The result showed that human insulin, acarbose, asiaticoside and madecassoside had hypoglycemic effect in Thai silkworm hemolymph with statistical significance ($P< 0.05$) comparing with 0.9% NaCl whereas ECa 233 did not showed glucose lowering effect.

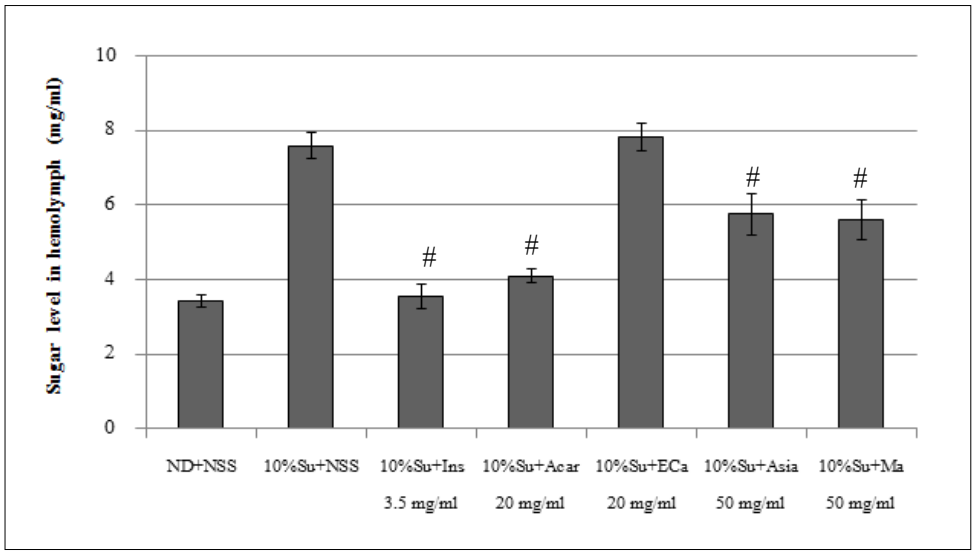


Figure 34 Hypoglycemic effect of ECa 233, asiaticoside and madecassoside in Thai silkworm by feeding 10% sucrose diet and each bar represents the mean ± S.E.M. (N=10), # $P< 0.05$ compared to 0.9% NaCl.

c) Maltose diet

After feeding the 5th instar larva silkworm with 10% maltose diet 5 g/group for 1 hr, then diet was taken out. The negative control groups (1st and 2nd group) were injected via intra-hemolymph with 50 µl of 0.9% NaCl and the treatment groups were also injected via intra-hemolymph with 50 µl of human insulin (3.5 mg/ml) and injected via intra-midgut with 50 µl of acarbose (20 mg/ml), ECa 233 (20 mg/ml), asiaticoside (50 mg/ml) and madecassoside (50 mg/ml), respectively. Silkworm larvae were kept at 27 ± 2 °C for 5 hrs, then hemolymph was collected and sugar levels were analyzed. The result showed that human insulin, acarbose, asiaticoside and madecassoside had hypoglycemic effect in Thai silkworm hemolymph with statistical significance ($P< 0.05$) comparing with 0.9% NaCl whereas ECa 233 showed glucose lowering effect but not with statistical significance comparing with 0.9% NaCl.

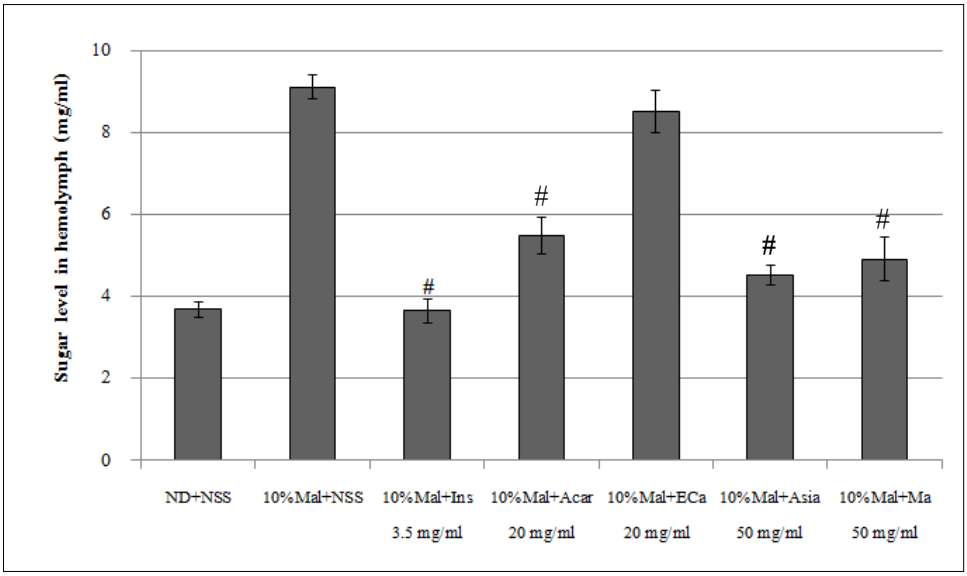


Figure 35 Hypoglycemic effect of ECa 233, asiaticoside and madecassoside in Thai silkworm by feeding 10% maltose diet and each bar represents the mean ± S.E.M. (N=10), # $P< 0.05$ compared to 0.9% NaCl.

8.2 Injection via intra-hemolymph

a) Glucose diet

After feeding the 5th instar larva silkworm with 10% glucose diet 5 g/group for 1 hr, then diet was taken out. The negative control groups (1st and 2nd group) were injected via intra-hemolymph with 50 µl of 0.9% NaCl and the treatment groups were also injected via intra-hemolymph with 50 µl of human insulin (3.5 mg/ml), ECa 233 (5 mg/ml), asiaticoside (50 mg/ml) and madecassoside (50 mg/ml), respectively. Silkworm larvae were kept at 27 ± 2 °C for 5 hrs, then hemolymph was collected and sugar levels were analyzed. The result showed that human insulin, ECa 233, asiaticoside and madecassoside had hypoglycemic effect in hemolymph with statistical significance ($P< 0.05$) comparing with 0.9% NaCl.

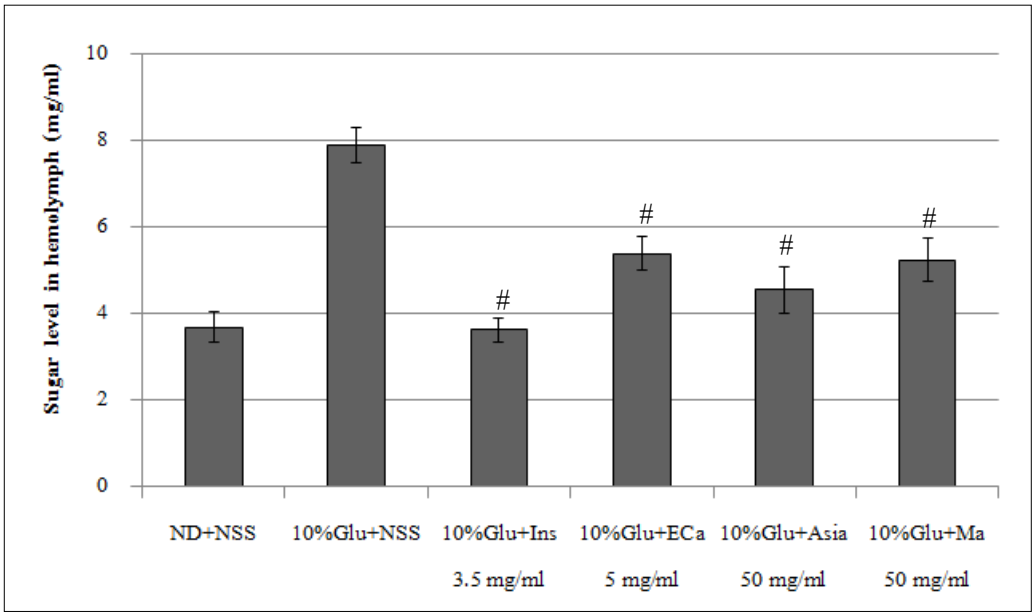


Figure 36 Hypoglycemic effect of ECa 233, asiaticoside and madecassoside in Thai silkworm by feeding 10% glucose diet and each bar represents the mean ± S.E.M. (N=10), # $P< 0.05$ compared to 0.9% NaCl.

b) Sucrose diet

After feeding the 5th instar larva silkworm with 10% sucorse diet 5 g/group for 1 hr, then diet was taken out. The negative control groups (1st and 2nd group) were injected via intra-hemolymph with 50 µl of 0.9% NaCl and the treatment groups were also injected via intra-hemolymph with 50 µl of human insulin (3.5 mg/ml), ECa 233 (5 mg/ml), asiaticoside (50 mg/ml) and madecassoside (50 mg/ml), respectively. Silkworm larvae were kept at 27 ± 2 °C for 5 hrs, then hemolymph was collected and sugar levels were analyzed. The result showed that human insulin, asiaticoside and madecassoside had hypoglycemic effect in Thai silkworm hemolymph with statistical significance ($P< 0.05$) comparing with 0.9% NaCl whereas ECa 233 showed glucose lowering effect but not with statistical significance comparing with 0.9% NaCl.

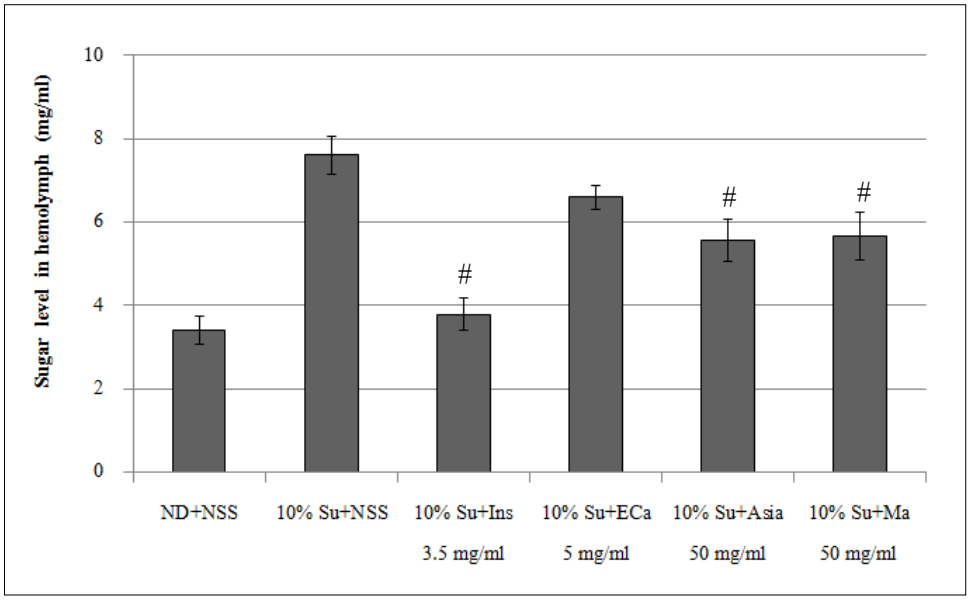


Figure 37 Hypoglycemic effect of ECa 233, asiaticoside and madecassoside in Thai silkworm by feeding 10% sucrose diet and each bar represents the mean ± S.E.M. (N=10), # $P< 0.05$ compared to 0.9% NaCl.

c) Maltose diet

After feeding the 5th instar larva silkworm with 10% maltose diet 5 g/group for 1 hr, then diet was taken out. The negative control groups (1st and 2nd group) were injected via intra-hemolymph with 50 µl of 0.9% NaCl and the treatment groups were also injected via intra-hemolymph with 50 µl of human insulin (3.5 mg/ml), ECa 233 (5 mg/ml), asiaticoside (50 mg/ml) and madecassoside (50 mg/ml), respectively. Silkworm larvae were kept at 27 ± 2 °C for 5 hrs, then hemolymph was collected and sugar levels were analyzed. The result showed that human insulin, ECa 233, asiaticoside and madecassoside had hypoglycemic effect in Thai silkworm hemolymph with statistical significance ($P< 0.05$) comparing with 0.9% NaCl.

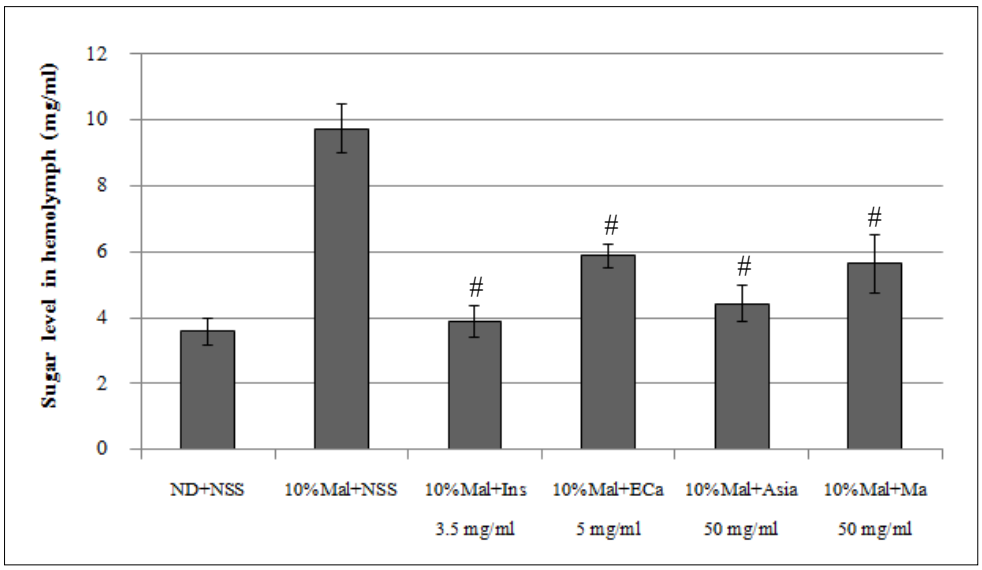


Figure 38 Hypoglycemic effect of ECa 233, asiaticoside and madecassoside in Thai silkworm by feeding 10% maltose diet and each bar represents the mean ± S.E.M. (N=10), # $P< 0.05$ compared to 0.9% NaCl.

CHAPTER IV

DISCUSSION AND CONCLUSION

Previousely, silkworm models was tested for screening the hypoglycemic activity of compounds including acarbose, gliblenclamide and ECa 233 (Chaingsom, 2011). It was found that ECa 233 could reduce blood sugar levels in hemolymph, but the mechanism of action remains unknown. In this study we aimed to investigate further the inhibition effects of ECa 233, asiaticoside and madecassoside on α-glucosidase both *in vitro* and *in vivo* studies. From *in vitro* inhibition study, the ability of test compounds for inhibiting the α-glucosidase activity has been determined using yeast α-glucosidase and intestinal α-glucosidase from rat. Acarbose, ECa 233, asiaticoside and madecassoside showed inhibition of yeast α-glucosidase with IC_{50} value 0.0008, >500, 92.42 and 68.89 mg/ml, respectively, as well as inhibition of intestinal-rat α-glucosidase with IC_{50} value 0.005, 205.61, 21.81 and 50.52 mg/ml, respectively. The result indicated that the inhibitory activity of acarbose, ECa 233, asiaticoside and madecassoside is more pronounced in intestinal-rat α-glucosidase than yeast α-glucosidase. The study of Loh and Hadira (2011) showed that *Centella asiatica* in hexane extract exhibited the inhibitory activity against yeast α-glucosidase with 48.45% inhibition. The previous studies showed that α-glucosidase inhibitors are phytoconstituents, such as flavonoids, alkaloids, phenolic compounds as well as terpenoid (Marles and Farnsworth, 1995; Bedekar *et al*., 2010; Wongsa *et al*., 2012). Our results revealed that all test compounds have inhibitory activity agianst α-glucosidase but potency of the inhibition less than the positive control, acarbose, both in yeast and mammalian α-glucosidase.

In *in vivo* toxicity study, the LD_{50} value of ECa 233, asiaticoside and madecassoside injection via intra-midgut in Thai silkworm was 178.51, >125 and >250 mg/ml, respectively and the LD_{50} value of ECa 233, asiaticoside and madecassoside injection via intra-hemolymph in Thai silkworm was 46.92, >125 and 174.58 mg/ml, respectively. Since the LD_{50} value is very useful to set up the concentration range utilized in the study of hypoglycemic effect of test compounds and to be sure that all of them are safe for silkworm larva model, in this study, therefore, we used the concentration for all test compounds that were not showing toxicity to silkworm larvae for the hypoglycemic activity study.

In *in vivo* hypoglycemic activity study, the result showed that in positive control group, human insulin, blood sugar level reduced in all sugar diet including glucose, sucrose and maltose, whereas acarbose can reduce blood sugar level only in sucrose and maltose diet. This may be to the fact that mechanism of action of acarbose is breaking of α-glucosidic bond in disaccharide including sucrose and maltose but insulin is working by stimulating the uptake of glucose into the fat body and muscle in silkworm. Thus, when disaccharide remains intact as a result of acarbose activity, there is not much monosaccharide absorbed into hemolymph. Then there is less monosaccharide to be detected by phenol-sulfuric acid reaction. For ECa 233 injection via intra-midgut, it was not so effective in lowering sugar levels in hemolymph in all sugar diet fed. On the contrary, injection via intra-hemolymph, ECa 233 at concentration 0.05, 0.5, 5 mg/ml can reduce sugar level when fed with 10% glucose with statistical significance (P<0.05). For asiaticoside and madecassoside injected via intra-midgut, they have hypoglycemic effect with statistical significance (P< 0.05) comparing with 0.9% NaCl in both 10% sucrose and 10% maltose diet. This result is consistent with the result from the study of Shigeru (2007) who reported that both extract of *C. asiatica* and asiaticoside (one compound in the extract of *C. asiatica*) could lower fasting blood sugar level significantly after 14 days of oral administration in mice. Taken into account for all the results, asiaticoside and madecassoside exhibit glucose lowering effect mainly only on disaccharide diet indicating that the inhibition of α-glucosidase activity may play the important role in their antidiabetic activity. Silkworm induced hyperglycemia by feeding high sugar diet may simulate the condition like the oral glucose tolerance test (OGTT) (American Diabetes Association, 2008). In time profile for different sugar diet fed, the sucrose diet has reached the highest level faster than glucose and maltose (at 4, 5 and 5 hour, respectively), it may due to the fact that sucrose has the higher rate of hydrolysis than maltose (Van Handel, 1968; Crailsheim 1988). In silkworm, glucose stimulates the release of bombyxin into hemolymph, and hemolymph bombyxin titers are decreased under starvation condition (Masumura *et al*., 2000). Injection of synthetic Bombyxin II into neck-ligated larvae resulted in a dose-dependent reduction of hemolymph sugar (trehalose) level accompanied by elaevalted trehalase activity in muscle and midgut tissue. Increased trehalase activity has been suggested to facilitate trehalose uptake into tissue and therby to be causal for the observed reduction of hemolymph trehalose levels. In addition, glycogen storage in the fat body was lowered and glycogen phosphorylase was activated

in fat body tissue after bombyxin II injection (Satake *et al*. 1997). Hence, function of bombyxin is to promote the consumption of carbohydrate store and not accumulation of reserves, contrary to insulin function in mammal. For injection via intra-hemolymph, the result show that all test compounds can reduce sugar level in hemolymph. The mechanism of action may be through the stimulation of bombyxin releasing from the brain and resulting in the carbohydrate metabolism.

In conclusion, hyperglycemic Thai silkworm model may be applied as an alternative model for screening of antidiabetic activity in test compounds especially such activity contributing for inhibition of α-glucosidase and ECa 233 may need further investigation to elucidate the hypoglycemic activity in mammalian model such as rat and mice.

REFERENCES

Aekplakorn W, Stolk RP, Neal B, Suriyawongpaisal P, Chongsuvivatwong V, Cheepudomwit S, et al. The prevalence and management of diabetes in Thai adults: the international collaborative study of cardiovascular disease in Asia. Diabetes Care. 2003 Oct;26(10):2758-63.

Alberti KG, Zimmet P, Shaw J. The metabolic syndrome-a new worldwide definition. Lancet. 2005 Sep 24-30;366(9491):1059-62.

Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med. 1998 Jul;15(7):539-53.

Amos AF, McCarty DJ, Zimmet P. The rising global burden of diabetes and its complications: estimates and projections to the year 2010. Diabet Med. 1997;14 Suppl 5:S1-85.

American Diabetes Association. Standards of medical care in diabetes-2008. Diabetes Care. 2008 Jan;31 Suppl 1:S12-54.

Bedekar A, Shah K, Koffas M. Natural Products for Type II Diabetes Treatment. Adv Appl Microbiol. 2010;71C:53-.

Bernal A, Kimbrell DA. Drosophila Thor participates in host immune defense and connects a translational regulator with innate immunity. Proc Natl Acad Sci U S A. 2000 May 23;97(11):6019-24.

Best PJ, Reddan DN, Berger PB, Szczech LA, McCullough PA, Califf RM. Cardiovascular disease and chronic kidney disease: insights and an update. Am Heart J. 2004 Aug;148(2):230-42.

Bischoff H. Pharmacology of alpha-glucosidase inhibition. Eur J Clin Invest. 1994 Aug;24 Suppl 3:3-10.

Bischoff H. The mechanism of alpha-glucosidase inhibition in the management of diabetes. Clin Invest Med. 1995 Aug;18(4):303-11.

Ceriello A. Postprandial hyperglycemia and diabetes complications: is it time to treat? Diabetes. 2005 Jan;54(1):1-7.

Ceriello A, Davidson J, Hanefeld M, Leiter L, Monnier L, Owens D, et al. Postprandial hyperglycaemia and cardiovascular complications of diabetes: an update. Nutr Metab Cardiovasc Dis. 2006 Oct;16(7):453-6.

Chiangsom A. DEVELOPMENT OF THAI SILKWORM MODEL FOR SCREENING GLUCOSE LOWERING EFFECT. Master's Thesis, Department of Pharmacology and Physiology Faculty of Pharmaceutical Sciences Chulalongkorn University. 2011.

Chiasson JL. Acarbose for the prevention of diabetes, hypertension, and cardiovascular disease in subjects with impaired glucose tolerance: the Study to Prevent Non-Insulin-Dependent Diabetes Mellitus (STOP-NIDDM) Trial. Endocr Pract. 2006 Jan-Feb;12 Suppl 1:25-30.

Chiasson JL, Gomis R, Hanefeld M, Josse RG, Karasik A, Laakso M. The STOP-NIDDM Trial: an international study on the efficacy of an alpha-glucosidase inhibitor to prevent type 2 diabetes in a population with impaired glucose tolerance: rationale, design, and preliminary screening data. Study to Prevent Non-Insulin-Dependent Diabetes Mellitus. Diabetes Care. 1998 Oct;21(10):1720-5.

Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M. Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial. Lancet. 2002 Jun 15;359(9323):2072-7.

Chiu CJ, Taylor A. Dietary hyperglycemia, glycemic index and metabolic retinal diseases. Prog Retin Eye Res. 2011 Jan;30(1):18-53.

Choi SW, Benzie IF, Ma SW, Strain JJ, Hannigan BM. Acute hyperglycemia and oxidative stress: direct cause and effect? Free Radic Biol Med. 2008 Apr 1;44(7):1217-31.

Crailsheim K. Intestinal transport of sugars in the honeybee (Apis mellifera L.). Journal of Insect Physiology. 1988;34(9):839-45.

Davis TM, Stratton IM, Fox CJ, Holman RR, Turner RC. U.K. Prospective Diabetes Study 22. Effect of age at diagnosis on diabetic tissue damage during the first 6 years of NIDDM. Diabetes Care. 1997 Sep;20(9):1435-41.

DeFronzo RA. Pathogenesis of type 2 diabetes mellitus. Med Clin North Am. 2004 Jul;88(4):787-835, ix.

Gray GM. Carbohydrate digestion and absorption. Role of the small intestine. N Engl J Med. 1975 Jun 5;292(23):1225-30.

Gray GM. Starch digestion and absorption in nonruminants. J Nutr. 1992 Jan;122(1):172-7.

Hamamoto H, Kamura K, Razanajatovo IM, Murakami K, Santa T, Sekimizu K. Effects of molecular mass and hydrophobicity on transport rates through non-specific pathways of the silkworm larva midgut. International Journal of Antimicrobial Agents. 2005;26(1):38-42.

Hanefeld M, Pistrosch F, Koehler C, Chiasson JL. Conversion of IGT to type 2 diabetes mellitus is associated with incident cases of hypertension: a post-hoc analysis of the STOP-NIDDM trial. J Hypertens. 2012 Jul;30(7):1440-3.

Horton ES. NIDDM--the devastating disease. Diabetes Res Clin Pract. 1995 Aug;28 Suppl:S3-11.

Hussain A, Claussen B, Ramachandran A, Williams R. Prevention of type 2 diabetes: a review. Diabetes Res Clin Pract. 2007 Jun;76(3):317-26.

Iwami M. Bombyxin: An Insect Brain Peptide that Belongs to the Insulin Family. Zoolog Sci. 2000 Nov 1;17(8):1035-44.

Kaito C, Kurokawa K, Matsumoto Y, Terao Y, Kawabata S, Hamada S, et al. Silkworm pathogenic bacteria infection model for identification of novel virulence genes. Mol Microbiol. 2005 May;56(4):934-44.

Klowden MJ. Chapter 6 - Metabolic Systems. Physiological Systems in Insects (Second Edition). San Diego: Academic Press; 2008. p. 293-355.

Lawal M. Management of diabetes mellitus in clinical practice. Br J Nurs. 2008 Sep 25-Oct 8;17(17):1106-13.

Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA. The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults. Cell. 1996 Sep 20;86(6):973-83.

Loh SP, Hadira O. In vitro inhibitory potential of selected Malaysian plants against key enzymes involved in hyperglycemia and hypertension. Malays J Nutr. 2011 Apr;17(1):77-86.

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 (Tokyo). 2007 Jun;53(3):267-76.

Marles RJ, Farnsworth NR. Antidiabetic plants and their active constituents. Phytomedicine : international journal of phytotherapy and phytopharmacology. 1995;2(2):137-89.

Masumura M, Satake SI, Saegusa H, Mizoguchi A. Glucose Stimulates the Release of Bombyxin, an Insulin-Related Peptide of the Silkworm Bombyx mori. General and Comparative Endocrinology. 2000;118(3):393-9.

Matsumoto Y, Sumiya E, Sugita T, Sekimizu K. An invertebrate hyperglycemic model for the identification of anti-diabetic drugs. PLoS One. 2011;6(3):e18292.

Mazzola N. Review of current and emerging therapies in type 2 diabetes mellitus. Am J Manag Care. 2012 Jan;18(1 Suppl):S17-26.

Mohamed Sham Shihabudeen H, Hansi Priscilla D, Thirumurugan K. Cinnamon extract inhibits α-glucosidase activity and dampens postprandial glucose excursion in diabetic rats. Nutrition & Metabolism. 2011 2011/06/29;8(1):1-11.

Nagasawa H. Neuropeptides of the silkworm, Bombyx mori. Experientia. 1992 May 15;48(5):425-30.

National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes. 1979 Dec;28(12):1039-57.

Nosadini R, Tonolo G. Relationship between blood glucose control, pathogenesis and progression of diabetic nephropathy. J Am Soc Nephrol. 2004 Jan;15 Suppl 1:S1-5.

Palanuvej C, Hokputsa S, Tunsaringkar T, Ruangrungsi N. In Vitro Glucose Entrapment and Alpha-Glucosidase Inhibition of Mucilaginous Substances from Selected Thai Medicinal Plants. Sci Pharm. 2009;77:837–49.

Pallardy SG. Chapter 7 - Carbohydrates. Physiology of Woody Plants (Third Edition). San Diego: Academic Press; 2008. p. 199-215.

Rafamantanana MH, Rozet E, Raoelison GE, Cheuk K, Ratsimamanga SU, Hubert P, et al. An improved HPLC-UV method for the simultaneous quantification of triterpenic glycosides and aglycones in leaves of Centella asiatica (L.) Urb (APIACEAE). J Chromatogr B Analyt Technol Biomed Life Sci. 2009 Aug 1;877(23):2396-402.

Rathmann W, Giani G. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004 Oct;27(10):2568-9; author reply 9.

Shigeru T. Hypoglycemic and antihypertensive activities of *Centella asiatica*. Associate Journal of Japanese Society for Medical Use of Functional Foods. 2007;4:109-14.

Terra WR, Ferreira C. Insect digestive enzymes: properties, compartmentalization and function. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry. 1994;109(1):1-62.

Terra WR, Ferreira C. 11 - Biochemistry and Molecular Biology of Digestion. In: Lawrence IG, editor. Insect Molecular Biology and Biochemistry. San Diego: Academic Press; 2012. p. 365-418.

Thompson SN. Trehalose – The Insect 'Blood' Sugar. Advances in Insect Physiology: Academic Press; 2003. p. 205-85.

Treherne JE. Gut absorption. Annu Rev Entomol. 1967;12:43-58.

Triplitt C, McGill JB, Porte D, Jr., Conner CS. The changing landscape of type 2 diabetes: the role of incretin-based therapies in managed care outcomes. J Manag Care Pharm. 2007 Dec;13(9 Suppl C):S2-16; quiz S7.

Triplitt CL. Managing diabetes in patients with diabetes of long duration. Diabetes Educ. 2012 Jul-Aug;38(4 Suppl):23S-30S; quiz 1S.

Tsujimoto T, Shioyama E, Moriya K, Kawaratani H, Shirai Y, Toyohara M, et al. Pneumatosis cystoides intestinalis following alpha-glucosidase inhibitor treatment: a case report and review of the literature. World J Gastroenterol. 2008 Oct 21;14(39):6087-92.

Unger J. Reducing oxidative stress in patients with type 2 diabetes mellitus: A primary care call to action. Insulin. 2008;3(3):176-84.

Van de Laar FA, Lucassen PL, Akkermans RP, Van de Lisdonk EH, Rutten GE, Van Weel C. Alpha-glucosidase inhibitors for type 2 diabetes mellitus. Cochrane Database Syst Rev. 2005(2):CD003639.

Van Dijk JW, Manders RJ, Hartgens F, Stehouwer CD, Praet SF, van Loon LJ. Postprandial hyperglycemia is highly prevalent throughout the day in type 2 diabetes patients. Diabetes Res Clin Pract. 2011 Jul;93(1):31-7.

Van Gaal LF, De Leeuw IH. Rationale and options for combination therapy in the treatment of Type 2 diabetes. Diabetologia. 2003 Mar;46 Suppl 1:M44-50.

Van Handel E. Utilization of injected maltose and sucrose by insects: Evidence for non-intestinal oligosaccharidases. Comparative Biochemistry and Physiology. 1968;24(2):537-41.

Vichayanrat A, Ploybutr S, Tunlakit M, Watanakejorn P. Efficacy and safety of voglibose in comparison with acarbose in type 2 diabetic patients. Diabetes Res Clin Pract. 2002 Feb;55(2):99-103.

Waugh N, Scotland G, McNamee P, Gillett M, Brennan A, Goyder E, et al. Screening for type 2 diabetes: literature review and economic modelling. Health Technol Assess. 2007 May;11(17):iii-iv, ix-xi, 1-125.

Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004 May;27(5):1047-53.

Wongsa P, Chaiwarit J, Zamaludien A. In vitro screening of phenolic compounds, potential inhibition against α-amylase and α-glucosidase of culinary herbs in Thailand. Food Chemistry. 2012;131(3):964-71.

Wyatt GR, Kale GF. The chemistry of insect hemolymph. II. Trehalose and other carbohydrates. J Gen Physiol. 1957 Jul 20;40(6):833-47.

Yamagishi S. Cardiovascular disease in recent onset diabetes mellitus. J Cardiol. 2011 May;57(3):257-62.

Yamagishi S, Imaizumi T. Diabetic vascular complications: pathophysiology, biochemical basis and potential therapeutic strategy. Curr Pharm Des. 2005;11(18):2279-99.

APPENDIX

APPENDIX

TABLES OF EXPERIMENTAL RESULTS

Table 1 The percentage of inhibition of yeast α-glucosidase with different concentration of acarbose (mg/ml). Value represents means ± S.D. of three-independent experiments.

Acabose (mg/ml)	% Yeast α-glucosidase inhibition
0.0002	40.02 ± 2.70
0.002	52.86 ± 0.98
0.02	69.11 ± 3.10
0.2	73.34 ± 3.02
2	88.69 ± 1.27
20	91.93 ± 1.12

Table 2 The percentage of inhibition of yeast α-glucosidase with different concentration of ECa 233 (mg/ml). Value represents means ± S.D. of three-independent experiments.

ECa 233 (mg/ml)	% Yeast α-glucosidase inhibition
5	-44.15 ± 1.76
25	-28.33 ± 4.66
75	-18.07 ± 3.33
125	6.64 ± 2.00
250	12.13 ± 0.07
500	31.31 ± 1.46

Table 3 The percentage of inhibition of yeast α-glucosidase with different concentration of asiaticoside (mg/ml). Value represents means ± S.D. of three-independent experiments.

Asiaticoside (mg/ml)	% Yeast α-glucosidase inhibition
15	14.09 ± 1.87
25	30.72 ± 2.84
50	33.99 ± 4.60
75	38.64 ± 2.75
150	77.57 ± 2.94

Table 4 The percentage of inhibition of yeast α-glucosidase with different concentration of madecassoside (mg/ml). Value represents means ± S.D. of three-independent experiments experiments.

Madecassoside (mg/ml)	% Yeast α-glucosidase inhibition
15	8.49 ± 3.13
25	30.37 ± 1.57
50	38.68 ± 3.34
75	49.23 ± 5.62
125	99.16 ± 1.98

Table 5 IC_{50} values for *in vitro* yeast α-glucosidase of acarbose, ECa 233, asiaticoside and madecassoside

	Acarbose (mg/ml)	**ECa 233** (mg/ml)	**Asiaticoside** (mg/ml)	**Madecassoside** (mg/ml)
IC_{50}	0.0008	>500.00	92.42	71.21

Table 6 The percentage of inhibition of intestinal α-glucosidase from rat with different concentration of acarbose (mg/ml). Value represents means ± S.D. of three-independent experiments.

Acabose (mg/ml)	% inhibition of intestinal α-glucosidase from rat
0.0002	27.09 ± 3.49
0.002	41.61 ± 3.65
0.02	62.596± 7.09
0.2	69.97 ± 3.55
2	81.19 ± 1.74
20	85.08 ± 4.69

Table 7 The percentage of inhibition of intestinal α-glucosidase from rat with different concentration of ECa 233 (mg/ml). Value represents means ± S.D. of three-independent experiments.

ECa 233 (mg/ml)	% inhibition of intestinal α-glucosidase from rat
5	13.50 ± 4.54
25	29.26 ± 4.68
75	37.50 ± 6.79
125	48.17 ± 7.71
250	50.84 ± 8.98
500	57.26 ± 4.48

Table 8 The percentage of inhibition of intestinal α-glucosidase from rat with different concentration of asiaticoside (mg/ml). Value represents means ± S.D. of three-independent experiments.

Asiaticoside (mg/ml)	% inhibition of intestinal α-glucosidase from rat
15	31.69 ± 3.48
25	62.67 ± 3.48
50	65.53 ± 2.65
75	72.59 ± 6.91
150	88.10 ± 1.85

Table 9 The percentage of inhibition of intestinal α-glucosidase from rat with different concentration of madecassoside (mg/ml). Value represents means ± S.D. of three-independent experiments.

Madecassoside (mg/ml)	% inhibition of intestinal α-glucosidase from rat
15	8.36± 3.94
25	14.04 ± 6.19
50	49.47 ± 6.80
75	76.89 ± 3.96
125	94.68 ± 5.34

Table 10 IC_{50} values for *in vitro* intestinal α-glucosidase from rat of acarbose, ECa 233, asiaticoside and madecassoside

	Acarbose (mg/ml)	**ECa 233** (mg/ml)	**Asiaticoside** (mg/ml)	**Madecassoside** (mg/ml)
IC_{50}	0.005	205.62	21.82	50.52

Table 11 Mortality rate of Thai silkworm 48 hrs after injection ECa 233 via intra- midgut at different concentrations

ECa233 concentrations (mg/ml)	**Number of Subjects**	**Number of Thai silkworm death (%mortality)**
0	10	0 (0%)
5	10	0 (0%)
25	10	0 (0%)
50	10	1 (10%)
100	10	4 (40%)
200	10	6 (60%)
400	10	9 (90%)

Table 12 Mortality rate of Thai silkworm 48 hrs after injection asiaticoside via intra- midgut at different concentrations

Asiaticoside concentrations (mg/ml)	**Number of Subjects**	**Number of Thai silkworm death (%mortality)**
0	10	0 (0%)
2.5	10	0 (0%)
12.5	10	0 (0%)
25	10	0 (0%)
75	10	0 (0%)
125	10	0 (0%)

Table 13 Mortality rate of Thai silkworm 48 hrs after injection madecassoside via intra-midgut at different concentrations

Madecassoside concentrations (mg/ml)	**Number of Subjects**	**Number of Thai silkworm death (%mortality)**
0	10	0 (0%)
25	10	0 (0%)
50	10	0 (0%)
75	10	0 (0%)
125	10	0 (0%)
250	10	0 (0%)

Table 14 Mortality rate of Thai silkworm 48 hrs after injection ECa 233 via intra-hemolymph at different concentrations

ECa233 concentrations (mg/ml)	**Number of Subjects**	**Number of Thai silkworm death (%mortality)**
0	10	0 (0%)
5	10	0 (0%)
25	10	1 (10%)
50	10	5 (50%)
75	10	10 (100%)
100	10	10 (100%)

Table 15 Mortality rate of Thai silkworm 48 hrs after injection asiaticoside via intra-hemolymph at different concentrations

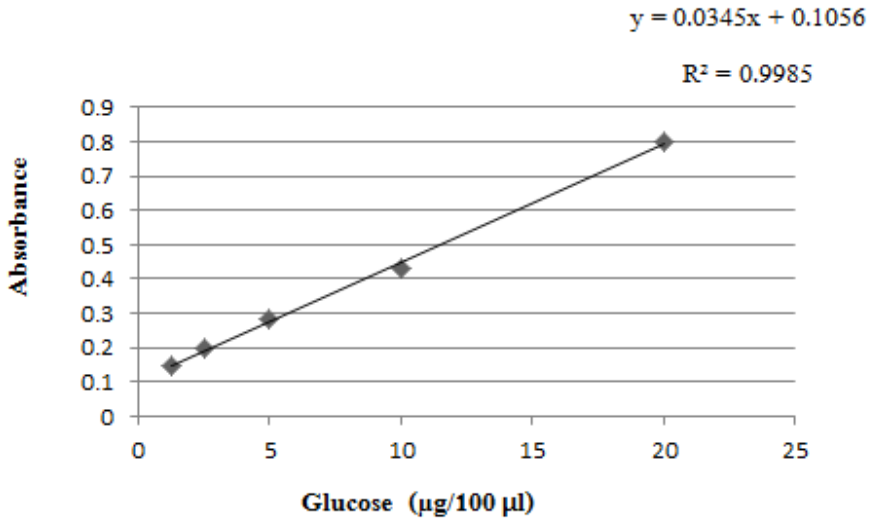
Asiaticoside concentrations (mg/ml)	**Number of Subjects**	**Number of Thai silkworm death (%mortality)**
0	10	0 (0%)
2.5	10	0 (0%)
12.5	10	0 (0%)
25	10	0 (0%)
75	10	0 (0%)
125	10	0 (0%)

Table 16 Mortality rate of Thai silkworm 48 hrs after injection madecassoside via intra-hemolymph at different concentrations

Madecassoside concentrations (mg/ml)	**Number of Subjects**	**Number of Thai silkworm death (%mortality)**
0	10	0 (0%)
5	10	0 (0%)
50	10	0 (0%)
100	10	2 (20%)
125	10	3 (30%)
150	10	6 (60%)
250	10	7 (70%)

Table 17 Glucose serial dilution for standard curve and absorbance

Glucose concentration (µg/100 µl)	OD_{490}
1.25	0.148
2.5	0.199
5	0.282
10	0.433
20	0.802



Standard curve of glucose

Table 18 Time profile of sugar levels in Thai silkworm hemolymph after receiving normal diet, value represents mean ± S.E.M. (N=10).

N	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
1	0.82	1.58	2.45	1.69	0.91	2.82	1.49
2	0.33	1.34	2.91	1.58	2.33	2.77	1.61
3	0.48	1.17	1.49	3.14	1.14	3.00	2.91
4	0.68	1.34	1.69	1.69	3.32	3.14	3.32
5	0.48	0.91	1.69	1.81	2.24	2.97	0.91
6	1.49	1.43	1.49	2.56	2.77	3.08	0.97
7	1.81	1.63	1.32	2.82	2.77	2.68	3.20
8	1.81	1.81	1.23	2.85	2.62	3.00	3.69
9	1.40	2.10	1.37	2.77	3.23	2.82	2.77
10	1.69	1.61	1.32	2.10	2.48	3.00	3.72
mean	1.10	1.49	1.70	2.30	2.38	2.93	2.46
S.E.M.	0.19	0.11	0.17	0.19	0.25	0.05	0.35

Table 19 Time profile of sugar levels in Thai silkworm hemolymph after receiving 10%glucose diet, value represents mean ± S.E.M. (N=10).

N	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
1	2.68	4.94	2.82	5.00	5.52	8.21	6.53
2	2.85	4.10	5.55	5.20	5.14	7.92	6.27
3	3.00	3.14	4.27	5.06	4.59	6.53	7.00
4	3.03	2.94	5.08	6.45	7.40	6.30	7.11
5	2.97	2.48	4.45	5.55	6.85	6.50	7.14
6	3.00	3.75	5.26	4.13	5.95	7.29	5.61
7	2.48	4.36	4.50	5.72	6.62	6.79	6.07
8	2.62	3.58	6.39	6.45	6.82	7.52	7.26
9	2.68	4.33	7.46	5.11	5.26	6.33	5.11
10	2.74	5.34	4.68	4.45	7.03	6.82	4.91
mean	2.80	3.90	5.05	5.31	6.12	7.02	6.30
S.E.M.	0.06	0.28	0.40	0.24	0.30	0.21	0.27

Table 20 Time profile of sugar levels in Thai silkworm hemolymph after receiving 10%sucrose diet, value represents mean ± S.E.M. (N=10).

N	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
1	5.26	7.11	7.17	8.07	11.63	7.92	7.61
2	5.26	7.11	7.17	8.07	11.63	7.92	7.61
3	3.43	6.62	7.37	7.23	8.39	7.03	7.46
4	10.07	3.29	7.84	7.11	8.36	7.46	7.00
5	6.97	7.23	6.94	7.92	7.69	7.58	7.46
6	3.20	7.20	7.66	8.04	8.04	7.78	6.79
7	6.94	6.97	7.84	7.72	8.36	7.43	6.85
8	3.55	7.29	7.90	8.04	8.27	7.46	6.79
9	3.26	7.17	7.66	7.92	8.36	7.43	6.85
10	5.81	7.32	6.71	8.07	8.27	7.52	7.06
mean	5.39	6.74	7.48	7.82	8.60	7.46	7.13
S.E.M.	0.70	0.39	0.13	0.11	0.35	0.09	0.10

Table 21 Time profile of sugar levels in Thai silkworm hemolymph after receiving 10%maltose diet, value represents mean ± S.E.M. (N=10).

N	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
1	2.77	3.34	4.85	6.79	6.01	13.72	9.84
2	2.91	4.94	2.77	6.56	8.24	7.55	8.33
3	3.52	4.10	3.03	5.58	8.33	10.56	8.36
4	3.14	6.07	4.68	6.33	10.74	7.58	7.58
5	2.94	3.00	4.45	6.27	7.29	8.04	11.29
6	2.48	3.92	6.21	10.56	11.40	11.20	8.48
7	2.68	3.17	5.03	8.94	8.19	9.17	7.95
8	3.14	2.97	8.04	8.13	11.20	10.65	8.79
9	5.55	2.50	6.16	5.75	10.65	7.52	9.23
10	3.58	3.20	5.66	7.40	7.52	9.20	7.87
mean	3.27	3.72	5.09	7.23	8.96	9.52	8.77
S.E.M.	0.28	0.34	0.49	0.50	0.60	0.64	0.35

Table 22 Glucose concentration profile in Thai silkworm hemolymph after receiving diet at different glucose concentrations, value represents mean ± S.E.M. (N=10), # $P < 0.05$ compare to control group.

	Normal diet	5%Glucose diet	10%Glucose diet	20%Glucose diet
1	2.56	3.00	8.79	10.59
2	2.79	4.65	7.55	13.75
3	3.00	5.08	5.08	13.98
4	3.34	3.14	6.04	9.63
5	2.13	8.65	8.65	11.69
6	3.20	5.55	8.45	11.69
7	2.24	6.13	6.13	15.61
8	2.04	5.20	5.20	15.17
9	3.46	5.52	8.42	11.26
10	2.39	6.30	6.30	9.92
mean	2.72	5.32#	7.06#	12.33#
S.E.M.	0.16	0.51	0.46	0.68

Table 23 Sucrose concentration profile in Thai silkworm hemolymph after receiving diet at different sucrose concentrations, value represents mean ± S.E.M. (N=10), # $P < 0.05$ compare to control group.

	Normal diet	5% Sucrose diet	10% Sucrose diet	20% Sucrose diet
1	2.82	5.40	7.43	11.20
2	2.68	8.01	9.11	13.81
3	3.43	5.49	8.27	11.29
4	4.45	5.49	6.39	10.71
5	3.63	7.92	7.72	7.92
6	3.49	4.36	7.55	10.16
7	5.17	6.59	7.40	12.39
8	3.37	4.42	5.90	10.21
9	3.03	7.32	8.48	13.11
10	3.72	4.88	6.65	10.68
mean	3.58	5.99#	7.49#	11.15#
S.E.M.	0.24	0.44	0.31	0.53

Table 24 Maltose concentration profile in Thai silkworm hemolymph after receiving diet at different maltose concentrations, value represents mean ± S.E.M. (N=10), # $P < 0.05$ compare to control group.

	Normal diet	5% Maltose diet	10% Maltose diet	20% Maltose diet
1	4.33	4.19	8.48	11.37
2	3.11	4.74	8.56	11.46
3	3.58	4.04	8.77	11.66
4	3.08	6.27	8.74	13.66
5	4.16	6.68	10.94	10.94
6	2.21	4.91	11.08	11.08
7	3.69	9.81	11.11	11.11
8	4.68	6.56	10.62	10.62
9	3.14	6.79	9.92	9.92
10	3.58	8.45	10.62	10.62
mean	3.56	6.24#	9.88#	11.25#
S.E.M.	0.23	0.59	0.36	0.31

Table 25 Sugar levels in hemolymph after receiving the different concentration of ECa 233 via intra-midgut, Thai silkworm were receiving 10%glucose diet (a), 10%sucrose diet (b) or 10%maltose diet (c). Value represents mean ± S.E.M. (N=10), # $P < 0.05$ compare to control group.

(a) 10%glucose diet

	Normal diet	0.9% NaCl	Insulin 3.5 mg/ml	Acarb 20 mg/ml	Eca 0.02 mg/ml	Eca 0.2 mg/ml	Eca 2 mg/ml	Eca 20 mg/ml
mean	3.33	7.63	3.69#	7.55	11.44	11.17	11.10	9.42
S.E.M	0.21	0.61	0.30	0.65	0.27	0.44	0.70	0.70

(b) 10%sucrose diet

	Normal diet	0.9% NaCl	Insulin 3.5 mg/ml	Acarb 20 mg/ml	Eca 0.02 mg/ml	Eca 0.2 mg/ml	Eca 2 mg/ml	Eca 20 mg/ml
mean	3.07	7.88	3.65#	4.48#	10.44	10.14	8.84	8.36
S.E.M	0.10	0.78	0.44	0.43	0.70	0.78	0.73	0.59

(c) 10% maltose diet

	Normal diet	0.9% NaCl	Insulin 3.5 mg/ml	Acarb 20 mg/ml	Eca 0.02 mg/ml	Eca 0.2 mg/ml	Eca 2 mg/ml	Eca 20 mg/ml
mean	3.20	9.20	3.74#	4.44#	10.94	10.01	9.37	9.32
S.E.M	0.24	0.48	0.36	0.26	0.08	0.54	0.58	0.69

Table 26 Sugar levels in hemolymph after receiving the different concentration of asiaticoside via intra-midgut, Thai silkworm were receiving 10%glucose diet (a), 10%sucrose diet (b) or 10%maltose diet (c). Value represents mean ± S.E.M. (N=10), # $P < 0.05$ compare to control group.

(a) 10%glucose diet

	Normal diet	0.9%NaCl	Insulin 3.5 mg/ml	Acarb 20 mg/ml	Asia 0.05 mg/ml	Asia 0.5 mg/ml	Asia 5 mg/ml	Asia 50 mg/ml
mean	3.68	7.53	3.49#	7.32	7.79	7.22	7.21	7.00
S.E.M	0.29	0.61	0.42	0.56	0.34	0.85	0.67	0.56

(b) 10%sucrose diet

	Normal diet	0.9%NaCl	Insulin 3.5 mg/ml	Acarb 20 mg/ml	Asia 0.05 mg/ml	Asia 0.5 mg/ml	Asia 5 mg/ml	Asia 50 mg/ml
mean	3.49	7.57	3.82#	3.96#	10.79	9.27	7.72	5.60#
S.E.M	0.27	0.55	0.64	0.40	0.28	0.70	0.89	0.38

(c) 10% maltose diet

	Normal diet	0.9%NaCl	Insulin 3.5 mg/ml	Acarb 20 mg/ml	Asia 0.05 mg/ml	Asia 0.5 mg/ml	Asia 5 mg/ml	Asia 50 mg/ml
mean	3.64	8.64	3.54#	4.48#	6.32	5.22#	4.76#	4.65#
S.E.M	0.38	0.48	0.28	0.26	0.67	0.67	0.60	0.71

Table 27 Sugar levels in hemolymph after receiving the different concentration of madecassoside via intra-midgut, Thai silkworm were receiving 10%glucose diet (a), 10%sucrose dciet (b) or 10%maltose diet (c). Value represents mean ± S.E.M. (N=10), # $P < 0.05$ compare to control group.

(a) 10%glucose diet

	Normal diet	0.9%NaCl	Insulin 3.5 mg/ml	Acarb 20 mg/ml	Made 0.05 mg/ml	Made 0.5 mg/ml	Made 5 mg/ml	Made 50 mg/ml
mean	3.32	7.23	3.38#	7.51	7.57	7.57	7.43	7.26
S.E.M	0.12	0.40	0.41	0.79	0.31	0.90	0.61	0.56

(b) 10%sucrose diet

	Normal diet	0.9%NaCl	Insulin 3.5 mg/ml	Acarb 20 mg/ml	Made 0.05 mg/ml	Made 0.5 mg/ml	Made 5 mg/ml	Made 50 mg/ml
mean	3.37	7.76	3.24#	3.90#	9.18	6.34	5.79#	5.68#
S.E.M	0.17	0.61	0.12	0.20	0.36	0.45	0.55	0.65

(c) 10% maltose diet

	Normal diet	0.9%NaCl	Insulin 3.5 mg/ml	Acarb 20 mg/ml	Made 0.05 mg/ml	Made 0.5 mg/ml	Made 5 mg/ml	Made 50 mg/ml
mean	3.48	9.08	3.32#	4.28#	6.66#	5.80#	5.51#	5.02#
S.E.M	0.22	0.39	0.20	0.11	0.62	0.61	0.54	0.35

Table 28 Sugar levels in hemolymph after receiving the different concentration of ECa 233 via intra-hemolymph, Thai silkworm were receiving 10%glucose diet (a), 10%sucrose diet (b) or 10%maltose diet (c). Value represents mean ± S.E.M. (N=10), # $P < 0.05$ compare to control group.

(a) 10%glucose diet

	Normal diet	0.9% NaCl	Insulin 3.5 mg/ml	ECa 0.005 mg/ml	ECa 0.05 mg/ml	ECa 0.5 mg/ml	ECa 5 mg/ml
mean	3.69	7.61	3.74#	6.89	5.56#	5.38#	4.75#
S.E.M	0.14	0.48	0.38	0.61	0.53	0.40	0.37

(b) 10%sucrose diet

	Normal diet	0.9% NaCl	Insulin 3.5 mg/ml	ECa 0.005 mg/ml	ECa 0.05 mg/ml	ECa 0.5 mg/ml	ECa 5 mg/ml
mean	3.36	7.50	3.79#	8.77	8.15	7.46	6.42#
S.E.M	0.33	0.64	0.32	0.12	0.62	0.53	0.72

(c) 10% maltose diet

	Normal diet	0.9% NaCl	Insulin 3.5 mg/ml	ECa 0.005 mg/ml	ECa 0.05 mg/ml	ECa 0.5 mg/ml	ECa 5 mg/ml
mean	3.52	9.53	3.70#	9.05	8.18	6.30#	5.39#
S.E.M	0.17	0.46	0.37	0.37	0.29	0.45	0.44

Table 29 Sugar levels in hemolymph after receiving the different concentration of asiaticoside via intra-hemolymph, Thai silkworm were receiving 10%glucose diet (a), 10%sucrose diet (b) or 10%maltose diet (c). Value represents mean ± S.E.M. (N=10), # $P < 0.05$ compare to control group.

(a) 10%glucose diet

	Normal diet	0.9%NaCl	Insulin 3.5 mg/ml	Asia 0.05 mg/ml	Asia 0.5 mg/ml	Asia 5 mg/ml	Asia 50 mg/ml
mean	3.54	7.35	3.76#	6.58	6.10	4.96#	4.61#
S.E.M	0.32	0.86	0.42	0.56	0.57	0.30	0.32

(b) 10%sucrose diet

	Normal diet	0.9%NaCl	Insulin 3.5 mg/ml	Asia 0.05 mg/ml	Asia 0.5 mg/ml	Asia 5 mg/ml	Asia 50 mg/ml
mean	3.18	7.76	3.55#	8.42	7.79	6.34	5.63#
S.E.M	0.19	0.43	0.40	0.67	0.30	0.54	0.39

(c) 10% maltose diet

	Normal diet	0.9%NaCl	Insulin 3.5 mg/ml	Asia 0.05 mg/ml	Asia 0.5 mg/ml	Asia 5 mg/ml	Asia 50 mg/ml
mean	3.60	9.61	3.63#	6.38#	5.32#	4.86#	4.24#
S.E.M	0.35	0.56	0.34	0.63	1.04	0.86	0.53

Table 30 Sugar levels in hemolymph after receiving the different concentration of madecassoside via intra-hemolymph, Thai silkworm were receiving 10%glucose diet (a), 10%sucrose dciet (b) or 10%maltose diet (c). Value represents mean ± S.E.M. (N=10), # $P < 0.05$ compare to control group.

(a) 10%glucose diet

	Normal diet	0.9%NaCl	Insulin 3.5 mg/ml	Made 0.05 mg/ml	Made 0.5 mg/ml	Made 5 mg/ml	Made 50 mg/ml
mean	3.13	7.79	3.57#	6.34	5.75	5.32	5.08#
S.E.M	0.21	0.42	0.32	0.69	0.95	0.72	0.39

(b) 10%sucrose diet

	Normal diet	0.9%NaCl	Insulin 3.5 mg/ml	Made 0.05 mg/ml	Made 0.5 mg/ml	Made 5 mg/ml	Made 50 mg/ml
mean	3.41	7.86	3.85#	7.98	6.43	5.95	5.59#
S.E.M	0.27	0.51	0.36	0.58	0.59	0.59	0.64

(c) 10% maltose diet

	Normal diet	0.9%NaCl	Insulin 3.5 mg/ml	Made 0.05 mg/ml	Made 0.5 mg/ml	Made 5 mg/ml	Made 50 mg/ml
mean	3.43	9.99	3.92#	7.06#	6.59#	6.49#	5.54#
S.E.M	0.29	0.52	0.28	0.55	0.91	1.00	0.52

Table 31 Sugar levels in hemolymph after receiving the test substances via intra-midgut, Thai silkworm were receiving 10%glucose diet (a), 10%sucrose diet (b) or 10%maltose diet (c). Value represents mean ± S.E.M. (N=10), # $P < 0.05$ compare to control group.

(a) 10%glucose diet

	Normal diet	0.9% NaCl	Insulin 3.5 mg/ml	Acarbose 20 mg/ml	ECa 20 mg/ml	Asia 50 mg/ml	Made 50 mg/ml
mean	3.15	7.74	3.43#	7.09	9.14	7.34	7.23
S.E.M	0.22	0.27	0.25	0.27	0.26	0.34	0.44

(b) 10%sucrose diet

	Normal diet	0.9% NaCl	Insulin 3.5 mg/ml	Acarbose 20 mg/ml	ECa 20 mg/ml	Asia 50 mg/ml	Made 50 mg/ml
mean	3.42	7.59	3.54#	4.10#	7.81	5.76#	5.62#
S.E.M	0.18	0.34	0.34	0.19	0.36	0.55	0.53

(c) 10% maltose diet

	Normal diet	0.9% NaCl	Insulin 3.5 mg/ml	Acarbose 20 mg/ml	ECa 20 mg/ml	Asia 50 mg/ml	Made 50 mg/ml
mean	3.68	9.09	3.65#	5.48#	8.50	4.52#	4.91#
S.E.M	0.19	0.29	0.29	0.44	0.52	0.24	0.54

Table 32 Sugar levels in hemolymph after receiving the test substances via intra-hemolymph, Thai silkworm were receiving 10%glucose diet (a), 10%sucrose diet (b) or 10%maltose diet (c). Value represents mean ± S.E.M. (N=10), # $P < 0.05$ compare to control group.

(a) 10%glucose diet

	Normal diet	0.9%NaCl	Insulin 3.5 mg/ml	ECa 5 mg/ml	Asia 50 mg/ml	Made 50 mg/ml
mean	3.68	7.89	3.61#	5.37#	4.54#	5.23#
S.E.M	0.35	0.42	0.28	0.39	0.55	0.49

(b) 10%sucrose diet

	Normal diet	0.9%NaCl	Insulin 3.5 mg/ml	ECa 5 mg/ml	Asia 50 mg/ml	Made 50 mg/ml
mean	3.41	7.61	3.79#	6.60	5.56#	5.66#
S.E.M	0.34	0.45	0.39	0.28	0.51	0.56

(c) 10% maltose diet

	Normal diet	0.9%NaCl	Insulin 3.5 mg/ml	ECa 5 mg/ml	Asia 50 mg/ml	Made 50 mg/ml
mean	3.58	9.74	3.89#	5.87#	4.43#	5.65#
S.E.M	0.40	0.72	0.50	0.35	0.53	0.89

VITA

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