ฤทธิ์ลดน้ำตาลในเลือดหนูขาวที่เป็นเบาหวานและฤทธิ์ต้านจุลินทรีย์ของสารสกัดด้วยน้ำจากดายขัด

นาย ชัยสิทธิ์ สิทธิเวช

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

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HYPOGLYCEMIC EFFECT IN DIABETIC RATS AND ANTIMICROBIAL ACTIVITY OF *MALVASTRUM COROMANDELIANUM* GARCKE AQUEOUS EXTRACT

Mr.Chaiyasit Sittiwet

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Faculty of Pharmaceutical Sciences
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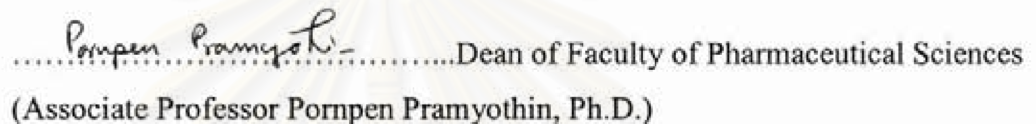
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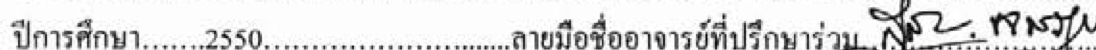
ชัยสิทธิ์ สิทธิเวช : ฤทธิ์ลดน้ำตาลในเลือดหนูขาวที่เป็นเบาหวานและฤทธิ์ต้านจุลินทรีย์ของสารสกัดด้วยน้ำจากคายขัด (HYPOGLYCEMIC EFFECT IN DIABETIC RATS AND ANTIMICROBIAL ACTIVITY OF *MALVASTRUM COROMANDELIANUM* GARCKE AQUEOUS EXTRACT)
อ.ที่ปรึกษา: รศ.ดร. สุนันท์ พงษ์สามารถ, อ.ที่ปรึกษาร่วม: รศ.ดร.สุกัญญา เจษฎานนท์
จำนวนหน้า 94 หน้า.

การศึกษาแสดงให้เห็นฤทธิ์ลดระดับน้ำตาลในเลือดของสารสกัดด้วยน้ำจากคายขัด (*Malvastrum coromandelianum* Garcke) (ME)ในหนูเพศผู้พันธุ์ Wistar ที่เหนี่ยวนำให้เกิดอาการเบาหวานโดย Streptozotocin (STZ), ฤทธิ์ลดน้ำตาลในเลือดของสารสกัดด้วยน้ำจากคายขัดในหนูปรกติ โดยวิธี glucose tolerance test พบว่าสารสกัดในทุกขนาดที่ให้คือ 5, 10, และ20 มิลลิกรัม/น้ำหนักตัว 1 กิโลกรัม 1 ครั้ง สามารถลดระดับน้ำตาลในเลือด อย่างมีนัยสำคัญทางสถิติ ($p<0.05$) ที่เวลา 30 นาที เมื่อเปรียบเทียบกับกลุ่มควบคุมซึ่งป้อนน้ำกลั่น หลังจากป้อนสารละลายกลูโคส ขนาด 1 กรัม/น้ำหนักตัว 1 กิโลกรัม การป้อน MEในหนูเบาหวาน ขนาด 10, 50 และ 100 มิลลิกรัม/น้ำหนักตัว 1 กิโลกรัม 1 ครั้ง ในหนูที่เป็นเบาหวานในสภาวะไม่อดอาหาร พบว่า ME ขนาด 50 และ 100 มิลลิกรัม/น้ำหนักตัว 1 กิโลกรัม สามารถลดระดับน้ำตาลในเลือดได้อย่างมีนัยสำคัญทางสถิติหลังการป้อน 1 ชั่วโมง และรักษาการลดระดับน้ำตาลในเลือดได้นานถึง 6 ชั่วโมง และมีฤทธิ์การลดน้ำตาลใกล้เคียงกับการฉีดอินสุลิน 5 IU/น้ำหนักตัว 1 กิโลกรัม การให้สารสกัด ME ในหนูเบาหวานในขนาด 50, 100 และ 500 มิลลิกรัม/น้ำหนักตัว 1 กิโลกรัม วันละ 1 ครั้ง เปรียบเทียบกับกลุ่มฉีดอินสุลินขนาด 5 IU/น้ำหนักตัว 1 กิโลกรัม และกลุ่มควบคุมให้น้ำกลั่นเป็นเวลา 30 วัน พบว่ากลุ่มของหนูเบาหวานที่ได้รับสารสกัด ME ทุกขนาด มีระดับน้ำตาลในเลือดลดลงอย่างมีนัยสำคัญทางสถิติ ในวันที่ 15 และ 30 เมื่อเปรียบเทียบกับกลุ่มควบคุม ยังพบว่าหนูเบาหวานที่ให้อินสุลิน 5 IU/น้ำหนักตัว 1 กิโลกรัมต่อวัน และป้อน ME 50, และ 500 มิลลิกรัม ต่อน้ำหนักตัว 1 กิโลกรัม ต่อวัน สามารถลดระดับไตรกลีเซอไรด์ในเลือดในวันที่ 30 เมื่อเทียบกับหนูทดลองกลุ่มที่ป้อนน้ำกลั่น นอกจากนี้ยังพบอีกว่าปริมาณของโคเลสเตอรอลในเลือดลดลงในหนูกลุ่มที่ได้รับสารสกัด 50, 100 และ 500 มิลลิกรัมต่อน้ำหนักตัว 1 กิโลกรัม ต่อวันเป็นเวลา 30 วัน พยาธิวิทยาของทั้งสี่กลุ่มข้างต้นไม่พบว่าเกิดรอยโรคในอวัยวะภายในของสัตว์ทดลอง การศึกษาผลของการยับยั้งการดูดซึมกลูโคสผ่านผนังลำไส้เล็กโดยวิธีทดสอบนอกกายโดยใช้ลำไส้เล็กกลับด้านพบว่า ME ไม่มีผลต่อการดูดซึมกลูโคสผ่านผนังลำไส้เล็ก นอกจากนั้นการศึกษาความเป็นพิษแบบเฉียบพลันในหนูทดลองปกติโดยให้สาร ME ในขนาด 100 มิลลิกรัม, 10 กรัม และ 20 กรัม ต่อน้ำหนักตัว 1 กิโลกรัม ไม่พบว่าเกิดความเป็นพิษเฉียบพลันในวันที่ 5 ผลการศึกษาความเป็นพิษกึ่งเฉียบพลันเมื่อให้ต่อเนื่องวันละ 1 ครั้งนาน 30 วัน พบว่า การให้สารสกัด 100 มิลลิกรัม, 10 กรัม และ 20 กรัม ต่อน้ำหนักตัว 1 กิโลกรัม ต่อวันในหนูปกติไม่เกิดความเป็นพิษ นอกจากนี้การทดสอบฤทธิ์ต้านจุลินทรีย์ของสารสกัดด้วยน้ำจากคายขัดต่อ *Staphylococcus aureus* ATCC 25923, *Escherichia. coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, and *Pseudomonas aeruginosa* ATCC 27853 นั้นพบว่าสารสกัดสามารถยับยั้งได้เฉพาะ *S. aureus* ATCC 25923 เมื่อทดสอบโดยใช้วิธี Agar disc diffusion และยังพบว่าจากเชื้อ MSSA และ MRSA ที่ใช้ทดสอบจากทั้งหมด 15 สายพันธุ์ในแต่ละกลุ่ม ME สามารถยับยั้งได้ 6 สายพันธุ์ในแต่ละกลุ่ม และการทดสอบโดยใช้วิธี Broth macrodilution method เพื่อหาค่า MICs และ MBCs ของ ME ต่อ MSSA พบว่าอยู่ช่วง 20-40 มิลลิกรัมต่อมิลลิลิตรและ 40-80 มิลลิกรัมต่อมิลลิลิตรและ ค่า MICs และ MBCs ของ ME ต่อ MRSA อยู่ในช่วง 20-40 มิลลิกรัมต่อมิลลิลิตรและ 40-80 มิลลิกรัมต่อ มิลลิลิตร

ภาควิชา.........ชีวเคมี.........................ลายมือชื่อนิสิต

สาขาวิชา.........ชีวเวชเคมี.....................ลายมือชื่ออาจารย์ที่ปรึกษา

ปีการศึกษา.......2550..........................ลายมือชื่ออาจารย์ที่ปรึกษาร่วม


4576954933 : MAJOR BIOMEDICINAL CHEMISTRY
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CHAIYASIT SITTIWET : HYPOGLYCEMIC EFFECT IN DIABETIC RATS AND ANTIMICROBIAL ACTIVITY OF *MALVASTRUM COROMANDELIANUM* GARCKE AQUEOUS EXTRACT. THESIS ADVISOR : ASSOC. PROF. SUNANTA PONGSAMART, Ph.D., THESIS COADVISOR : ASSOC. PROF. SUKANYA JESADANONT, Ph.D. 94 pp.

Hypoglycemic effect of *Malvastrum coromandelianum* Garcke extract (ME), in normal and streptozotocin (STZ)-induced diabetic male Wistar rats was demonstrated. For glucose tolerance test in normal rats, ME fed at doses 5, 10, and 20 mg/kg body weight significantly decreased blood glucose concentration at 30 min after feeding 1 g/kg body weight glucose. Feeding ME in non-fasted, diabetic rats at single doses of 10, 50 or 100 mg/kg body weight showed that ME at doses 50 and 100 mg/kg body weight significantly decreased blood glucose concentration at 1 hr and lasted through 6 hr to a level comparable to that of insulin injection, i.p., 5 IU/ kg body weight or of normal rats. Repeated-doses of ME were given orally to STZ-induced diabetic rats at 50, 100 and 500 mg/kg body weight/day or insulin injection at 5 IU/kg body weight/day or control diabetic group fed distilled water, for 30 days. ME fed daily in diabetic rats significantly decreased fasting blood glucose concentration observed on day 15 and 30 compared with diabetic rats treated with water in control group. In diabetic rat treated with insulin 5 IU/kg body weight/d., i.p. or 50 mg ME/kg body weight and 500 mg ME/kg body weight/day, p.o., showed significant decreased fasting plasma triglyceride level after treatment for 30 days. Fasting plasma cholesterol also decreased after daily treatment for 30 days of 50, 100 and 500 mg ME/kg. Histopathological examination did not show any abnormalities of the collected organs that could be attributed to toxicity of the extract either in normal rats or diabetic rats for such repeated doses. *In vitro* study indicated that ME showed no inhibitory effect on glucose absorption using everted rat's intestinal sac model. In acute toxicity test, feeding single doses of 100 mg, 10 and 20 g of ME/Kg body weight in normal rats showed similar profile in biochemical and hematological analysis of blood in treated and control groups on day 5. In subacute toxicity test, daily feeding of 100 mg, 10 g and 20 g of ME/kg body weight for 30 day in normal rats showed similar profile in biochemical and hematological analysis. ME was tested against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 27853, the result showed inhibition zone against only *S. aureus* ATCC 25923 by using agar diffusion method. ME gave inhibition zone against 6 out of 15 tests strains of each group of MSSA and MRSA. The broth macrodilution test showed that MICs and MBCs of ME against MSSA were 20 – 40 and 40-80 mg/ml, respectively. MICs and MBCs of ME against MRSA were 20 – 40 and 40 – 80 mg/ml respectively.

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จุฬาลงกรณ์มหาวิทยาลัย

Department.......Biochemistry..................Student's signature Chaiyasit Sittiwet.
Field of study....Biomedicinal Chemistry....Advisor's signature............
Academic year...2007...........................Co-advisor's signature. S. Jesadanont.

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ABBREVIATIONS

ME	*Malvastrum coromandelianum* Garcke water extract
%	Percentage
°C	Degree Celsius (centigrade)
g	Gram (s)
hr	Hour (s)
mg	Milligram (s)
dl	Deciliter (s)
min	Minute (s)
ml	Milliliter (s)
nm	Nanometer (s)
mm	Millimeter (s)
mM	Milli Mole
wt	weight
d	day (s)
i.p.	intraperitoneal
IU	Insulin Unit (s)
STZ	Streptozotocin
S.E.M.	Standard error of mean
FDA	United States Food and Drug Administration
EPA	United States Environmental Protection Agency
ATCC	American type culture collection
HPLC	High performance liquid chromatography
OGTT	Oral dose in glucose tolerance test
MSSA	Methicillin-sensitive *Staphylococcus aureus*
MRSA	Methicillin-resistant *Staphylococcus aureus*

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER I

GENERAL BACKGROUND

Introduction

The global prevalence of diabetes in the year 2000 (as used in the World Health Organization [WHO] Global Burden of Disease Study) and projections for 2030 has been reported. The estimates are based on demographic changes alone with the conservative assumption that other risk factor levels such as obesity and physical activity remain constant (in developed countries) or are accounted for by urbanization (in less developed countries). The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030. The prevalence of diabetes is higher in men than woman, but there are more women with diabetes than men. The urban population in developing countries is projected to double between 2000 and 2030. The most important demographic change to diabetes prevalence across the world appears to be the increase in the proportion of people >65 years of age (Wild et. al, 2004). Diabetic prevalence is also parallel with obesity prevalence although this study has reported that the obesity prevalence remains stable until 2030 (Wild et. al, 2004).

Diabetes is likely to be an important determinant of the vascular disease burden in countries such as Thailand where coronary heart disease has been the leading cause of death for over decade (Aekplakorn, *et.al*, 2003). The estimated national prevalence of diabetes in Thai adults was 9.6% (2.4 million people), which included 4.8% previously diagnosed and 4.8% newly diagnosed. The prevalence of impaired fasting glucose was 5.4% (1.4 million people). Diagnosed diabetes, undiagnosed diabetes, and impaired fasting glucose were associated with greater age, body mass index (BMI), Waist-to-hip ratio, systolic blood pressure, total cholesterol, and serum creatinine levels. The majority of individuals with diagnosed diabetes had received dietary or other behavioral advice, and 82% were taking oral hypoglycemic

therapy. Blood pressure – lowering therapy was provided to 67% of diagnosed diabetes patients with concomitant hypertension (Aekplakorn, *et al*. 2003).

Plants based drugs have been used against various diseases since time memorial. The primitive man used herbs as therapeutic agents and medicament, which they were able to procure easily. The nature has provided abundant plant wealth for all living creatures, which posses medicinal virtues. The essential values of some plants have long been published but a large number of them remain unexplored as yet. So there is a necessity to explore their uses and to conduct pharmacognostic and pharmacological studies to ascertain their therapeutic properties.

Despite considerable progress in the management of diabetes mellitus by conventional synthetic drugs, the search for natural anti-diabetic plant products for controlling diabetes is going on. Approximately 343 plants of the world have been tested for the blood glucose lowering effect in the laboratory experiments. Of these plants 158 are claimed to be used in the ayurved (Ahmad, *et.al*., 2007)

Malvastrum coromandelianum Garcke is a member of family Malvaceae, has been traditionally used as hypoglycemic herb (อุไรวรรณ เพิ่มพิพัฒน์, 2534, สุชิต นาคพันธ์, 2544, Andrade-Cetto and Heinrich, 2005), and for its antipyretic activity, including affecting smooth muscle activity (relaxant effect) and ulcero-protective activity (Dahanukar, *et al.,* 2000). Rattanajarasroj, *et al*. have reported that the dried powder of water extract showed hypoglycemic activity only in crude water extract but not in separated fractions with chloroform or ethanol. It was also showed that crude drug at dose of 0.2, 2 and 20 g/kg body weight/day given orally to alloxan induce diabetic rabbit showed no toxicity of the extract (Attawish, 1998). Due to the conflict results, hypoglycemic activity of *M. coromandelianum* still needs more investigation which would supply the scientific data for its herbal use. This study also includes in vitro anti-microbial test and toxicity of the plant water extract.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Literatures review

1. Diabetes mellitus

Diabetes mellitus (DM) is a metabolic disorder characterized by disturbance in carbohydrate, lipid and protein metabolism. These metabolic derangement results from a combination of insulin deficiency and/or insulin resistance and lead to a

variety of acute and chronic complications. Type 1 diabetes is characterized by an immune – mediated, selective destruction of insulin secreting β - cell. Individuals with Type 1 diabetes therefore require regular insulin injections to control blood sugar levels. The hypoglycemia that is associated with type 2 diabetes results from both an impaired insulin secretary response to glucose and decreased insulin effectiveness (insulin resistance) (Berkow, 1992).

1.1 The foot in diabetes mellitus

Diabetes is the commonest cause of amputation of the foot in civilian life. A background to such a foot management program, the physician needs to understand the pathology and sequence of events that commonly lead to amputation. There are five factors in such a sequence: vascular, neuropathic, mechanical, infective and metabolic. The common sequence of events:

1. The diabetic may have a reduced peripheral needed to combat gross infection.
2. The patient has diminished sensation – not total anesthesia, but a change threshold of perception of pain and pressure.
3. He or she the suffers a break in the skin from external mechanical force, often associated with improper footwear.
4. In the absence of pain, the patient continues to walk on this open wound, pressing on the infected tissues and spreading the infection until it becomes a gross cellitis and osteomyelitis.
5. Now feeling ill, and with pus in his shoe, the patient goes to a surgeon or to an emergency room. The surgeon finds that he is a diabetic, checks his glucose level, and finds him out of control (perhaps due to the infection). He assumes that such a foot in a danger to the limb or even to the life of the patient, and amputates below or above the knee it is stage 3 and 4 that early intervention can often prevent the need for amputation and restore the patient to a normal life (Brand, 1981).

1.2 Methicilllin-resistant *Staphylococcus aureus* (MRSA) in diabetic foot infection

In 2004, Memmel, H., has reported that common organisms in diabetic burn infections were *Streptococcus pyogenes*, *Proteus mirabilis*, *Pseudomonas*

aeruginosa, *Candida albicans* and MRSA. Peripheral neuropathy may have precipitated and delayed medical treatment in lower-extremely burns of diabetic patients. Hospitalized diabetic burn patients were also at an increased risk for nosocomial infections, which prolonged hospitalization (Memmel, 2004). Due to the report of diabetic bacterial infection, most pathogens are susceptible strains except MRSA which was known as drug resistant bacterial strain. This quite reasonable for people to concern about MRSA treatment in people with diabetic.

2. Animal models in type 1 diabetes mellitus study

It is important selecting animal model used in researches which intend to reflect the human symptoms or diseases. The selection of species of animal depends on several factors such as cost and the size of animal. The smaller the animal is, the more manageable and cheaper is the experiment. Previously, surgical method to remove pancreas was used to make animal diabetes mellitus.

Recently, the substances with diabetogenic effect have been used. Hypoglycemia inducing agent such as streptozotocin and alloxan was used as non-surgical diabetes mellitus method. Type 1 diabetes mellitus in humans is characterized by specific destruction of the pancreatic β cells, commonly associated with immune-mediated damage.

2.1 Chemical induced diabetes mellitus in animal model

There are many kinds of substance used to induce diabetes mellitus in animal model such as alloxan, streptozotocin, vacor, dithizone, 8-hydroxyquinolone (Ree and Alcolado, 2005). Alloxan (2,4,5,6,-tetraoxypyrimidine; 5,6-dioxyuracil) was used to cause a specific necrosis of pancreatic islets (Szkudelski, 2001). Its mechanism has been intensively studied. Superoxide radicals are generated after cell uptake of alloxan and converted to dialuric acid. The target of reactive oxygen species is DNA of pancreatic islets cell. Reactive oxygen species cause the DNA fragmentation. This was concluded that alloxan causes β cell necrosis (Szkudelski, 2001). Streptozotocin (STZ, 2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose) is a toxin which synthesized by *Streptomycetes achromogenes*. STZ was uptaken by pancreatic β cells *via* glucose transporter GLUT2. It was found that the main reason of STZ-induced β cell death is alkylation of DNA (Szkudelski, 2001).

2.2 Non-obese diabetic (NOD) mouse and Bio breeding (BB) rats

Non-obese diabetic (NOD) mouse and bio breeding (BB) rats develops an autoimmune, insulin dependent diabetic. Several genes contribute to the development of diabetes in BB rats. The *iddm* loci that have been identified, the homozygosity mutation of *iddm*1 luci leads to 5-10 fold reduction of peripheral $CD4^+$, $TcR\alpha\beta^+$ T cells and a virtual absence of $CD8^+$, $TcTR\alpha\beta^+$ T cells. The mitotic activity of BB rat T cell is antigen driven and stimulated by the small size of the peripheral blood lymphocyte of these animals. Activated macrophages are the first mononuclear cells to infiltrate the pancreatic islets of BB rats and NOD mice. Early depletion of macrophages prevents the onset of diabetes. "The macrophage produces an excessive amount of nitric oxide, which has been shown to be toxic for β cells in vitro. Further, the incidence of diabetes is decreased in BB rats treated with inhibitors of nitric oxide synthase" (Ramanathan and Poussier, 2001).

Due to the convenience, lower cost and effectiveness of chemical induces diabetic rats, streptozotocin was frequently used in many studies (Yang *et.al*., 2007, El-Hilaly *et.al.*, 2007, Andrade-Cetto *et.al*., 2007).

3. Potential plants used in the treatment of diabetes mellitus and MRSA infection in diabetic foot

3.1 Anti-diabetic plants

It was obviously that the treatment of people with diabetes is focused on blood sugar and following its complication. Recently, there are many kinds of medicine but almost all are chemical or biochemical agents which have side-effect such as hypoglycemia, lactic acid intoxication and gastrointestinal upset. The drugs commonly used in clinic to treat or control diabetes are showed in Table 1.

3.2 Plant as anti MRSA

The foot ulceration in diabetes mellitus is a serious complication which accounts for 20% of diabetes-related hospital admissions in the UK (Tentolouris, *et.al*. 1999). Diabetic patients with MRSA infections develop severe

Table 1. The drugs commonly used in clinic to treat or control diabetes.

Groups of drugs	preparations
Insulin	There are many kinds of preparations
Sulfonylureas (SU)	Tolbutamide (D_{860}, Orinase), Glibenclamide (Glyburid, HB419, Micronase, Daonil), Gliclazide (Diamicron), Glibenese (Minidiab), Glurenorm (Gliquidone), Glutril (Glibornuride) and Glimepiride, etc.
Biguanide (BG)	Phenformin (Phenethyldiguanidi Hydrochloridum), Diabenide, DBI), Dimethylbiguanide (FluamineMetformin, Diaformin, Diabex, Mellitin, Obin, Melbine, Metformin hydrochloride, Glucophage, DMBG)
α-Glucosidase inhibitors (α-GDI)	Glucobay (Acarbose), Viglibose, Miglitol, Emiglitate, Glyset, Precose
Aldose reductase inhibitor (ARI)	Tolrestat, alredase, Epslstat, Kinedak, Imirestat, Opolrestat
Thiazolidinediones (TZD)	Troglitazone, Rosigitazone, Pioglitazone, Englitazone
Carbamoeylmethyl benzoic acid (CMBA)	Repaglinide
Insulin-like growth factor (IGF)	IGF-1
Others	Dichloroacetic acid

(Data from: Li, W.L. et. al. 2004. Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. J.Ethnopharmacol. 92: 1-21.)

Table 2. Mexican plants reported as Hypoglycemic in 2005 (Andrade-Cetto and Heinrich, 2005).

Scientific name	common name	Family	Plant part used and preparation	Pharmacological studies	Phytochemical information
Abutilon lignosum (Cav.) D. Don	Sacxiu	Malvaceae	Root infusion		
Abutilon trisulcatum (Jacq.) Urban.	Tronadora	Malvaceae	Leaf boil		
Acacia retinodes Schltdl.	Mimosa	Fabaceae	Leaf boil		
Acourtia thurberi (A. Gray) Reveal & R. M. King	Matarique	Asteraceae	Plant (aerial) infusion	Normal rabbits (+)	
Acrocomia mexicana Karw. ex Mart.	Coyol	Arecaceae	Root roasted, fruit raw	Alloxanic mice (+) Alloxanic mice (++) EtOH	Tetrahydropyrane, Coyolose
Agastache mexicana (Kunth) Lint et Epling	Toronjil	Lamiaceae	Plant (aerial) infusion		Essential oils
Agave atrovirens Karw. Ex Salm-Dyck	Maguey	Agavaceae	Steam macerated		Sapogenins
Agave lechuguilla Torr.	Lechuguilla	Agavaceae	Steam macerated		
Agave salmiana Otto ex Salm-Dyck	Maguey	Agavaceae	Steam macerated		
Ageratina petiolaris Moc. & Sessé ex DC.	Hierba del ángel o Yolochichotl	Asteraceae	Plant (aerial) infusion		Terpens
Ageratum conyzoides L.	Hierba dulce	Asteraceae	Plant (aerial) infusion		Flavonoids, essential oils, terpens
Allionia choisyi Standl.	Hierba de la hormiga	Nyctaginaceae	Plant (aerial) infusion		
Allium cepa L.	Cebolla	Liliaceae	Bulbs raw		Sulfuric compounds
Alloispermum integrifolium (DC.) H. Rob.	Prodijiosa	Asteraceae	Plant (aerial) infusion		
Aloe barbadensis Mill.	Sábila	Liliaceae	Steam roasted, juice of the leaves	Normal rabbits (−)	Polysaccharides, flavonoids
Aloe vera (L.) Burm. F	Sábila	Liliaceae	Mixed with Nopal taken orally before meals	Normal mice (+)	Polysaccharides A B, flavonoids, terpens
Ambrosia artemisiifolia L.	Artemisa	Asteraceae	Plant (aerial) infusion		Sesquiterpen lactones
Anacardium occidentale L.	Marañon	Anacardiaceae	Bark infusion		
Ananas comosus (L.) Merr.	Piña	Bromeliaceae	Juice of the fruit		Monoterpenoids, Carotenoids, Lactones
Annona cherimola Mill.	Chirimoya	Annonaceae	Bark infusion		Isoquinolin Alkaloids
Annona glabra L.	Anona silvestre, palo de corcho	Annonaceae	Juice of the fruit root infusion		Diterpens, Alkaloids
Annona muricata L.	Guanabana	Annonaceae	Fruit raw		
Apodanthera buraeavi Cogn.	Pisto	Cucurbitaceae	Plant (aerial) infusion		
Aporocactus flagelliformis (L.) Lem.	Flor de junco	Cactaceae	Flowers infusion, steam infusion		
Arachis hypogaea L.	Cacahuate	Fabaceae	Seeds and oil		Sterols, flavonoids
Arceuthobium vaginatum (Humb. & Bonpl. ex Willd.) J. Presl	Injerto	Loranthaceae	Plant infusion		
Arctostaphylos pungens Kunth	Pingüica	Ericaceae	Leaves infusion, roots infusion		Tannins
Argemone mexicana L.	Chicalote, Cardo lechero.	Papaveraceae	Plant (aerial) infusion		Alkaloids, flavonoids
Argemone ochroleuca Sweet	Chicalote	Papaveraceae	Plant (aerial) infusion		Alkaloids
Argemone platyceras Link & Otto	Chicalote	Papaveraceae	Plant (aerial) infusion		
Aristolochia asclepiadifolia Brandegee	Guaco	Aristolochiaceae	Plant infusion EtOH		
Aristolochia malacophylla Standl.	Guaco	Aristolochiaceae	Flowers infusion		
Aristolochia sericea Benth.	Guaco	Aristolochiaceae	Steam infusion		

Table 2. (Continued)

Scientific name	common name	Family	Plant part used and preparation	Pharmacological studies	Phytochemical information
Artemisia absinthium L.	Ajenjo	Asteraceae	Leaf boil		Sesquiterpen lactones, flavonoids
Artemisia ludoviciana Nutt.	Estafiate	Asteraceae	Plant (aerial) infusion		
Artemisia vulgaris L.	Ajenjo	Asteraceae	Leaf boil		Sesquiterpens flavonoids
Asclepias linaria Cav.	Romerillo	Asclepiadaceae	Plant (aerial) infusion		Sterols, triterpenoids
Barosma betulina Bartl. & H.L. Wendl.	Buchú	Rutaceae	Leaves infusion		
Bauhinia divaricata L.	Pata de vaca	Fabaceae	Leaf boil, flowers boil	Normal rabbits (+)	
Begonia heracleifolia Schltdl. & Cham.	Mano de león	Begoniaceae	Steam infusion		
Berberis moranensis Schult. & Schult. f.	Palo muerto	Berberidaceae	Bark infusion		Cucurbitacines
Beta vulgaris L.	Betabel	Chenopodiceae	Juice of the leaves		Alkaloids, flavonoids
Bidens aurea (Aiton) Sherff	Té de milpa	Asteraceae	Plant (aerial) infusion		Essential oils
Bidens leucantha (L.) Willd.	Rosilla	Asteraceae	Plant (aerial) infusion	Alloxanic mice (++)	
Bidens odorata Cav.	Aceitilla, Mosote blanco	Asteraceae	Plant (aerial) infusion		Flavonoids, triterpens
Bidens pilosa L.	Aceitilla	Asteraceae	Plant (aerial) infusion	Alloxanic mice (+)	Flavonoids, triterpens
Bocconia arborea S. Watson	Llora sangre	Papaveraceae	Leaves infusion		Alkaloids
Peumus boldus Molina J. A. Schultes & J. H. Schultes in J. J. Roemer & J. A. Schultes	Boldo	Monimiaceae	Plant (aerial) infusion		
Bouvardia ternifolia (Cav.) Schltdl.	Trompetilla	Rubiaceae	Leaves, steam infusion		Bouvardin
Brickellia cavanillesii (Cass.) A. Gray	Prodigiosa	Asteraceae	Plant (aerial) infusion	Normal rabbits (+)	Essential oils, brikelin
Brickellia squarrosa B.L. Rob. & Seaton	Aamla	Asteraceae	Plant (aerial) infusion	Normal rabbits (+)	Flavonoids
Brosimum alicastrum Sw.	Ojite	Moraceae	Bark infusion		Benzoquinones
Buchnera pusilla Kunth	Chichibe	Scrophulariaceae	Bark infusion		
Buddleia stachyoides Cham. & Schltdl.	Hierba del perro	Loganiaceae	Leaves infusion		Flavonoids, alkaloids, essential oils
Buddleja Americana L.	Tepozán	Loganiaceae	Leaves infusion		Flavonoids, alkaloids
Buddleja cordata Kunth	Tepozán	Loganiaceae	Leaves infusion		Alkaloids
Bursera simaruba (L.) Sarg.	Cuajiote	Burseraceae	Bark infusion		Tannins
Byrsonima crassifolia (L.) Kunth	Nanche	Malpighiaceae	Fruit, bark infusion		Triterpenoids
Cacalia decomposita A. Gray	Matarique	Asteraceae	Root infusion	Alloxan Mice (++)	Alkaloids, polysaccharides
Cacalia peltata Kunth	Matarique	Asteraceae	Root infusion	Normal rabbits (++)	Polysaccharides
Calamintha macrostema Benth.	Tabaquillo	Lamiaceae	Root infusion	Alloxanic mice (+)	
Calea hypoleuca B.L. Rob. & Greenm.	Prodigiosa	Asteraceae	Plant (aerial) infusion		
Calea integrifolia (DC.) Hemsl.	Prodigiosa	Asteraceae	Stem, infusion		Sesquiterpen lactones
Calea zacatechichi Schltdl.	Prodigiosa	Asteracae	Leaves infusion	Normal rabbits (+)	
Calliandra anomala (Kunth) J.F. Macbr.	Cabello de ángel	Fabaceae	Leaves infusion		Triterpenoid saponins
Callicarpa acuminata Kunth	Xpuk'im	Verbenaceae	Root, infusion		
Capraria biflora L.	Sabadilla	Scrophulariaceae	Leaves infusion	Alloxanic mice (+)	Alkaloids, loiflorin
Carica papaya L.	Papaya	Caricaceae	Latex		Monoterpenoids
Carya Nutt.	Nogal	Juglandaceae	Leaves infusion		
Casimiroa edulis La Llave & Lex.	Zapote blanco	Rutaceae	Leaves infusion, bark infusion		Alkaloids, casimiroin, edulein, edulinin
Cassia fistula L.	Caña Fistula	Fabaceae	Fruit		

Table 2. (Continued)

Scientific name	common name	Family	Plant part used and preparation	Pharmacological studies	Phytochemical information
Cassia skinneri Benth.	Frijolillo	Fabaceae	Leaves infusion		
Cassia tomentosa L. f.	Retama cimarrona	Fabaceae	Leaves infusion		
Castela texana (T. & G.) Rose	Chaparro amargoso	Simaroubaceae	Bark infusion		Steroids
Castela tortuosa Liebm.	Venenilo	Simaroubaceae	Bark infusion		
Castilleja Mutis ex L. f.	Hierba del gato	Scrophulariaceae	Plant (aerial) infusion		
Catharanthus roseus (L.) G. Don	Vicaria	Apocinaceae	Root infusion		
Cecropia peltata L.	Guarambo	Cecropiaceae	Leaves infusion		
Ceiba pentandra (L.) Gaertn.	Ceiba, Pochote	Bombacaceae	Bark infusion		Essential oils
Centaurium brachycalyx Standl. & L.O. Williams	Tlanchalahua	Gentianaceae	Leaves infusion		
Centaurium calycosum (Buckley) Fernald	Tlanchalagua	Gentianaceae	Leaves infusion		
Chamaecrista hispidula (Vahl) H.S. Irwin & Barneby	Frijolillo	Fabaceae	Leaves infusion		
Chamaecrista hispidula (Vahl) H.S. Irwin & Barneby	Frijolillo	Fabaceae	Leaves infusion		
Chenopodium glaucum L.	Hierba del puerco	Chenopodiceae	Plant (aerial) infusion		
Chromolaena bigelovii (A. Gray) R.M. King & H. Rob	Ambula	Asteraceae	Plant (aerial) infusion		
Cirsium mexicanum DC.	Cardo santo	Asteraceae	Root infusion		
Cirsium rhaphilepis (Hemsl.) Petr.	Cardo santo	Asteraceae	Flower infusion		
Cissampelos pareira L.	Guaco	Menispermaceae	Root raw		Alkaloids, isoquinolin
Citrus aurantifolia (Christm.) Swingle	Limón	Rutaceae	Fruit		Essential oils, sesquiterpen lactones
Citrus limetta Risso	Lima	Rutaceae	Fruit		
Citrus sinensis (L.) Osbeck	Flor de azahar	Rutaceae	Ripe fruit infusion		Essential oils, flavonoids
Cnidoscolus aconitifolius (Mill.) I.M. Johnst.	Chaya	Euphorbiaceae	Leaves infusion		Polysaccharides
Cnidoscolus multilobus (Pax) I.M. Johnst.	Mala mujer	Euphorbiaceae	Leaves infusion		Triterpenoids, flavonoids, tannins
Cnidoscolus chayamansa Mc Vaugh	Chayamansa	Euphorbiaceae	Leaves infusion		Flavonoids glycosides
Coix lacryma-jobi L.	Lágrima de San Pedro	Poaceae	Plant (aerial) infusion	Normal rabbits (+)	
Combretum farinosum Kunth	Bejuco de Carape	Combretaceae	Sap raw		
Conyza filaginoides (D.C.) Hieron.	Simonillo	Asteraceae	Plant (aerial) infusion		Alkaloids, lenecin
Conyza gnaphalioides Kunth	Cimonillo, zacachichitl	Asteraceae	Leaves infusion		Terpens
Cordia elaeagnoides A. DC.	Cuerano	Boraginaceae	Bark infusion		Terpens
Cordia tinifolia Willd. Ex Roem. & Schult.	Palo mulato	Boraginaceae	Bark infusion		
Coriandrum sativum L.	Cilantro	Apiaceae	Plant (aerial) infusion		Coumarins, flavonoids, sesquiterpenoids, steroids
Costus mexicanus Liebm. ex Petersen	Caña de Jabalí	Zingiberaceae	Plant (aerial) infusion		
Costus rubber C. Wright ex Griseb.	Caña agria	Zingiberaceae	Plant (aerial) infusion		
Costus spicatus (Jacq.) Sw.	Caña de Jabalí	Zingiberaceae	Plant (aerial) infusion		
Crataegus mexicana Moc. & Sessé ex DC.	Tejocote	Rosaceae	Root infusion		
Crataegus pubescens (C. Presl) C. Presl	Tejocote	Rosaceae	Root infusion	Normal rabbits (++)	Tannins, flavonoids
Crotalaria acapulcensis Hook. & Arn.	Retama	Fabaceae	Leaves infusion		

Table 2. (Continued)

Scientific name	common name	Family	Plant part used and preparation	Pharmacological studies	Phytochemical information
Croton draco Schltdl.	Sangre de Grado	Euphorbiaceae	Cortex infusion, latex		Diterpens
Croton torreyanus Müll. Arg.	Salvia	Euphorbiaceae			
Cucurbita maxima Duchesne	Calabaza	Cucurbitaceae	Fruit juice		Sterols, flavonoids
Cucurbita mexicana Damm	Calabaza, Melón	Cucurbitaceae	Leaves infusion fruit juice	Normal rabbits (++)	
Cuscuta jalapensis Schltdl.	Sacapal	Convolvulaceae	Steam infusion		
Cyathea fulva (M. Martens & Galeotti) Fée	Árbol de la vida	Cyatheaceae	Root infusion		
Cyathea fulva (Martens & Galeotti) Fée.	Árbol de la vida	Filicaceae	Leaves infusion		
Cynara scolymus L.	Alcachofa	Asteraceae	Fruit infusion, flowers infusion		Flavonoids, sesquiterpen lactones, fenolic acids
Cynodon dactylon (L.) Pers.	Grama	Poaceae	Plant (aerial) infusion	Normal rabbits (+)	Flavonoids, terpens
Daucus carota L.	Zanahoria	Apiaceae	Root juice		Cumarines, flavonoids, essential oils, fenolic acids
Diospyros digyna Jacq.	Zapote negro	Ebenaceae	Fruit		
Dorstenia contrajerva L.	Contrayerba	Moraceae	Leaves boiled		Alkaloids, cardenolids
Dyssodia micropoides (DC.) Loes.	Hierba pelotazo	Asteraceae	Plant (aerial) infusion		
Elaphoglossum sp. Schott ex J. Sm.	Hierba del pastor	Lomariopsidaceae	Plant (aerial) infusion		
Equisetum giganteum L.	Limpia plata	Equisetaceae	Plant (aerial) infusion		Flavonoids
Equisetum hyemale L.	Cola de caballo	Equisetaceae	Plant (aerial) infusion		Flavonoids, alkaloids
Eriobotrya japonica (Thunb.) Lindl.	Nispero	Rosaceae	Leaves infusion, flowers infusion	Normal rabbits (–)	Sesquiterpens, flavonoids
Eucalyptus globulus Labill	Eucalipto	Myrtaceae	Leaves infusion	Alloxanic mice (+)	Flavonoids, terpens
Euphorbia maculata L.	Hierba de la Golondrina	Euphorbiaceae	Leaves infusion		
Euphorbia prostrata Aiton	Hierba de la Golondrina	Euphorbiaceae	Leaves infusion		Flavonoids
Eysenhardtia polystachya (Ortega) Sarg.	Palo dulce	Fabaceae	Plant (aerial) infusion, bark infusion	Alloxanic mice (+)	Flavonoids, triterpens
Foeniculum vulgare Mill.	Hinojo	Apiaceae	Plant (aerial) infusion		Essential oils, flavonoids
Fouquieria splendens Engelm.	Albarda	Fouquieriaceae	Leaves infusion		
Fraxinus alba Marshall	Fresno	Oleaceae	Leaves infusion bark infusion		
Gnaphalium oxyphyllum DC.	Gordolobo	Asteraceae			Diterpens, flavonoids
Guaiacum coulteri A. Gray	Guayacan	Zygophyllaceae	Bark infusion		Alkaloids
Guaiacum sanctum L.	Guayacan	Zygophyllaceae	Bark infusion		
Guardiola angustifolia (A. Gray ex S. Watson) B.L. Rob.	Chintuza	Asteraceae			
Guardiola tulocarpus A. Gray	Chintuza	Asteraceae	Leaves infusion		
Guazuma ulmifolia Lam.	Guázima	Sterculiaceae	Bark infusion		Alkaloids, tannins
Haematoxylon brasiletto H. Karst.	Palo Brazil	Fabaceae	Bark infusion		
Hamelia patens Jacq.	Balletilla	Rubiaceae	Leaves infusion		Tannins
Haplopappus venetus (Kunth) S.F. Blake	Xapulli	Asteraceae	Plant (aerial) infusion		
Hechtia melanocarpa L. B. Sm.	Maguey agrio	Bromeliaceae	Steam raw		Flavonoids, alkaloids
Heterotheca inuloides Cass.	Arnica	Asteraceae	Leaves infusion		Flavonoids, essential oils
Hibiscus rosa-sinensis L.	Tulipán	Malvaceae	Plant (aerial) infusion		Sterols, flavonoids
Hidalgoa ternata La Llave	Mozote de monte	Asteraceae	Plant (aerial) infusion		
Hintonia latiflora (Sesse & Moc. ex DC.) Bullock	Copalquín, Cáscara sagrada.	Rubiaceae	Bark infusion	Alloxanic mice (++)	Neoflavonoid, coutareagenin.

Table 2. (Continued)

Scientific name	common name	Family	Plant part used and preparation	Pharmacological studies	Phytochemical information
Hippocratea excelsa Kunth	Cancerina	Hippocrateaceae	Root infusion		Sesquiterpens
Ipomoea stans Cav.	Tumba vaquero	Convolvulaceae	Plant (aerial) infusion		Essential oils
Jatropha dioica Cerv.	Sangre de grado	Euphorbiaceae	Root infusion		
Jatropha elbae J. Jiménez Ram.	Sangre de grado	Euphorbiaceae	Bark infusion		Terpens, flavonoids
Juliania adstringens (Schltdl.) Schltdl.	Cuachalalate	Julianiaceae	Bark infusion		Triterpens
Justicia spicigera Schltdl	Muicle	Acanthaceae	Leaves infusion		Flavonoids
Kalanchoe pinnata (Lam.) Pers.	Tronador	Crassulaceae	Plant (aerial) infusion		Flavonoids
Karwinskia humboldtiana (Willd. ex Roem. & Schult.) Zucc.	Tullidora	Rhamnaceae	Leaves infusion		
Kohleria sp. Regel	Tlanchichinoli	Gesneriaceae	Leaves infusion		Triterpens
Larrea tridentata (Sessé & Moc. ex DC.) Coville	Gobernadora	Zygophylaceae	Plant (aerial) infusion		Terpens, lignans
Lepechinia caulescens (Ortega) Epling	Bretónica	Lamiaceae	Leaves infusion	Alloxanic mice (++)	Terpens
Lepidium virginicum L.	Lentejilla	Brassicaceae	Leaves infusion		
Leucaena leucocephala (Lam.) de Wit	Guaje	Fabaceae	Seed raw		Tannins
Leucophyllum texanum Benth.	Cenicillo	Scrophulariaceae	Plant (aerial) infusion		
Ligusticum porteri J.M. Coult. & Rose	Raiz de cochino	Apiaceae	Root infusion		Essential oils
Ligustrum japonicum Thunb.	Fresno	Oleaceae	Leaves infusion		
Loeselia coccinea (Cav.) G. Don	Hoja de la virgen	Polemoniaceae	Leaves infusion		Alkaloids, saponins
Loeselia mexicana (Lam.) Brand	Hierba de la virgen.	Polemoniaceae	Leaves infusion	Alloxanic mice (+)	Alkaloids, essential oils
Lonchocarpus cruentus Lundell	Guayacán	Fabaceae	Bark infusion		
Lopezia racemosa Cav.	Perilla	Onagraceae	Plant (aerial) infusion		
Lophocereus schottii (Engelm.) Britton & Rose	Muso	Cactaceae	Steam infusion		Alkaloids
Lysiloma acapulcense (Kunth.) Benth	Tepehuaje	Fabaceae	Leaves infusion, bark infusion		Tannins
Malmea depressa (Baillon) Fries	Elemuy	Anonaceae	Root infusion		Flavonoids
Malvastrum coromandelianum (L.) Garcke	Marvavisco	Malvaceae	Leaves infusion		Tannins
Mangifera indica L.	Mango	Anacardiaceae	Bark infusion leaves infusion		Flavonoids, essential oils, terpens
Marrubium vulgare L.	Marrubio	Lamiaceae	Leaves infusion, root infusion	Normal rabbits (++)	Terpens, flavonoids
Melothria pendula L.	Sandiita	Cucurbitaceae	Plant (aerial) infusion		
Mentha piperita L.	Hierbabuena	Lamiaceae	Leaves infusion		Essential oils, terpens, flavonoids
Mentha rotundifolia (L.) Huds.	Mostranza	Lamiaceae	Leaves infusion		Essential oils, terpens
Mentha suaveolens Ehrh.	Mastranzo	Lamiaceae	Leaves infusion		
Mimosa zygophylla Benth.	Gatuño	Fabaceae	Leaves infusion		
Mirabilis jalapa L.	Maravilla	Nyctaginaceae	Plant (aerial) infusion		Triterpens, flavonoids
Momordica charantia L.	Cundeamor,	Cucurbitaceae	Leaves infusion		Terpens, steroids, flavonoids
Morus nigra L.	Moral negro	Moraceae	Leaves infusion		
Musa sapientum L.	Flor de plátano	Musaceae	Root infusion		
Nasturtium officinale R. Br.	Berro	Brassicaceae	Plant (aerial) infusion		Flavonoids, alkaloids, terpens
Nopalea cochenillifera (L.) Salm-Dyck	Nopal	Cactaceae	Steam raw		

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Table 2. (Continued)

Scientific name	common name	Family	Plant part used and preparation	Pharmacological studies	Phytochemical information
Nopalea inaperta Schott ex Griffiths	Nopal	Cactaceae	Stem raw		
Olea europaea L.	Hierba de oliva	Oleaceae	Leaves infusion		Alkaloids, flavonoids, terpens
Opuntia atropes Rose	Nopal blanco	Cactaceae	Stem raw		
Opuntia ficus-indica (L.) Mill.	Nopal	Cactaceae	Stem raw		Alkaloids, flavonoids
Opuntia fulgida Engelm.	Choya	Cactaceae	Stem raw		
Opuntia guilanchi Griffiths	Nopal blanco	Cactaceae	Stem raw		
Opuntia imbricata (Haw) DC.	Xoconostle	Cactaceae	Stem raw, fruit		
Opuntia leucotricha DC.	Duraznillo	Cactaceae	Stem		
Opuntia megacantha Salm-Dyck	Nopal blanco	Cactaceae	Stem raw		
Opuntia streptacantha Lem.	Nopal	Cactaceae	Stem raw	Normal rabbits (+)	
Pachira aquatica Aubl.	Zapote de agua	Bombacaceae	Bark infusion		
Pachycereus marginatus (DC.) Britton & Rose	Organo, Sahuaro	Cactaceae	Stem raw		
Pachycereus pringlei (S. Watson) Britton & Rose	Cardón	Cactaceae	Stem raw		
Packera candidissima (Greene) W.A. Weber & A. Löve	Lechugilla	Asteraceae	Plant (aerial) infusion		
Parathesis lenticellata Lundell	Chagalapoli	Myrsinaceae	Leaves infusion		
Parkinsonia aculeata L.	Bagote	Fabaceae	Leaves infusion		Flavonoids, triterpens
Parthenium hysterophorus L.	Escobilla	Asteraceae	Plant (aerial) infusion		Alkaloids, partenin
Pavonia schiedeana Steud.	Cadillo	Malvaceae	Leaves infusion	Normal rabbits (–)	Tannins
Persea americana Mill.	Aguacate	Lauraceae	Leaves infusion		Sterols, flavonoids
Petroselinum crispum (Mill.) Nyman ex A.W. Hill	Perejil	Apiaceae	Plant (aerial) infusion		Essential oils, flavonoids
Phaseolus vulgaris L.	Frijol	Fabaceae	Fruit infusion	Normal rabbits (+)	Essential oils, flavonoids, alkaloids
Phlebodium aureum (L.) J. Sm.	Calahuala	Polypodiaceae	Root infusion		Steroids
Phoradendron bolleanum (Seem.) Eichler	Injerto	Viscaceae	Plant (aerial) infusion		
Phoradendron tomentosum (DC.) Engelm. ex A. Gray	Muicle	Viscaceae	Plant (aerial) infusion		Phoratoxins
Phragmites australis (Cav.) Trin. ex Steud.	Carrizo	Poaceae	Plant (aerial) infusion		
Physalis coztomatl Dunal	Costomate	Solanaceae	Leaves infusion		
Physalis philadelphica Lam.	Tomate	Solanaceae	Fruit roasted	Normal rabbits (–)	
Piper auritum Kunth	Acoyo	Piperaceae	Leaves infusion		Terpens, flavonoids, essential oils
Piper hispidum Sw.	Cordoncillo	Piperaceae	Leaves infusion		
Piper sanctum (Miq.) Schltdl. ex C. DC.	Hierba Santa	Piperaceae	Leaves infusion		Essential oils, alkaloids
Piper schiedeanum Steud.	Tlatalinaut	Piperaceae	Leaves infusion		
Pithecellobium dulce (Roxb.) Benth.	Guamuchil	Fabaceae	Bark infusion		
Plantago australis Lam.	Gusanillo	Plantaginaceae	Plant (aerial) infusion		Lignans
Plantago major L.	Llante	Plantaginaceae	Plant infusion		Flavonoids, terpens
Plumbago scandens L.	Plumbago	Plumbaginaceae	w/i		Flavonoids
Plumeria rubra L.	Flor de mayo	Apocynaceae	Flowers infusion		
Polygonum acre Lam.	Sanguinaria	Polygonaceae	Leaves infusion		
Populus alba L.	Abedul	Salicaceae	Leaves infusion		

Table 2. (Continued)

Scientific name	common name	Family	Plant part used and preparation	Pharmacological studies	Phytochemical information
Porophyllum punctatum (Mill.) S.F. Blake	Piojillo	Asteraceae	Flowers infusion		
Portulaca denudata Poelln.	Verdolaga	Portulacaceae	Plant (aerial) infusion		
Portulaca oleracea L.	Verdolaga	Portulacaceae	Plant (aerial) infusion		Alkaloids, terpens
Pouteria hypoglauca (Standl.) Baehni	Baehni	Sapotaceae	Leaves infusion		
Prosopis juliflora (Sw.) DC.	Mezquite	Fabaceae	Fruit raw		
Prunus serotina subsp. *capuli* (Cav.) McVaugh	Capulin	Rosaceae	Fruit infusion		Terpens
Psacalium sinuatum (Cerv.) H. Rob. & Brettell	Matarique	Asteraceae	Root infusion		
Pseudosmodingium perniciosum (Kunth) Engl.	Cuajilote	Anacardiaceae	Root infusion bark infusion		
Psidium guajava L.	Guayaba	Myrtaceae	Fruit		Terpens, flavonoids
Psidium yucatanense Lundell	Pach	Myrtaceae	Bark infusion		
Psittacanthus calyculatus (DC.) G. Don	Muérdago	Loranthaceae	Plant infusion, flowers infusion	Alloxanic mice (++)	
Quassia amara L.	Cuasia	Simaroubaceae	Leaves infusion		Alkaloids, terpens
Quercus acutifolia Neé	Encino	Fagaceae	Bark infusion		Terpens, flavonoids
Quercus rugosa Neé	Encino	Fagaceae	Bark infusion		
Randia echinocarpa Moc. & Sessé ex DC.	Grangel	Grangel	Leaves infusion		
Randia echinocarpa Moc. & Sessé ex DC.	Granjil	Rubiaceae	Fruit		
Raphanus sativus L.	Rábano	Brassicaceae	Root infusion		
Rhipsalis baccifera (J.S. Muell.) Steam	Nigrilla	Cactaceae	Stem infusion, fruit raw		
Rhizophora mangle L.	Mangle	Rhizophoraceae	Bark infusion		Tannins
Ricinus communis L.	Higuerilla	Euphorbiaceae	Leaves infusion		Flavonoids, terpens
Rosa centifolia L.	Rosa de castilla	Rosaceae	Leaves infusion		
Rubus adenotrichus Schltdl.	Zarzamora	Rosaceae	Leaves infusion		
Russelia equisetiformis Schltdl. & Cham.	Cola de caballo	Scrophulariaceae	Plant (aerial) infusion		
Salix taxifolia Kunth	Taray	Salicaceae	Steam infusion		
Salpianthus arenarius Humb. & Bonpl.	Catarinita	Nyctaginaceae.	Leaves infusion	Normal rabbits (++)	
Salvia leucantha Cav.	Salvia morada	Lamiaceae	Plant (aerial) infusion		Terpens
Sanvitalia procumbens Lam.	Ojo de gallo	Asteraceae	Plant (aerial) infusion		Terpens
Saurauia pringlei Rose	Picon	Actinidaceae	Leaves infusion		
Sechium edule (Jacq.) Sw.	Chayote	Cucurbitaceae	Fruit raw		Flavonoids
Sedum dendroideum Moc. & Sessé ex DC.	Siempreviva	Crassulaceae	Plant (aerial) infusion		Sedoheptulose
Sedum moranense HBK.	Siempreviva	Crassulaceae	Plant (aerial) infusion		
Sedum praealtum A. DC.	Siempreviva	Crassulaceae	Leaves infusion		
Selaginella lepidophylla (Hook. & Grev.) *Spring*	Doradilla	Selaginellaceae	Plant (aerial) infusion		Essential oils
Selaginella pallescens (C. Presl) Spring	Flor de piedra	Sellaginaceae	Plant (aerial) infusion		
Selloa plantaginea Kunth	Diente de elefante	Asteraceae	Plant (aerial) infusion		
Senecio albo-lutescens Sch. Bip.	Matarique	Asteraceae	Root infusion		
Senecio palmeri A. Gray	Matarique	Asteraceae	Root infusion		
Senecio peltiferus Hemsl.	Matarique	Asteraceae	Root infusion		
Senna multiglandulosa (Jacq.) H.S. Irwin & Barneby	Retama china	Fabaceae	Leaves infusion		

Table 2. (Continued)

Scientific name	common name	Family	Plant part used and preparation	Pharmacological studies	Phytochemical information
Senna obtusifolia L. (L.) H.S. Irwin & Barneby	Pa xojk	Fabaceae	Leaves infusion		Antraquinones, emodin
Senna occidentalis (L.) Link	Frijolillo	Fabaceae	Root infusion		Flavonoids, sterols
Serjania racemosa Schumach.	Bejuco tres en uno.	Sapindaceae	Plant (aerial) infusion		
Serjania triquetra Radlk.	Bejuco de tres C.	Salicaceae	Bark infusion		
Simira sp. Aubl	Quina roja, cáscara sagrada	Rubiaceae	Bark infusion		
Smilax aristolochiifolia Mill.	Zarzaparrilla	Similicaceae	Root infusion		Sapogenins
Solandra nitida Zuccagni	Flor de guayacán	Solanaceae	Flower infusion		
Solanum americanum Mill.	Hierba mora	Solanaceae	Plant (aerial) infusion		Alkaloids, solanin
Solanum brevistylum Wittm	Malabar	Solanaceae	Plant (aerial) infusion		
Solanum diversifolium Dunal	Malabar	Solanaceae	Leaves infusion	Normal rabbit (++)	
Solanum nigrescens M. Martens & Galeotti	Hierba mora	Solanaceae	Plant (aerial) infusion		
Solanum rostratum Dunal	Duraznillo	Solanaceae	Plant (aerial) infusion		
Solanum torvum Sw.	Berenjena	Solanaceae	Root infusion		
Solanum verbascifolium C.B. Wright	Berenjena	Solaneceae	Plant (aerial) infusion		Steroidal, alkaloids
Sonchus oleraceus L.	Lechuguilla	Asteraceae	Leaves infusion		Flavonoids
Spartium junceum L.	Retama	Fabaceae	Leaves infusion		
Sphaeralcea angustifolia (Cav.) G. Don	Hierba del negro	Malvaceae	Plant (aerial) infusion		
Stachytarpheta jamaicensis (L.) *Vahl*	Verbena	Verbenaceae	Plant (aerial) infusion		Terpens
Stenocereus marginatus (DC.) Berger & Buxb	Organo de Zopilote	Cactaceae	Steam roasted		
Struthanthus densiflorus (Benth.) Standl.	Injerto	Loranthaceae	Leaves infusion		
Swietenia humilis Zucc.	Zopilote	Meliaceae.	Seed raw		
Tagetes erecta L.	Cempasuchil o Flor de muerto,	Asteraceae	Plant (aerial) infusion		Terpens, essential oils
Tamarindus indica L.	Tamarindo	Fabaceae	Pulp of fruit raw		Flavonoids
Taraxacum officinale Weber ex F.H. Wigg.	Diente de león	Asteraceae	Leaves infusion		Terpens
Taxodium mucronatum Ten.	Ahuehuete	Taxodiaceae	Leaves infusion		Flavonoids
Tecoma stans (L.) Juss. ex Kunth	Tronadora	Bignoniaceae	Leaves infusion, plant infusion plant infusion	Alloxanic mice (++) Normal Dogs (++)	Alkaloids, terpens
Terminalia catappa L.	Castaño	Combretaceae	Fruit		
Teucrium cubense Jacq.	Agrimonia	Lamiaceae	Leaves infusion	Normal rabbits (+)	
Thryallis glauca (Cav.) Kuntze	Amargoso	Malphigiaceae	Root infusion		Flavonoids, terpens
Tillandsia usneoides (L.) L.	Heno	Bromeliaceae	Plant (aerial) infusion	Alloxanic mice (++)	Flavonoids
Tournefortia hirsutissima L.	Lagrima de San Pedro.	Boraginaceae	Steam infusion		
Tournefortia petiolaris DC.	Lagrima de San Pedro.	Boraginaceae	Steam infusion		
Tradescantia pendula (Schnizl.) D.R. Hunt	Comellina	Commelinaceae	Leaves infusion		Flavonoids
Trigonella foenum-graecum L.	Fenogreco	Fabaceae	u/i		
Tropaeolum majus L.	Mastuerzo	Tropaeoleaceae	Leaves infusion		
Turnera diffusa Willd ex Schult.	Damiana.	Turneraceae	Leaves infusion		Flavonoids, terpens
Urtica dioica L.	Ortiga	Urticaceae	Plant (aerial) infusion	Normal rabbits (–)	Flavonoids, coumarins
Urtica mexicana Liebm.	Ortiga	Urticaceae	Leaves infusion		

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Table 2. (Continued)

Scientific name	common name	Family	Plant part used and preparation	Pharmacological studies	Phytochemical information
Valeriana edulis Nutt. ex Torr. & A. Gray	Valeriana	Valerianaceae	Root infusion		
Valeriana procera Kunth	Valeriana	Valerianaceae	Root infusion	Alloxanic mice (−)	
Verbesina crocata (Cav.) Less.	Capitaneja	Asteraceae	Leaves infusion	Alloxanic mice (+)	
Verbesina persicifolia DC.	Huichin	Asteraceae	Plant (aerial) infusion	Alloxanic mice (+)	Sesquiterpens
Zaluzania augusta (Lag.) Sch. Bip.	Limpia tuna	Asteraceae	Root infusion		
Zanthoxylum fagara L.	Tankasché	Rutaceae	Leaves infusion		Alkaloids
Zea mays h.	Pelos de elote	Poaceae	Fruit infusion		
Zexmenia gnaphalioides A. Gray	Peonia	Asteraceae	Root infusion		
Zizyphus acuminate Benth	Corongoro, amol	Rhamnaceae	Plant (aerial) infusion		

* In the Animal studies +, indicates activity and the level of it, while—mean no observed activity for the tested extract.
** The phytochemical information, refers about the reports for the plant no the active compounds.

infections. Antibiotics commonly prescribed were trimethoprim-sulphamethoxazole, amoxicillin-clavulanic acid, second-generation cephalosporins and clindamycin, all of which were prescribed on an outpatient basis (Tentolouris, *et.al.* 2006). However, the use of antibiotic not only causes side effect but also leads to severe symptom in patient with allergy.

Because of the side effects of currently used anti-hyperglycemic drugs and anti-MRSA drug, it is interesting to develop an alternative medicine with low side effect. Some traditional medicine demonstrated a good practice and shows a very interesting outcome. Thus, it is important to understand the traditional therapeutics and natural medicine for diabetes treatment.

4. Ethnopharmacology plant in Family Malvaceae

The hypoglycemic effect of plants in this family has been reported in international journals such as in *Hibiscus rosa Sinensis* Linn. (Sechdewa *et al.*, 2001a, Sechdewa *et al*. 2001b, Sechdewa and Khemani, 2003) and *Abutilon inducum* at dose of 400 mg/kg body weight (Seetharam *et al*., 2002).

CHAPTER II

MATERIALS AND METHODS

Materials

1. Equipments

- Accutrend® meter GCT (Roche Diagnostics, Ltd., Germany)
- Balance (AX 205 DeltaRange, Mettler Toledo, Switzerland)
- Spectrophotometer Spectronic® Genesys™ (Milton Roy, USA)
- HPLC (Shimadzu SCL-10A VP, Japan)
- HPLC Column (Inertsil® ODS-3 5 µm x 250 mm, 4EI86275, GL Sciences Inc. Japan)
- Colorimeter (Biomerieux Vitex), HACH company, USA

2. Chemicals

- Glucose, anhydrous (analytical grade, Fluka, Switzerland)
- Streptozotocin (STZ) (Sigma Chemicals Co., Ltd., Germany)
- Accutrend® Glucose (blood) (Roche Diagnostics, Ltd., Germany)
- Accutrend® Triglyceride (blood) (Roche Diagnostics, Ltd., Germany)
- Accutrend® Cholesterol (blood) (Roche Diagnostics, Ltd., Germany)
- Humulin® R (Eli Lilly, USA)
- Diethyl ether (analytical grade, E.Merck, Germany)
- Acetonitrile (HPLC grade, Lab-Scan analytical sciences, Ireland)
- Normal Saline (0.9% sodium chloride injection) (General hospital products Public Co., Ltd., Pathum Thani, Thailand)
- Commercial pellet food for rats (C.P. Mice Feed; S.W.T. Co. Ltd., Samutprakarn, Thailand)
- Mueller Hinton Agar (Merck KGaA, Germany)
- Mueller Hinton Broth (Merck KGaA, Germany)
- Oxacillin 1µg/disc (BD BBL™ Sensi-Disc™, Ref 231319, Becton, Dickinson and Company, USA)
- Glucose Oxidase (β-D- Glucose: oxygen 1-oxido-reductase; EC 1.1.3.4) (Sigma® Chemical company, Lot 34F-95801, USA)
- Peroxidase (POD, Donor: hydrogen peroxide oxido-reductase; EC

1.11.1.7) (Sigma® Chemical company, Lot 31F-9500, USA)

- o-Dianisidine Dihydrochloride (3,3'-Dimethoxybenzidine; Fast Blue B) (Sigma®Chemical company, Lot 062K5304, USA)

3. Plant material

Plant materials, *Malvastrum coromandelianum* Garcke was identified by Royal forest department of Thailand. Plant material used was provided by Mr. Kamol Vichitpan.

4. Bacteria

Staphylococcus aureus ATCC 25923, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 25212 and *Pseudomonas aeruginosa* ATCC 27853 were used as standard strains. Clinical isolates of *S. aureus* including Methicillin sensitive *S. aureus* (MSSA) 15 strains and Methicillin resistant *S. aureus* (MRSA) 15 strains were kindly provided by Associated Professor Dr. Pintip Pongpech from Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand. MRSA were screened by using Agar Disc diffusion method. The strains of *S. aureus* which showed no inhibition zone against Oxacillin disc (BBL tm, USA) on Mueller Hinton agar medium were defined as MRSA.

5. Preparation of agar and broth media

All agar and broth media were dissolved in water and sterilized in autoclave for 20 minute at 15 lb/inch^2 (121° C). Mueller Hinton agar (MHA) and Mueller hinton broth (MHB) were used for agar diffusion susceptibility test and broth microdilution susceptibility test, respectively. All test bacteria were tested on MHA and MHB.

Methods

1. Water extraction of Plant material

The aerial part of *M. coromandelianum* was dried at 60 ^oC until constant weight was obtained. Dried plant was ground and sieved to obtain uniform powder. Boil 10 kilograms of powder in 100 liters deionized water for 5 min and simmered for

7-8 hr at 80 ^{o}C. Filter and boil residue twice each with 50 liters water. Collect all three filtrates and spray-dried to obtain spray-dried powder of *M. coromandelianum* extract (ME) with yield of 8-10 %.

The spray-dried powder (ME) was dissolved in distilled water to prepare the ME solution used in testing for hypoglycemic activity and antibacterial activity.

2. HPLC profile and stability of ME

The 10 mg/ml of ME was analyzed by using HPLC in optimum chromatographic conditions. HPLC is equipped with a UV-VIS detector. HPLC column is a C_{18} column.

The Ultra-pure water and Acetonitrile were filtered using polyamide membrane with 0.45 μm diameter. Column was washed with 100% acetonitrile for 1 hr and equilibrated with 50:50 (acetonitrile:water) for 1 hr. Column was then rinsed with water for 1 hr. The running time was 45 min. with flow rate 1.0 ml/min, absorbance detection at 280 nm using UV-VIS detector. Running program was showed as follows.

0 – 5 min	0% acetonitrile	100% water
5-25 min	0-70%acetonitrile	100-30% water (gradient mode)
25-40 min	70% acetonitrile	30% water (step wise mode)
40-45 min	70%-0% acetonitrile	30-100% water (gradient mode)

Column was then washed with water for 1 hr and equilibrated with 50:50 (acetonitrile:water) for 1 hr. The column was rinsed with 100% acetonitile for 1 hr. before being disconnected and stored.

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3. Antimicrobial susceptibility tests of ME

3.1 Agar diffusion susceptibility test

Antibacterial susceptibility test of ME was performed as described in the standard guideline technique (Lorian, 1991). All test bacteria were inoculated on tryptic soy agar (TSA) slant and incubated at 37 ºC overnight. Bacteria were washed from surface agar slant with 5 ml sterile normal saline solution (0.9% NaCl) then adjusted to match turbidity of standard Mcfarland No. 0.5 (equal to approximately 1 x 10^{8} CFU/ml) by using Colorimeter ((Biomerieux Vitex), HACH company, USA) and the bacteria were used as inoculum. Twenty ml of Mueller

Hinton agar (MHA) was pipetted into Petri dish (inner diameter 100 mm). The media were cooled to room temperature overnight and stock bacterial suspension was inoculated on agar surface by swab technique.

ME was dissolved in sterile distilled water to prepare 10, 20 and 40 mg/ml solution. Sterile stainless steel cylinders (6 mm inner diameter and 10 mm height) were placed on the inoculated agar surface. Plant extract solutions were filled into cylinders (300 µl/cylinder). After pre-diffusion at room temperature for 1 hour, plates were incubated at 37 ºC for 19 hrs. NSS was used as control and Oxacillin disc was used as control drug in the same inoculate plate.

3.2 Broth macrodilution test for determination of minimum inhibitory concentration (MICs) and minimum bactericidal concentration (MBCs) of ME

Susceptibility test of ME solution against all bacteria strains were performed by the broth macrodilution method according to the modified method from NCCLS (NCCLS, 1999). ME was dissolved in sterile water to prepare 5 ml of 320 mg/ml. Five ml of 320 mg/ml ME solution was pipetted into 4.8 ml of steriled Muller-Hinton broth and mixed. Five ml of the first tube was pipetted into 4.8 ml of Muller-Hinton broth of second tube and repeated this process to make serial two fold dilution. The final concentrations of extract in the medium were 160, 80, 40, 20 and 10 mg/ml. The 200 µl of stock bacterial suspension culture (1x108 CFU/ml) were pipetted into each tube to make the final volume of 5 ml. The control tube was 200 µl normal saline solution replaced stock bacterial suspension. The tubes of inoculated and control were incubated at 37°C for 24 hrs and the MIC was determined as the lowest concentration of the extract showed no visible growth of each strain. MBC was determined by sub-culturing from tubes showing no visible growth with plant extract on the MHA plates after incubation at 37°C for 24 hours. Plates of the lowest concentration of ME with no bacterial growth represented MBC value of ME. All determinations were performed in triplicate.

4. Test for hypoglycemic effect of ME

4.1. Preparation of animal model

Male Wistar rats weighed 70-100 g were obtained from National Laboratory Animal Centre, Mahidol University, Bangkok, Thailand. Animals were acclimatized

for 1 week before used under a constant 12 hr light: dark cycle, 22-25 °C and humidity 55 ± 5%. Food and distilled water were given ad libitum. All animals were weighed and placed in individual stainless steel cage (8x10 inch) at the day the experiment started.

4.2 Diabetes mellitus induction in animal model

Normal male Wistar rats were fasted overnight (15-18 hr). They were injected a single dose streptozotocin (STZ) which was freshly dissolved in normal saline at a dose of 85 mg/kg body weight, intraperitoneally (Brosius, 2003). Normal saline was injected in control rats. Animals in all groups consumed water and food *ad libitum*. Twenty-four hours after STZ injection, blood glucose concentration was determined. Diabetes mellitus was confirmed in STZ – treated rats by measuring fasting blood glucose concentration within 48-72 hr after STZ injection. Rats having blood glucose concentration above 150 mg/dl were considered diabetes in this study.

4.3 Blood collection and determination

Blood sample was collected by cutting tip of rat tail. Blood glucose concentration was determined by Glucose – oxidase/mediator reaction test strips (Accutrend® Glucose, Roche Diagnostics, Ltd, Germany) using Accutrend® meters GCT. Blood glucose concentration was expressed in mg/dl.

4.4 Blood collection for clinical biochemical analysis and hematological analysis

At the end of experiments, rats were anaesthetized with diethyl ether. Blood samples (3 ml) were obtained from the heart and collected in heparinized form or EDTA form. Heparinized form of blood was centrifuged at 3,000 rpm for 5 min. Supernatant (plasma) was separated for blood clinical biochemistry analysis by Flexor E automation. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN) and creatinine were determined. Blood which was collected in EDTA form was mixed for 5 min by Hematology series cell mixer (Baker instrument, Thailand) and Complete Blood Counts (CBC) [total red cell counts, total white cell counts, differential white cell counts, hemoglobin (Hb) and Hematocrit (Hct) measurement] were measured by automate monitor (DANAM HC 5710, USA).

4.5 The hypoglycemic effect of crude water extract from *M. coromandelianum* (ME) in normal male Wistar rats

4.5.1 Single – oral dose of ME in glucose tolerance test (OGTT)

A total of 24 rats were divided into 4 groups, each group of 6 rats and fasted overnight (15-18 hrs).

- Control group. (Distilled water 1 ml/kg body weight)
- Treatment group 1. (5 mg ME /kg body weight)
- Treatment group 2. (10 mg ME /kg body weight)
- Treatment group 3. (20 mg ME /kg body weight)

Blood samples were collected from tail vein at 30 min-intervals for determination of fasting blood glucose concentration. Single doses of ME or water were fed at 30 min prior to feeding of glucose at 1 g/kg body weight, where blood was collected just before glucose feeding. A blood glucose concentration was determined at time -30, 0, 30, 60, 90, 120, 150 and 180 min after feeding glucose, by cutting vein at the tip of rat tail.

4.6 The hypoglycemic effect of ME in diabetic male Wistar rats

4.6.1 Single – oral dose of ME in diabetic rats; non- fasting blood glucose concentration analysis

A total of 36 rats were divided into 6 groups, each group of 6 rats.

- Normal control group
- Diabetic rats fed water;
- Diabetic rats treat with insulin (Humulin® R) 5 IU/kg body weight, i.p.;
- Diabetic rats fed 10 mg ME/kg body weight;
- Diabetic rats fed 50 mg ME/kg body weight;
- Diabetic rats fed 100 mg ME/kg body weight;

Non-fasting blood glucose concentration was determined before administration of ME (at 0 hr). The treated groups were fed ME orally or injected Humulin® R intra-peritoneally. Blood glucose concentration was measured at 1, 2, 3, 4, 5 and 6 hrs after treatment.

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4.6.2 Repeated-oral doses of ME in diabetic rats; fasting blood glucose concentration analysis

A total of 36 rats were divided into 6 groups, each of 6 rats.

- Normal control group fed daily with distilled water;
- Diabetic rats fed daily with distilled water;
- Diabetic rats injected with insulin (Humulin® R) 5 IU/kg body weight/d, i.p.;
- Diabetic rats fed 50 mg/kg body weight/d;
- Diabetic rats fed 100 mg/kg body weight/d;
- Diabetic rats fed 500 mg/kg body weight/d;

Diabetic rats or normal rats were fasted overnight (15-18 hrs) before day 0, and blood glucose concentrations were determined. The treatments were given daily for 30 days. On day 15th and 30th, fasting blood glucose concentration was determined at 22 hrs after treatment. Rats were sacrificed, where blood samples and organs were collected (liver, exocrine pancreas, kidney, eyes, aorta vascular, femoral nerve and brain). Relative liver weight was calculated by this equation.

$$\text{Relative liver weight} = \frac{\text{Rat's liver weight}}{\text{Rat's body weight}} \times 100$$

And relative weight gain was calculated by this equation.

$$\text{Relative weight gain} = \frac{\text{Rat's follow-up weight} - \text{Rat's initial weight}}{\text{Rat's initial weight}} \times 100$$

5. *In vitro* test for glucose absorption in rat jejunum: everted rat intestine (jejunum) sac model

Male Wistar rats (200-250 g) were fasted for 19 h, anesthetized with ether, sacrificed and removed the small intestine. The small intestines were cut at about 10 centrimeter of intestine distal to the pyrorus (the area of jejunum). The small intestine sac was transferred to a Petri dish filled with cold Krebs-Ringer saline buffer pH. 7.2, and quickly washed 3 times with cold Krebs- Ringer saline buffer pH. 7.2 before

everted. A simple procedure for eversion and preparation of an intestinal sac was showed in Figure 1. One end of the small intestine was tied tightly. A glass rod was inserted to push the tied end of the sac to evert the mucosal surface out. The everted intestine was cut 6 cm long and quickly rinsed with Krebs-Ringer saline buffer pH. 7.2 and tied at one end. A syringe with needle was used to fill the sac with Krebs-Ringer saline buffer pH. 7.2 for 1 ml. The syringe was pulled out and quickly tied the other end of intestinal sac tightly. The small intestinal sac was kept in cold Krebs – Ringer saline buffer pH. 7.2 before used.

The intestinal sac was incubated in organ chamber (Figure 2) filled with 10 ml of 200 mg% glucose in Krebs-Ringer saline buffer pH 7.2 for 1 h, at 37°C, with oxygen supplied by bubbling of oxygen throughout the chamber. At the end of incubation period, the sac was removed from the organ chamber. One end of sac was cut and collected all the buffer inside the sac. The buffer with glucose in the chamber was also collected. The buffer solution inside and outside the sac were assayed for glucose concentration by using glucose oxidase test.

6. Acute toxicity test in normal rats (United States Environmental Protection Agency [EPA], 1998)

Normal male Wistar rats were divided into 4 groups, each of 6 rats.

- Control group: normal rats fed distilled water;
- Treatment group 1, fed a single dose of 100 mg ME/kg body weight;
- Treatment group 2, fed a single dose of 10 g ME/kg body weight;
- Treatment group 3, fed a single dose of 20g ME/kg body weight;

The effective dose of 100 mg ME/kg body weight with significant hypoglycemic activity in diabetic rats was selected to test for toxicity. In addition, the excessive doses of 10 and 20 g ME/kg body weight were also tested. Rats were orally given single dose of ME (treated groups) or distilled water (control group). General behavior, clinical signs and any toxic effects within 1-4 hr after administration were observed, i.e., fur, mucous, respiratory system, and lethargy. Rat weight was recorded on day 0 before feeding ME and on day 5 after feeding ME. On day 5, rats were sacrificed by sedative method, blood was drawn from heart and test for biochemistry and hematological analysis.

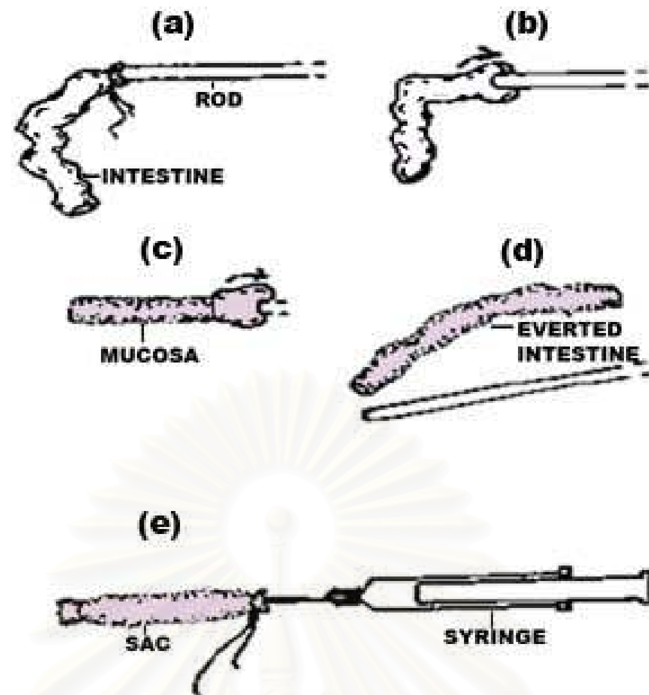

Figure 1. The procedure outlined of eversion and preparation of an intestinal sac (Deyrup-Olsen and Linder, 1979).

Figure 2. Organ chamber using in everted intestine sac experiment (picture from iWorx/CB Sciences-One, Washington street, Suite 404, Dover NH-03820)

7. Subacute (30 days) toxicity test in normal rats (United States Food and Drug Administration [FDA], 2003)

Normal male Wistar rats were divided into 4 groups, each of 6 rats.

- Control group: normal rats fed daily with distilled water;
- Treatment group 1, fed daily with 100 mg ME/kg body weight;
- Treatment group 2, fed daily with 10 g ME/kg body weight;
- Treatment group 3, fed daily with 20g ME/kg body weight;

The effective dose of 100 mg ME/kg body weight with significant hypoglycemic activity in diabetic rats was selected to test for toxicity. In addition, the excessive doses of 10 and 20 g ME/kg body weight were also tested. Rats were orally given ME or distilled water daily for 30 days. General behavior, clinical signs and any toxic effects within 1-4 hr after administration were observed, i.e., fur, mucous, respiratory system, and lethargy. Rat weight was recorded on day 0 before feeding ME and weekly after feeding ME. On day 30, rats were sacrificed by sedation method. Blood was withdrawn from heart and tested for biochemistry and hematological analysis. Liver, kidney and spleen were collected and carried out for histopathological examination.

8. Histopathological Evaluation

On day 30 of experiment of repeated –oral doses of ME in diabetic rats and subacute (30 days) toxicity test in normal rats, organs were collected for histopathological examination. For repeated – oral doses of ME in diabetic rats experiment liver, exocrine pancreas, kidney, eyes, aorta, femoral nerve and brain were collected. In subacute (30 days) toxicity test in normal rats, spleen, liver and kidney were collected. Rats were anesthetized with ether. The organs were kept in 10% formalin.

8.1 Preparation of Histological Sections (Luna, 1968)

8.1.1 Fixation

The organs were fixed in the fresh fixative using an aqueous 10% neutral buffered formalin for at least 24 hrs.

8.1.2 Processing

The organs processing procedure was operated by the followings process.

Washing: After fixation, the specimens were washed in running water for 30 mins.

Dehydrating: the specimens were dehydrated by transferring through a series of ethyl alcohol of increasing concentrations using 80% ethyl alcohol for 30 mins, 2 times; 95% ethyl alcohol for 30 mins, 2 times; and 100% ethyl alcohol for 40 mins, 2 times, respectively.

Clearing: the specimens were transferred to a clearing agent such as xylene, which is miscible with both 100% ethyl alcohol and paraffin. The specimens were infiltrated in xylene for 30 mins, 2 times.

Infiltration: following the replacement of alcohol by clearing reagent, the tissues were immersed in melted paraffin (60°C), which infiltrates the tissues for 30 mins, 2 times.

Embedding and blocking: when infiltration was completed, the specimens were transferred to fresh melted paraffin and embedded in a cubical paraffin mold. After cooling, the melted paraffin was hardened. The paraffin block was removed and excess paraffin was trimmed away.

8.1.3 Sectioning

A slide of section was prepared by the following steps: the block of paraffin was secured to the microtome and oriented appropriately with respect to the knife. With each revolution of the microtome handle, the specimen moved through the blade and a section of the desired 4-6 μm thickness was produced. Each successive section adhered to the proceeding one, forming a continuous ribbon. Subsequently, one or more sections were carefully separated from the ribbon and transferred to the surface of warm water in a water bath at 40-45 °C to produce softness of paraffin and flatness of the section as well as eliminating wrinkles. The flattened section was floated onto a slide, which was left for air drying in room temperature. As the preparation dried, the section adhered to the surface of the slide (Bacha and Wood, 1990).

8.1.4 Staining

After the section on the slide was dried. The sections were stained with hematoxylin-eosin reagents.

Hematoxylin and Eosin (H&E) staining procedure:

The paraffin was removed with xylene for 10 mins, the same procedure was repeated. The specimens were dehydrated by passing through a gradual series of decreasing concentrations at absolute, 95% and 70% ethyl alcohol, respectively, for 2 min with each alcohol concentration. The specimens were washed in running water for 5 min and stained with Harris hematoxylin solution for 6 mins. The sections were a bluish-violet color and washed in running water for 5 mins. The sections were removed the excess hematoxylin in 1% acid alcohol 1 dip, and washed the excess acid in running water for 5 mins. The sections were then neutralized by dipping into saturated lithium carbonate (Li_2CO_3) for 4 dips and washed in running water for 5 mins. Counterstain the sections with eosin working solution for 1 min to produce a pink or red color. After stained, the specimens were dehydrated by passing through a gradual series of increasing concentrations of 95% ethyl alcohol 5 dips and absolute ethyl alcohol twice for 2 min of each. The specimens were cleared (made transparent) with xylene for 5 min, 3 times. Permanent mounting prepared by covering the specimens with a resinous mounting medium (DPX solution) and topped with a cover slip (Luna, 1968).

8.2 Histopathological Analysis

The lesions were given a score ranging from 0 (no remarkable lesions), 1 (mild), 2 (moderate) and 3 (severe).

9. Statistical analysis

Results were expressed as mean ± standard error of mean (S.E.M.) excepted the antimicrobial activity part was expressed as mean ± standard deviation (SD). Statistical comparison were made using one way analysis of variance (ANOVA), followed by Duncan's Multiple range test (DMRT). From six rats in each group, p – value < 0.05 were considered significant.

CHAPTER III

RESULTS AND DISCUSSIONS

The biological activity of *M. coromandelianum* water extract (ME) on hypoglycemic and anti-microbial activity was studied. The hypoglycemic effect of ME was investigated in both normal and diabetic rats. Firstly, ME was tested in normal rats by using single-oral dose in glucose tolerance test (OGTT) to determine the effect on glucose tolerance in normal rats with normal function of beta cell and insulin action. Secondly, ME was tested in diabetic induced rats by using single oral dose and repeated oral dose in streptozotocin-induced diabetic rats to determine hypoglycemic effect of ME on diabetic rats with streptozotocin-induced beta cell death. Toxicity of ME in diabetic rats was also studied. Thirdly, the acute and subacute (30 days) toxicity of ME was determined by histopathological and biochemical analysis. Finally, the anti-microbial activity of ME was tested against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 25212 and *Pseudomonas aeruginosa* ATCC 27853.

1. Crude water extraction of *M. coromandelianum* (ME)

Aerial part of ME were dried by sunlight and pulverized. Dried plant was extracted in boiling water and spray dried. Filtrates were collected. The yield of dried powder was 8-10% of dried plant.

In this study, one batch of spray-dried extract was used. Sterile water was used as vehicle to dissolve spray-dried ME powder, which was freshly prepared at indicated concentration before use.

2. HPLC profile and stability of ME

The use of chromatographic fingerprinting for herbal drugs tends to focus on identification and assessment of the stability of the chemical constituents in herbal extracts.

The HPLC profile of ME was used to determine the consistency of plant constituents. ME was freshly prepared before each run on HPLC. This study used only one batch of ME and the HPLC chromatograms of ME after storage as powder at ambient temperature for 1, 30 and 180 days and freshly prepared as solution before analysis were shown in Figure 3.

3. Antimicrobial susceptibility tests of ME

According to the evidence that this plant has been used for wound healing in diabetic foots complication patients in Thailand, the wounds usually infect with bacteria. It is well known that one of the seriouse diabetic foot complication is infection which leads to amputation of extremities of in diabetic patient. Common organisms in diabetic burn infections are *Streptococcus*, *Proteus*, *Pseudomonas*, *Candida* species and MRSA (methicillin– resistant *Staphylococcus aureus*) (Memmel; 2004). In this study, ME was tested for its antimicrobial activity against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 25212 and *Pseudomonas aeruginosa* ATCC 27853 by using agar disc diffusion susceptibility test and broth macro dilution test.

3.1 Agar diffusion susceptibility test

Agar diffusion susceptibility test was performed to test antibacterial activity of ME against *S. aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *E. Faecalis* ATCC 29212 and *Ps. aeruginosa* ATCC 27853. ME solution showed inhibition zone only against *S. aureus* ATCC 25923 (9.0 ± 0.7 mm with 40 mg ME/ml) as shown in Table 3. It was found that 40 mg ME/ml showed inhibition zone against 6 strains out of each group of 15 tested strains of MSSA and MRSA (Table 3.). Inhibition zone against *S. aureus* ATCC 25923 was 9.0±0.7 mm with 40 mg ME/ml and 8.0±0.5 mm with 20mg ME/ml. It also showed inhibition zone against MSSA and MRSA. Inhibition zone against MSSA were 14.5 - 20.0 mm for 40 mg ME/ml and 10.2-13.5 mm for 20 mg ME/ml. Inhibition zone against MRSA were 11.0 - 17.0 mm for 40 mg ME/ml and 15.0±0.7 mm for 20 mg ME/ml in only one strain among 15 tested strains (Table 3.). The results from agar disc diffusion method showed that ME did not inhibit all clinical isolates of *S. aureus* but at least 6 strains of MRSA out of 15 strains were susceptible to ME at 40 mg/ml concentration.

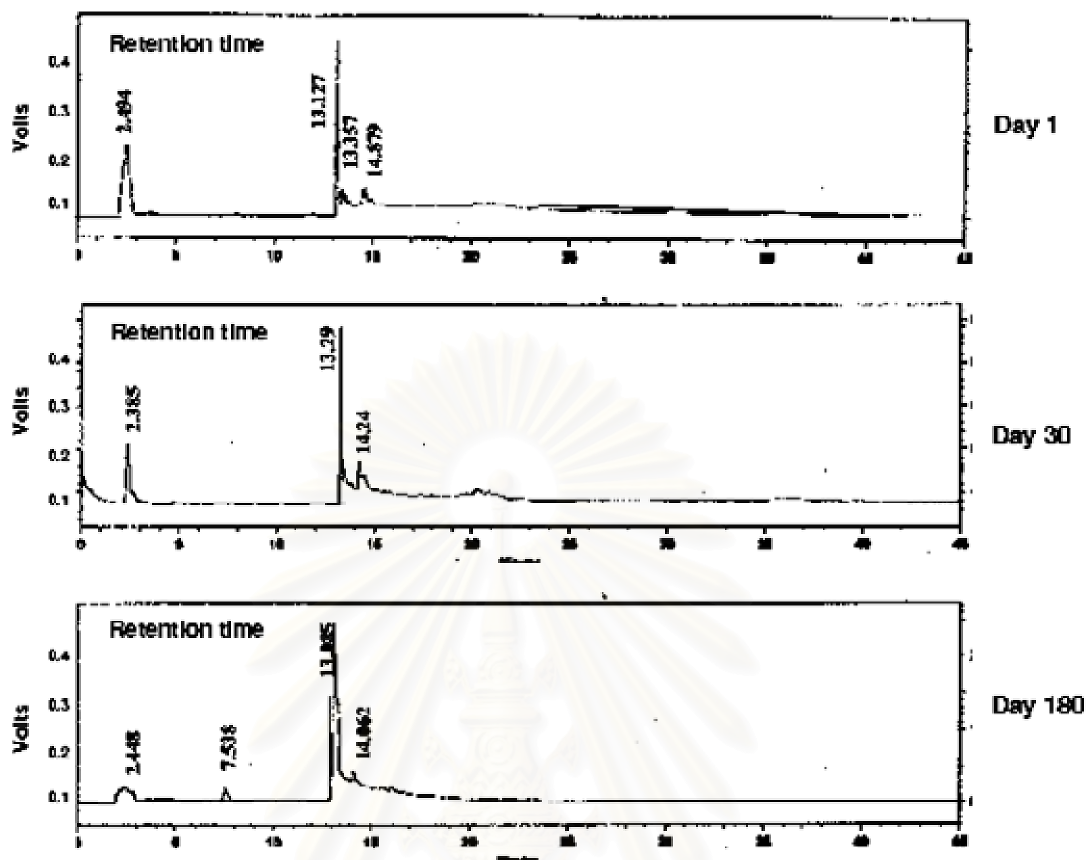

Figure 3. HPLC chromatograms of ME solution in water, on day 1, 30 and 180 of storage at ambient temperature.

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Table 3. Inhibition of bacteria growth by *M. coromandelianum* water extract solution using agar diffusion method.

Bacteria	Clear zone diameter (mm)		
	40 mg/ml plant extract	20 mg/ml plant extract	Oxacillin 1 μg
S. aureus ATCC 25923	9.0 ± 0.7	8.0 ± 0.49	21.0 ± 0.2
MSSA1	14.5 ± 0.7	10.5 ± 0.7	22.7 ± 2.1
MSSA2	16.5 ± 2.1	10.2 ±3.4	20.2 ± 0.7
MSSA3	20.0 ± 5.7	NZ	20.2 ± 0.7
MSSA4	NZ	NZ	23.2 ± 0.7
MSSA5	NZ	NZ	19.0 ± 0.7
MSSA6	16.5 ± 3.3	11.5 ± 2.1	22.3 ± 1.4
MSSA7	17.5 ± 0.7	13.5 ± 0.7	21.5 ± 2.1
MSSA8	NZ	NZ	19.0 ± 2.1
MSSA9	NZ	NZ	18.3 ± 0.7
MSSA10	NZ	NZ	22.1 ± 0.7
MSSA11	NZ	NZ	17.1 ± 2.1
MSSA12	NZ	NZ	20.0 ± 1.4
MSSA13	NZ	NZ	19.0 ± 1.4
MSSA14	NZ	NZ	21.5 ± 0.7
MSSA15	16.0 ± 1.4	NZ	25.3 ± 3.7
MRSA1	11.0 ± 0.7	NZ	NZ
MRSA2	NZ	NZ	NZ
MRSA3	NZ	NZ	NZ
MRSA4	NZ	NZ	NZ
MRSA5	NZ	NZ	NZ
MRSA6	NZ	NZ	NZ
MRSA7	12.0 ± 0.7	NZ	NZ
MRSA8	NZ	NZ	NZ
MRSA9	17.0 ± 0.1	15.0 ± 0.7	NZ
MRSA10	11.0 ± 0.7	NZ	NZ
MRSA11	NZ	NZ	NZ
MRSA12	13.5 ± 0.7	NZ	NZ
MRSA13	NZ	NZ	NZ
MRSA14	NZ	NZ	NZ
MRSA15	12.0 ± 0.70	NZ	NZ

Data are mean ± S.D.

ME showed inhibitory effect against the clinical isolates of *S. aureus* six out of 15 test strains MSSA were inhibited by 40 mg/ml of ME and 6 out of 15 test strains of methicillin-resistant *S. aureus* were inhibited by 40 mg/ml of ME.

The clear inhibition zone from ME against *S. aureus* ATCC 25923, MSSA 1 and MRSA 9 were demonstrated in Figure 4, 5 and 6, respectively.

3.2 Determination of minimum inhibitory concentration (MICs) and minimum bactericidal concentration (MBCs) of ME by Broth macrodilution method.

By using Broth macrodilution method, MICs of ME against *S. aureus* ATCC 25923 was 40 mg/ml, MSSA was 20 – 40 mg/ml(6 strains among 15 test strains) and MRSA was 20 – 40 mg/ml(6 strains among 15 test strains). MBCs of ME against *S. aureus* ATCC 25923 was 80 mg/ml, against MSSA was 40-80 mg/ml and against MRSA was 40-80 mg/ml, respectively (Table 4).

MIC and MBC of ME against *S. aureus* ATCC 25923 and patient's isolated strains were determined by broth macrodilution method. MIC of *M. coromandelianum* against *S. aureus* ATCC 25923 was 40 mg/ml while MBC was 80 mg/ml. For MSSA, ME showed MICs at 20-40 mg/ml and MBCs at 40-80 mg/ml. Similarly MICs and MBCs of ME against MRSA were 20-40 mg/ml and 40-80 mg/ml, respectively. ME was shown to be bacteriostatic agent at the lower concentration and bactericidal agent at higher concentration.

The antimicrobial active compound of *M. coromandelianum* had never been reported. The related plant such as *Abutilon indicum* and *Hibiscus rosa sinensis* Linn. have Myricetin which showed hypoglycemic activity. Myricetin was also reported of its antimicrobial properties against different bacterial strains (el-Gammal and Mansour, 1986).

Thus, ME can inhibit growth of swamp and abscess causing bacteria (*S. aureus*) which is usually found in diabetic foot infections. It was very interesting to further investigate antibacterial activity of ME against bacteria which also found in diabetic foot infections such as *Streptococcus spp*., *Proteus spp*. and *Candida spp*. Therefore, ME is beneficial for diabetic patient not only to decrease blood glucose concentration but also to inhibit growth of bacterial complication involved diabetic foot infection.

4. Test for hypoglycemic effect of ME

4.1. Preparation of animal model and diabetes mellitus induction in animal model

The protocol of this study has been approved by ethic committee of The Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand (No. 181/2006). Rats of weight about 70-100 g were used in this study. Rats were induced with STZ at dose of 85 mg/kg body weight by single intraperitoneal injection (Brosius, 2003). Rats having fasting blood glucose concentration higher than 150 mg/dl were considered diabetes in this study. Rats showed moderate diabetes, i.e. fasting blood glucose concentration of 200-300 mg/dl were used.

4.2 The hypoglycemic effect of crude water extract from *M. coromandelianum* (ME) in normal male Wistar rats

4.2.1 Single-oral doses of ME in glucose tolerance test (OGTT) in normal rats

Four groups of each 6 normal Wistar rats were fasted overnight (15-18 hrs). Single dose of plant extract solution (ME) or distilled water (control) were fed at 30 min prior to feeding of glucose at 1 g/kg body weight. Treatment groups 2, 3, and 4 were fed solution of ME orally at dose 5, 10, and 20 mg/kg body weight, respectively; and group 1 (control group) fed distilled water, at 30 min before glucose feeding. ME-treated rats showed significantly decrease ($p<0.05$) in blood glucose concentration compared with control group at 30 min after glucose feeding (Figure 7.).

Blood glucose concentration at 30 min of treatment group 2, 3 and 4 were 138.2 ± 7.41, 124.3 ± 5.93 and 109.6 ± 5.12 mg/dl, respectively; whereas that of control group was 155.1 ± 5.09 mg/dl. Percentage blood glucose concentration decrease was 10.9, 19.8, and 29.3%, respectively. All concentration of 5, 10, 20 ME mg/kg body weight significantly decreased blood glucose concentration ($p < 0.05$) in normal rats.

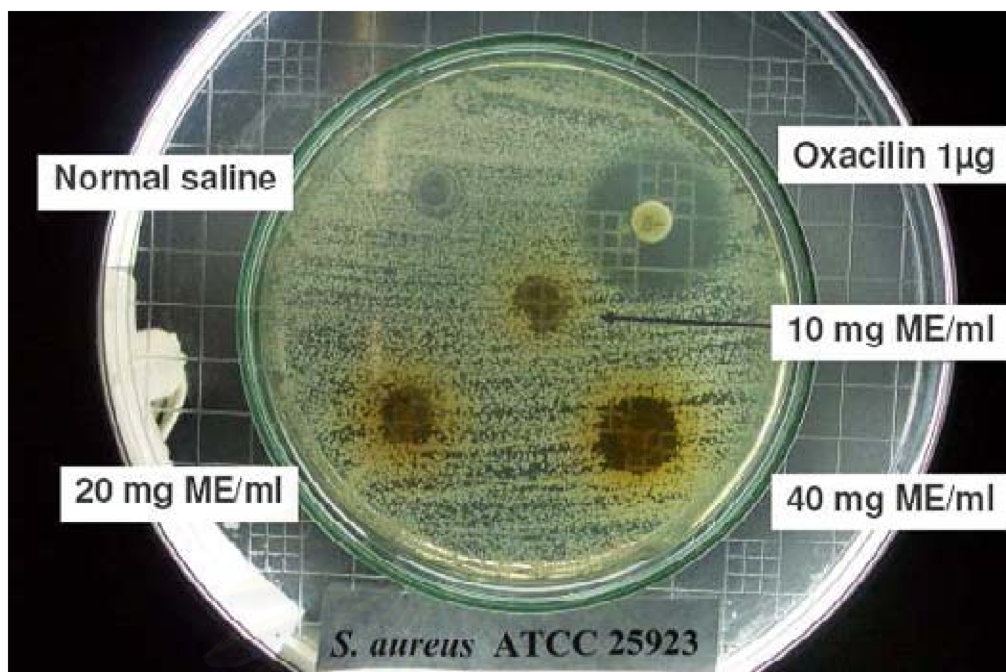

Figure 4. Agar diffusion susceptibility test of ME against *S. aureus* ATCC 25923.

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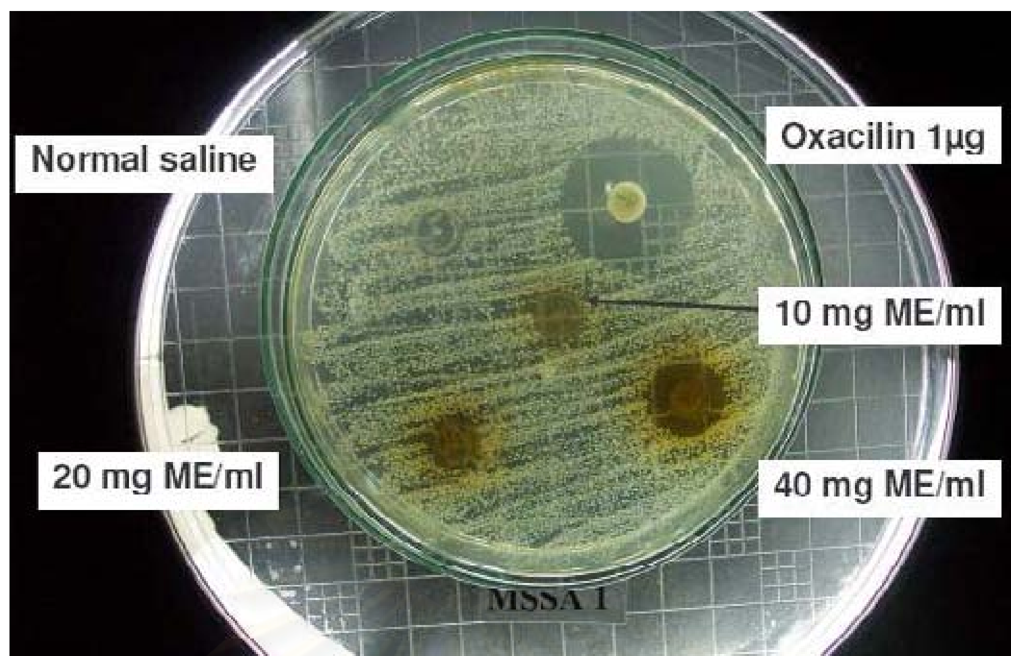

Figure 5. Agar diffusion susceptibility test of ME against MSSA 1.

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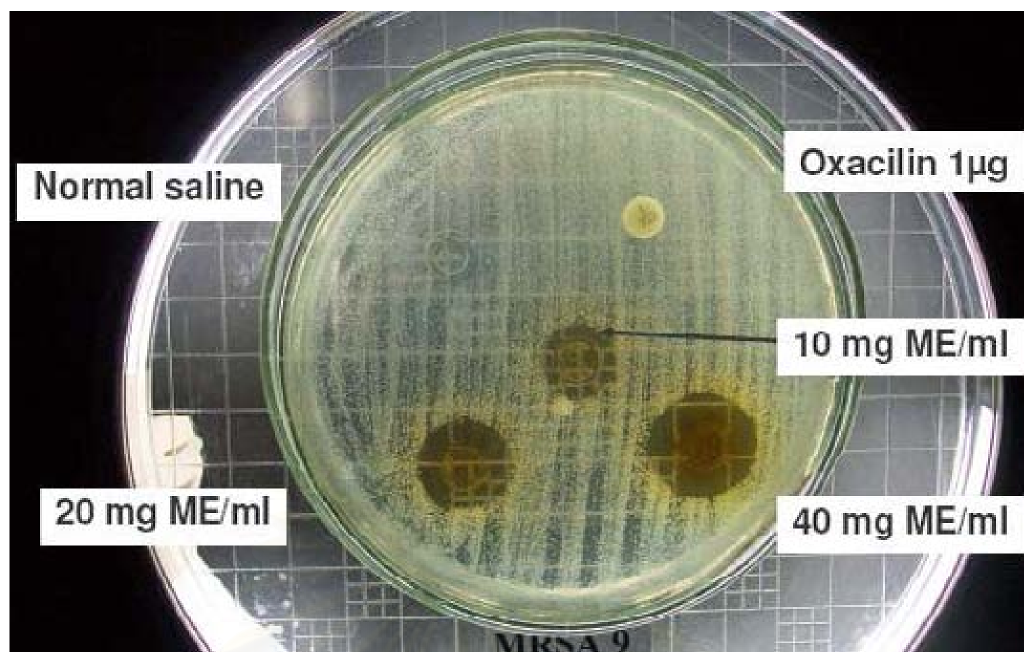

Figure 6. Agar diffusion susceptibility test of ME against MRSA 9.

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Table 4. MICs and MBCs of *M. coromandelianum* water extract against *S. aureus.*

Bacteria	MIC (mg/ml)	MBC (mg/ml)
S. aureus ATCC 25923	40	80
MSSA1	40	80
MSSA 2	40	80
MSSA 3	20	40
MSSA 6	40	80
MSSA 7	40	80
MSSA 15	40	80
MRSA 1	40	80
MRSA 7	40	80
MRSA 9	20	40
MRSA 10	40	80
MRSA 12	40	80
MRSA 15	40	80

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ME showed hypoglycemic effect in normal rats. Then the hypoglycemic effect of ME was further studied in streptozotocin induced hypoglycemic rats.

4.3 The hypoglycemic effect of ME in diabetic male Wistar rats

4.3.1 Single-oral doses of ME in diabetic rats; non-fasting blood glucose concentration analysis

Effect of single-oral dose of ME on fasting streptozotocin induce diabetic rats was studied to determine the optimum dose that reduces blood glucose concentration. Treatment groups 4, 5, and 6 were given ME orally at dose 10, 50, and 100 mg ME/kg body weight, respectively. Rats were given orally ME or distilled water or intraperitoneal 5 IU/kg body weight of insulin injection at 0 hrs. Blood were collected to determine blood glucose concentration at 0 hr and every 1 hr for 6 hrs. The result showed in Figure 8. In all treatment groups of 10, 50, and 100 mg ME/kg body weight, non-fasting blood glucose concentration decreased significantly at 1 hr after feeding ME in diabetic rats ($p<0.05$) compared with diabetic rats fed distilled water (control group). ME showed good hypoglycemic effect corresponds to 5 IU /kg body weight of insulin injection where duration of action of 50 and 100 mg ME/kg body weight lasted for 6 hrs similar to that of insulin.

The oral ME administration of a single oral dose of 10, 50, and 100 mg/kg body weight in diabetic rats significantly decreased non-fasting blood glucose concentration within 1 hr after ME feeding. Only 50 and 100 mg ME/kg body weight showed the decreased blood glucose concentration equivalent to that of normal level with long duration of hypoglycemic action (Figure 8).

ME, thus, can reduce non-fasting blood glucose concentration in normal rats or in streptozotocin-induced diabetic rats whose insulin was decreased. Thus, in normal rats whose glucose homeostasis still functions, ME may function through elevation of insulin secretion or extra-pancreatic actions influencing glucose metabolism such as, stimulation of glucose uptake by peripheral tissue. In this model of diabetic rats, however, insulin may be almost absent, therefore, decrease in non-fasting blood glucose concentration may reflex only the peripheral uptake of glucose.

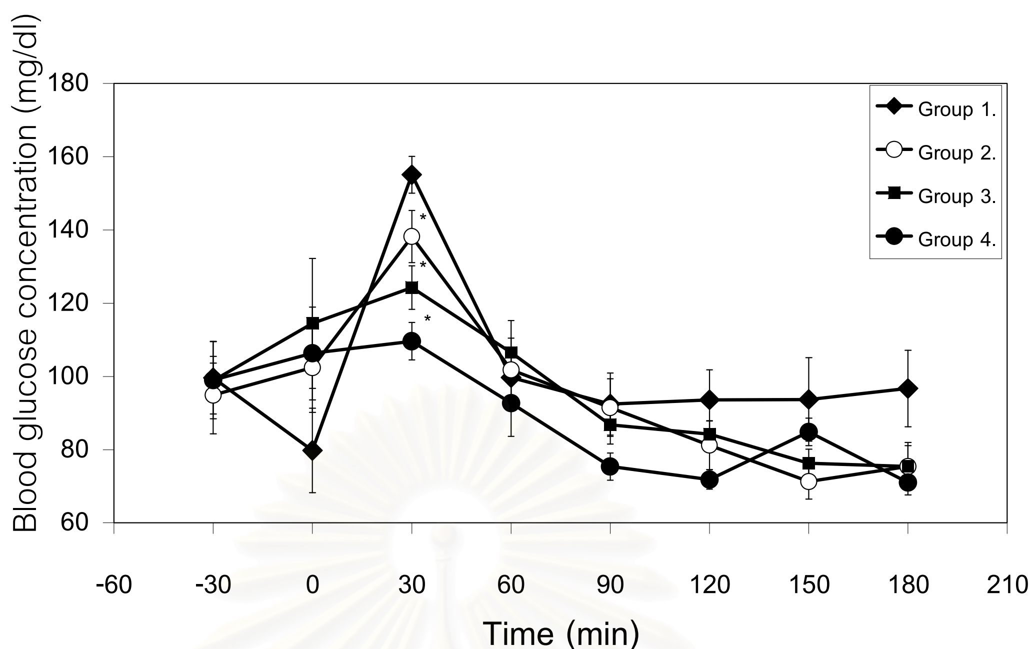

Figure 7. Effect in normal rats of feeding at time -30 minute of single oral doses of ME from *M. coromandelianum* in glucose tolerance test.

Glucose, 1 g/Kg body weight, was given orally to each rat at time 0.

Symbols represents mean ± S.E.M. ; * significant at $p<0.05$

Group 1. Normal control group fed distilled water (N = 6);

Group 2. Normal rats fed 5 mg ME/kg body weight (N= 6);

Group 3. Normal rats fed 10 mg ME/kg body weight (N=6);

Group 4. Normal rats fed 20 mg ME/kg body weight (N=6).

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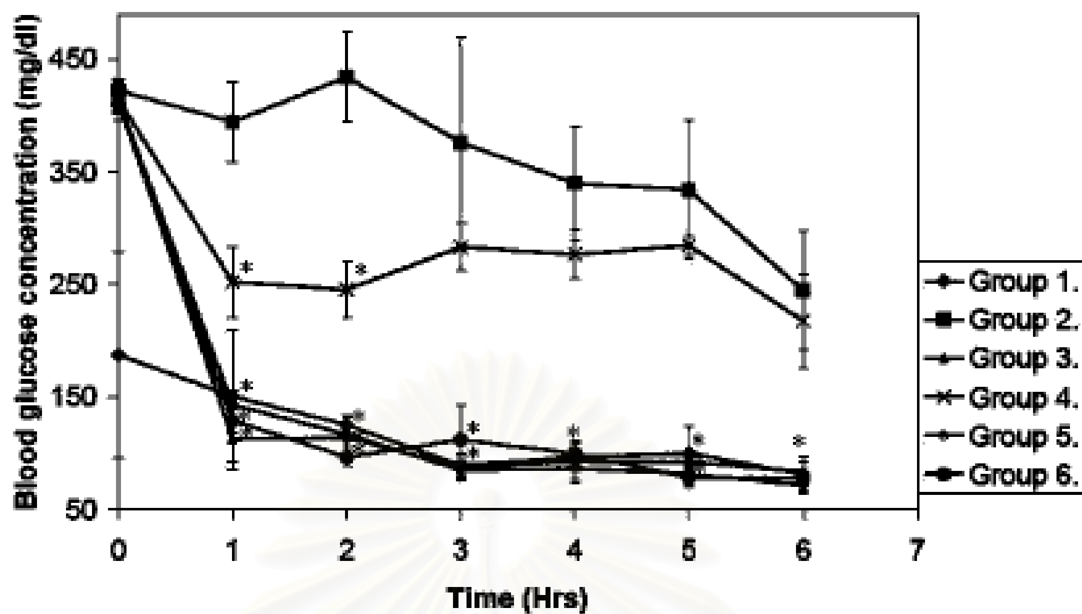

. Figure 8. Effect of single oral doses of ME in non-fasting diabetic rats.
*significant at $p< 0.05$, compared to diabetic rats fed water.

Symbols represents mean ± S.E.M.

Group 1. Non – diabetic rats (N=6);

Group 2. Diabetic rats fed distilled water (N=6);

Group 3. Diabetic rats, insulin (Humulin®) 5 IU/kg body wt., i.p. (N=6);

Group 4. Diabetic rats fed 10 mg ME/kg body wt. (N=6);

Group 5. Diabetic rats fed 50 mg ME/kg body wt. (N=6);

Group 6. Diabetic rats fed 100 mg ME/kg body wt. (N=6).

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4.3.2 Repeated-oral doses of ME in diabetic rats; fasting blood glucose concentration analysis

The effect of ME using repeated-oral doses for 30 days was investigated in diabetic rats. Diabetic or normal rats were fasted overnight (15-18 hrs) prior treatment day 0, and blood glucose concentrations were determined. The treatment groups were given ME daily for 30 days. On day 15th and 30th fasting blood glucose concentrations were determined at 22 hrs after treatment. The result showed in Figure 9. Rats were sacrificed, where blood samples and organs were collected (liver, exocrine pancreas, kidney, eyes, aorta vascular, femoral nerve and brain) for histopathological evaluation.

Repeated oral doses of 100 and 500 mg ME/kg body weight/days showed significant reduction of blood glucose compared with diabetic rats treated with water(control group) on day 15 and 30 (Figure 9.). A dose of 50 mg ME/kg body weight/day also showed significant reduction of blood glucose on day 15. The histopathological examination revealed non - remarkable lesions of the liver, exocrine pancreas, kidney, eyes, aorta vascular, femoral nerve and brain of treated rats sacrificed after experiment ended on day 30. Figure.20 showed degeneration of β-cells caused by streptozotocin in diabetic rats compare to non-diabetic rats. Diabetic rats fed ME showed relative liver weight comparable to that of normal rats, while relative liver weight of diabetic rats fed distilled water decreased significantly. This suggests protective effect of ME to maintain the liver weight in diabetic condition. In addition, blood parameter in diabetic rats did not showed any significant differences when compared with normal rats in repeated oral doses experiment feeding 50, 100, and 500 mg ME/kg body weight/day for 30 days (Table 5.).

Feeding single oral doses of ME in non-fasting diabetic rats as well as feeding repeated oral doses in diabetic rats showed significant blood glucose reduction ME reduced blood glucose concentration level in streptozotocin-induced diabetic rats whose pancreatic β-cells were damaged and very low level of insulin is anticipated to remain. Thus, ME decreased blood glucose level of the animal either in the presence or in the absence of insulin or at much lower level of insulin.

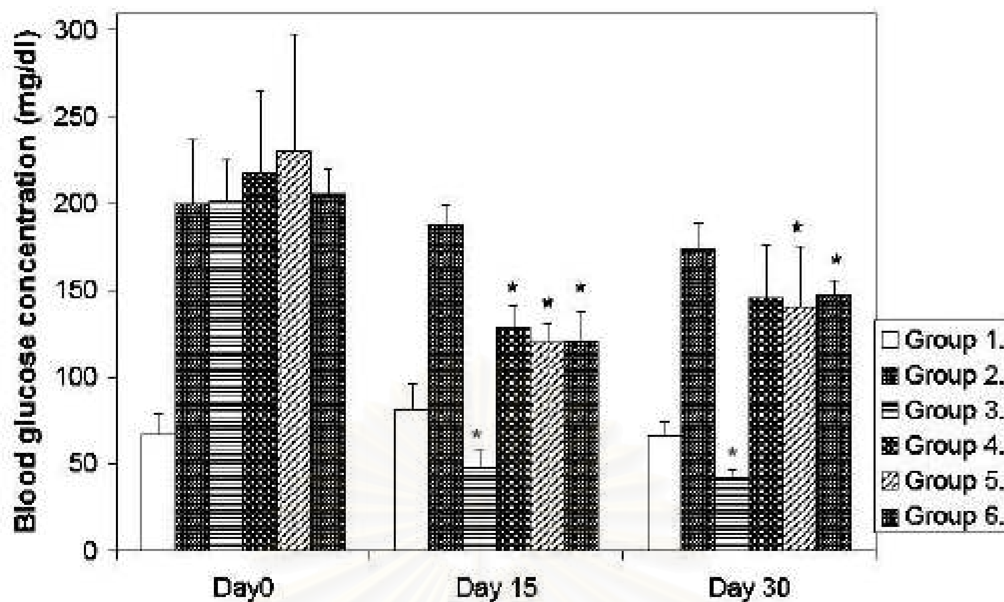

Figure 9. Effect of repeated oral doses of ME in diabetic rats on fasting blood glucose concentration. * significant at $p < 0.05$, compared to diabetic rats fed water.

Symbols represents mean ± S.E.M.

Group 1. Non – diabetic rats (N=6);

Group 2. Diabetic rats fed distilled water (N=6);

Group 3. Diabetic rats, insulin (Humulin®) 5 IU/kg body wt/d., i.p. (N=6);

Group 4. Diabetic rats fed 50 mg ME/kg body wt/d. (N=6);

Group 5. Diabetic rats fed 100 mg ME/kg body wt/d. (N=6);

Group 6. Diabetic rats fed 500 mg ME/kg body wt/d. (N=6).

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Table 5. Relative liver weight on day 30 after daily oral repeated – doses of ME in diabetic male Wistar rats.

Group	Relative liver weight (Mean%±S.E.M)
Non – diabetic rats (N=6)	3.4±0.34
Diabetic rats fed distilled water (N=6)	2.7±0.24*
Diabetic rats, insulin (Humulin®) 5 IU/kg body wt/d., i.p. (N=6)	3.6±0.18
Diabetic rats fed 50 mg ME/kg body wt/d. (N=6)	3.4±0.13
Diabetic rats fed 100 mg ME/kg body wt/d. (N=6);	3.5±0.12
Diabetic rats fed 500 mg ME/kg body wt/d. (N=6);	3.4±0.54

* Significant difference at $p < 0.05$ when compared to normal control group fed water (N= 6 for each group).

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Table 6. Biochemical and hematological analysis of blood in diabetic male Wistar rats after daily feeding water extract of *M. coromandelianum* (ME) for 30 days.

Group	RBC (x 10^6/µl)	WBC (cell/µl)	MCV (fl)	Platelets (x 10^3/µl)	Neu (cell/µl)	Lymp (cell/µl)	ALT (unit/ml)	AST (unit/ml)	ALP (unit/ml)	BUN (mg/dl)	Creatinine (mg/dl)
Normal control rats	6.8±0.2	931.3±389.4	67.8±1.3	49.3±17.6	463.8±97.4	1285.3±239.6	45.8±6.4	155.4±34.9	388.6±53.3	15.4±1.2	0.6±0.04
Diabetic fed distilled water	6.3±1.1	1745.0±586.4	65.8±0.9	67.2±7.1	662.1±81.4	1872.5±308.2	79.8±42.5	147.5±34.3	369.2±29.0	23.3±1.9	0.6±0.05
Diabetic rats, insulin (Humulin®) 5 IU/kg body wt., i.p.	7.2±0.2	1640.0±491.5	65.8±0.6	63.5±13.2	442.2±117.4	1573.1±454.6	106.6±55.3	158.0±26.1	731.6±212.6	32.8±9.3	0.5±0.04
Diabetic fed 50 mgME/kg body wt/d	5.6±0.9	1868.3±332.5	65.8±1.0	46.5±6.4	421.9±220.9	1628.9±225.8	42.5±4.5	139.7±27.5	361.3±51.0	20.7±2.1	0.6±0.01
Diabetic fed 100mgME/kg body wt/d	5.8±0.8	2521.7±577.3	65.8±0.7	41.7±5.4	547.4±123.5	2250.3±395.9	39.0±2.8	115.8±17.5	399.7±37.2	17.2±1.6	0.6±0.04
Diabetic fed 500mgME/kg Body wt/d	6.1±0.2	2350.0±511.0	64.7±1.0	32.2±7.3	505.0±121.5	1817.4±385.9	48.3±5.6	148.2±22.0	465.7±52.0	28.0±1.4	0.6±0.05

No significant difference was observed when compared to normal control group fed water (N= 6 for each group).

Data are Mean ± S.E.M.

RBC = Red blood cell count Lymp= Lymphocytes Neu = Neutrophils

WBC = White blood cell count ALT = Alanine transminase (E.C.2.6.1.2) ALP = Alkaline phosphatase(E.C.3.1.3.1)

MCV = Mean corpuscular volume AST = Aspatate transminase (E.C. 2.6.1.1) BUN = Blood urea nitrogen

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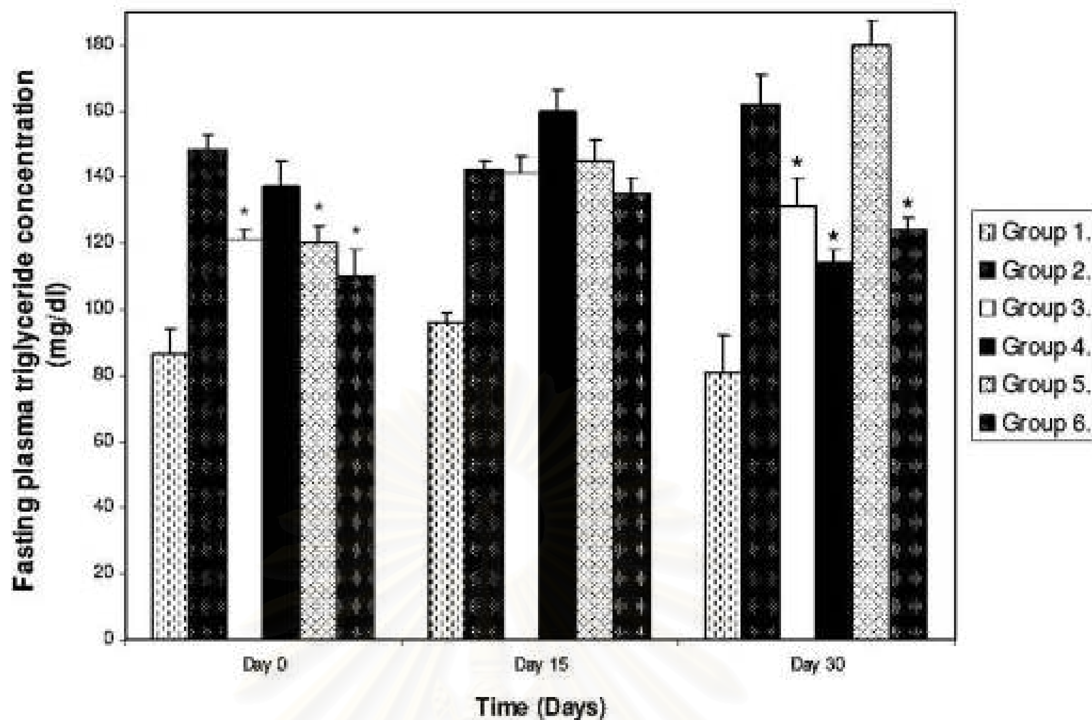

Figure 10. Effect of repeated oral doses of ME in diabetic rats on fasting plasma triglyceride concentration. * significant at $p< 0.05$ (multiple range test), compared to diabetic rats fed water (group 2).

Symbols represents mean ± S.E.M.

Group 1. Non – diabetic rats (N=6);

Group 2. Diabetic rats fed distilled water (N=6);

Group 3. Diabetic rats, insulin (Humulin®) 5 IU/kg body wt/d., i.p. (N=6);

Group 4. Diabetic rats fed 50 mg ME/kg body wt/d. (N=6);

Group 5. Diabetic rats fed 100 mg ME/kg body wt/d. (N=6);

Group 6. Diabetic rats fed 500 mg ME/kg body wt/d. (N=6).

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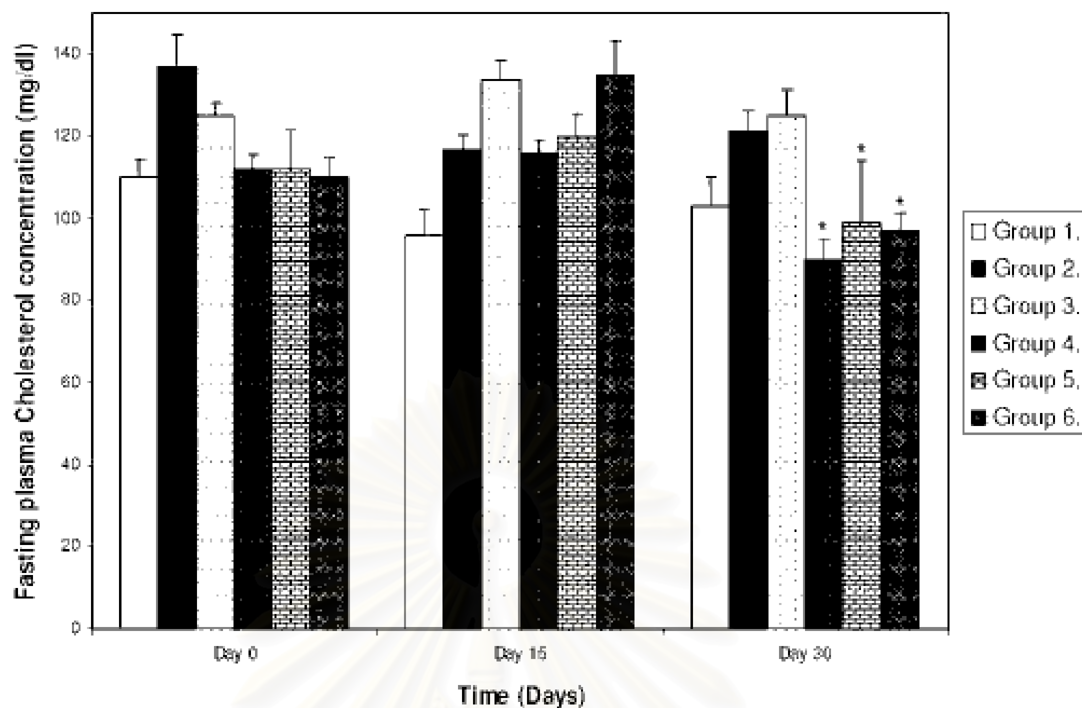

Figure 11. Effect of repeated oral doses of ME in diabetic rats on fasting plasma cholesterol concentration. * significant at $p< 0.05$ (multiple range test), compared to diabetic rats fed water (group 2).

Symbols represents mean ± S.E.M.

Group 1. Non – diabetic rats (N=6);

Group 2. Diabetic rats fed distilled water (N=6);

Group 3. Diabetic rats, insulin (Humulin®) 5 IU/kg body wt/d., i.p. (N=6);

Group 4. Diabetic rats fed 50 mg ME/kg body wt/d. (N=6);

Group 5. Diabetic rats fed 100 mg ME/kg body wt/d. (N=6);

Group 6. Diabetic rats fed 500 mg ME/kg body wt/d. (N=6).

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Triglyceride and Cholesterol analysis in Figures 10 and 11, respectively, showed mild hypolipidemic effect of ME in diabetic rats (rat's normal fasting triglyceride 105.2±26.4 mg/dl; fasting cholesterol 92.90±23.11 mg/dl (Ringler and Debich, 1979)). In diabetic rat treated with Humulin® 5 IU/kg body wt/d., i.p., 50 mg ME/kg body weight and 500 mg ME/kg body weight showed significant decreased fasting plasma triglyceride after treatment daily for 30 days (Figure 10). In parallel, fasting plasma cholesterols also decreased after treatment daily for 30 days of 50 mg ME/kg, 100 mg ME/kg and 500 mg ME/kg (Figure 11). It is interesting and might be beneficial that fasting plasma triglyceride and cholesterol decreased after subacute daily treatment for 30 days.

Relative liver weight of rats was determined by comparing liver weight and body weight in term of percentage. Table 5. show that diabetic rats in control group fed distilled water showed significant decreased relative liver weight compared to non-diabetic rat (normal control group fed distilled water). The reduction of body weight was observed in diabetic rats (Figure 12 and 13.). This might due to effect of diabetes which might inhibit normal growth of rats. On the other hand, in groups treated with insulin (Humulin®) 5 IU/kg body wt/d., i.p., and treated with 50, 100 and 500 mg ME/kg body weight showed normal relative liver weight compared with non-diabetic rats(normal control group) suggested the protective effect of ME.

Hematological parameter and blood chemistry parameters in Table 6. showed that all parameters were comparable to that of normal control group except high level of ALP in the insulin treated diabetic group. This also supported by the fact that gross lesion was not found in all treated groups. Histopathological examination of pancreas showed the degeneration of β-cell in streptozotocin-induced diabetic rat groups compared with non-diabetic groups as showed in Figure 20. Histopathological examination of liver showed non-remarkable lesion between groups (Figure 21.).

5. *In vitro* test of glucose absorption in rat jejunum using everted rat intestinal (jejunum) sac model

There are many possible of mechanisms of action for hypoglycemic activity of ME such as the elevation of insulin secretion or extra – pancreatic actions influencing glucose metabolism such as, stimulation of glucose uptake by peripheral tissue, correction of insulin resistance, inhibition of the endogenous glucose

production or activation of the glycogenesis pathway by stimulating glycogen synthetase activity and inhibition of intestinal glucose absorption. In this study, we selected to investigate the hypoglycemic effect of ME on inhibition of glucose absorption by using everted rat intestinal (jejunum) sac model.

Glucose transported from everted intestinal sac was measured by using glucose oxidase test and standard curve of standard glucose concentration vs. rate of reaction (Figure 14) was used to determine glucose concentration inside and outside intestinal sac.

ME in Krebs Ringer saline solution showed no effect on glucose absorption using everted rat's intestinal sac model as showed in Figure 15, although a positive control using agent capable of inhibiting glucose absorption should be included in the same experiment.

6. Acute toxicity test in normal rats

The effective dose of 100 mg ME/kg body weight with significant hypoglycemic activity in diabetic rats was selected to test for toxicity. In addition, the excessive doses of 10 and 20 g ME/kg body weight were also tested. Rats were given orally single doses of ME or distilled water. Behavior of treated animal was observed for 5 days. Acute toxicity test in normal male Wistar rats demonstrated that ME did not induce any sign of toxicity within 5 days. Noticeable changes in diet and water consumption was not observed. Relative liver weight of the ME-treated rats showed no significant difference from the control group (Table 7.).

The physical examination was also observed every day after treatment. There were no changes of skin, fur, eyes, mucous membranes, respiratory function, and general behavior as visually observed. Body weight was measured before treatment and after treatment for 5 days. Body weight was not significantly different between treatment groups and control group fed distilled water (Figure 16). Hematological and biochemical analysis of blood was observed in Table 8. There was no significant difference of all hematological and biochemical parameters between treated and control groups. The enzymes AST, ALT, and ALP were not higher than that of normal control.

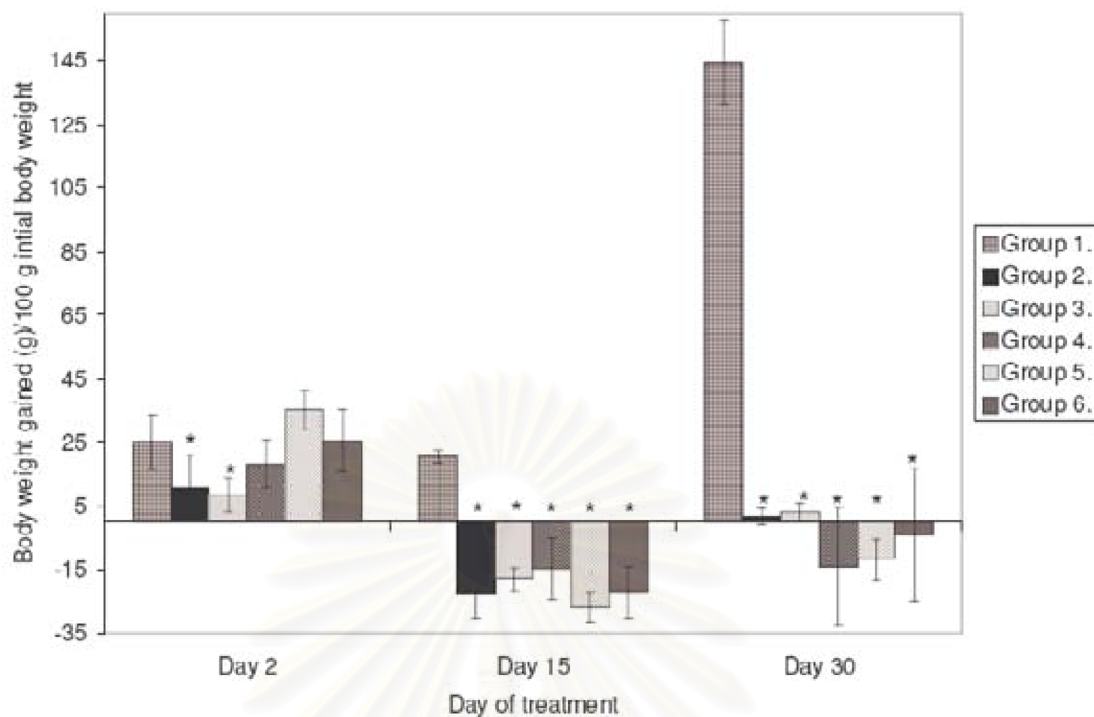

Figure 12. Percentage of relative body weight gained on day 14 after daily oral repeated-does of ME in diabetic male Wistar rats. * significant at $p < 0.05$ (multiple range test), compared to diabetic rats fed water.

Symbols represents mean ± S.E.M.

Group 1. Non – diabetic rats (N=6);

Group 2. Diabetic rats fed distilled water (N=6);

Group 3. Diabetic rats, insulin (Humulin®) 5 IU/kg body wt/d., i.p. (N=6);

Group 4. Diabetic rats fed 50 mg ME/kg body wt/d. (N=6);

Group 5. Diabetic rats fed 100 mg ME/kg body wt/d. (N=6);

Group 6. Diabetic rats fed 500 mg ME/kg body wt/d. (N=6).

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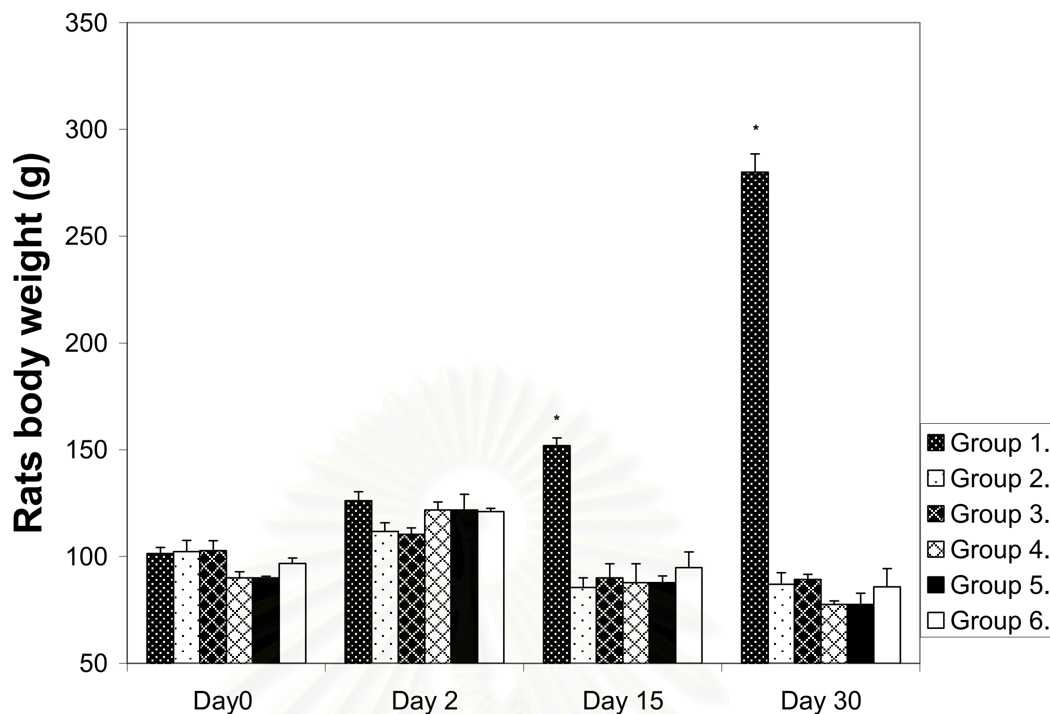

Figure 13. Effect of repeated oral doses of ME in diabetic rats on rat's body weight.

* significant at $p< 0.05$, compared to diabetic rats groups.

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Symbols represents mean ± S.E.M.

Group 1. Non – diabetic rats (N=6);

Group 2. Diabetic rats fed distilled water (N=6);

Group 3. Diabetic rats, insulin (Humulin®) 5 IU/kg body wt/d., i.p. (N=6);

Group 4. Diabetic rats fed 50 mg ME/kg body wt/d. (N=6);

Group 5. Diabetic rats fed 100 mg ME/kg body wt/d. (N=6);

Group 6. Diabetic rats fed 500 mg ME/kg body wt/d. (N=6).

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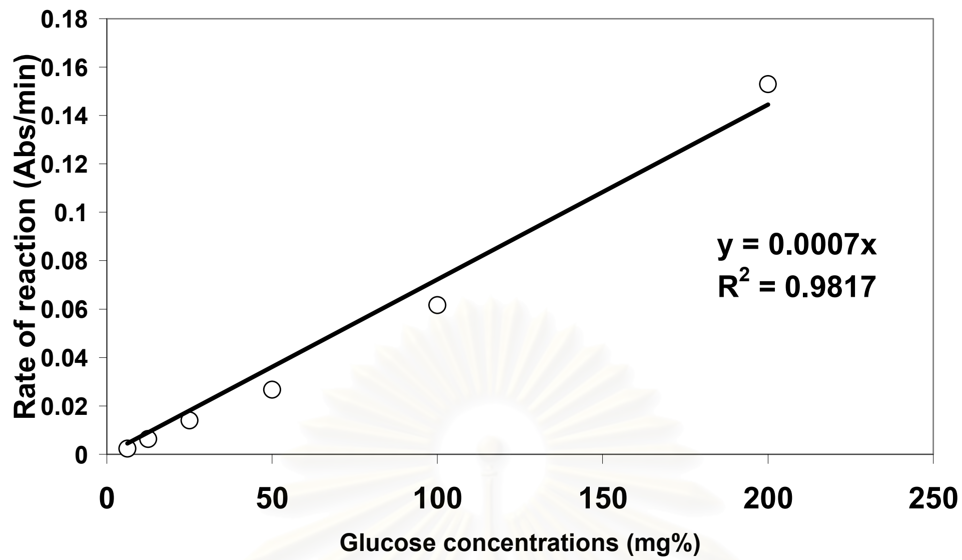

Figure 14. Linear correlation between rate of glucose oxidase (coupling with peroxidase) and glucose concentration.

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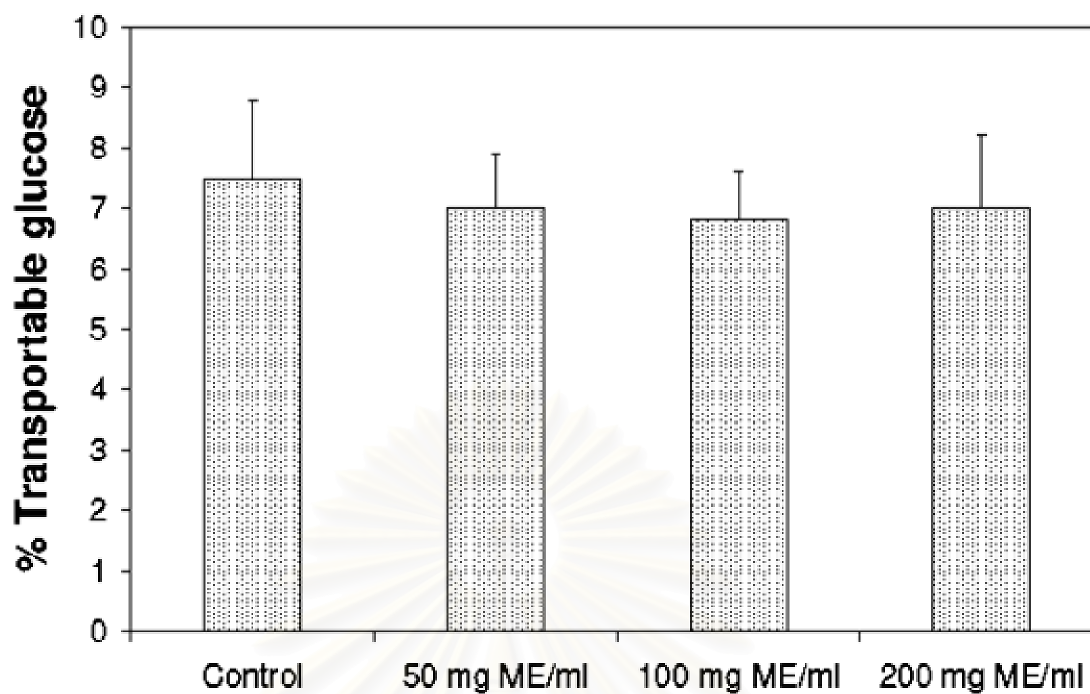

Figure 15. Study of glucose absorption inhibition of ME on everted rat's small intestinal sac (Data are mean ± S.E.M.; no significant between group ($p > 0.05$)).

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Table 7. Relative liver weight in normal rats on day 5 post-treatment with ME at high oral single-dose.

Group	Relative liver weight (Mean%±S.E.M)
Normal control fed distilled water (N=6)	4.9±0.28
Normal rats treated 100 mg ME/kg body weight (N=6)	4.8±0.17
Normal rats treated 10 g ME/kg body weight (N=6)	4.8±0.30
Normal rats treated 20 g ME/kg body weight (N=6)	4.8±0.31

No significant difference was observed when compared the treated groups to control group (N= 6 for each group).

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7. Subacute toxicity test in normal rats

Toxicity of repeated dose were studied for potential adverse effects of ME. Repeated dose studies may be of varying duration, generally 1 to 4 weeks for subacute studies, 3 months for sub-chronic studies, and 6-12 months for chronic studies (Nelson *et al*. 2001). Subacute toxicity with rodents is generally conducted for 14 or 28 days (one month). Results of these studies help to predict appropriate doses of the test substance correspond to future sub-chronic or chronic toxicity studies (FDA, 2003). The study of subacute toxicity of ME for 30 days was investigated in normal rats using effective dose of 100 mg/kg body weight/day and excessive dose of 10, and 20 g ME/kg body weight/day.

The physical examination of rats was recorded everyday until the experiment ended. Skin, fur, eyes, mucous membranes, respiratory function and general behavior were observed before and after daily feeding of ME in treated groups or distilled water. In contrast, no significant changes in diet and water consumption were observed. There were no sign of toxicity visually observed on skin, fur, eyes, mucous membranes, respiratory function and general behavior. Body weight was measured everyday before feeding of ME or distilled water. The rat's relative body weight gain and body weight were showed in Figures 17. and 18. where significant difference was not found in the treated groups compared with control rats fed with distilled water. Treated rats group fed 100 mg ME/kg body weight/day showed the reduction of relative liver weight compared with control rats fed distilled water (Table 9.), however, the excessive dose of 10 and 20 g ME/kg body weight/day showed no significant difference of liver weight compared with control rats fed distilled water.

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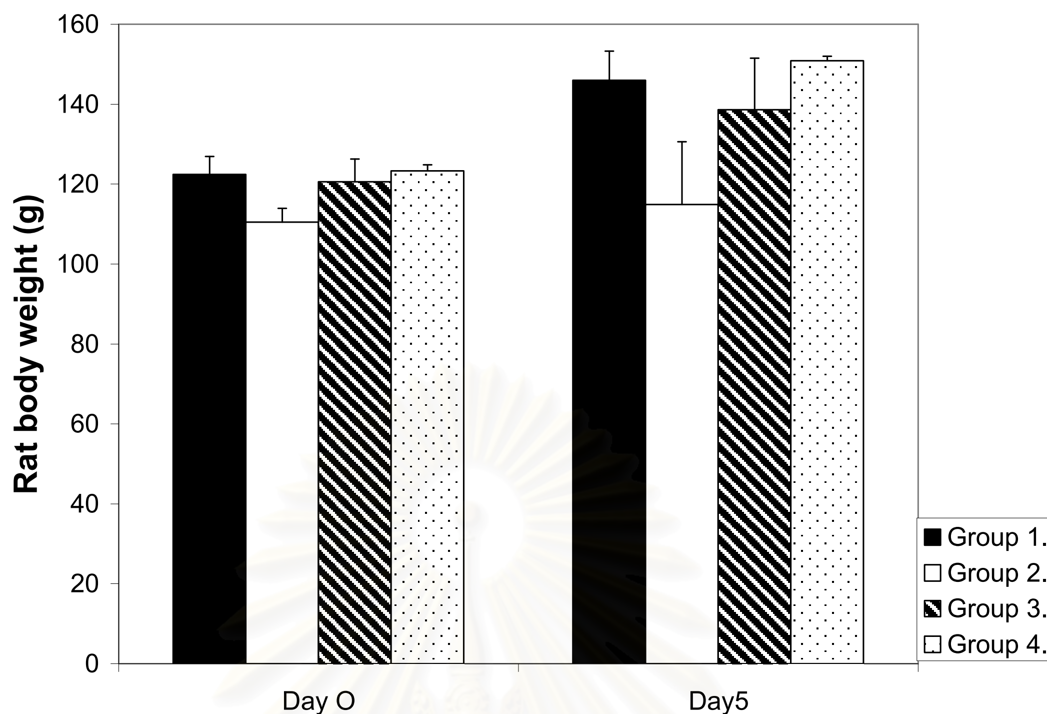

Figure 16. Rats total body weight in acute toxicity test.

No significant difference compared to control group 1.

Symbols represents mean ± S.E.M.

Group 1. Normal rats fed distilled water as control (N=6);

Group 2. Normal rats fed 100 mg ME/kg body weight (N=6);

Group 3. Normal rats fed 10 g ME/kg body weight (N=6);

Group 4. Normal rats fed 20 g ME/kg body weight (N=6);

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Table 8. Hematological and biochemical analysis of blood in normal rats in acute toxicity test (feeding single oral dose) of crude water extract of *M. coromandelianum* (ME).

Group	RBC (x 10^6/µl)	WBC (cell/µl)	MCV (fl)	Platelets (x 10^3/µl)	Neu (cell/µl)	Lymp (cell/µl)	ALT (unit/ml)	AST (unit/ml)	ALP (unit/ml)	BUN (mg/dl)	Creatinine (mg/dl)
Normal control rats	5.2±0.3	2582.5±444.3	68.5±1.2	176.5±27.6	154.9±14.2	2169.3±16.4	46.4±19.7	329.8±94.8	930.5±80.2	32.5±2.9	0.6±0.03
Normal rats Fed100 mgME/kg body weight	4.7±0.2	2001.7±499.1	71.5±1.0	198.3±25.6	110.1±3.6	1731.5±11.2	50.2±2.8	116.5±11.4	787.8±61.5	32.8±2.3	0.5±0.03
Normal rats Fed 10 gME/kg body weight	5.2±0.2	2652.5±168.4	69.5±1.0	203.2±16.0	193.6±1.3	2262.2±3.0	59.7±7.1	149.2±35.7	769.6±60.2	35.6±3.9	0.5±0.04
Normal rats Fed 20 gME/kg body weight	4.8±0.1	2506.0±198.9	68.4±0.6	168.8±28.1	195.5±3.2	2090.0±4.8	60.7±9.7	171.2±47.7	661.5±33.8	33.6±1.1	0.5±0.02

No significant difference was observed when compared the treated groups to control group (N= 6 for each group).

Data are Mean ± S.E.M.

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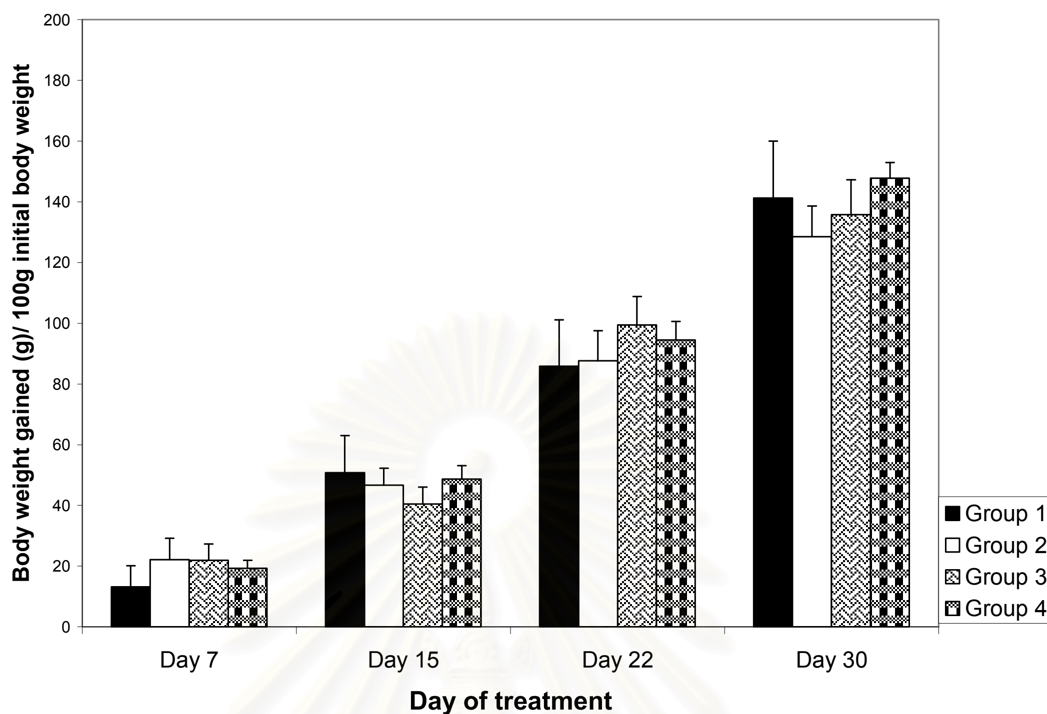

Figure 17. Percentage of relative body weight gained on day 30 post-treatment with ME subacute (30 days) toxicity test. No significant compared the treated groups to control group (N= 6 for each group).

Symbols represents mean ± S.E.M.

Group 1. Normal rats fed distilled water (Control) (N=6);

Group 2. Normal rats fed 100 mg ME/kg body weight (N=6);

Group 3. Normal rats fed 10 g ME/kg body weight (N=6);

Group 4. Normal rats fed 20 g ME/kg body weight (N=6);

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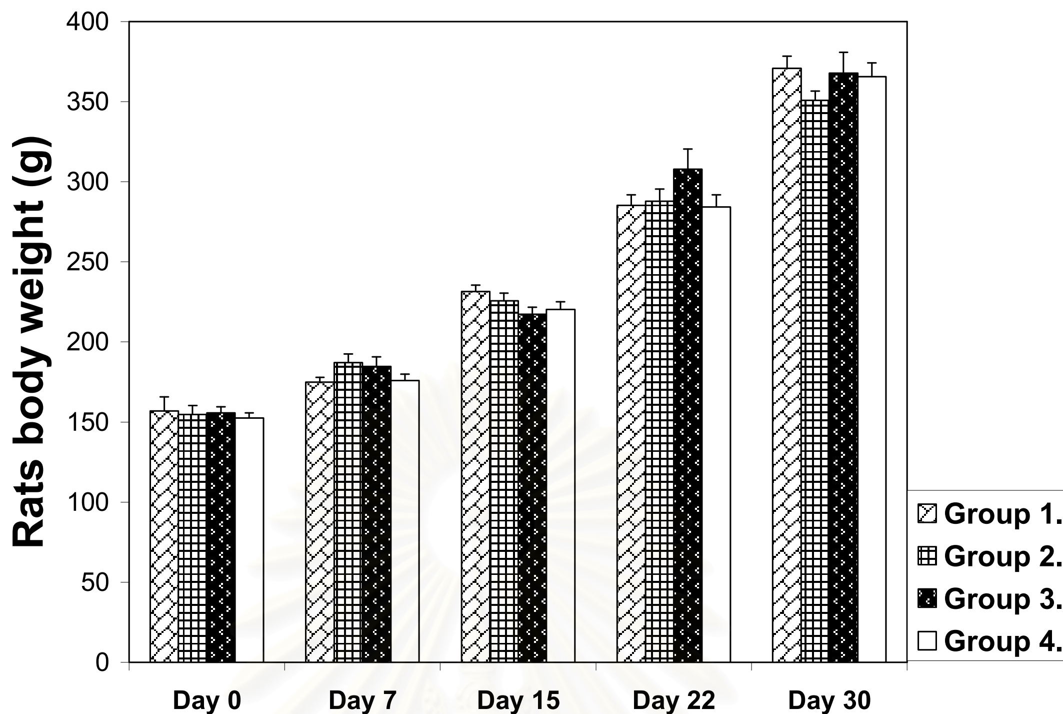

Figure 18. Rats body weight in subacute (30 days) toxicity test.

Total body weights were not significantly different between groups.

Symbols represents mean ± S.E.M.

Group 1. Normal rats fed distilled water (Control) (N=6);

Group 2. Normal rats fed 100 mg ME/kg body weight (N=6);

Group 3. Normal rats fed 10 g ME/kg body weight (N=6);

Group 4. Normal rats fed 20 g ME/kg body weight (N=6);

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Table 9. Relative liver weight in normal rats treated with ME at high oral – dose daily for 30 days.

Group	Relative liver weight (Mean%±S.E.M)
Normal fed distilled water (Control) (N=6)	4.2±0.21
Normal rats treated 100 mg ME/kg body weight/day (N=6)	3.7±0.04*
Normal rats treated 10 g ME/kg body weight/day (N=6)	3.9±0.08
Normal rats treated 20 g ME/kg body weight/day(N=6)	3.8±0.08

* Significant at $p < 0.05$ compared the treated groups to control group (N= 6 for each group).

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Table 10. Histological parameters and biochemical analysis of blood in normal rats in subacute toxicity test (30 days) of crude water extract of *M. coromandelianum* (ME)

Group	RBC ($x 10^6/\mu l$)	WBC (cell/µl)	MCV (fl)	Platelets ($x 10^3/\mu l$)	Neu (cell/µl)	Lymp (cell/µl)	ALT (unit/ml)	AST (unit/ml)	ALP (unit/ml)	BUN (mg/dl)	Creatinine (mg/dl)
Normal control rats	5.1±0.08	3140.0±389.4	70.4±0.7	972.2±65.9	460.5±107.7	2487.0±305.4	51.4±4.6	126.4±22.1	536.8±107.3	31.6±2.0	0.6±0.07
Normal rats Fed100 mgME/kg body weight/d	5.2±0.07	3801.0±653.4	70.8±0.5	914.8±37.3	672.7±231.2	3564.3±179.6	47.2±3.8	105.2±13.5	498.8±51.7	27.2±1.0	0.6±0.06
Normal rats Fed 10 gME/kg body weight/d	5.1±0.1	2978.3±575.0	69.6±1.2	940.8±36.7	369.9±101.2	2346.4±501.3	39.3±1.9	84.8±4.8	535.3±54.1	27.5±0.8	0.6±0.05
Normal rats Fed 20 gME/kg body weight/d	5.0±0.2	3420.0±996.7	68.0±0.7	580.2±171.4	621.5±262.8	3405.6±913.4	64.2±7.3	111.8±26.8	408.3±82.3	22.1±1.5	0.5±0.89

No significant difference was observed when compared the treated groups to control group (N= 6 for each group).

Data are Mean ± S.E.M.

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Hematological and biochemical parameters of whole blood and serum of normal rats showed no sign of toxicity in all treated groups compared to control, they are all in normal range (Canandian Council on Animal care, 1980) (Table 10).

8. Histophathological evaluations

Histopathological lesions were evaluated after ME feeding daily for 30 day of both experiments on repeated-oral doses of ME in diabetic rats and in subacute (30 days) toxicity test in normal rats.

Figure 19. showed the β-cells in normal pancreas and degenerative of necrosis β-cells in pancreatic islet in diabetic rats. Effect of repeated-oral doses of ME was investigated in diabetic rats. First, the investigation of the pancreatic histopathological lesions was monitored to confirm the effect of STZ for induction of diabetes. Histopathological findings showed the lesion of β-cell necrosis by the effect of STZ diabetic induction (Figure 20) in group 2, 3, 4, 5, and 6. No histopathological lesion after feeding ME daily for 30 days in both normal and diabetic rat at dose of 50, 100 and 500 mg ME/kg body weight/day. The toxicity of ME on diabetic rats was determined by histopathological examination of liver. Non-remarkable lesion in liver of all diabetic groups were observed in comparison to non-diabetic group (Figure 21).

In subacute (30 days) toxicity test in normal rats, liver and spleen were examine for histopathological lesion as shown in Figures 22 and 23. No remarkable lesion was found in liver (Figure 22). Daily feeding ME for 30 days at doses 100 mg, 10 and 20 g ME/kg body weight showed no remarkable histopathological lesion compared to control. On the other hand, excessive dose of ME caused partial lymphoid depletion in spleen at dose of 10 g ME/kg body weigh/day and 20 g ME/kg body weight/ day which was shown by decreasing of white pulp of spleen although not too severe (Figure 23).

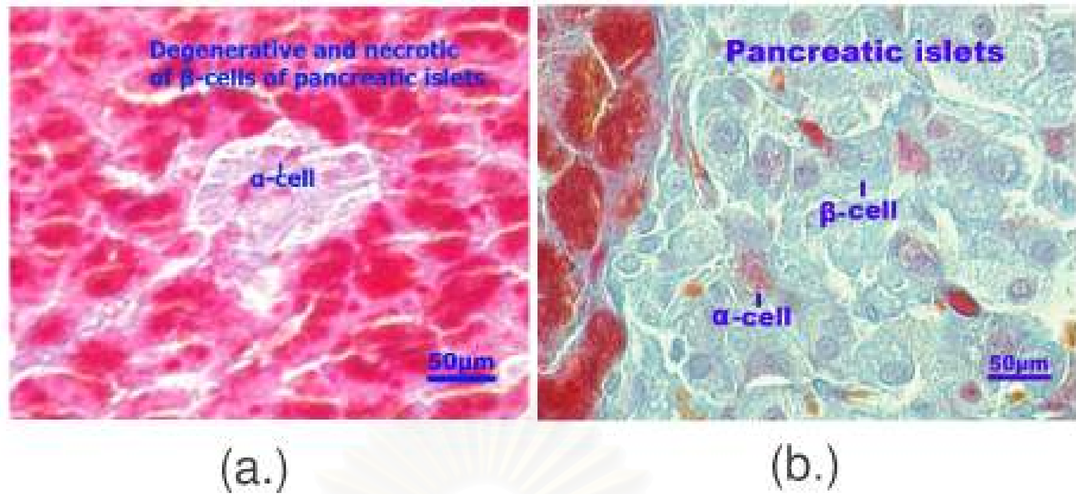
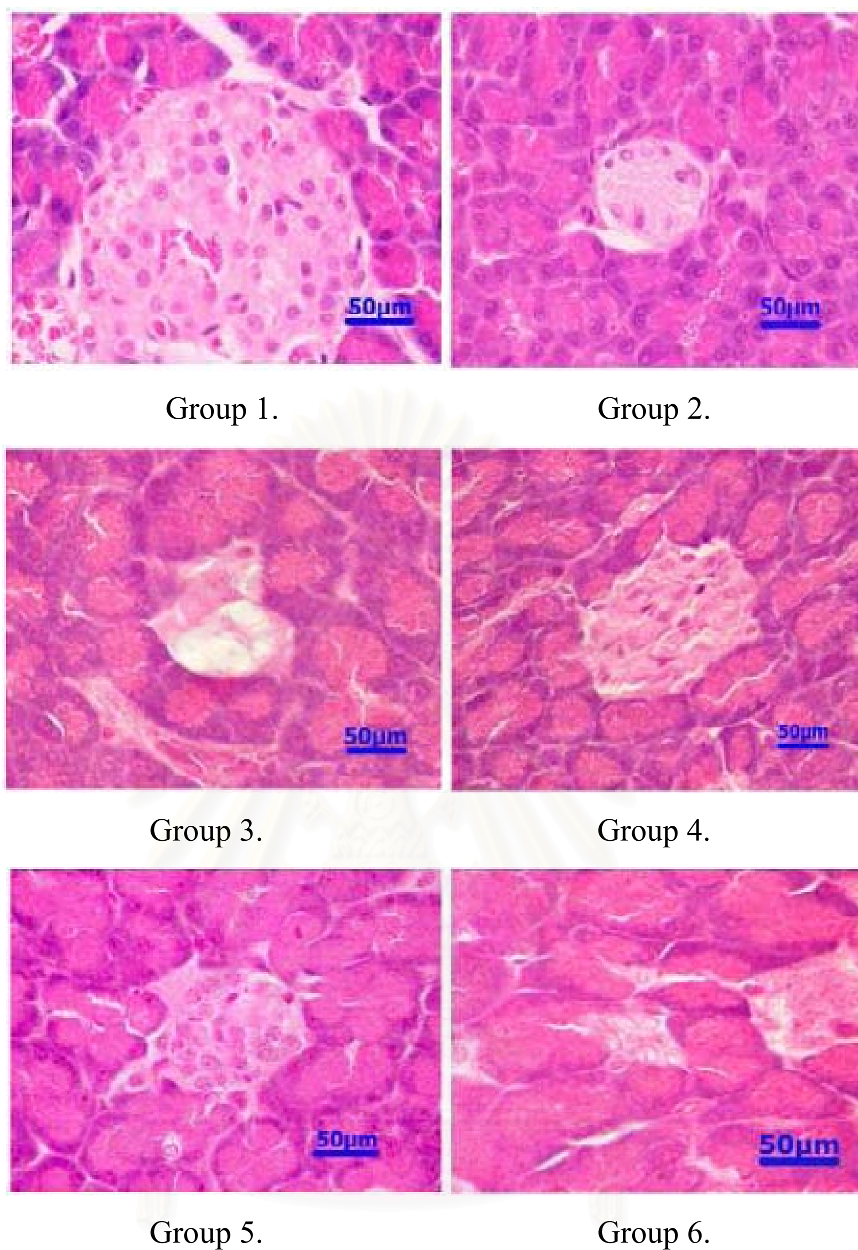


Figure 19. Photomicrographs of pancreatic islets at different states. (a.) Degenerative and necrotic β-cells of pancreatic islets. A few α-cells contains pink cytoplasmic granules were marked in STZ-induced diabetic rats. (b.) Normal pancreatic islets revealed majority β-cells contain blue cytoplasmic granules and a few α-cells contain pink cytoplasmic granules. No-remarkable lesions of exocrine pancreas. Gomori's stain x400



Group 1. Group 2.

Group 3. Group 4.

Group 5. Group 6.

Figure 20. Photomicrographs of rat pancreas after repeated-oral dose treatment of ME in STZ induced diabetic rats for 30 days. The light-staining apical portion of the pancreatic exocrine cells contains zymogen granules, the base dark-staining ergastoplasm. Degenerative and necrotic pancreatic islets cells were marked in STZ induced diabetic groups 2,3,4,5 and 6 (Hematoxylin & Eosin stain x400).

Group 1. Non – diabetic rats (N=6);

Group 2. Diabetic rats fed distilled water (N=6);

Group 3. Diabetic rats, insulin (Humulin®) 5 IU/kg body wt., i.p. (N=6);

Group 4. Diabetic rats fed 50 mg ME/kg body wt. (N=6);

Group 5. Diabetic rats fed 100 mg ME/kg body wt. (N=6);

Group 6. Diabetic rats fed 500 mg ME/kg body wt. (N=6).

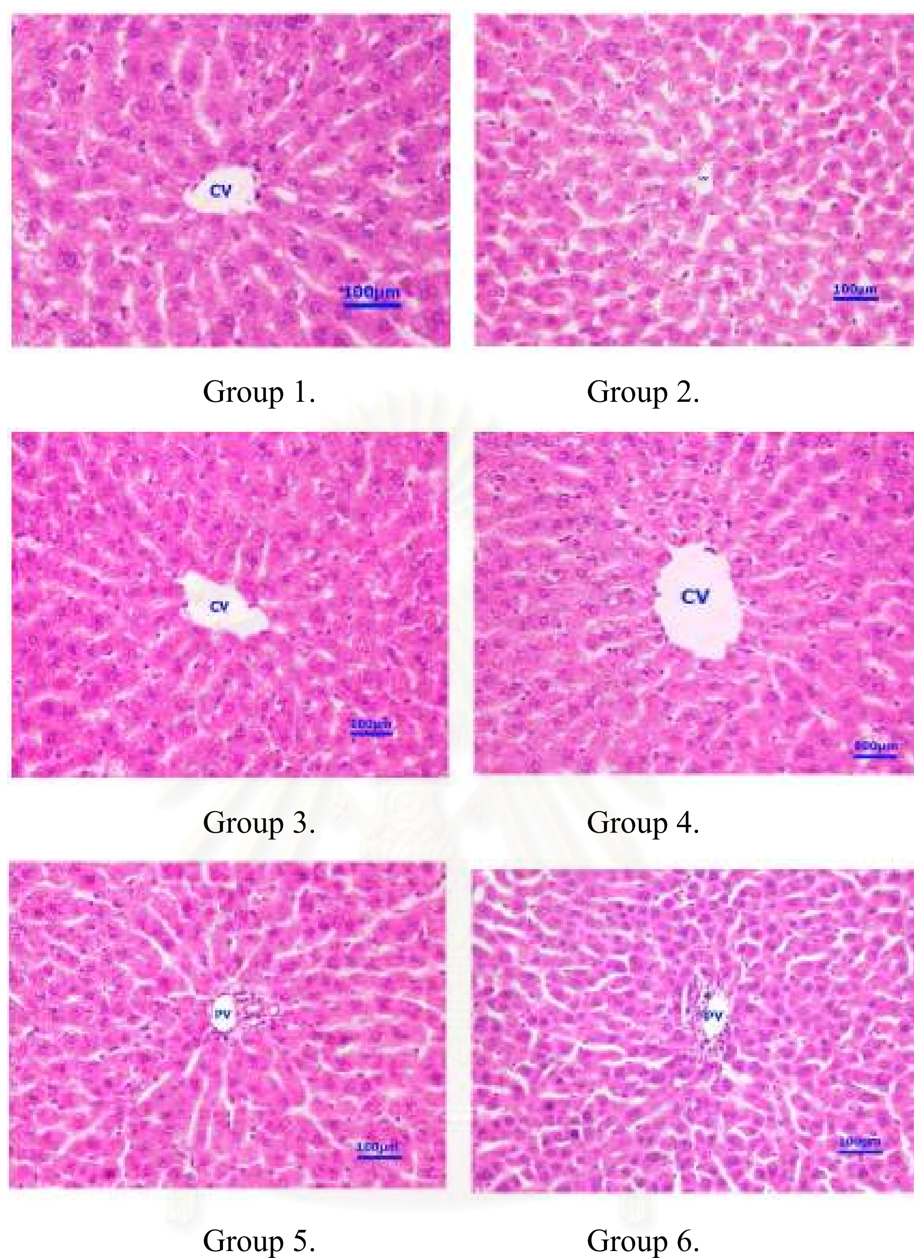


Figure 21. Photomicrographs of rat liver after repeated-oral dose treatment of ME in STZ induced diabetic rats for 30 days. The liver cell cords converge toward the central vein. Non-remarkable lesions of liver were illustrated except for mild vacuolar degeneration in diabetic rats fed distilled water. (Hematoxylin & Eosin stain x200)

Group 1. Non – diabetic rats (N=6);

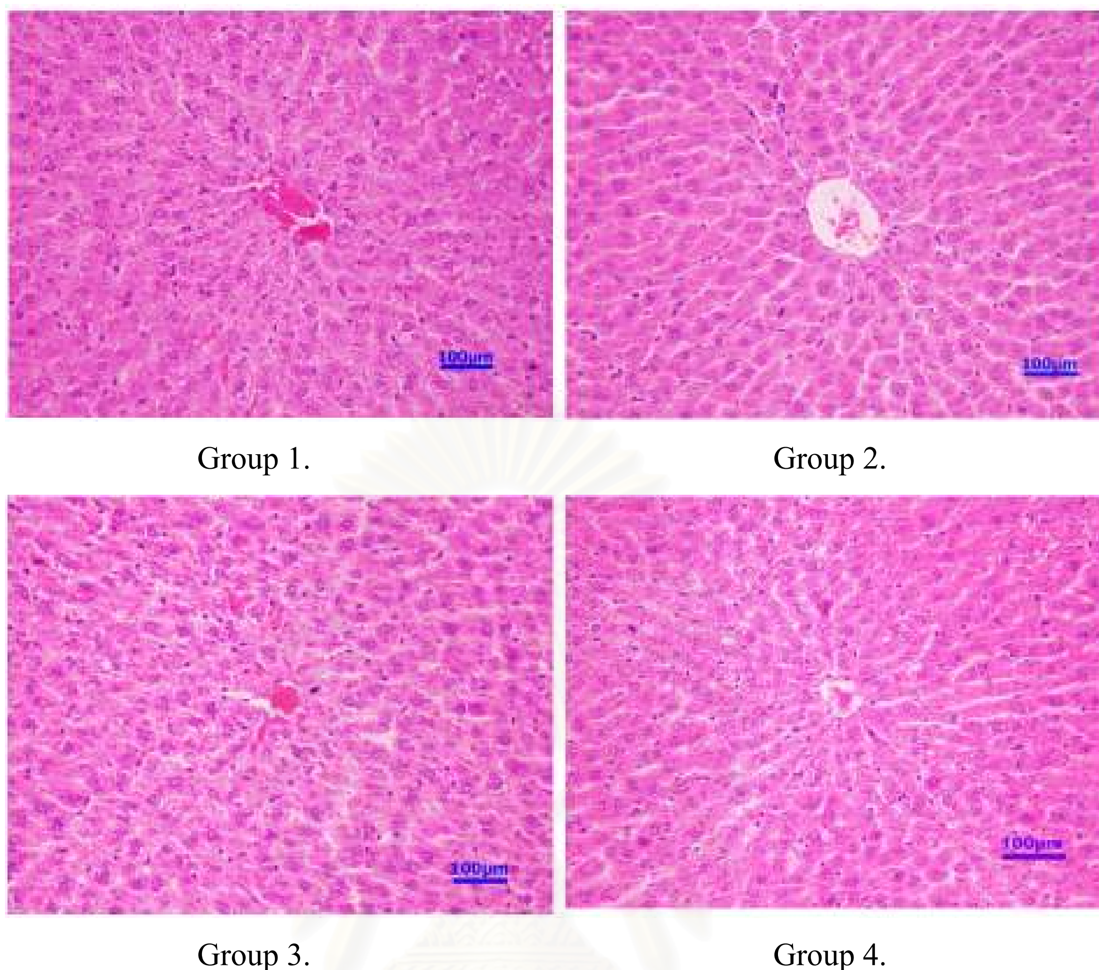
Group 2. Diabetic rats fed distilled water (N=6);

Group 3. Diabetic rats, insulin (Humulin®) 5 IU/kg body wt., i.p. (N=6);

Group 4. Diabetic rats fed 50 mg ME/kg body wt. (N=6);

Group 5. Diabetic rats fed 100 mg ME/kg body wt. (N=6);

Group 6. Diabetic rats fed 500 mg ME/kg body wt. (N=6).



Group 1. Group 2.

Group 3. Group 4.

Figure 22. Photomicrographs of rat liver after a subacute (30 days) toxicity test, feeding daily of ME or distilled water (control) for 30 days in normal rats. Hematoxylin & Eosin stain x200, non-remarkable lesions of liver were illustrated.

Group 1. Normal rats fed distilled water (Control) (N=6);

Group 2. Normal rats fed 100 mg ME/kg body weight (N=6);

Group 3. Normal rats fed 10 g ME/kg body weight (N=6);

Group 4. Normal rats fed 20 g ME/kg body weight (N=6);

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Group 1.

Group 2.

Group 3.

Group 4.

Figure 23. Photomicrographs of rat spleen after a subacute (30 days) toxicity test, feeding daily of ME or distilled water for 30 days in normal rats. Mild to moderated lymphocytic depletion were illustrated in rats fed single-dose of ME at 10 g ME/kg and 20 g ME/kg body weight/day daily for 30 days in comparison to control group. (Hematoxylin & Eosin stain x100)

Group 1. Normal rats fed distilled water (Control) (N=6);
Group 2. Normal rats fed 100 mg ME/kg body weight (N=6);
Group 3. Normal rats fed 10 g ME/kg body weight (N=6);
Group 4. Normal rats fed 20 g ME/kg body weight (N=6);

CHAPTER IV

CONCLUSIONS

ME extraction using boiling in water and spray-drying method gave a yield of approximately 10%. Crude water extract of *M. coromandelianum* was tested against *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *E. faecalis* ATCC 29212 and *Ps. aeruginosa* ATCC 27853 using agar diffusion method inhibition zone was observed only against *S. aureus* ATCC 25923. 40 mg/ml of plant extract shown inhibition zone against 6 out of 15 test strains of each group of MSSA and MRSA. MICs and MBCs of plant extract against MSSA are 20 – 40 mg/ml and 40-80 mg/ml, respectively using Broth macrodilution method. And MICs and MBCs of plant extract against MRSA are 20 – 40 mg/ml and 40 – 80 mg/ml.

For glucose tolerance test in normal rats, crude water extract (ME) from whole plant of *M. coromandelianum*, feeding at doses 5, 10, and 20 mg/kg body weight significantly decreased blood glucose concentration at 30 min after feeding glucose 1 g/kg body weight.

Feeding ME in non-fasted, diabetic rats at single doses of 10, 50 or 100 mg/kg body weight showed that ME either at 50 and 100 mg/kg body weight significantly decreased blood glucose concentration at 1 hr and lasted through 6 hr to a level comparable to that of insulin injection, i.p., 5 IU/ kg body weight or of normal rats.

Repeated-doses of ME given orally to rats at 50 mg, 100 mg and 500 mg/kg body weight/day or insulin injection at 5 IU/kg body weight/day or control group fed distilled water, for 30 days significantly decreased fasting blood glucose concentration on day 15 and 30 compared with diabetic rats treated with water. In diabetic rat treated with Insulin 5 IU/kg body wt/d., i.p., 50 mg ME/kg body weight and 500 mg ME/kg body weight showed significantly decreased fasting plasma triglyceride after treatment daily for 30 days. Fasting plasma cholesterol also decreased after daily treatment for 30 days of 50 mg ME/kg, 100 mg ME/kg and 500 mg ME/kg. Histopathological examination did not show any abnormalities of the liver that could be attributed to toxicity of the extract either in normal rats or diabetic

rats for such repeated doses for 30 days. No sign of toxic effect was observed in acute and subacute toxicity test in normal rats at doses 100 mg, 10g and 20g.

Histopatholigical examination of pancrease liver and spleen in rats fed repeated-oral doses of ME for 30 days. Diabetic-complicated organ such as kidney, eyes, aorta vascular, femoral nerve, spleen and brain showed no histopathological lesion after feeding ME daily for 30 day in both normal and diabetic rat at doses of 50, 100 and 500 mg ME/kg body weight/day.

Daily feeding of ME for 30 days of effective dose 100 mg/kg body weight caused no remarkable histopathological lesion in liver and spleen. On other hand, excessive dose of ME cause various degree of lymphoid depletion in splenic white pulp at dose of 10 g ME/kg body weigh/day and 20 g ME/kg body weight/ day.

In conclusion ME effectively decreases blood glucose concentration in normal and diabetic rats and appear not to have toxic effect on liver.

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APPENDICES

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Appendix A

Reagent

1. Krebs-Ringer Saline. This is prepared from stock solutions on each day of experimentation (the solution may deteriorate, even if stored in the refrigerator) (Deyrup – Olsen, 1979).

Sodium chloride, 0.75 M

Potassium chloride, 0.75 M

Calcium chloride, 0.75 M

Magnesium chloride, 0.75 M

Phosphate buffer: 2ml 1 N HCl + 1.78 g Na_2HPO_4 (or 2.68 g $Na_2HPO_4.7H_2O$) made up to 100 ml with distilled water; this solution to be prepared on the day of the experiment.

: Mix 100 ml NaCl + 4 ml KCl + 3 ml $CaCl_2$ + 2 ml $MgCl_2$; to 100 ml of the mixture add 21 ml of phosphate buffer, and make up to 500 ml. For experiments on glucose transport, add 1g glucose (glucose concentration = 200 mg %; other transportable solutes may, of course, be substituted for glucose, but at least 100 mg % glucose should be present even in such cases, to serve as a substrate for the tissue). The pH of this solution is 7.3.

2. Enzymatic assay of glucose oxidase (E.C. 1.1.3.4)

A. 50 mM Sodium Acetate Buffer, pH 5.1 at 35 °C (Prepare 200 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 5.1 at 35 °C with 1 M HCl.)

B. 0.21 mM o-Dianisidine Solution (Dissolve the content of one 50 mg vial of o-Dianisidine Dihydrochloride, Sigma Stock No. 510-50, in 7.6 ml of deionized water. Dilute 1.0 ml to 100 ml with Reagent A.)

C. 10% (w/v) β-D (+) Glucose substrate solution (Prepare 10 ml in deionized water using β-D (+) Glucose, Sigma Prod. No. G-5250.)

D. 0.17 mM o-Dianisidine and 1.72% (w/v) Glucose solution (Reactoin Cocktail) (Immediately before use, prepare 29 ml by combining 24.0 ml of Reagent B

with 5.0 ml of Reagent C. Equilibrate to 35°C and adjust to pH 5.1 if necessary with 1 M HCl or 1 M NaOH. (prepare fresh)

E. Peroxidase Enzyme Solution (POD) (Immediately before use, prepare a solution containing 60 Purpurogallin units/ml of Peroxidase, Type II, Sigma Prod. No. P-8250, in cold deionized water.)

F. Glucose oxidase enzyme solution (For all Glucose oxidase product numbers, except for crude products (Sigma Prod. Nos. G-6766) prepare an initial solution of 20 – 40 units/ml in cold reagent A. Then immediately prior to use, further dilute to 0.4 -0.8 unit in cold reagent A. For crude products (Sigma Prod. Nos. G-6766 and G-1262), immediately prior to use prepare a solution of 0.4 – 2 units/ml in cold Reagent A.)

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Appendix B.

Table 11. Hematological and blood chemical value of repeated – oral dose of ME in diabetic rats

NO	RBC	WBC	Hb	Ht	MCV	Platlets	Neu	Eos	Baso	Lymp	Mono	ALT	Alk	Bun	Creati	AST	Notice
	x10^6/µl	cell/µl	g/dl	%	fl	x10^3/ µl	%	%	%	%	%	U/ml	U/ml	U/ml	mg/dl	mg/dl	
1	6.5	1,190	15	45	69	35	22	-	-	78	-	45	549	14	0.6	114	
2	6.8	-	-	-	-	-	-	-	-	-	-	58	443	15	0.5	280	หนูตาย
3	7.4	330	14	44	70	101	-	-				62	233	20	0.5	173	
4	-	2403	16	50	67	102	-	-				35	392	14	0.7	134	
5	7.0	-	-	-	66	-	-	-	-	-	-	-	-	-	-	-	หนูตาย
6	7.8	1890	15	49	63	98	42	1	-	55	2	29	326	14	0.5	76	
7	6.9	1750	16	52	67	22	28	-	-	72	-	176	910	22	0.5	218	
8	6.3	2280	14	46	67	40	27	1	-	72	-	10	295	17	0.5	114	
9	9.5	430	13	40	65	48	-	-	-	-	-	18	1689	32	0.6	156	
10	6.9	920	20	64	64	71	-	-	-	-	-	351	362	78	0.5	4	
11	6.7	790	14	46	69	69	-	-	-	-	-	12	680	29	0.6	214	
12	1.5	3670	14	44	63	62	24	1	-	74	1	73	454	19	0.3	88	
13	6.6	1210	3	10	66	39	32	-	-	68	-	36	398	21	0.5	188	
14	1.2	1850	14	45	68	90	18	1	-	80	1	292	268	23	0.7	300	
15	6.9	370	2	7	65	64	-	-	-	-	-	42	326	22	0.5	102	
16	6.6	4330	15	46	68	79	21	-	-	77	2	30	337	17	0.6	83	
17	6.9	600	14	45	67	47	24	-	-	76	-	36	450	26	0.8	87	

Table 11. Hematological and blood chemical value of repeated – oral dose of ME in diabetic rats (cont.)

NO	RBC	WBC	Hb	Ht	MCV	Platlets	Neu	Eos	Baso	Lymp	Mono	ALT	Alk	Bun	Creati	AST	Notice
	x10^6/µl	cell/µl	g/dl	%	fl	x10^3/ µl	%	%	%	%	%	U/l	U/l	U/l	mg/dl	mg/dl	
18	6.9	2110	14	45	65	79	16	-	-	84	-	43	437	31	0.6	125	
19	6.0	2900	13	41	68	47	21	-	-	79	-	43	432	23	0.6	118	
20	7.8	1610	16	52	67	49	23	-	-	77	-	26	273	12	0.6	88	
21	6.0	770	12	37	62	31	-	-	-	-	-	53	184	20	0.7	152	
22	6.5	2690	14	43	66	56	17	3	-	79	1	38	530	18	0.6	124	
23	6.9	1920	14	44	64	69	22	-	-	76	2	39	424	26	0.6	88	
24	6.2	1320	13	42	68	27	19	2	-	78	1	56	325	25	0.6	268	
25	6.5	1500	13	44	67	45	15	1	-	84	1	38	312	14	0.4	107	
26	6.5	3290	14	44	68	31	26	-	-	72	1	33	348	17	0.6	80	
27	6.9	4640	15	46	66	66	20	1	-	80	-	41	440	19	0.6	104	
28	6.4	1870	13	40	63	39	20	2	-	79	-	49	429	15	0.7	141	
29	6.2	3080	13	40	65	30	18	-	-	79	1	43	320	14	0.5	189	
30	2.0	750	4	13	66	39	-	1	-	-	-	30	549	24	0.6	74	
31	6.2	2320	13	41	66	51	22	1	-	77	-	31	331	26	0.6	70	
32	6.4	4570	14	43	68	50	23	1	-	76	-	37	387	25	0.5	90	
33	5.7	1190	11	36	64	40	25	1	-	74	-	54	610	30	0.6	197	
34	6.7	2440	14	43	65	6	19	1	-	80	-	70	366	31	0.5	180	

Table 11. Hematological and blood chemical value of repeated – oral dose of ME in diabetic rats (cont.)

NO	RBC	WBC	Hb	Ht	MCV	Platlets	Neu	Eos	Baso	Lymp	Mono	ALT	AST	ALP	BUN	Creati	Notice
	$x10^6$/µl	cell/µl	g/dl	%	fl	$x10^3$/ µl	%	%	%	%	%	U/l	U/l	U/l	mg/dl	mg/dl	
35	6.2	2470	12	38	61	24	21	2	-	77	-	52	366	24	0.5	172	
36	5.3	1110	11	34	64	22	17	1	-	82	-	46	473	32	0.8	180	

Table 12. Hematological and blood chemical value of acute toxicity of ME in rats

NO	RBC	WBC	Hb	Ht	MCV	Platlets	Neu	Eos	Baso	Lymp	Mono	ALT	AST	ALP	BUN	Creati	Notice
	$x10^6/\mu l$	cell/μl	g/dl	%	fl	$x10^3/\mu l$	%	%	%	%	%	U/l	U/l	U/l	mg/dl	mg/dl	
1	5.4	3660	11	36	66	218	16	4	-	76	4	-	417	1008	-	-	
2	-	-	-	-	-	-	-	-	-	-	-	118	531	540	36	-	
3	-	-	-	-	-	-	1	-	-	83	-	16	-	1045	-	-	Hemolysis
4	5.86	5.86	12	42	71	229	1	1	-	83	15	50	101	927	28	0.6	
5	4.24	4.24	9	30	70	118	1	-	-	98	1	5	497	1010	39	0.6	Hemolysis
6	5.31	5.31	11	36	67	141	11	-	-	80	9	43	103	1053	27	0.5	Polychromasia
7	4.24	4.24	10	30	70	310	7	1	-	85	7	60	111	753	38	0.6	Small number of wbc
8	4.27	4.27	9	30	70	224	4	-	-	93	4	46	105	790	39	0.6	Platelet aggregation
9	5.21	5.21	12	39	74	148	7	1	-	85	7	57	160	581	35	0.6	
10	5.53	5.53	12	38	68	199	2	1	-	92	5	44	95	960	25	0.5	
11	4.16	4.16	9	31	74	144	7	-	-	76	17	50	140	682	32	0.6	
12	5.19	5.19	11	38	73	165	6	-	-	88	6	44	88	958	28	0.4	
13	4.62	4.62	11	32	69	225	5	-	-	87	8	48	88	745	49	0.6	
14	4.54	5.45	12	37	67	160	8	1	-	89	2	60	106	672	32	0.5	
15	-	-	-	-	-	-	-	-	-	-	-	88	-	643	-	-	Hemolysis
16	-	-	-	-	-	-	-	-	-	-	-	46	136	981	39	0.4	Hemolysis
17	5.38	2500	12	39	72	198	7	3	-	84	6	44	128	925	31	0.5	

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Table 12. Hematological and blood chemical value of acute toxicity of ME in rats (cont.)

NO	RBC	WBC	Hb	Ht	MCV	Platlets	Neu	Eos	Baso	Lymp	Mono	ALT	AST	ALP	BUN	Creati	Notice
	$x10^6/\mu l$	cell/µl	g/dl	%	fl	$x10^3/\mu l$	%	%	%	%	%	U/l	U/l	U/l	mg/dl	mg/dl	
18		3010	12	39	72	198	7	3	-	81	6	72	288	652	27	-	
19		3250	10	30	66	111	6	3	-	88	3	43	100	721	36	0.5	
20		2510	11	34	69	95	5	2	-	86	7	106	405	688	30	0.4	
21		2440	12	37	69	208	6	3	-	88	3	67	107	647	36	0.5	
22		2180	11	33	69	191	14	3	-	78	5	48	112	773	36	0.6	
23		2150	10	33	69	239	8	7	-	77	8	46	160	588	32	0.5	
24	-	-	-	-	-	-	-	-	-	-	-	54	143	552	32	0.5	

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Table 13. Hematological and blood chemical value of short - term toxicity of ME in rats

NO	RBC	WBC	Hb	Ht	MCV	Platlets	Neu	Eos	Baso	Lymp	Mono	ALT	AST	ALP	BUN	Creati	Notice
	$x10^6/\mu l$	cell/μl	g/dl	%	fl	$x10^3/\mu l$	%	%	%	%	%	U/l	U/l	U/l	mg/dl	mg/dl	
1	5.4	2400	13	38	70	869	13	1	-	84	2	39	85	563	26	0.5	
2	5.2	2000	13	35	68	1214	10	1	-	85	4	56	129	519	32	0.7	
3	-	-	-	-	-	-	-	-	-	-	-	66	208	142	33	0.4	
4	4.9	3700	12	36	72	912	15	-	-	85	-	51	89	748	38	0.8	
5	5.0	3800	13	36	72	1008	14	-	-	86	-	45	121	712	29	0.6	
6	5.2	3800	12	36	70	858	21	-	-	78	-	-	-	-	-	-	
7	5.2	3400	13	37	71	790	9	1	-	90	-	54	159	449	30	0.6	
8	5.4	4300	13	38	70	942	9	-	-	90	1	45	99	740	30	0.8	
9	4.9	1006	13	36	73	1006	15	-	-	84	1	41	77	384	26	0.7	
10	5.3	5300	13	37	70	875	15	1	-	83	1	63	130	475	28	0.4	
11	5.1	3500	13	36	71	1023	13	1	-	83	3	41	75	520	24	0.6	
12	5.0	5300	12	35	70	853	25	1	-	71	3	39	91	425	25	0.5	
13	5.5	4700	14	38	70	1064	11	-	-	88	1	33	73	433	29	0.8	
14	5.0	3200	13	37	74	808	7	1	-	91	1	45	75	534	30	0.7	
15	5.3	3900	12	35	66	964	18	-	-	79	3	40	100	462	28	0.5	
16	4.8	3700	12	33	69	1005	14	-	-	85	1	35	75	444	24	0.5	
17	4.8	4300	12	32	67	903	16	-	-	79	3	44	90	689	27	0.6	

Table 13. Hematological and blood chemical value of short - term toxicity of ME in rats (cont.)

NO	RBC	WBC	Hb	Ht	MCV	Platlets	Neu	Eos	Baso	Lymp	Mono	ALT	AST	ALP	BUN	Creati	Notice
	$x10^6/\mu l$	cell/μl	g/dl	%	fl	$x10^3/ \mu l$	%	%	%	%	%	U/l	U/l	U/l	mg/dl	mg/dl	
18	5.2	2300	13	37	72	901	16	-	-	81	3	39	96	650	27	0.7	
19	5.2	2400	12	36	68	435	20	-	-	79	1	69	190	369	33	0.6	
20	4.5	3500	11	30	67	946	28	1	-	67	44.8	46	84	587	24	0.6	
21	4.8	2500	11	32	66	46	2	1	-	97	-	73	134	487	30	0.7	
22	5.74	7200	14	38	69	515	13	-	-	83	4	48	106	600	32	0.6	
23	-	-	-	-	-	-	-	-	-	-	-	56	156	350	34	0.7	
24	5.0	1500	13	35	70	959	6	-	-	93	1	57	580	580	30	0.6	

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BIOGRAPHY

Mr.Chaiyasit Sittiwet was born on January 19, 1978 in Saraburi Province. He was graduated in Bachelor of Science (Chemistry) in 1999 from Mahasarakham University and Master's degree of Science (Biochemistry) in 2001 from Chulalongkorn University. He worked as a lecturer at Department of Chemistry, Faculty of Science, Mahasarakham Univerisity.

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