THE TOXICITY STUDY OF *MORUS ALBA* L. LEAF EXTRACT

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE IN PHARMACY
(PHARMACOLOGY)
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY
2004

ISBN 974-04-5217-5

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THE TOXICITY OF *MORUS ALBA* L. LEAF EXTRACT

was submitted to the Faculty of Graduate Studies, Mahidol University
For the degree of Master of Science in Pharmacy (Pharmacology)
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ACKNOWLEDGEMENTS

I would like to express my deepest and sincere gratitude to Associate Professor Srichan Phornchirasilp, my major advisor, for her kindness, invaluable advice, great attention, assistance with consultant encouragement throughout my study.

I am also deeply grateful to my co-advisor, Associate Professor Omboon Luanratana, Professor Vorachai Sirikulchayanonta and Associate Professor Chongkol Tiengda for their helpful guidance, meaningful consultant and constructive comment.

I wish to thank Mr.Koset Pinpradap and lab staff, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, for their friendship and helpful in my work.

Unforgettably, I would like to thank Miss Nipapun Buchakul, Miss Panee Litthilert and Miss Saisamorn Yenphasuk for all their help and wonderful friendship throughout my study. I also wish to thank Mr. Chattiya Vorakhunpinij, who has helped me with the herbal extraction.

My indefinite gratitude must be extended to my parents and friends, who are always on my mind. Their perpetual love forwards me to have full confidence and strength during the wayward attention.

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ABSTRACT

Morus alba L., a member of Moraceae family, commonly known as mulberry (Thai name : Mon), is widely cultivated in the north and northeast region of Thailand. *Morus alba* L. leaf extract can reduce plasma glucose level in streptozotocin-induced diabetic rats. Thus, it is possible to develop an antidiabetic drug from this extract.

The objective of this study was to establish acute and subchronic toxicity of *Morus alba* L. leaf extract. In the acute toxicity study, the *Morus alba* L. leaf extract was administered intraperitoneally and orally into mice and Wistar rats. Following a single i.p. dose, the LD_{50} of *Morus alba* L. leaf extract in the mice and Wistar rats were approximately 4 and 5 g/kg, respectively. But when this extract was administered orally, doses as high as 5 g/kg did not cause any significant toxic effects. There were also no deaths in these groups of animals. The only abnormal signs and symptoms were CNS depression and respiratory depression. Moreover, all animals recovered within 15-30 minutes. In the subchronic toxicity study, the *Morus alba* L. leaf extract was administered orally into Wistar rats for 60 days at doses 1,2 and 3 g/kg/day. Almost all doses of *Morus alba* L. leaf extract did not significantly affect blood chemistry and hematologic values when compared to the control group. Only the dose of 3g/kg BW/day of female rats increased blood glucose level significantly. Microscopic examination of major organs indicated no significant histopathological abnormalities.

In conclusion, our results suggested that *Morus alba* L. leaf extract is safe for use. This toxicity study might provide enough evidence to consider further development of pharmacological dosage form.

KEY WORDS : *MORUS ALBA* L., WATER EXTRACT , ACUTE TOXICITY , SUBCHRONIC TOXICITY

132 pp. ISBN 974-04-5217-5

การศึกษาความเป็นพิษของสารสกัดจากใบหม่อน (THE TOXICITY STUDY OF *MORUS ALBA* L. LEAF EXTRACT)

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บทคัดย่อ

Morus alba L. อยู่ในวงศ์ Moraceae ภาษาอังกฤษเรียก mulberry ภาษาไทยเรียกหม่อน เป็นพืชที่ปลูกอย่างแพร่หลายทางภาคเหนือและภาคตะวันออกเฉียงเหนือของประเทศไทย พบว่า สารสกัดจากใบหม่อน สามารถลดระดับน้ำตาลในเลือดในหนูที่ถูกเหนี่ยวนำให้เป็นเบาหวานด้วย streptozotocin ได้ ดังนั้นจึงมีความเป็นไปได้ในการพัฒนาเพื่อเป็นยารักษาเบาหวานต่อไป

งานวิจัยนี้มีจุดมุ่งหมาย เพื่อศึกษาความเป็นพิษแบบเฉียบพลันและกึ่งเรื้อรังของสารสกัดจากใบหม่อน วิธีการศึกษาความเป็นพิษแบบเฉียบพลัน คือ ใช้สัตว์ทดลอง 2 สายพันธ์ หนูถีบจักรพันธุ์ Swiss albino และหนูขาวพันธุ์ Wistar โดยให้สารสกัดจากใบหม่อนแก่สัตว์ทดลอง 2 ทาง คือ การฉีดเข้าช่องท้องและการกิน ในการศึกษาความเป็นพิษแบบเฉียบพลัน พบว่า เมื่อฉีดสารสกัดเข้าทางช่องท้องของสัตว์ทดลอง ขนาดยาที่ทำให้สัตว์ทดลองตาย 50 เปอร์เซนต์ ของหนูถีบจักร Swiss albino และหนูขาวพันธ์ Wistar คือประมาณ 4 และ 5 กรัมต่อกิโลกรัมตามลำดับ แต่เมื่อให้สารสกัดโดยการกินพบว่า ในขนาดยาที่มากกว่า 5 กรัมต่อกิโลกรัม ไม่ทำให้สัตว์ทดลองตายในระหว่างการทดลอง อาการผิดปกติที่พบคือ การหายใจช้าลง ซึม และไม่เคลื่อนไหว แต่อาการดังกล่าวสามารถกลับคืนสู่สภาวะปกติได้ภายใน 30 นาที ในการศึกษาความเป็นพิษแบบกึ่งเรื้อรัง คือ ใช้หนูขาวพันธ์ wistar ทั้งเพศผู้และเพศเมีย โดยให้สารสกัดจากใบหม่อนโดยการกินในขนาด 1, 2 และ 3 กรัมต่อกิโลกรัมต่อวัน เป็นเวลา 60 วัน พบว่าไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติในทางเคมีคลินิก และส่วนประกอบในเลือด เมื่อเปรียบเทียบกับกลุ่มควบคุม มีเพียงในขนาดยา 3 กรัมต่อกิโลกรัมต่อวัน เมื่อให้ในหนูเพศเมีย ทำให้ระดับน้ำตางในเลือดสูงขึ้นเมื่อเทียบกับกลุ่มควบคุม และผลทางพยาธิวิทยา ไม่พบความผิดปกติใดๆ ในทุกอวัยวะที่ทำการศึกษา

จากผลการทดลองแสดงว่า สารสกัดจากใบหม่อนมีความปลอดภัย เหมาะที่จะนำมาใช้เป็นยา และผลจากการศึกษาทางพิษวิทยาของสารสกัดจากใบหม่อนนี้ จะเป็นประโยชน์ในการประกอบการพิจารณาที่จะนำใบหม่อนมาพัฒนาเป็นยาต่อไป

132 หน้า ISBN 974-04-5217-5

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LIST OF ABBREVIATIONS

%	=	percentage
°C	=	degree celcius
cont.	=	continued
et.al.	=	et alli; and others
etc.	=	et cetera; and so on
fig.	=	figure
BW	=	body weight
IDDM	=	insulin-dependent diabetes mellitus
NIDDM	=	non insulin-dependent diabetes mellitus
ATP	=	adenosine triphosphate
PPAR	=	peroxisomal proliferators-activated receptor
ED_{50}	=	dose level in milligram per milliliter at 50 percent effective
IC_{50}	=	concentration giving 50 percent inhibition
LD_{50}	=	median lethal dose (dose giving 50 percent killing)
W/W	=	weight by weight
kg	=	kilogram
g	=	gram
mg	=	milligram
µg	=	microgram
ml	=	milliliter
mm	=	millimeter
cm.	=	centimeter
m.	=	meter
L	=	liter
hr	=	hour
U/L	=	units per liter
U/hr	=	unit per hour

LIST OF ABBREVIATIONS (Cont.)

ppm	=	part per million
µmol/L	=	micromole per liter
µU/ml	=	microunit per milliliter
µM	=	micromole
vs	=	versus
mg/dl	=	milligram per deciliter

CHAPTER 1
INTRODUCTION

Diseases are always constant problems of mankind. The curative properties of some plants have been discovered and exploited early in human history. Over the years, the knowledge of herbal medicine has grown. In this time, a wide range of plants have been sought for their healing effects and used more and more. In many developing countries, traditional medicine is still the mainstay of health care, and most of the drugs used come from plants. Thailand has a very long history of Thai traditional medicine. At present, there are many medicinal plants that have already been promoted to use in primary health care and classified according to their pharmacological actions such as treatment of stomach pain, respiratory problem, anti-herpetic, etc. The major advantage of using Thai traditional plants is its availability in our own country. Thus, allow easier accessment with lower cost. However , most of the traditional used medicinal plants have not been sufficiently evaluated for their pharmacological and toxicological effects.

Morus alba L. is one of the Thai medicinal plant that has been claimed to be effective in lowering blood sugar. However, it needs to be confirmed scientifically.

M. alba L. belongs to the Moraceae family, commonly known as white mulberry. *Morus* is the old Latin name ; *alba* refers to the white fruits. White mulberry is routinely used to feed the silkworms. It is native to China. It is cultivated throughout the world wherever silkworms are raised , and is occasionally cultivated elsewhere in Europe , North America, Africa and Asia. In Thailand , it is widely cultivated in the north and northeast region. Its leaves have been used in Thai traditional medicine for the treatment of cough, relieve sore throat, relieve thirst, fever, reddening of the eye , diaphoretic and used as sedative. Some of its common uses in most countries are as antipyretics , anti-inflammatories , antibacterials and antivirals. Moreover, the mulberry leaves is used for the treatment of diabetes in oriental medicine.

Diabetes mellitus is a metabolic disorder of the endocrine system. The disease is found in all parts of the world and is rapidly increasing in most parts of the world (1).

It is a heterogeneous group of disorders characterized by varying degrees of insulin hyposecretion and/or insulin insensitivity. Regardless of cause, it is associated with hyperglycemia (2), altered metabolism of lipids, carbohydrates and proteins and an increase risk of complications from vascular disease (3).

There are two types of diabetes, namely type 1 and type 2. Type 1 , insulin-dependent diabetes mellitus (IDDM), in which the body does not produce any insulin, most often occurs in children and young adults. People with type 1 diabetes must take daily insulin injections to stay alive. Type 1 diabetes accounts for 5-10% of diabetes. Type 2 , noninsulin-dependent diabetes mellitus (NIDDM), in which the body does not properly use insulin, is the most common form of the disease, accounting for 90-95 % of diabetes. Type 2 diabetes is nearing epidermic proportions, due to an increased number of elderly people, and a greater prevalence of obesity and sedentary lifestyles (1) .The symptoms are similar in type 1 and type 2, but vary in their intensity. Common symptoms include polyuria and polydipsia, which are a consequence of osmotic diuresis secondary to sustained hyperglycaemia. Weight loss, despite normal or increased appetite, is also a common feature. Persistent hyperglycemia and hypertension are the two major controllable factors that influence the development of diabetic complications. Some of other chronic complications are blurring of vision, open-angle glaucoma, retinopathy, urinary tract infections, nephropathy, neuropathy, muscle pain, myocardial infarction, foot ulcers, musculoskeletal problems and dermatological conditions (2).

Diabetes is estimated to affect more than 150 million adults worldwide, and this number is expected to double in the next 25 years, encompassing 5.4% of the total adult population (4). WHO predicts a 170% increase in numbers of individuals with diabetes in developing countries compared with a 42% rise in developed countries (5). Most people with diabetes in the developed world are older than 65 years of age, whereas most of those in developing countries are only 45 to 64 years old (4).

Treatment of diabetes spent vast amount of resources including medicines, diets, physical training and so on in all countries. Diabetes requires long term treatment as well as causes serious complications which could result in high death-rate. The present treatment of diabetes is focused on controlling and lowering blood glucose to a normal level. The drugs commonly used to control diabetes are insulin and oral antidiabetic

agents. Insulin therapy afford effective glycemic control, but it has disadvantages such as ineffectiveness on oral administration, short shelf life and requirement of constant refrigeration. Current oral treatment options can be subdivided into the hypoglycemic drugs (sulfonylureas and benzoic acid derivatives) and antihyperglycemic drugs (biguanide, α-glucosidase inhibitors and thiazolidinediones) and others (dichloroacetic acid aldose reductase inhibitor, carbamoylmethyl benzoic acid and insulin-like growth factor) (6-8). The effect of these drugs is only aimed to lower the level of blood glucose. Although, oral hypoglycemic agents/insulin are the mainstay for the treatment of diabetes and are effective in controlling hyperglycemia, they have prominent side effects and fail to significantly alter the course of diabetic complications (9).

Many plants are used in Thai traditional medicinal as anti-hyperglycemic agents. But, there are insufficient scientific data supporting their pharmacological and toxicological actions. Therefore , it is worth to study the toxicity of *M. alba* L. leaf extract. Its toxicological data will support the safety use of this plant as well as encourage further development of this extract to pharmaceutical dosage form.

Objective of this study

1. To determine the acute toxicity of the water extract of *M. alba* L. leaf in mice and Wistar rats by intraperitoneal and oral administration.
2. To determine the subchronic toxicity of the water extract of *M. alba* L. leaf administered orally to Wistar rats for 60 days.

CHAPTER 2
LITERATURE REVIEW

1. Diabetes mellitus

Diabetes mellitus is a complex and heterogeneous metabolic disease that has reached epidemic proportions. Type 1 diabetes is an autoimmune disorder characterized by the destruction of pancreatic endocrine β-cells, whereas in type 2 diabetes, both an insulin secretory defect and a condition termed insulin resistance coexist. Both forms of diabetes have shown an increase in their incidence in the past decade, and those numbers are expected to increase in the future (10).

Complications such as eyes and cardiovascular diseases are frequently present at diagnosis. Conventional medicine has some effective ways to control blood glucose but requires significant self-care on the part of the individual. It is the natural course of type 2 diabetes for the majority of individuals to eventually require insulin, even if they are initially commenced on diet or oral hypoglycaemic agents (11).

Type 1 diabetes

Type 1 Diabetes is much less common than type 2 diabetes and typically affects younger individuals. The pathophysiology of "typical" type 1 diabetes is thought to be due to an absolute insulin deficiency without significant insulin resistance that results in hyperglycemia, spontaneous ketosis, and if untreated, diabetic ketoacidosis. Type 1 diabetic individuals require the daily administration of exogenous insulin to avoid diabetic ketoacidosis and hence to preserve life. This absolute insulin deficiency is thought to be caused by autoimmune β-cell destruction, triggered by unknown mechanisms. Markers of autoimmunity (such as insulin autoantibodies [IAA], and glutamic acid decarboxylase enzyme [GAD]) are present in the plasma in 65% to 90% of Caucasians (of Northern European decent) with new onset type 1 diabetes. Although these patients present with absolute insulin deficiency at diagnosis, they often have a variable β-cell functional recovery within the first years after diagnosis, a period called the "honeymoon period." The recovery of β-cell function usually lasts

only a few months. In adults with Type 1 diabetes, C-peptide secretion is usually completely absent within 5 years after diagnosis.(12)

Type 2 diabetes

Type 2 diabetes is one of the most common chronic diseases and is associated with co-morbidities, such as obesity, hypertension, hyperlipidemia (increased VLDL triglycerides and decreased HDL cholesterol), and cardiovascular disease, which, taken together, comprise the 'Metabolic Syndrome'. Type 2 diabetes is characterized by fasting and postprandial hyperglycemia and relative insulin insufficiency. If left untreated, hyperglycemia may cause long-term microvascular and macrovascular complications, such as nephropathy, neuropathy, retinopathy and atherosclerosis. This disease causes significant morbidity and mortality at considerable expense to patients, their families and society.

Environmental and genetic factors influence insulin sensitivity, lipid metabolism, adiposity and the onset of diabetes. Obesity, sedentary lifestyles and diets that are rich in fats are known risk factors for diabetes. Both central obesity and high fat diets induce insulin resistance, which leads to hyperinsulinemia, the compensatory response to insulin resistance. Certain ethnic groups such as those of African, Hispanic, Native American and Asian descent are particularly vulnerable to diabetes and its complications. In genetically susceptible individuals, weight gain, increased insulin resistance, decreased acute insulin secretory response and hyperinsulinemia accompany the progression from normal glucose tolerance to impaired glucose tolerance (IGT), a state in which hyperglycemia occurs following a glucose load but is not as pronounced as in diabetes. Hyperinsulinemia is often associated with further weight gain, which exacerbates hyperglycemia and leads to chronic over-production of insulin. (13)

Drug therapy of diabetes mellitus

Insulin therapy

Insulin, specifically produced by pancreatic β-cells, is one of the most important regulators of glucose homeostasis. When the glucose concentration rises, insulin is rapidly released from storage granules, and the level of insulin mRNA increases

through transcriptional activation and insulin mRNA stabilisation. β-Cells secrete insulin in response to glucose, not through a cell surface receptor for glucose, but through the metabolism of glucose, which generates intracellular ATP. Intracellular ATP concentrations directly affect the behaviour of ion channels in the β-cell membrane such that at high intracellular ATP concentrations, a potassiumchannel is blocked, leading to the opening of a voltage-sensitive calcium channel. The rapid influx of calcium causes the release of granules containing insulin from the β-cell .

In nondiabetic individuals, insulin secretion can be divided into two basic components, basal and stimulated. Basal insulin is secreted continuously between meals and throughout the night at a rate of 0.5–1 U/hr in adults. Basal insulin secretion provides serum concentrations of 5–15 μU/mL. Normally, stimulated insulin secretion occurs in response to a meal and results in insulin concentrations of 60–80 μU/mL from just after to 30 minutes after the meal. Concentrations return to basal levels in 2–4 hours. Regimens of regular insulin attempt to mimic the stimulated insulin secretory pattern.

Insulin is used for treatment of all type 1 diabetes and many type 2 diabetes patients. Insulin treatment of type 2 diabetes is indicated for initial stabilisation of patients with severe hyperglycaemia, established patients with hyperglycaemic crises, and patients in whom oral hypoglycaemic therapy has failed. When injected subcutaneously, commercial insulin formulations have time-action profiles that do not closely approximate normal physiologic insulin secretion. With so-called rapid-acting regular human insulin, onset of activity is 0.3–0.7 hours after injection, peak concentration is reached within 2–4 hours, and duration of action is up to 8 hours .

The most recent advance is the development of insulin analogues produced by recombinant DNA, peptide synthesis, and enzyme-catalysed semisynthesis. Recent evidence of an increased risk of macrovascular disease associated with hyperinsulinaemia is still under discussion, particularly in patients with type 2 diabetes. Attention has also been focused on the desirability of using the lowest possible dose of insulin to achieve glycaemic control .(14)

Oral antidiabetic agents

Orally administered drugs available for the treatment of type 2 diabetes include the sulfonylureas, the biguanide metformin, the α-glucosidase inhibitor, and the thiazolidinedione. These agents reduce blood glucose levels by different mechanisms.

Sulfonylureas

The sulfonylureas (SFU) are the first oral agents available for the treatment of type 2 diabetic subjects. Sulfonylureas promote insulin secretion through direct stimulation of pancreatic beta cells. They bind to ATP-sensitive potassium channels and inhibit potassium efflux. The resulting depolarization allows influx of calcium through the voltage-dependent calcium channels. The release of insulin is triggered by the rise of intracytosolic calcium concentration. Secondary failure of sulfonylureas-induced insulin secretion is result from beta cell "exhaustion", occurs in approximately 10% of patients per year. After 10 years of sulfonylureas-treatment, approximately 50% of treated patients become non-responder(15).

All sulfonylureas are able to augment insulin secretion in patients with type 2 diabetes who still have pancreatic insulin reserve. These drugs have different durations of action, dose responses, and side effects. Sulfonylureas reduce fasting glucose concentrations 20% to 30%. They have a substantial primary and secondary failure rate. Hypoglycemia, weight gain, and hyperinsulinemia are potential side effects of sulfonylureas. Thus, it is relatively uncommon to achieve persistent normoglycemia with a sulfonylurea alone. Sulfonylureas may be used in combination with other oral agents or insulin. Although this approach has been shown to decrease insulin requirements in some patients with type 2 diabetes, it is not effective in all patients with type 2 diabetes and may result in episodes of hypoglycemia (16).

Meglitinides

These agents are non-sulfonylureas secretagogues, with a sulfonylureas-like mechanism of action. They target a different binding site on pancreatic beta cells, leading to a similar cascade of events triggering insulin release. Repaglinide (a benzoic acid derivative) and, the newer agent, nateglinide (a phenylalanine derivative)

are examples of drugs belonging to this class of agents. Their pharmacokinetic profile is favorable in terms of targeting postprandial hyperglycemia.

Repaglinide is completely metabolized by the liver and eliminated mostly by biliary route (90%). The drug elimination kinetics is widely variable; its half-life ranges between 0.5 hours and 8 hours. In elderly patients with type 2 diabetes mellitus (reduced creatinine clearance), the mean diurnal plasma concentration was found to be significantly higher than healthy controls. Similar results were noted in patients with moderate to severe liver disease. Nateglinide should also be used cautiously in the context of renal and/or hepatic impairment. Studies have proved repaglinide as efficacious as sulfonylureas . Nateglinide, however, appears to be less potent than sulfonylureas. Another drawback in using these agents is the frequent pre-meal dosing that may affect compliance. Although it was hoped that weight gain and hypoglycemia would occur less often with meglitinides than with sulfonylureas, a recent clinical trial found no major clinical advantage when repaglanide was compared to glipizide. (15)

Biguanides

Metformin is the biguanide prescribed most worldwide. It is much safer than earlier biguanides, phenformin and buformin. The latter agents were withdrawn from the market because of their tendency to cause lactic acidosis. Its principal action is to reduce hepatic gluconeogenesis. Excessive hepatic glucose production is then diminished at any given level of plasma insulin. To a lesser degree, possibly an indirect effect, metformin increases insulin mediated glucose uptake and utilization in peripheral tissues, especially in the muscle. Metformin is, therefore, an antihyperglycemic agent preferred in obese patients with insulin resistance. Metformin is not metabolized and mostly renally excreted (90%) in 12 hours. It is 50–60% bioavailable, absorbed mainly from the small intestine and has an estimated plasma half-life of 1.5–4.9 hours. It has negligible binding to plasma proteins. Metformin is beneficial at a low dose of 500 mg/day with meals and demonstrates a progressive response to doses up to 2000 mg/day. Although metformin is approved for up to 2550 mg/day, little additional benefit is seen beyond 2000 mg dose. It is not associated with weight gain and reduces adiposity. Metformin also has a modest antihypertensive and

antihyperlipidemic effect (slight increase in HDL, decrease in triglycerides and LDL) (15).

As monotherapy, it does not cause hypoglycemia. It can be used as a single agent with efficacy equivalent to that of the sulfonylureas, or it can be used in combination with a sulfonylurea. The combination of metformin with a sulfonylurea has the potential of achieving normoglycemia in patients with type 2 diabetes without concomitant weight gain and hyperinsulinemia. Metformin may also be used with acarbose or insulin. Of the newer drugs, studies indicate that metformin is best for normalizing abnormal blood lipids. Contraindications to the use of metformin include kidney disease (serum creatinine level greater than 130 μmol/L in men and 120 μmol/L in women), diabetic ketoacidosis, and pregnancy(16).

Thiazolidinediones

This class of agents is relatively new. It is the most expensive class of antidiabetic agents. Troglitazone, the first compound in this class, was withdrawn from the market because of its association with unpredictable fulminant hepatic failure. The two currently available agents, rosiglitazone and pioglitazone, do not appear to have the same tendency to cause hepatotoxicity.

Thiazolidinediones enhance glucose uptake and utilization in peripheral tissues, mainly skeletal muscle. Thiazolidinediones bind to the peroxisomal proliferator-activated receptor (PPAR-γ). The nuclear receptor most highly expressed not only in adipose tissue but also found in skeletal muscle, liver, intestine, kidney, vascular smooth muscle, heart and macrophages. Once activated, PPAR-γ receptor binds to DNA leading to transcriptional modulation of genes involved in carbohydrate and lipid metabolism. Thiazolidinediones decrease insulin resistance in peripheral tissues with only a minor effect on hepatic glucose production at high doses. Given the abundance of PPAR-γ receptors in adipocytes compared to myocytes, the predominant action of thiazolidinediones on skeletal muscle may be through an indirect interaction between fat and muscle cells.

Thiazolidinediones do not cause hypoglycemia when used as alone. Rosiglitazone and pioglitazone have a half-life ranging between 3 and 7 hours. Some active metabolites of pioglitazone have longer half-lives. These agents are 99% protein

bound. Rosiglitazone and pioglitazone have minimal drug–drug interaction. While pioglitazone partially induces the cytochrome P450 system (metabolized through the CYP2C8 isoenzyme and CYP3A4), rosiglitazone is metabolized through the CYP2C8 isoenzyme only and does not induce the cytochrome P450 system. Thiazolidinediones are hepatically metabolized and eliminated by biliary route. The dose of the glitazones does not need to be adjusted for renal insufficiency. But in the context of hepatic failure, thiazolidinediones must be discontinued. Nevertheless rosiglitazone and pioglitazone have not demonstrated an increased rate of liver enzyme abnormality so far. Based on the troglitazone experience, the current recommendation is still to monitor liver enzymes at baseline, every other month in the first year and periodically thereafter.

Thiazolidinediones also have non-hypoglycemic effect. Both rosiglitazone and pioglitazone slightly raise HDL cholesterol. LDL cholesterol increases with both agents although more so with rosiglitazone. Pioglitazone but not rosiglitazone may have a triglyceride lowering effect possibly because of its additional PPAR-γ activity. Thiazolidinediones also slightly reduce blood pressure, enhance fibrinolysis, improve endothelial function and decrease in vitro vascular inflammation.(15)

Alpha-glucosidase inhibitors

There are three agents in this category that are currently marketed worldwide. These include acarbose, miglitol and voglibose. These agents primarily target postprandial hyperglycemia. Their action on fasting blood sugar is minimal. Despite treatment with oral antidiabetic agents, over half of patients with type 2 diabetes have postprandial hyperglycemia. Postprandial hyperglycemia has been linked with cardiovascular mortality.

Alpha-glucosidase inhibitors have a unique mechanism of action. These agents competitively inhibit alpha-glucosidases, the brush border enzymes of the proximal small intestinal epithelium. This reversible inhibition delays hydrolysis of polysaccharides into absorbable monosaccharides (e.g. glucose). Carbohydrate absorption occurs over a greater portion of the small intestine, blunting postprandial glucose excursions.

Hypoglycemia may occur when alpha-glucosidase inhibitors are used in combination therapy with either insulin or sulfonylureas. In that context, glucose rather than sucrose or complex carbohydrates should be used. Glucose is readily absorbed and will promptly correct hypoglycemia.

Alpha-glucosidase inhibitors are safe and may be suitable agents in elderly diabetic patients either as monotherapy or in combination therapy. The need for multiple pre-meal dosing, gastrointestinal side effects and cost may, however, limit their use. (15)

In general, when proper glycemic control is not achieved despite diet, exercise and the use of oral antidiabetic agents, insulin therapy is often necessary.

All the above antidiabetic drugs that are available for clinical use also cause certain adverse drug reactions. Thus, in order to search for better agents, some plants that are previously claimed to be capable in reducing elevated blood glucose levels have been explored for their efficacy and safety.

2. Plants with anti-diabetic potential

***Allium cepa:* Onion (English)**

Onion is an important dietary constituent. Various ether soluble fractions of onion as a single oral dose (0.25 mg/kg) showed significant hypoglycemic effect in normal fasted rabbits. Ethyl ether extract is the most potent hypoglycemic action. Petroleum ether insoluble fraction of the ether extract of dried onion powder (100 mg/kg) given orally for 7 days to alloxan-treated diabetic rabbits (180 mg/kg) caused a significant anti-hyperglycemic effect. Oral administration of 250 mg/kg of ethanol, petroleum, chloroform and acetone extract of powder dried onion showed maximal reduction of 18.57, 8.35, 3.0 and 3.20% in fasting blood glucose of alloxan-treated diabetic rabbits (150 mg/kg, administered intraperitoneally). In a preliminary study of seven different fractions obtained from onion bulb, only petroleum ether and chloroform extracts significantly lowered blood sugar in oral glucose tolerance test (2 g/kg) in rabbits. Feeding of diet containing 3% freeze-dried onion powder for 8 weeks produced a significant hypoglycemia along with partial reversion of abnormal plasma albumin, urea, creatinine and inorganic phosphorus in streptozotocin-treated diabetic albino rats. The effect proposed to be the result of its anti-oxidant and hypolipidemic activity.(9)

Administration a sulfur containing amino acid isolated from *A. cepa* Linn. called *S*-methyl cysteine sulphoxide (SMCS) (200 mg/kg for 45 days) to alloxan-treated diabetic rats also significantly controlled blood glucose and lipids in serum and tissues and normalized the activities of liver hexokinase, glucose 6-phosphatase and HMG CoA reductase. The effect was comparable to that of glibenclamide and insulin. Moreover, oral administration of SMCS to alloxan-treated diabetic rats for a month provided a benificial effect to the diabetic condition, being characterized by glucose intolerance, weight loss and liver glycogen. In addition, SMCS also decreased the hyperglycemic peak in subcutaneous glucose tolerance tests conducted in rabbits .(9)

Oral administration of 25, 50, 100 and 200 g of aqueous onion extract to overnight fasted healthy volunteers (n=5/group) 30 minutes before, after or simultaneously with oral glucose (50 g) significantly and in a dose-dependent manner increased glucose tolerance. The effect was comparable to tolbutamide. In addition, adrenaline (0.5 ml of 1:1000, administered subcutaneously) induced hyperglycemia was also inhibited in these patients. In the same experiment, there was no difference in

the anti-hyperglycemic effect of raw and boiled onion extract in these human volunteers. Beneficial effects of fresh onion (3×20 g) as a dietary aid has also been shown in a crossover designed clinical study. The onion diet decreased or maintained blood sugar levels. (9)

Daily intraperitoneal administration of a high dose of aqueous onion (500 mg/kg) extract to rats caused extensive damage to the lungs and liver. Lungs of these animals exhibited thickening of the alveolar walls and aggregation of many red blood cells within the alveoli. The livers of these rats appeared to be clearly damaged with vacuolation especially near the organ surface, and considerable numbers of red blood cells aggregated intercellularly. Cellular organization of the liver was thereby disrupted and local hemorrhages were observed. In contrast, daily oral administration of a high dose of onion to rats caused a lesser degree of destruction to both lung and liver tissue when compared to those animals receiving an equivalent dose administered intraperitoneally. Rats which received low administered intraperitoneally doses of onion (50 mg/kg) exhibited minimal levels of lung and liver damage. Animals which were administered a low daily dose of onion orally had no significant lung damage, while the livers in these animals appeared similar to those receiving the low dose of onion administered intraperitoneally. (17)

Allium sativum: **Garlic (English)**

Garlic is a perennial herb and is commonly used as a food ingredient. Oral administration of 0.25 g/kg of ethanol, petroleum ether, ethyl ether extract of *A. sativum* causes 18.9, 17.9, 26.2% reduction in blood sugar in alloxan-treated diabetic rabbits (150 mg/kg, injected intravenously). Oral administration of 0.25 g/kg allicin (isolated from *A. sativum*) produced hypoglycemia comparable to tolbutamide in mildly diabetic rabbits (glucose levels ranging from 180 to 300 mg%) while it showed no such effect in severely diabetic animals (blood sugar >350 mg%). Aqueous homogenate of garlic (10 ml/kg/day) administered orally to sucrose fed rabbits (10 g/kg/day in water for 2 months) significantly increased hepatic glycogen and free amino acid contents, decreased fasting blood sugar, triglyceride levels in serum, liver and aorta and protein levels in serum and liver, in comparison to sucrose controls. In subcutaneous glucose tolerance test in rabbits, garlic decreased the hyperglycemic

peak. Pretreatment with aged garlic extract (AGE) (5 and 10 ml/kg, orally) in stress-induced hyperglycemia model of mice significantly prevented adrenal hypertrophy, hyperglycemia and elevation of cortisone without altering serum insulin levels. The efficacy of AGE was the same as that of diazepam (5 mg/kg, orally). Thus, AGE may prevent stress-induced risk of diabetes and its progression. (9)

Daily oral feeding of garlic extract (100 mg/kg) increased cardiovascular functions in streptozotocin-treated diabetic rats, prevented abnormality in lipid profile and increased fibrinolytic activities with decreased platelet aggregation. Plasma insulin level increased with concomitant decrease in plasma glucose levels. In addition, daily oral feeding of the same dose for 16 weeks showed anti-atherosclerotic effects in streptozotocin-treated diabetic rats. Thus, garlic may prevent diabetic cardiovascular complications. Ethanolic (95%) extract (45 mg/kg body weight/day for 28 days) of garlic in alloxan-treated diabetic mice exerted anti-nociceptive effects in tail-flick, hotplate, allodynia and formalin tests with concomitant decrease in serum glucose levels. However, administration of garlic as 6.25% by weight in the diet and as infusions (1 g/400 ml) in place of drinking water for 12 days to normal mice did not alter plasma glucose and insulin concentrations. Moreover, it reduced hyperphagia and polydipsia but not hyperglycemia or hypoinsulinemia in streptozotocin-treated diabetic mice (200 mg/kg administered intraperitoneally). (9)

SACS, a sulfur containing amino acid, is the precursor of allicin and garlic oil. Various studies in experimental diabetes have shown beneficial effect of SACS. Administration of SACS (200 mg/kg) significantly decreased the concentration of serum lipids, blood glucose and activities of serum enzymes like alkaline phosphatase, acid phosphatase and lactate dehydrogenase and liver glucose-6-phosphatase. It also significantly enhanced liver and intestinal HMG CoA reductase activity and liver hexokinase activity. In another study, oral administration of SACS to alloxan-treated diabetic rats for 1 month ameliorated glucose intolerance, weight loss, depletion of liver glycogen in diabetic rats, in comparison to glibenclamide and insulin. SACS also controlled lipid peroxidation better than glibenclamide and insulin and ameliorated diabetic condition almost to the same extent as they did. Furthermore, SACS significantly stimulated in vitro insulin secretion from isolated β-cells of normal rats .(9)

Daily intraperitoneal administration of a high dose of aqueous garlic (500 mg/kg) extract to rats caused extensive damage to the lungs and liver. Lungs of these animals exhibited thickening of the alveolar walls and aggregation of many red blood cells within the alveoli. The livers of these rats appeared to be clearly damaged with vacuolation especially near the organ surface, and considerable numbers of red blood cells aggregated intercellularly. Cellular organization of the liver was thereby disrupted and local hemorrhages were observed. In contrast, daily oral administration of a high dose of garlic to rats caused a lesser degree of destruction to both lung and liver tissue when compared to those animals receiving an equivalent administered intraperitoneally dose. Rats which received low administered intraperitoneally doses of garlic (50 mg/kg) exhibited minimal levels of lung and liver damage, while animals which were administered a low daily dose of garlic orally had no significant lung damage. (18)

***Aloe vera* or *Aloe barbadensis:* Ghee Kunwar and Kumar panthu (Hindi)**

The dried sap of the plant (half a teaspoonful daily for 4–14 weeks) has shown significant hypoglycemic effect both clinically as well as experimentally.

Extracts of aloe gum effectively increased glucose tolerance in both normal and diabetic rats. Chronic but not single administration of the exudate of the leaves of *Aloe barbadensis* (500 mg/kg, orally) showed significant hypoglycemic effect in alloxan-treated diabetic mice. Morever, single as well as chronic administration of the bitter principle (5 mg/kg, administered intraperitoneally) showed significant hypoglycemic effect in the same model. The hypoglycemic effect of single dose of the bitter principle was extended over a period of 24 hours with the maximum hypoglycemia observed at 8 hours. But chronic administration (exudate twice daily and the bitter principle once a day for 4 days) induced a maximum reduction in plasma glucose level at the fifth day. Regarding to the mode of action, the hypoglycemic effect of aloe and its bitter principle is proposed to be mediated through stimulation of synthesis and/or release of insulin from the β-cells of Langerhans (9)

In addition to their hypoglycemic effet, both *A. vera* and *A. gibberellin* (over a dose range of 2–100 mg/kg) were also effective in inhibiting inflammation in a dose-

response manner and improving wound healing in streptozotocin-treated diabetic mice. (9)

Mangifera indica: **Mango (English)**

Its seeds and fruits are used for treatment of various ailments. Regarding to the anti-diabetic property, oral administration of aqueous extract of the leaves (1 g/kg) failed to alter the blood glucose levels in normoglycemic or streptozotocin-treated diabetic rats. In contrast, this extract showed anti-diabetic activity when given 60 minutes before or concurrently with glucose. This action of the extract could be resulted from a reduction in intestinal absorption of glucose. However, the possibility of other mechanism can not be excluded. (9)

Momordica charantia: **Bitter Gourd (English)**

Bitter gourd is a very common folklore remedy for diabetes. Extract of fruit pulp, seed, leaves and whole plant of *M. charantia* has been demonstrated to possess hypoglycemic effect in various animal models .

In a preliminary study, *M. charantia* exhibited the hypoglycemic as well as antihyperglycemic activity in laboratory animals. Polypeptide-p, isolated from fruit, seeds, and tissue of *M. charantia* showed potent hypoglycemic effect when administered subcutaneously to gerbils, langurs, and humans. Aqueous extracts of *M. charantia* improved oral glucose tolerance test after 8 hours in normal mice and reduced hyperglycemia by 50% after 5 hours in alloxan-treated diabetic mice. In addition, chronic oral administration of aqueous extract to normal mice for 13 days improved oral glucose tolerance test while no significant effect was seen on plasma insulin levels. Ethanolic extract of *M. charantia* (250 mg/kg dose orally) significantly lowered blood sugar in fasted as well as glucose loaded non-diabetic rats. Oral administration of acetone extract of fruit powder of *M. charantia* for 15–30 days to alloxan-treated diabetic rats reduced the blood sugar and serum cholesterol levels to normal range and the blood sugar was found normal even after 15 days of treatment discontinuation. Thus, ethanolic extract of *M. charantia* (200 mg/kg) can produce anti-hyperglycemic as well as hypoglycemic effect in normal and streptozotocin-treated diabetic rats as evident by 23% ($P<0.01$) and 27% ($P<0.001$) decrease in blood sugar,

respectively. The mechanisms which were responsible for these actions of *M. charantia* extract was believed to be an inhibition of glucose-6-phosphatase and fructose-1,6-bisphosphatase in the liver and stimulation of red-cell and hepatic glucose-6-phosphate dehydrogenase activities. When fed orally, aqueous extract of *M. charantia* exhibited anti-hyperglycemic and hypoglycemic effect in cyproheptadine-induced hyperglycemic and normoglycemic mice, respectively. In addition to the whole plant, the pulp juice and saponin free methanolic extract of pulp juice also exerted significant hypoglycemic effect in fasting and post-prandial states of normal and NIDDM rats but not in IDDM rats. The effect was more pronounced in case of saponin free methanol extract. Moreover, Charantin, a peptide resembling insulin isolated from *M. charantia* (50 mg/kg) when administered orally, was capable in lowering blood glucose by 42% at the fourth hour with a mean fall of 28% during 5 hours. Furthermore, administration of homogenized suspension of the vegetable pulp of *M. charantia* to 100 cases of moderate NIDDM subjects has been shown to cause a significant reduction (P<0.001) of post-prandial serum glucose in 86% cases and fasting glucose in 5% cases .Aqueous juice of *M. charantia* fruit also exhibited anti-hyperglycemic effect in pancreas of streptozotocin-treated diabetic mice. Moreover, this juice was demonstrated to possess anti-oxidant activity.(9)

The effects of dietary *M. charantia* freeze-dried powder on serum glucose level and lipid parameters of the serum and liver were studied in rats. Diets supplemented with and without cholesterol, as well as *M. charantia* freeze-dried powder in various percentage were fed for 14 days, the diet used were *M. charantia* freeze-dried powder at 0.5, 1 and 3% without an added dietary cholesterol and *M. charantia* at the level of 1% with or without 0.5% cholesterol and 0.15% bile acid. Dietary *M. charantia* resulted in a consistent decrease in serum glucose levels in rats fed with cholesterol-free diets, but not in those fed with cholesterol-enriched diets.The result indicated that *M. charantia* had little effect on serum lipid parameters, except for high density lipoprotein (HDL)-cholesterol. HDL-cholesterol levels tended to decrease by dietary cholesterol, while they were consistently elevated by dietary *M.charantia* both in the presence and absence of dietary cholesterol, indicating an antiatherogenic activity of *M. charantia.* In addition, *M. charantia* caused a marked reduction in the hepatic total cholesterol and triglyceride levels both in the presence and absence of dietary

cholesterol; the reduction of triglyceride levels in the absence of dietary cholesterol was in a dose-dependent manner. (9)

Streptozotocin-treated diabetic albino rats (50 mg/kg administered intraperitoneally) fed with 0.5% diet containing *M. charantia* for 6 weeks did not show any beneficial hypoglycemic effect and neither prevented diabetes related abnormalities in the levels of protein, urea and creatinine. In another study, feeding of 0.02, 0.1 and 0.5% w/w diet containing *M. charantia* for 8 weeks did not affect blood sugar, food intake, growth, organ weights and hematological parameters of normal adult rats. Notably, 0.5% diet caused a significant hypo-cholestrolemic effect. Single or repeated oral administration of *M. charantia* juice (10 ml/kg for 30 days) did not affect the results of oral glucose tolerance test in streptozotocin-treated diabetic rats. In addition, glycosylated hemoglobin concentration remained unchanged in treated and untreated diabetic rats. Results of that study were suggestive of the fact that viable beta cells were required to manifest the hypoglycemic activity of *M. charantia*. (9)

Oral administration of different *M. charantia* extracts showed a varying pattern of anti-hyperglycemic effect without altering the insulin response, suggesting a mechanism of action which is independent of intestinal glucose absorption and probably involves an extra-pancreatic effect. In addition, oral feeding of *M. charantia* juice to normal rats prior to glucose loading increased hepatic and muscle glycogen content while triglyceride content was not altered. In vitro study, the fruit juice increased glucose uptake by tissues without concomitant increase in tissue respiration. Moreover, aqueous extract of unripe fruits of *M. charantia* has also been demonstrated to partially stimulate insulin release from isolated beta-cell of obese-hyperglycemic mice. However, this effect of *M. charantia was* differed from D-glucose and other insulin secretagogues agent. It was not suppressed by L-epinephrine but was potentiated by the removal of Ca^{2+} which led to the insulin-releasing action is the result of perturbations of membrane functions. (9)

With regards to diabetic complication, daily administration of extract of *M. charantia* fruit (4 g/kg/day) for 2 months to alloxan-treated diabetic rats (120 mg/kg) delayed the development of cataract. The respective blood sugar level in the two groups was 307±81 and 66.37 mg%. (9)

Since *M. charantia* is quite valuable for diabetic control, its toxicological aspect is also important, prior to introducing this plant for clinical use.

M. charantia has been proven to be safe (no signs of nephrotoxicity and hepatotoxicity and any adverse influence on the food intake, growth organ weights and hematological parameters) in experimental animals when administered orally in low doses up to 2 months. However, relatively low toxicity of all parts of this plant was also reported when administered orally. Higher toxic effects were found in laboratory animals when higher doses were administered intravenously or intraperitoneally. In addition to its general toxicity, *M. charantia* also possess abortifacient activity. In this aspect, the fruit and seeds of this plant were more toxic than the leaf or aerial parts. The documented adverse effects of *M. charantia* are hypoglycemic coma and convulsions in children, reduced fertility in mice, a favism-like syndrome, increases in gamma-glutamyltransferase and alkaline phosphatase levels in animals, and headaches.(19)

Musa sapientum: **Banana (English)**

Bananas are used for different medicinal purposes including diabetes. Intragastric administration of fresh flower decoction (4 ml/kg) to hyperglycemic rabbits significantly decreased the hyperglycemic peak and/or the area under the glucose tolerance curve. In addition , oral administration of various doses (150, 200 and 250 mg/kg) of chloroform extract of *M. sapientum* flowers for 30 days also significantly reduced blood glucose and glycosylated hemoglobin as well as increased total hemoglobin in alloxan-treated diabetic rats (150 mg/kg administered intraperitoneally). The effect was highly significant at the dose of 250 mg/kg .(9)

Nelumbo nucifera: **Lotus (English)**

Oral administration of ethanolic extract of *N. nucifera* rhizome (400 mg/kg) significantly reduced the blood sugar level of normal, glucose fed hyperglycemic and streptozotocin-treated diabetic rats after 1 hour . The extract also improved glucose tolerance and potentiated the action of exogenously injected insulin. The activity of extract was 73 and 67% of that of tolbutamide in normal and diabetic rats, respectively (9)

***Syzigium cumini* (*Eugenia jambolana*): Black Berry (English)**

S. cumini seeds and decoction of dry leaves also have hypoglycemic effect. Oral feeding of *E. jambolana* (170, 240 and 510 mg/rat for 15 days) caused 50% reduction in blood glucose of normal fasted rats while chlorpropamide produced only 52% reduction. In addition, there was a 2.4–6.8-fold and 9.2-fold increase in cathepsin B activity (proteolytic conversion of proinsulin to insulin) by this plant extract and chlorpropamide, respectively. Oral administration of pulp extract of the fruit of *S. cumini* to normoglycemic and streptozotocin-treated diabetic rats exerted hypoglycemic activity in 30 min. This effect might be mediated by insulin secretion. In addition, the extract inhibited insulinase activity from liver and kidney. Oral administration of dried alcoholic extract of the seeds caused hypoglycemia and reduced glycosuria. While the aqueous extract of seeds of *S. cumini* (2.5 and 5.0 g/kg for 6 weeks) showed hypoglycemic (>glibenclamide) and anti-oxidant activity. The hypoglycemic effect was most prominent at the dose of 5.0 gm/kg whereas no significant effect was observed at 7.5 gm/kg dose. Daily administration of lyophilized powder of *E. jambolana* (200 mg/kg) caused maximum reduction of 73.51, 55.62 and 48.81% as compared to their basal values in mild (plasma sugar >180 mg/dl, duration 21 days), moderate (plasma sugar >280 mg/dl, duration 120 days) and severe (plasma sugar >400 mg/dl, duration 60 days) diabetic rats. In addition, the treatment also partially restored altered hepatic and skeletal muscle glycogen content and hepatic glucokinase, hexokinase, glucose-6-phosphate and phosphofructokinase levels. (9)

Tinospora cordifolia:

T. cordifolia is widely used as tonic, vitalizer and as a remedy for diabetes and metabolic disorders. Oral administration of 400 mg/kg of aqueous extract of *T. cordifolia* for 15 weeks showed a maximum hypoglycemic of 70.37, 48.81 and 0% of plasma sugar in mild (plasma sugar >180 mg/dl), moderate (plasma sugar >280 mg/dl) and severe (plasma sugar >400 mg/dl) diabetic rats, respectively. The hypoglycemic effect depended upon the functional status of the pancreatic beta cells. When water extract of root was used to study its effect on various parameters , 2.5, 5 and 7.5 mg/kg dose caused a significant reduction in blood glucose, brain lipid level, hepatic glucose-6-phosphatase, serum acid phosphatase, alkaline and lactate dehydrogenase and

increase in body weight, total hemoglobin and hepatic hexokinase in alloxanized diabetic rats (150 mg/kg, administered intraperitoneally) .(9)

***Vinca rosea* (*Catharanthus roseus*): Madagascar periwinkle (English)**

Oral administration of water-soluble fraction of ethanolic extract of *V. rosea* leaves (100, 250, 500 and 1000 mg/kg) showed a significant with dose-dependent reduction in blood sugar at 4 hour by 26.22, 31.39, 35.57 and 33.37%, respectively, in normal rats. In addition, oral administration of 500 mg/kg 3.5 hours before oral glucose tolerance test (10 g/kg) and 72 hours after streptozotocin-treated diabetic rats (50 mg/kg administered intraperitoneally) showed significant anti-hyperglycemic effects. No gross behavioral changes and toxic effect were observed up to 4 g/kg administered intraperitoneally .(9)

3. *Morus alba* L.

The nomenclature of this plant has been known in under various synonyms; *Morus alba* L., *Morus indica* L., *Morus atropurpurea* Roxb., *Morus merettiana* Jacq. Ex Burr. (20)

M. alba L. belongs to Moraceae family (division : Spermatophyta, class : Angiospermae, subclass : Dicotyledoneae, group : Thalamiflorae, order : Urticales). The common names of this plant are white mulberry, Russian mulberry, silkworm mulberry, China mulberry.

Botanical description and cultivation (21-25)

M. alba L. is a deciduous tree, small to medium-sized monoecious or diecious shrub or tree, up to 15 m. tall, wide-spreading, round-topped, trunk attaining 60 cm. in diameter. The stem is slender, yellowish green to brownish gray, smooth, more or less shining, slightly sweetish if chewed. The bark is light brown to gray and smooth, becoming devided into narrow scaly ridges. The twigs are thin, light-brown and glabous. The sap is milky. The leaf is alternate, simple, stipulate, viable in shape and degree of lobing, sometime unlobed but often 3- or 5- lobed, coarsely serrate or dentate, ovate to broad ovate, 6-15 cm. long and 5-10 cm. wide, acute or short acuminate, rounded or cordate at base, dark green and usually smooth above, pubescent on veins beneath or nearly glabous, long-petioled, 12*8 cm. on fruiting branched, up to 25*20 cm. on vigorous non-fruiting branches. The bud are imbricate, terminal-absent, laterals-small. 0.3-0.7 cm. long, ovoid, 3-6 scales, appressed, sharp- or blunt-pointed, light brown to reddish brown, often set oblique to leaf scar, margins of bud scales somewhat finely hairy. The flowers are small, greenish-yellow, in dense spikes to 2 cm. long, sepals 4, stamens 4, staminate and pistillate on different tree. Pistils with two styles ; staminate spike soon decidous ; pistillate spiks maturing into an aggregate fruit (syncarp) of drupelets. The fruits are small, 1-seed, numerous, ovoid to oblong-cylindric, crowed into cluster 10-20 mm. long, inkish or purplish to nearly black, edible long before ripe, sweet, but insipid. The seeds are brown, 1-1.2 mm. long. The pictures of *M. alba* L. were shown in fig. 1-3.

Fig. 1 *Morus alba* L.

1. fruiting twig; 2. infructescence; 3. female flower; 4. male inflorescence; 5. male flower (PROSEA)

(1)

(2)

(3)

Fig. 2 *M. alba* L.
(1) and (2) tree (3) fruits

(From Institute of Thai Traditional Medicine, Ministry of Public Health , Nonthaburi , Thailand)

Fig. 3 *M. alba* L. leaves

(From Institute of Thai Traditional Medicine, Ministry of Public Health , Nonthaburi , Thailand)

Ethnobotanical Information of *Morus alba* L.

M. alba L. have been consumed as food in Thailand and other countries . The various parts of mulberry have been used in traditional medicine by local people in many countries.

1. Leaves

The leaves of *M. alba* L. is one of the best known Oriental medicinal herbs. Medications based on the leaves of *M. alba* L. have been found to be effective as a hypoglycemic (26), astringent (27), diuretic , antihypertensive and antispasmodic (28).

In Thailand , the decoction and oral administration of leaves have been used for cough , fever , relieve sore throat , relieve thirst , sedative , reddening of the eyes and diaphoretic (29-34)

In Jordan, a decoction of leaf is used as diuretic and vermifuge (35)

In The island of Rodrigues, a decoction of leaf is used in case of hypertension and hernia (36).

In Yogoslavia and Peru , the hot water extract of leaves is used for antidiabetic.(37, 38)

In Spain , the hot water extract of leaves is used for hypoglyclemia and astringent (26).

2. Root bark

The root bark of *M.alba* L. has been traditionally used in Asian countries for medicinal purposes. It exhibits anti-inflammatory , hypoglycemic , antibacterial activities (39) , expectorant , diuretic , laxative (40), antiphlogistic (41) , antitussive agent to treat severe asthamtic attacks (42), a tonic (43), antipyretic actions (44) .

In Thailand , the decoction of root bark is used as laxative and antihelmintic . (29-34)

In China , the hot water extract of root bark is used orally as expectorant , diuretic , antitussive , to relieve fever and antiphlogistic.(45, 46)

3. Cortex of the root bark

The cortex of the root bark of *M. alba* L. is used for removing heat from the lung, relieving asthma and inducing diuresis (47).

4. Fruits

The fruits of *M. alba* L. are used to treat diseases of the liver and kidney (48), as a tonic , sedative (49).

In Thailand , the decoction and fruit are used orally as expectorant , laxative and to relieve sore throat. (29-34)

In China , the hot water extract and fruits are used orally as antiarthritis and to alleviate arthritic pain (45, 46)

In the Turkey , the fruits are used to treat reddening of the eyes , internally , concentrated fruit fuice is drunk 2-3 times a day for 3-4 days to cure reddening of the eyes. During condensation on fire , it is advised to expose the eyes to the vapour (49).

5. Bark

The bark of *M. alba* L. is used for antiphlogistic , diuretic (50).

6. Root

The whole root of *M. alba* L. is used for treatment of hypertension , rheumatism, eye problems and spastic children (48).

7. Branch

The young branch of the tree is used for treatment of hypertension and paralysis of arms and legs in China (48).

Chemical constituents

The chemical compounds present in various parts of *M. alba* L. are

Leaf

The chemical compounds presented in the leaf of *M. alba* L. are

1. Alkaloid compounds : (51)

1-deoxynojirimycin (fig.4) , N-methyl-1-deoxynojirimycin (fig.4) , 2-O-α-D-galactopyranosyl (GAL-DNJ) , fagomine , 1,4-dideoxy-1,4-imino-D-arabinitol and calystegin B_2 (fig.)

2. Flavonoid compounds : (52-54)

chalcomoracin (fig. 5) , quercitin , quercetin-3,O-β- D-glucopyranoside , quercetin-3-O-(6”-O-acetyl)β-D-glucopyranoside , quercetin-3-O-β-Dglucopyranosyl-(1-6)-β-D-glucopyranoside, quercetin-3-O-α-L-rhamnopyranosyl-(1-6)-β-D-glucopyranoside (rutin) , quercetin-3,7-di-O-β- D-glucopyranoside , isoquercitin , astragalin (kaempferol-3-O-β-D-glucopyranoside) (fig. 5),kaempferol-3-O-(6”-O-acetyl)β-D-glucopyranoside , kaemperol-3-O-α-L-rhamnopyranosyl-(1-6)-β-D-glucopyranoside , moracetin and mulberroside F

3. Other : (55-57)

protein (urease), coumarin (scopolin , skimmin), sesquiterpene (roseoside II), alcohol (3,5-dinitrobenzoates, n-butanol and β-γ-hexanol) , acid (ρ-phenyl phenacyl esters , 5-hydroxypipecolic acid) and aldehyde and ketone (methyl-ethyl ketone , 2,4-dinitrophenylhydrazones , n-butylaldehyde isobutylaldehyde and n-valeraldehyde)

Moreover , *M. alba* L. leaf contain high level of crude proteins, amino acids, copper, manganese, vitamin D , ascorbic acid , Vitamin B1 and folic acid (28).

Root bark

The chemical compounds presented in the root bark of *M. alba* L. are

1. Alkaloid compounds : (58)

1-deoxynojirimycin (fig. 4) , N-methyl-1-deoxynojirimycin (fig. 4) , fagomine , 3-epi-fagomine , 4-O-β-D-glucopyranosyl-fagomine , 1,4-dideoxy-1,4-imino-D-arabinitol(fig. 4) ,1,4-dideoxy-1,4-imino-D-ribitol (fig. 4) , calystegin B_1 (fig. 4) , calystegin B_2 (1α,2β,3α,4β-tetrahydroxy-nor-tropane) (fig. 4) , 1,4-dideoxy-1,4-imino-(2-O-β-D-glucopyranosyl)-D-arabinitol, 2-O-α-D-galactopyranosyl-1-deoxynojirimycin , 2-O-α-D-glucopyranosyl-1-deoxynojirimycin , 3-O-β-D-glucopyranosyl-1-deoxynojirimycin , 4-O-β-D-glucopyranosyl-1-deoxynojirimycin , 6-O-β-D-glucopyranosyl-1-deoxynojirimycin and (2R,3R,4R)-2 hydroxymethyl-3,4-dihydroxypyrrolidine-N-propionamide

2. Flavonoid compounds : (45, 47, 59-75)

Compound A , cyclomorusin , cyclomulberrin , cyclomulberrochromene , kuwanon A-F, kuwanon G (fig. 5), kuwanon H-I , K , L , S , Y , Z , mulberrin (fig. 5), mulberrochromene , moracenin A-D , sanggenons A , B , C , D , E , L, M , N , O , P , morusin (fig. 5) , moralbanone , oxydihydromorusin (morusinol) , mulberroside C , eudraflavone B hydroperoxide , leachinone G , α-acetyl-amyrin, moran 20K

3. Stillbene compounds : (40, 42, 50)

cis- mulberroside A , mulberroside A (fig. 6), mulberroside C , oxyresveratrol , oxyresveratrol-2,3'-O-β-D-diglucoside , oxyresveratrol-2,-O-β-D-glucorunide-3'-O-sulfate , oxyresveratrol-3'-O-β-D-glucopyranoside , β-sitesterol

4. Oxygen heterocycles : (68, 76-81)

Oxygen heterocycle compounds presented in root bark of *M. alba* L. are mulberrofuran A (fig. 8) , mulberrofuran B (fig.) , mulberrofuran F , G , K , M , N , O , P , Q

Fruit

The chemical compounds presented in the fruit of *M. alba* L. are

1. Alkaloids compounds : (51, 58)

1-deoxynojirimycin (DNJ) (fig. 4) , N-methyl-1-deoxynojirimycin (fig. 4) ,fagomine (FAG) , calystegin B_2 (fig. 4), 4-O-α-D-galactopyranosyl- calystegin B_2 , 1,4-dideoxy-1,4-imino-D-arabinitol (D-ABA) ,1,4-dideoxy-1,4-imino-(2-O-β-D-glucopyranosyl)-D-arabinitol , 2α,3β-dihydroxynortropane , 2β,3β-dihydroxy nortropane , 2α,3β,6exo-trihydroxynortropane , 2α,3β,4α-trihydroxynortropane, 3β,6exo-dihydroxynortropane , 3β,6β-dihydroxy nortropane (fig. 4) , 2-O-α-D-galactopyranosyl-1-deoxynojirimycin and 6-O-β-D-glucopyranosyl-1-deoxy nojirimycin.

2. Flavonoids compounds **:** (82)

Cyanidin and chrysanthemin (cyanidin-3-monoglucoside).

3. Amino acid compounds **:** (51)

Morusinic A , B , C , D , E and F .

Root

The chemical compounds of the roots of *M. alba* L. are alkaloids. These alkaloids are 1-deoxynojirimycin (fig. 4) , N-methyl- 1-deoxynojirimycin (fig. 4) ,fagomine , 3-epi-fagomine , 1,4-dideoxy-1,4-imino-D-arabinitol (fig. 4) , 1,4-dideoxy-1,4-imino-D-ribitol (fig. 4), 1,4-dideoxy-1,4-imino-(2-O-β-D-glucopyranosyl)-D-arabinitol , calystegin B_2 (1α,2β,3α,4β-tetrahydroxy-nor-tropane) (fig. 4) ,calystegin C_1 (1α,2β,3α,4β,6α-pentahydroxy-nor-tropane) , 2-O- and 6-O-α-D-galactopyranosyl-1-deoxynojirimycin , 2-O- , 3-O- and 4-O-α-D-glucopyranosyl-1-deoxynojirimycin and 2-O- , 3-O- , 4-O- and 6-O-β-D-glucopyranosyl-1-deoxynojirimycin**.** (83)

Heartwood

The chemical compounds presented in heartwood of *M. alba* L. are

1. Flavonoids compounds : (61, 84)

Morin , maclurin , 2,4,6,4'-tetrahydroxybenzophenone , kaempferol , dihydrokaempferol , quercetin , dihydromorin and norartocarpanone (5,7,2',4'-tetrahydroxyflavanone)

2. Stillbene compounds : (84)

Resveratrol (fig.6), oxyresveratrol (fig.6) , dihydroresveratrol , 3,4'-dihydroxy
dihydrostillbene and 3,2',4'-trihydroxydihydrostillbene

3. Benzenoid compounds : (85)

Albactalol (fig.7) , β-resorcylaldehyde and resorcinol

4. Oxygen heterocycle compounds : (84)

6,3',5'-trihydroxy-2-phenylbenzofuran.

Stem and Bark

The chemical compounds presented in stem and bark of *M. alba* L. are flavonoids. These flavonoids are mulberrin (fig.5) , cyclomulberrin , mulberrochromene and cyclomulberrochromene (61). Moreover , Bark of *M. alba* L. presented albanol A , B , mulberranol and tannin (86).

Shoot epidermis

The compounds isolated from shoot epidermis of *M.alba* L. were phytoalexins and flavonoids. These phytoalexins are Albanin F (0.4%) and G (0.3%) . (87, 88). These flavonoids are Albafuran A(0.006%) , B (0.0003%) and C (0.004%) (89, 90). All compounds possess minor antifungal activities. They completely inhibited spore germination of *Bipolaris leeriae* at concentration of 10^{-4} -10^{-5} M (87, 89, 90).

Branches

Polysaccharides were extracted from young branches of *M. alba* L. It consisted of arabinose (20.5%) , galactose (12.1%) , glucose (19.9%) , mannose (16.4%) , rhamnose (14.4%) and xylose (16.6%) (91).

Callus tissue

The most abundant chemical compounds presented in callus tissue of *M. alba* L. are flavonoids. Flavanoids presented in callus tissue are chalcomoracin , kuwanon J, Q, R, V and mulberrofuran T (92-94). The other compounds are diglucosides (mullberroside D, E ,F) (94) , steroids (stigmast-5-en-3β-o-7-one , β-sitosterol , campesterol , dihydrobrassieasterol) (95) , kuwanol E (96) and mulberrofuran E (97).

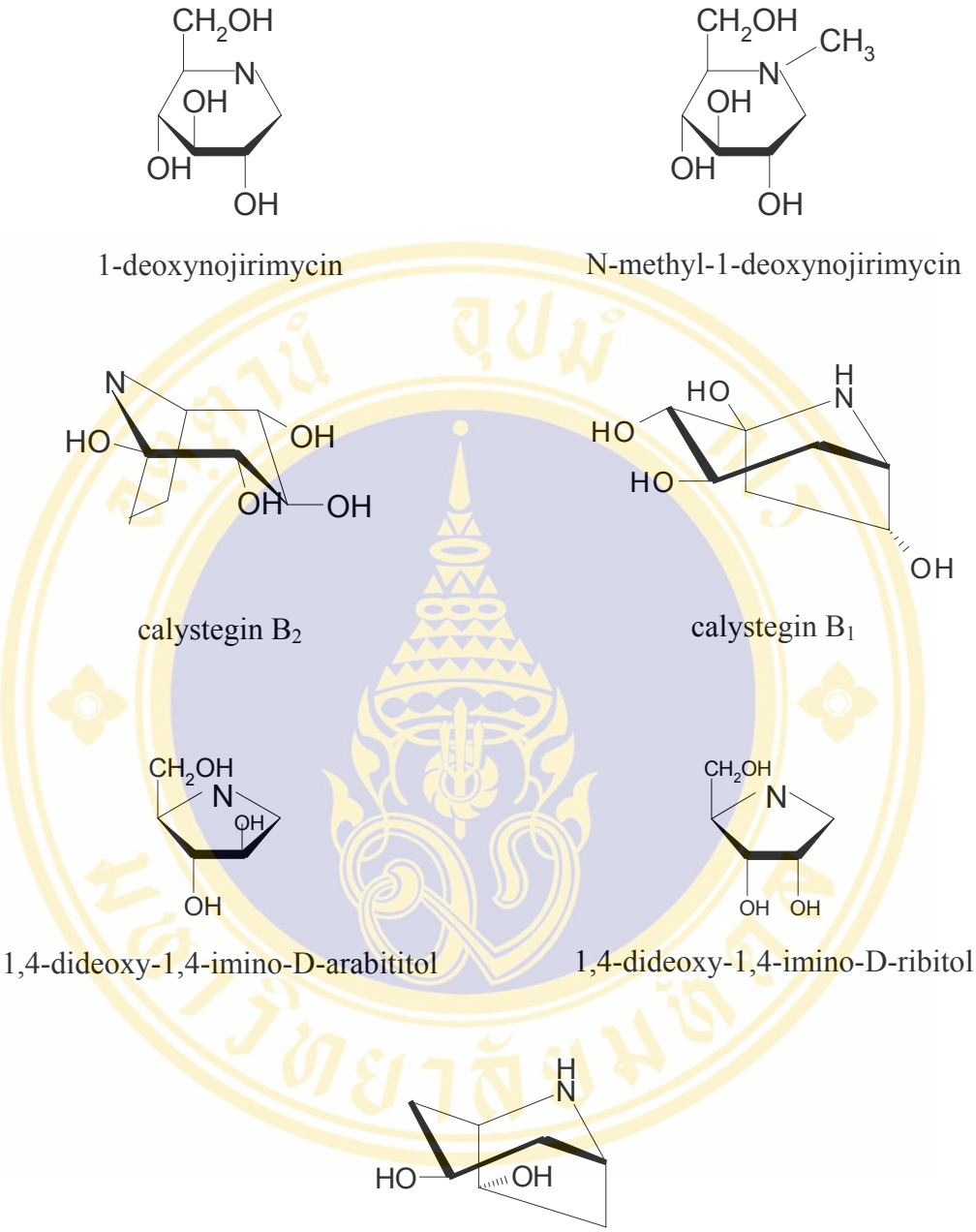


Fig. 4 structure of some alkaloid compounds of *M. alba* L.

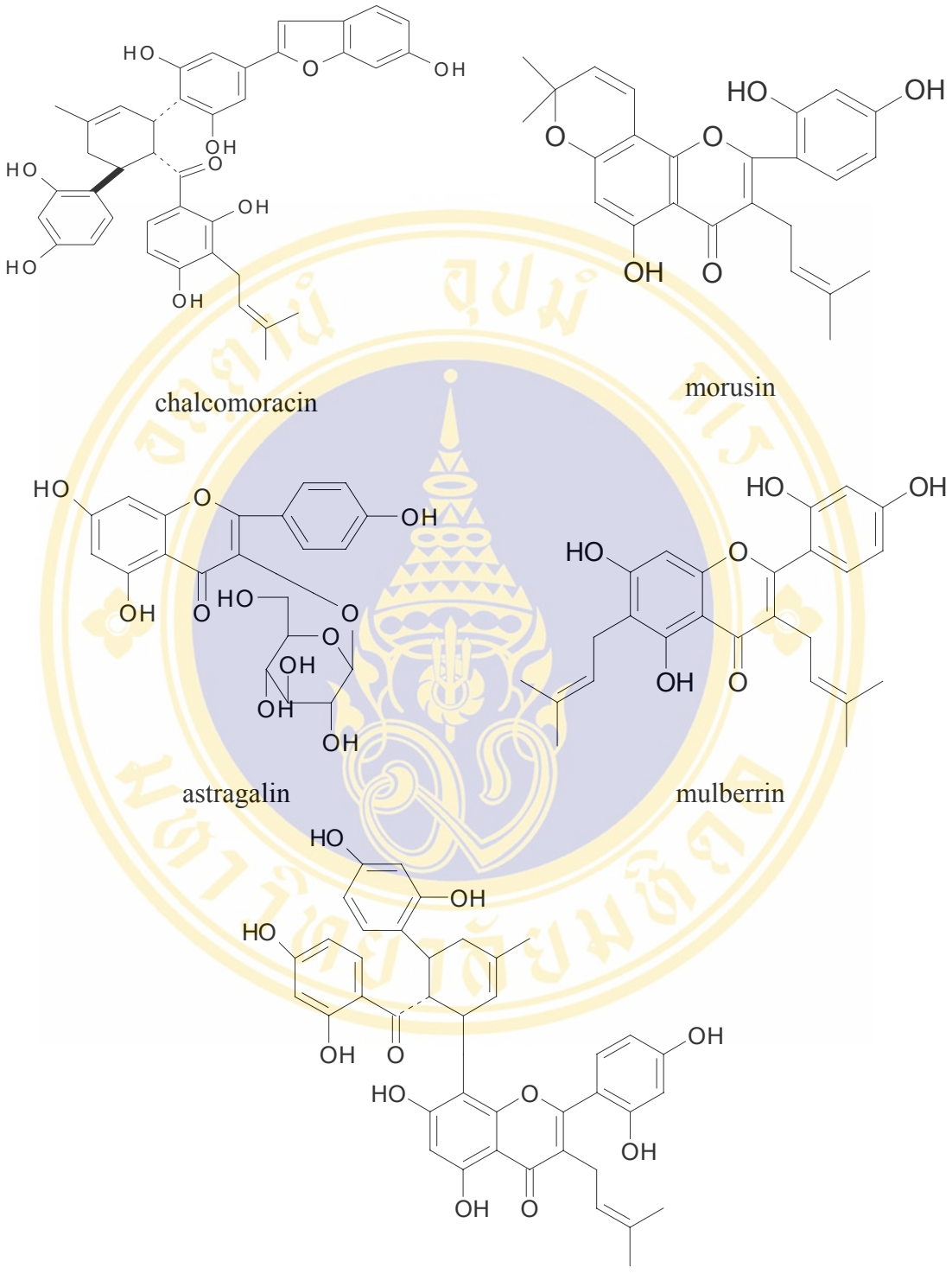
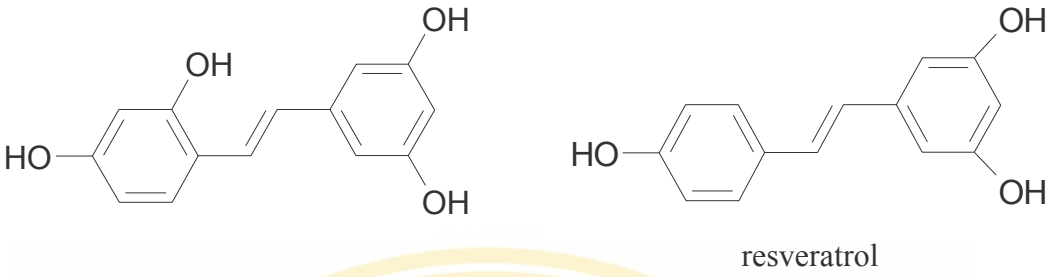
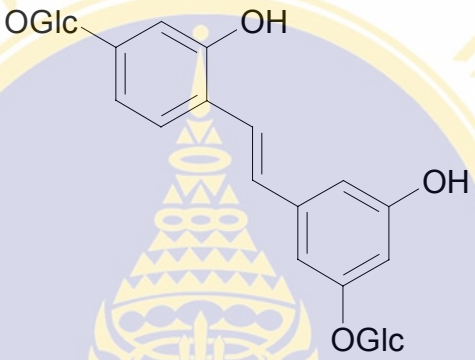


Fig. 5 structure of some flavonoid compounds of *M. alba* L.



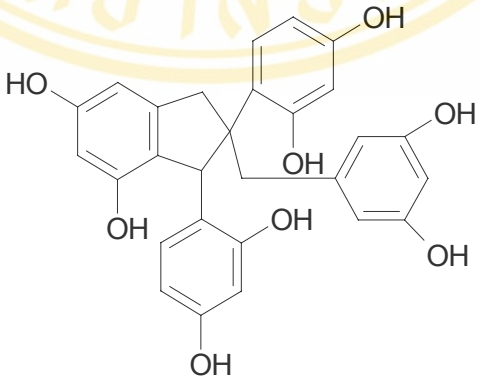
resveratrol

oxyresveratrol



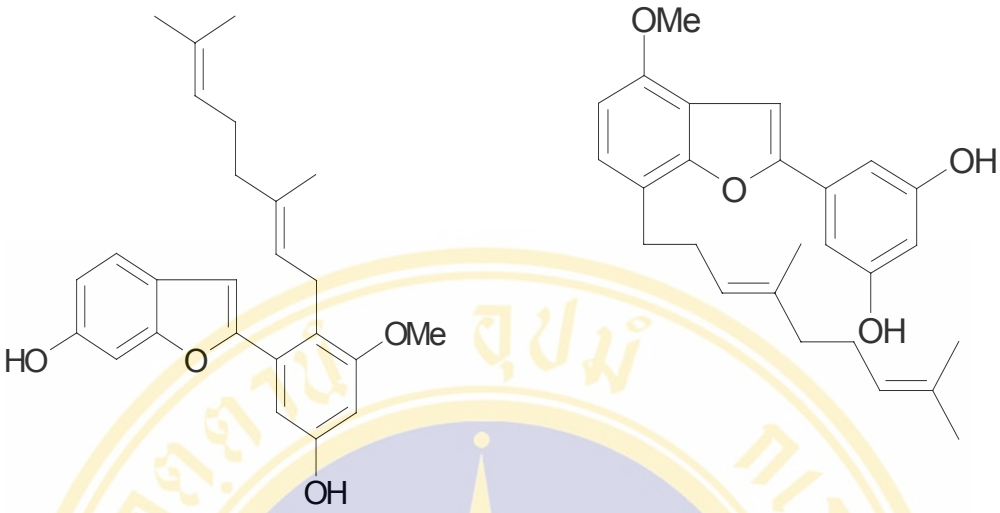
mulberroside A

Fig. 6 structure of some stillbene compounds of *M. alba* L.



albactalol

Fig. 7 structure of some benzenoid compounds of *M. alba* L.



mulberrofuran A mulberrofuran B

Fig. 8 structure of some oxygen heterocycle compounds of *M. alba* L.

Pharmacological evidences

The pharmacological effects of *M. alba* L. have been studied as follows ;

1. Antidiabetic activity

M. alba L. has been demonstrated to possess antidiabetic activity in experimental animals as well as in vitro studies. Evidence obtained from *M. alba* L. leaf were as follow.

The effect of leaves extracts were studied for 4 weeks in streptozotocin(STZ)-induced diabetic rats to investigate hypoglycemic and other effects including effects on body weight and food and fluid intakes. Oral administration of 150, 300 and 600 mg/kg of the ethanol extract once daily for 4 weeks resulted in reduction in plasma glucose, especially at the dose of 600 mg/kg ($p < 0.05$) at week 1. Moreover, hypoglycemic activity of the ethanol extract was dose-dependent. It also induced weight gain. In the other experiment, 150, 300 and 600 mg/kg of the freeze-dried water extract was orally administered once daily for 4 weeks. The dose of 300 mg/kg significantly decreased plasma glucose level ($p < 0.05$) at week 1. The freeze-dried water extract 150 mg/kg significantly decreased fasting plasma glucose level ($p < 0.05$) in a longer treatment period. Consequently, polydipsia and polyphagia were also improved in the freeze-dried water extract treated groups. The tea (2.34 g dried powder/kg), provided as a substitute for water ad libitum at 180 ml/rat/day in a concentration of 2.5 g/L, significantly decreased plasma glucose level ($p < 0.05$) at week 1 and 2. However, the plasma glucose reduction of tea was nearly the same as that of WM 300 mg/kg. (98)

The effect of leaf on the expression of nitric oxide synthase (NOS) in the hypothalamus of streptozotocin(STZ)-induced diabetic rats was investigated via nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) histochemistry. This extract at a concentration of 100 mg/kg was effective in limiting the enhancement in nitric oxide synthase (NOS) expression in streptozotocin(STZ)-induced diabetic rats. However, no significant effect on nitric oxide synthase (NOS) was observed in nondiabetic rats (26).

A single oral dose (500 mg/kg , dried plant) of *M. alba* L. leaves reduced blood glucose in alloxan-induced diabetic mice. But, in normoglycemic and glucose-induced

hyperglycemic mice, the blood glucose levels were not different in control and *M. alba* L. leave decoctions treated animals during 6 hours. The result indicated that *M. alba* L. leaves extracts only reduced blood glucose in alloxan-induced diabetic mice (93).

In another experiment , the hypoglycemic actions of the leaf were also examined using alloxan – induced diabetic rats. The effect on the fasting blood sugar , temporary hyperglycemia after adrenaline and temporary hyperglycemia after glucose were monitored. The result indicated that mulberry leaf when fed for 10 days to alloxan-induced diabetic rats slightly reduced the mean value of the sugar concentration in their blood. But, when it was given subcutaneously for 10 days, the 100% ethanol extract of leaf had no apparent effect on alloxan-induced diabetic rats and the mean value of the blood sugar concentration was nearly the same as that of controls. In fasting rats, an injection of mulberry leaf extract always contributed an apparent effect on the mean fasting blood sugar value. Moreover, it was capable of lowering both adrenaline or glucose hyperglycemia; the effect was more marked in adrenaline hyperglycemia. Elimination of glucose in the urine was always more rapid than the controls, so that urine became sugar free in the shorter time (99).

When leaf extract was evaluated for its effect on beta-cells of the endocrine pancreas of rabbits after subcutaneous and parenteral administration. The most manifested morphologic changes were observed after subcutaneous administration under chronic treatment. Degranulation of beta-cells of the Langerhans islets in these animals developed at various degree and was discovered by means of a light and electron microscope (100).

In addition, the 20% dried leaf infusion was evaluated in alloxan and streptozotocin induced hyperglycemic rats. A dose of 0.4 g/rat orally exhibited the hypoglycemic activity in alloxan-induced hyperglycemia whereas this effect was not found in streptozotocin-induced diabetic rats (101).

Since leaf extract exhibited some pharmacological important, the extract was then fractionated for evaluation. As expected, single intra-peritoneal dose of 200 mg/kg of ethanol insoluble fraction of hot water extract of leaf exhibited a potent hypoglycemic activity in fasted and nonfasted streptozotocin (150 mg/kg, injected intravenously) diabetic mice and the glucose level fell by 24.6±6% and 81.4±7.9%, respectively.

Increase in glucose uptake was postulated as the mechanism of hypoglycemic action (9).

The antihyperglycemic effects of six compound, N-containing sugars 1-deoxynojirmycin (DNJ), N-methyl-DNJ (N-Me-DNJ), 2-O-α-D-galactopyranosyl-DNJ (GAL-DNJ), fagomine, 1,4-dideoxy-1,4-imino-D-arabinitol (DAB), and 1,2α,3β,4α-tetrahydroxynortropane (calystegin B_2) derived from mulberry leaves were investigated in streptozocin (STZ)-induced diabetic mice 6 hour after injection. GAL-DNJ and fagomine lowered the blood glucose level in a dose-dependent manner. The ED_{50} values (95% confidence limits) were 115.0 (96.8-136.7) mmol/kg and 142.4 (130.5-155.3) mmol/kg, respectively. The ED_{50} values with 95% confidence limits were 41.0 (31.8-52.7) mg/kg for hot water extract and 33.9 (26.6-43.1) mg/kg for ethanol-insoluble extract from mulberry leaves. GAL-DNJ and fagomine are the most active among six compounds in producing antihyperglycemic effects. Fagomine (3 mM) but not GAL-DNJ (3 mM) potentiated 8.3 mM glucose-induced immunoreactive insulin release from isolated perfused rat pancreas. Thus, the mechanism of antihyperglycemic effect induced by fagomine may be mediated via the potentiation of the insulin release. (102).

In addition to the chemical constituent from leaf extract, compound from root bark was also studied for its hypoglycemic effect. Moran A (a compound of root bark) elicited remarkable hypoglycemic effects in normal and alloxan-induced diabetic mice. Moreover, an aqueous methanol (1:1) extracts of root bark at concentration 20 g/kg (crude drug equivalent) exhibited a significant hypoglycemic effect following intraperitoneally administration to normal mice. This effect hypoglycemic activity was exhibited in a dose-dependent manner at 7 hours after administration (46).

2. Antifungal activity

The water extraction of dried leaf was screened for antifungal activity against *Fusarium oxysporum* f. sp. *lentis*. A concentration of 1 g/ml of the extracts exhibited strong fungiotoxicity (96% myocardial inhibition of *F. oxysporum* f. sp. *lentis*) (103).

Chalcomoracin, is a major phyoalexin of the leaves. This compound completely inhibited germination of *Fusarium roseum* and *Bipolaris leesiae* at concentration of 10^{-4} -10^{-5} M (52).

The ethanol extract of root on *Trichophylon rubrum* cultivated in Sabouraud's glucose agar plate was examined. This extract inhibited fungal growth (104).

Mulberrofuran A , a compound from rootbark, was tested for its antimicrobial activity using the agal streak method . At concentration of 25 μg/ml, this compound was active against *Trichophyton mentagrophytes* (76).

Phytoalexins from *M. alba* infected by *Fusarium solani mori* S-26 were testes against 30 species of plant pathogenic fungi using the holeslide method and 24 species of bacteria using the cup method on their antifungal activity. Moracin A inhibited the growth of 27 fungi at 56 ppm while Moracin C and D inhibited growths of *Rosellina necatrix* and *Sclerotinia trifoliorum* at 3.5 ppm. Moreover, moracin M showed strong activity on *Xanthomonas* (105).

Leachinone G and mulberroside C, two flavonoids isolated from the root bark of *M. alba* L.. The antiviral activity of compounds were determined using that viral cytopathic effect assay. The antiviral activity was expressed in μg/ml as 50% inhibitory concentration (IC_{50}), and cytotoxicity on vero cells were expressed as 50% cytotoxic concentration (CC_{50}). Leachinone G showed potent antiviral activity (IC_{50} = 1.6 μg/ml, CC_{50} = 15.5 μg/ml), whereas mulberroside C showed weak activity (IC_{50} = 75.4 μg/ml, CC_{50} = 250 μg/ml) against herpes simplex type 1 virus (HSV-1) (106).

3. Anti-HIV activity

The water extraction of *M. alba* L. was tested for the ability to inhibit the activities of murine retroviral reverse transcriptase and human deoxyribonucleic acid (DNA) polymerase. A concentration of 80 μg/ml of extract exhibited weak activity to inhibit RNA reverse transcriptase and α-DNA polymerase (107).

4. Anti-inflammatory activity, Analgesic and antipyretic activity

The 100% methanol extracts of mulberry leaf was evaluated for their inhibiting effect of prostaglandin E_2 production (for COX-2 inhibitors) and nitric oxide formation (for iNOS inhibitors) in lipopolysaccharide (LPS)-stimulated RAW264.7 cells, a mouse macrophage cell line. The extract suggested the potential inhibitors of iNOS activity (more than 70% inhibition at the test concentration 10 μg/ml), but it had

a little effect on COX-2 activity (less than 20% inhibition at the test concentration 10 μg/ml) (108).

The mulberry leaf was tested for its antipyretic effect. Male rats were randomly distributed into four groups : the experimental groups (given a crude extract of 1.25 g/kg BW), the reference standard group (paracetamol 250 mg/5ml) , the positive control group (2 ml NSS) and the negative control group (given only food and water). Fever was induced by injecting subcutaneously with the fungi C. albicans suspended in NSS. Rectal temperature was taken in an interval of one hour after the drug administration which lasted for 11 hours. Significant antipyretic effects were observed between the second and sixth hours after the administration of drugs for both the crude extract and paracetamol (109).

Intraperitoneal administration of butanol and water extracts from rootbark at dose of 2 g/kg were found to have analgesic effect in acetic acid-induced stretching behavior in writhing test in mice. Oral administration of these extracts in the same dose were also active in acetic acid and tail pressure test in mice. Moreover , the butanol extract of root bark at dose of 5 g/kg (oral) had weak analgesic effect in dextran-induced edema in rats. But, water extract at the same dose exerted potent analgesic action in dextran and carrageenan-induces pedal edema (110) .

5. Antimicrobial activity

The in vitro antimicrobial activity of methanol extracts of *M. alba* L. dried plant was studied using the agar dilution method against selected microorganisms. The antimicrobial activity were screened and the minimal inhibitory concentrations (MIC) were established. The extract was capable in inhibiting *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Candida albicans* at concentration >11.0 g/l and against *Mycobacterium phlei* at concentration 11.0 g/l (27).

The root bark of *M. alba* L. also exhibited antimicrobial activity against food poisoning microorganisms. The chloroform fraction of the root bark was reported to possess strong inhibitory activity against *Bacillus subtitis* and the acetic acid fraction was effective against *S. aureus*, *B. Subtitis* and *Escherichia coli* (111).

The methanol extracts of root bark had potent antibacterial activity against *Streptococcus mutans*. Its active component was identified to be sanggenon C, and the

minimum inhibitory concentration (MIC) was 25 μg/ml. The inhibitory effect of this component on the cellular adherence of *S. mutans* to glass surface was greater than berberine (an alkaloid from *Hydrastis canadensis*, family Berberidaceae, has been shown to leave antimalarial and antipyretic activity) in the presence of glucosyltransferase and sucrose in vitro. These results indicated that sanggenon C may play an important role in inhibiting plague formation and caries incidence (112).

The hydrophobic solvents such as n-hexane extract of root bark has been used to inhibit the gram-negative bacteria associated with the peridontal disease. In addition, 1-menthol and/or 1-carvone may be added into the extracts to enhance the antibacterial activity (113).

Kuwanon G was isolated from the ethyl acetate fraction of methanol extract. It was evaluated for its antibacterial activity by the minimum inhibitory concentration (MIC) test and the viable cell count method. MIC of kuwanon G against *S. mutans*, the microbe causing dental caries, was 8.0 μg/ml. It completely inactivated *S. mutans* at the concentration 20 μg/ml in 1 min. It also possessed preferential antimicrobial activity when tested against other cariogenic oral bacteria *Streptococcus sobrinus , Streptococcus sanguis* and a periodontal pathogen *Porpyromonas gingivalis* with MIC value of 8.0 μg/ml. In contrast, no susceptible activity was noted against *C. albicans , Actinobacillus actimomycetemcomitans, Lactobacillus acidophilus* and *Lactobacillus casei* (MIC 1,000 , 1,000 , >1,000 and > 1,000 μg/ml, respectively). Thus, kuwanon G might be a potential oral care agent, preventing dental caries, particularly due to its fast bactericidal effect against *S. mutans* (114).

Mulberrofuran A , a compound from rootbark , was determined for its antimicrobial activity by the agal streak method. It was effective against Gram-positive bacteria but inactive against Gram-negative bacteria. The minimum inhibitory concentration against *S. aureus , Streptococcus faecalis , B. subtilis* and *Mycobacterium sp.* were 6.25 , 3.12 , 3.12 and 1.56 μg/ml , respectively (76).

6. Antioxidant activity

The antioxidative activity of butanol extract of leaf as well as of isoquercitin, the main compound of butanol extract of leaf have been investigated by determining their effect on a stable radical agent. Both butanol extract of leaf and isoquercitin were

found to scavenge the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. Then, the extract was tested with copper-induced oxidative modification of rabbit and human low-density lipoprotein (LDL). The oxidation of LDL was spectrophotometrically monitored by changing in absorbance at 234 nm accompanied by the formation of conjugated dienes and measured the formation of thiobarbiturate acid reacting substances (TBARS). It was found that both butanol extract of leaf and isoquercitin were capable in inhibiting the formation of conjugated dienes and TBARS in copper-induced oxidative modification of rabbit and human low-density lipoprotein (LDL). These results indicated that the mulberry leaf could inhibit the oxidative modification of LDL and suggested that it may prevent atherosclerosis (115).

In order to search for new active-oxygen scavengers from natural resource. The extracts of fruits was evaluated for its scavenging activity on superoxide anion radical ($O_2^{\bullet}$) and hydroxyl radical ($HO^{\bullet}$) by electron spin resonance (ESR) technique. The water and methanol extract of mulberry fruits exhibited an inhibitory effect of 88.1 and 51.0% on the formation of 5,5-dimethyl-1-pyrroline-N-oxide (DMPO)-OH adduct, respectively. Moreover , they also inhibited 20.9 and 25.5% the production of DMPO-OOH and/or DMPO-OH, respectively. Thus, the water extract exerted a strong scavenging activity against hydroxy radical ($HO^{\bullet}$)(116).

Oxyresveratrol and 5,7-dihydrocoumarin-7-methyl ether, the compounds isolated from the ethanol extracts of *M. alba* L. were superoxide scavenger with the IC_{50} values of 19.1±3.6 and 3.81±0.5 microM, respectively. Moreover, oxyresveratrol also exhibited DPPH free radical scavenging effects with the IC_{50} values of 23.4±1.5 microM (117).

To examine the radical scavenging effects of quercetin-3,-O-β-D-glucopyranoside and quercetin-3,7-di-O-β- D-glucopyranoside, two flavonoids from the leaves of *M. alba* L. were tested by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The radical scavenging activities (EC) of the flavonoids isolated were 17.6 and 14.6 μg/ml, respectively. Also, the antioxidant activity of these flavonoids wea determined by measuring lipid peroxides using TBA. Quercetin-3,7-di-O-β- D-glucopyranoside inhibited the lipid peroxidation by 29.2% and 21.0% at concentration of 10^{-1} and 10^{-2} mg/ml ,respectively. However ,quercetin-3,-O-β- D-glucopyranoside did not show any inhibitory activity of lipid peroxidation at a low concentration (54)

7. Antitumor activity

The polysaccharides of root bark in the mixed aqueous extracts had antitumor activity against sarcoma 180 in mice. Studies with various cellular components of the immune system suggested that the mixed aqueous extract stimulated the tumor-related immune system with no effects on the overall immune system (118).

Morusin, a flavonoid isolated from the root bark of *M. alba* L., had been reported as an antitumor promotor. In a two stage carcinogenesis experiment, it inhibited tumor promotion by teleocidin on mouse skin initiated with 7,12-dimethylbenz(a)anthracene (DMBA)(119).

The induction of the differentiation of human promyelocytic leukemia cell line (HL-60) by Quercetin-3,-O-β-D-glucopyranoside and quercetin-3,7-di-O-β- D-glucopyranoside, two flavonoids isolated from mulberry leaves, was examined with analysis of a cell surface antigen using fluorescence-activated cell sorter (FACS). These compounds exerted a significant inhibitory effect on the growth of HL-60 cell at the concentration of $2*10^{-4}$ M. Quercetin-3,7-di-O-β- D-glucopyranoside also induced differentiation at the HL-60 cell line to express CD66b and CD14 antigens. (54)

8. Cardiovascular and central nervous system activity

Administration of mulberry leaf or their 1-butanol extracts resulted in the suppression of the increases in serum lipids, hepatic enlargement and atheromatous thickening of arterial intima in hypercholesterolemia rabbits (115).

The effect of mulberry on the blood pressure was monitored in dogs on a kymographic drum paper. The extract solution was injected intravenously into the femoral vein. The blood pressure was recorded from the carotid artery through a cannula containing saturated sodium citrate solution by a lever floating on mercury in a manometer. The diluted ethanol leaf extracts when injected into the dogs, lowered the blood pressure which remained subnormal for a short time (28).

The 50% hot methanol leaf extracts were tested for its inhibitory effect on dog kidney angiotensin converting enzymes. The extracts were fractionated by MCI gel and Sephadex LH-20 gel chromatrograph to concentrate the possible inhibitors. At the concentration of 20 μg/ml, 86% inhibition of ACE activity was obtained (120).

The crude extract of the leaf was found to induce the agglutination of certain animal erythrocytes and the hemagglutination could be inhibited by N-glycolylneuraminic acid (121).

The ether extracts of the root bark at concentration 6 mg/kg (injected intravenously) and the methanol extracts of the root bark at concentration 3 mg/kg, injected intraveneously to rabbit produced a significant hypotensive effect. Moreover, intravenous injection of kuwanon G and H ,flavonoids isolated from root bark, at concentration 0.1-3.0 mg/kg led to an equal manner transient dose-dependent decrease in arterial blood pressure in anesthesized rabbit (65).

The water extracts of root bark intensely depressed the blood pressure in mouse (50).

The 90% ethanol of dried root cortex (1 kg) generally sedate the mice. The LD_{50} of extract injected intraveneously was 32.7 mg/19 g. It lowered the blood pressure of rabbits; the effect that was reversed by atropine. It also inhibited the movement of the isolated frog heart but the effect was counteracted by atropine. Regarding to the vascular effect, it dilated the ear vein of rabbits, while it constricted the blood vessels of the hind legs. It stimulated the movement of the isolated intestine and uterus of the rabbit and caused a slight rise in salivery secretion. By intravenous and intraarterial injections, it showed little effect on the nictitating membrane of the cat, but when directly applied to the cervical autonomic ganglion, it inhibited the contraction of the membrane (122).

The butanol extraction of rootbark had cardiotonic activity when tested in rat atrium at a concentration of 1 mg/ml (110). Moreover, the butanol extraction of rootbark showed hypotensive activity in rat at a dose of 50 mg/ml (IV) , but water extraction was weaker in the same dose (110).

Flavonoids were isolated from root bark and their hypotensive activity were explored. Kuwanon G (1 mg/kg, injected intravenously in rabbits) (123) , kuwanon H (1 mg/kg injected intravenously in rabbits) (124), sanggenons C (1 mg/kg , in rabbits) (67), sanggenons D (0.5-2.0 mg/kg, injected intravenously in rats) (125) , mulberrofuran F (126) , moracenin A(74) and moracenin B (73) were proposed to be the major substance involved in this hypotensive activity.

9. Cytotoxic activity and antimutagenic effect

The water extracts of *M. alba* L. root bark exhibited cytotoxic activity on K-562, B380 human leukemia cells and B16 mouse melanoma cells at concentration higher than 1 mg/ml. Result from studied on DNA fragmentation, PARP cleavage , and nuclear condensation assay indicated that those cells exposed to the extract underwent apoptosis.The extract induced apoptosis of tumor cells by inhibiting microtubule assembly (127).

The fruit of *M. alba* L. was also investigated for its antimutagenic activity in genotoxicants (X-rays, N-methylnitrosourea , cyclophosphamide , NaF) and aging mutants in bone marrow cell chromosomes from mice and rats. The extracts showed an ability to decrease the frequency of chromosome aberrations (128).

Moreover, Cudraflavone B and oxyresveratrol from the ethanol extracts of *M. alba* L. were hepatoprotective with EC_{50} value of 10.3 ±0.42 and 32.3±2.62 microM, respectively, on tacrine-induced cytotoxicity in human liver derived Hep G2 cells (117).

10. Estrogenic effect

The effect of leaf extracts on the uterus of the rat was examined, in vitro, by using an electrically heated glass jar bath for isolated organs. The movements of rat uterine strip were recorded on a smoked drum paper, slowly moving by a light metal lever.The water and ethanol leaf extracts produced stimulation of the uterine musculature. The effect was examined by using rat uterus at various stages of sex cycle. The action was more pronounced in oestrous but less marked in pregnant and non oestrous uterus (99).

11. Gastrointestinal tract activity

When water extract of rootbark was administed orally at a dose of 3 g/kg, a laxative effect in mice was obtained (110). In addition, The butanol extract of rootbark at variable concentrations was active smooth muscle relaxant in ileum, taenia caecum, antrum and vas deferens of guinea pig and rats (110).

The effect of ethanol leaf extracts on the intestine of the rat was also examined, in vitro, by using an electrically heated glass jar bath for isolated organs. The dose-

dependent inhibition of the intestinal movement was obtained the minimum effective concentration to produce these effects was 0.2 ml of a 20% solution added to 50 ml bath. (99).

The effects of hot water extracts and six compounds of N-containing sugar , 1-deoxynojirimycin , N-methyl-1- deoxynojirimycin , 2-O-α-D-galactopyranosyl-deoxynojirimycin , fagomine , 1,4-dideoxy-1,4-imino-D-arabitol and 1,2α,3β,4α-tetrahydroxynortropane (calystegin B_2) , derived from mulberry leaf were investigated on pilocarpine-induced saliva secretion in STZ-induced diabetic mice. The extracts (100 and 200 mg/kg, administered intraperitoneally) significantly potentiated the pilocarpine-induced salivary flow but not the protein content. The component compounds (37.5-300 μM/kg) potentiated the saliva secretion , and the potency order was 1,4-dideoxy-1,4-imino-D-arabitol > fagomine > 2-O-α-D-galactopyranosyl-deoxynojirimycin. Surprisingly, only fagomine significantly increased the protein content in the saliva (129).

12. Immunomodulating activity

A polysaccharide isolated from *M. alba* L. (PMA) root bark was used for examined for its the immunomodulating activity in murine splenic lymphocytes. In the presence of mitogens, PMA enhanced the proliferation of splenic lymphocytes in a synergistic manner. However, PMA suppressed primary IgM antibody production from B cells, which was activated by lipopolysaccharides, a polyclonal activator, or immunized with a T-cell dependent antigen sheep red blood cell. The immunomodulating activity of PMA seemed to be distinct from those of other plant-originated polysaccharides (130).

In contrast, the water extracts of polysaccharide isolated from branches was not active either in enhancing immunoactivity or in inhibitory immunoactivity when tested on its humoral , cellular and nonspecific immunity (91).

13. Renal activity

The butanol and water extract of rootbark were weak diuretic in rats when administered intraperitoneally and orally at a doses of 500 mg/kg (110).

14. Respiratory activity

The butanol and water extract of rootbark had slight antitussive action in guinea pigs when administered by intraperitoneally injection at a dose of 500 mg/kg (110).

15. Tyrosinase inhibitor

An 85% methanol extract of dried *M. alba* L. leaves inhibited the tyrosinase activity that convert dopa to dopachrome in the biosynthetic process of melanin. Mulberroside F(moracin M-6, 3'-di-O-β-D-glucopyranoside) which was obtained after the bioactivity-guided fractionation of the extract showed inhibitory effect on tyrosinase activity and on the melanin formation in melan-A cells. Moreover, this compound with a concentration of 100 μg/ml exhibited 51.6% inhibition on the dopa oxidase activity of mushroom tyrosinase, where as 50% inhibition was shown at concentration of 0.29 μg/ml. Tyrosinase activities in the HM3KO cell line treated with Mulberroside F also decreased in a manner correlating with mushroom tyrosinase. Mammalian tyrosinase activity was suppressed as well to 50% at a concentration of 68.3 μg/ml of Mulberroside F. These results suggest that Mulberroside F isolated from *M. alba* L. leaves might be used as a skin whitening agent .(131)

Similar result was obtained when the extraction of melberry leaf was evaluated for anti-tyrosinase activity spectrophotometrically. These extract exhibited a mark inhibitory effect on tyrosinase. The presence of oxyresveratrol seems to justify the high tyrosinase inhibition (132).

Besides leaf extract, the extracts of young twigs of *M. alba* L. also exhibited potent inhibitory activity against tyrosinase and melanogenesis (133).

In addition, the radicis cortex of *M. alba* L. was selected to test for their potent inhibitory effect on this enzyme activity too. These extract was subjected to sequential fractionations with methylene chloride, ethyl acetate, n-butanol, and polar residue. The inhibition effects on dopa oxidase activity of tyrosinase was found in ethyl acetate fraction. This fraction exhibited 50% inhibition on dopa oxidase activity of tyrosinase at the concentration of 12 μg/ml (134).

Oxyresveratrol (2,3',4,5'-tetrahydroxystillbene), a naturally occurring compound particularly found in *M. alba* L., exhibited a potent inhibitory effect on dopa oxidase activity of tyrosinase which catalyzes the rate-limiting steps of melanin biosynthesis.

Oxyresveratrol at concentration of 0.3-0.5 μM inhibited this enzyme potently and dose-dependently (25-84%), where as 50% of inhibition was obtained at the concentration of about 1 mM. Oxyresveratrol inhibited the dopa oxidase activity of tyrosinase via a non-competitive manner ($Ki = 9.1*10^{-7}$ M) when L-dopa was used as a substrate and was about 150-fold more potent than resveratrol (3,4',5-trihydroxystillbene) as tyrosinase inhibitor(135)

A cosmetic lotion that contain 1% by weight of *M. alba* L. essence has been evaluated to be capable in preventing UV-induced skin-darkening in guinea pigs (136).

As the result, Kuwanon P, X and Y extracted from the root bark are now used in cosmetic preparations as skin-whitening agent (137).

16. Miscellaneous effect

The water extract of dried fruit was examined for its effect on blood ethanol concentration in rat after oral administration of ethanol. The effect on the activities of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) in rat liver was also monitored. The extracts caused a rapid elimination of ethanol from the blood of normal rats when they were administered orally at 30 minutes before oral ethanol administration or simultaneously with ethanol. This rapid elimination of ethanol seemed to be resulted from the inhibitory effect of the extract on the decrease of ADH activity in rat liver cytosol, and in supplementing nicotinamide adenine dinucleotide (a coenzyme in ethanol metabolism). Thus, these effects were believed to be the protection against alcohol activity (138).

The effects of various flavonoids and related compounds isolated from the root bark of mulberry tree on rat platelet lipoxygenase and cyclooxygenase products formed from arachidonic acid were studied. The formations of 12-hydroxy-5,8,10-heptadecatrienoic acid (HHT) and thromboxane B_2 (cyclooxygenase products) were inhibited by morusin dose-dependently, while the formation of 12-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE) (12-lipoxygenase products) was slightly stimulated at low concentrations (10^{-6}–10^{-5} M) but was inhibited at high concentrations (10^{-4}–10^{-3} M). Thus, Morusin inhibited HHT and thromboxane B_2 more strongly than the formation of 12-HETE.

Oxydihydromorusin also inhibited the formations of HHT and thromboxane B_2 dose-dependently, while it showed no effect on the formations of 12-HETE. Furthermore, oxydihydromorusin inhibited the formations of thromboxane B_2 more strongly than the formation of HHT.

The formations of 12-HETE, HHT and thromboxane B_2 were inhibited by kuwanon C in a dose-dependent manner. Additionally, mulberrofuran A also found to inhibit the formations of HHT and thromboxane B_2 , through less effectively, while it stimulated the formations of 12-HETE from arachidonic acid in platelet homogenates at concentration (10^{-6}–10^{-3} M). Contrary, Mulberrofuran F did not effect arachidonate metabolism in rat platelet homogenates (139).

17. Toxicity assessment

The LD_{50} value of the ethanol/water (1:1) extracts of the entire plant administered intraperitoneally to mice was more than 1.0 g/kg (140).In addition, butanol and water extracts of rootbark administered orally, intraperitoneally and intravenously in rat at the dose of 10,10 and 5 g/kg, respectively, were not toxic (110).

CHAPTER 3
MATERIALS AND METHODS

Materials

The compound used in this study and their sources were as follow :

95% alcohol (The Government Pharmaceutical Organization, Thailand)

Aluminium potassium sulphate (BDH Chemicals LTD Poole, England)

Chloral hydrate (Merck Company)

Citric acid (BPH Chemicals LTD Poole, England)

Diethyl ether (BDH Chemicals LTD Poole, England)

Di-Sodium hydrogen phosphate anhydrous (Carlo erba Co.,Milano)

Eosin (Merck Company)

Ethyl alcohol absolute (Carlo erba Co.,Milano)

Ethylenediamine tetra-acetic acid disodium salt dehydrate (EDTA) (Fluka Guarantee)

Formaldehyde 40% w/v (Carlo erba Co.,Milano)

Gelatin powder (Gelatin edible xx40,Brazil)

Hematoxylin (Sigma Chemical Company, St.Louis, MO USA)

Paraplast plus (Oxford® Labware, St.Louis, MO USA)

Permount Solution (Fisher scientific)

Phloxine B (Merck Company)

Sodium dihydrogen phosphate dehydrate (Fluka Guarantee)

Sodium hydrogen carbonate (Carlo erba Co.,Milano)

Sodium iodide (Merck Company)

Xylene (BDH Chemicals LTD Poole, England)

Acide urique enzymatique PAP 150 kit (bioMerieux, France)

Albumine-kit (bioMerieux, France)

Cholesterol RTU kit (bioMerieux, France)

Creatinine cinetique kit (bioMerieux, France)

Enzyline® ASAT/GOT 20 kit (bioMerieux, France)

Enzyline® ALAT/GPT 20 kit (bioMerieux, France)

Enzyline® PAL standardize 50 kit (bioMerieux, France)

Glucose RTU kit (bioMerieux, France)

HDL Cholesterol Direct kit (bioMerieux, France)

Proteines-kit (bioMerieux, France)

Triglyceride Enzymatique PAP 150 kit (bioMerieux, France)

Uree cinetique kit (bioMerieux, France)

Equipments

Cover Slipper (Leica CV 5000, K.V. Science Co.,Ltd.,Thailand)

Disposible Micotome blades (model Feather® S35,Japan)

Embed Station

Float Bath Temperature Control (model FU-T 003)

Histomatic tisse processor (model Path Centre, Shandon, England)

Hot Plate

Lyophilizer (model Heto FD 8.0, Hetolab Equipment, Denmark)

Paraffin dispenser (model Lipshaw Paraffin Dispensor, Lishaw Manufacturing Company, Detroit, USA)

Rotary microtome (model M1R, Shandon, England)

Slide autostainer (Leica autostainer XL, K.V. Science Co.,Ltd.,Thailand)

Ultracentrifuge (Model H-103N, Kokusan Corperation, Japan)

Vitalab Flexor (Serial nr. 0-2225, Vital Scientific N.V., Netherland)

Methods

1. Animal and Treatment

1.1 Acute Toxicity Study

Swiss albino mice and Wistar rats, both sexes, were used in acute toxicity study. The animals were purchased from the National Laboratory Animal Center, Mahidol University, Salaya. Their weights were between 25-30 grams for Swiss Albino mice and 120-150 grams for Wistar rats, when they arrived. All animals were fed *ad libitum* with an animal pellet diet (C.P., Thailand) and had free access to tap water. For their intimation with environment, all animals were brought up in the animal room about 1-2 weeks and three animals were raised in one cage. Then, they were divided into 5 groups, each group contained 10-12 animals. The routes of administration were intraperitoneal injection and oral. The control group was treated with normal saline solution. Doses used for intraperitoneal injection were 1.66, 3.33, 5 and 6.66 g/kg body weight and for oral were as high as 5 g/kg. Five doses of the *M. alba* L. leaf extract were administered to determine the dose-related toxic effects, and the animals were observed for at least 24 hours to detect the possible delayed toxicity or death. The percent of death in 24 hours were used to determine LD_{50} value. The LD_{50} was the dose of *M. alba* L. leaf extract that produced death in 50% of the treated animals . The LD_{50} value was obtained from the probit plot.

1.2 Subchronic Toxicity Study

Male and female Wistar rats were purchased from the National Laboratory Animal Center, Mahidol University, Salaya. Their weights were between 80-100 grams when they arrived. The rats were fed *ad libitum* with an animal pellet diet (C.P.,Thailand) and had free access to tap water. For their intimation with environment, all animals were brought up in the animal room about 1-2 weeks and three animals were raised in one cage. Then, they were divided into 4 groups, each group contained 10 animals. The route of administration was oral. The control group was treated with normal saline solution. The three treated groups were administered *Morus alba* L. leaf extract in three different doses. *M. alba* L. leaf extract concentrations used were of 1,2 and 3 g/kg body weight /day. All animals were treated

chronically for 60 days. Body weight was monitored weekly during treatment period and the growth rate were then constructed. The rats were killed at the end of treatment. Blood samples were collected on the last day of treatment for the determination of blood glucose, blood urea nitrogen (BUN), creatinine, uric acid, alkaline phosphatase, aspartate aminotransferase (AST) , alanine aminotransferase (ALT), total protein, albumin, high-density lipoprotein (HDL), low-density lipoprotein (LDL), cholesterol, triglyceride as well as hematology. Microscopic examination of the major organs was also performed.

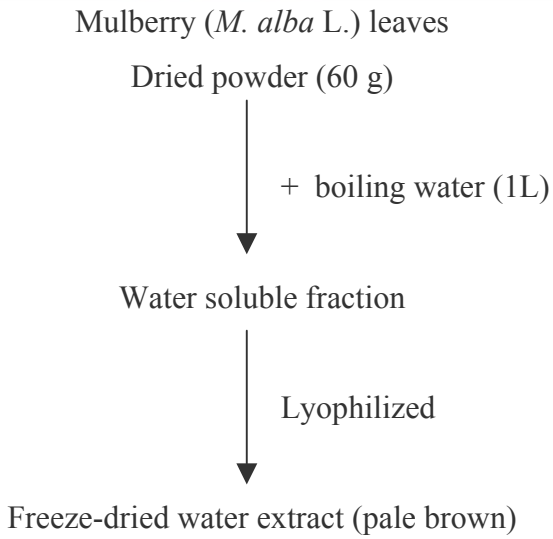
2. Plant Material

The leaf of *M. alba* L. (Nakhon Ratchasrima 60 strain) were provided and identified by Dr.Omboon Luanratana, Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University. The leave sample were collected from plants 2 years of age, in April-May 2002 from the Seri Cultural Research Center, Nakhon Ratchasrima Province

The freeze-dried water extract was provided by Mr.Chattiya Vorakunpinij, Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University.

Sixty grams of dried leave powder was steeped in 1 L. boiling water and stirred for 15 minutes. Then, allowed to cool at room temperature. The decoction was filtered through muslin cloth. The filtrate was freeze dried by lyophilizer.

The extraction and preparation of mulberry leaf extracts were shown in the following diagram.



M. alba L. leaf extract in lyophilized form were dissolved in normal saline solution and adjusted to the certain concentrations.

3. Blood Glucose Content Assay

Blood Glucose was determined by using Glucose RTU kit for Glucose assay. The content of glucose was expressed as milligram of glucose per 1 decilitre of blood (mg/dl).

4. Blood Urea Nitrogen (BUN) Content Assay

Blood Urea Nitrogen (BUN) was determined by using Uree cinetique UV 250 kit for BUN assay. The content of BUN was expressed as milligram of BUN per 1 decilitre of blood (mg/dl).

5. Creatinine Content Assay

Creatinine was determined by using Creatinine cinetique kit for Creatinine assay. The content of creatinine was expressed as milligram of creatinine per 1 decilitre of blood (mg/dl).

6. Uric Acid Content Assay

Uric acid was determined by using Acide urique enzymatique PAP 150 kit for Uric acid assay. The content of uric acid was expressed as milligram of uric acid per 1 decilitre of blood (mg/dl).

7. Alkaline Phosphatase Activity Assay

Alkaline phosphatase activity was determined by using Enzyline® PAL standardise 50 kit for Alkaline phosphatase assay. The activity of alkaline phosphatase was expressed as unit per liter (U/l).

8. Aspartate Aminotranferase Activity Assay

Aspartate aminotransferase (AST) activity was determined by using Enzyline® ASAT/GOT 20 kit for AST assay. The activity of AST was expressed as unit per liter (U/l).

9. Alanine Aminotransferase (ALT) Activity Assay

Alanine aminotransferase (ALT) activity was determined by using Enzyline® ALAT/GPT 20 kit for ALT assay. The activity of ALT was expressed as unit per liter (U/l).

10. Total Protein Content Assay

Total Protein was determined by using Proteines-kit for Total Protein assay. The content of total protein was expressed as gram of total protein per 1 decilitre of blood (g/dl).

11. Albumin Content Assay

Albumin was determined by using Albumine-kit for Albumin assay. The content of albumin was expressed as gram of albumin per 1 decilitre of blood (g/dl).

12. Cholesterol Content Assay

Cholesterol was determined by using Cholesterol RTU kit for Cholesterol assay. The content of cholesterol was expressed as milligram of cholesterol per 1 decilitre of blood (mg/dl).

13. High Density Lipoprotein (HDL) Content Assay

High Density Lipoprotein (HDL) was determined by using HDL Cholesterol Direct kit for HDL assay. The content of HDL was expressed as milligram of HDL per 1 decilitre of blood (mg/dl).

14. Triglyceride Content Assay

Triglyceride was determined by using Triglycerides enzymatique PAP 150 kit for Triglyceride assay. The content of triglyceride was expressed as milligram of triglyceride per 1 decilitre of blood (mg/dl).

15. Low Density Lipoprotein (LDL) Content

The content of low density lipoprotein (LDL) was expressed as milligram of triglyceride per 1 decilitre of blood (mg/dl) will be claculated from cholesterol, triglyceride and HDL level. The calculation is

LDL = cholesterol - HDL – (triglyceride/5)

16. Hematologic Examination

Hemaological parameters were analyzed by Baker ® Instruments Hematology series 150 Cell Counter. The parameter determined were red blood cell count, hemoglobin, white blood cell count and differential count (neutrophils, band, eosinophils, lymphocytes and monocytes).

17. Histopathological Examination

17.1. Tissue preparation and fixation

The rats were sacrificed at the end of treatmet. The brain, heart, lung, liver, spleen, kidney and bone marrow were isolated for histological determination.

Fixation preserved tissue samples by stopped autolytic change and allowed tissue to remain unchanged by subsequent treatment.(141)

All organs were immediately fixed in 10% buffer neutral formalin solution (see appendix) for 1 week. The specimens were then ready for tissue processing. Back skeleton bone was selected for bone marrow sample. After the back skeleton bone was fixed in 10% buffer neutral formalin solution for 1 week, it was then fixed in 10% EDTA (see appendix) for at least another week. The second step was for decalcification of bone.

17.2. Tissue processing

The three stages of tissue processing were dehydration, clearing and infiltration. The sequential steps designed to remove the extractable water from tissue specimens and replace it with a medium that solidifies to allow sectioning.

Ethyl alcohol was used for dehydration. The concentration of ethyl alcohol were 80%, 90% and 100% , respectively. Xylene was used for clearing and paraffin was used for infiltration.

If an automatic tissue processor was used, the total processing time were 14-16 hours. These included ;

1. 80% alcohol, 1 hour.
2. 90% alcohol, 3 changes, 1 hour each.
3. Absolute alcohol, 3 changes, 1 hour each.
4. Xylene, 3 changes, 1 hour each.
5. Paraffin, 3 changes, 1 hour each.
6. Paraffin, under vacuum, 1 hour.
7. Embed.

17.3. Embedding

Embedding was the process of surrounding tissue with a paraffin to facilitate the cutting of thin sections.

17.4. Sectioning

For tissue sectioning, the appropriate thickness of section was about 3-4 micrometers. Gelatin was used as section adhesives. After that, all the sectioned tissue slides were put into an oven at 170-180 °C for 15 minutes.

17.5. Staining

Mayer's hematoxylin and Eosin-Phloxine solution (see appendix) were used for staining. The staining steps was shown in Fig. 9

Fig. 9 Stain steps

The staining nuclei was blue, cytoplasm and most other tissue structures were pink to red.

17.6. Mounting

The final step in the preparation of a slide was to cover the portion containing the tissue with thin glass, a coverslip. This made the slide permanent and permits microscopic examination. Synthetic resins (Permount®) as mounting media was used for mounting.

Statistical Analysis

The results were expressed as mean ± the standard error of the mean (S.E.M). The data of drug-treated animals were compared to control group by using Student's *t*-test. A p-value of less than 0.05 ($p<0.05$) or 95% confidence limit was considered statistically significant difference.

CHAPTER 4
RESULTS

1. Acute Toxicity Study

Acute Toxicity Study in Rats

The *M. alba* L. leaf extract was administered intraperitoneally at doses of 1.66, 3.33 and 5 and 6.66 g/kg body weight. The abnormal signs and symptoms of animals that observed were sedation, respiratory depression and decrease in motor activity.The percent of death in 24 hours was used to determine LD_{50} value as shown in Fig. 10-12. The LD_{50} value was determined from a probit plot. The LD_{50} values, intraperitoneally route, were 4.742, 4.999 and 5.092 g/kg body weight for male, female and both sexes, respectively. As seen in figures, the dose of 1.66 g/kg body weight was not included. In our experiment, the dose did not cause any death. Thus, it is not convertable to probit value for plotting.

Acute Toxicity Study in Mice

The *M. alba* L. leaf extract was administered intraperitoneally at doses of 1.66, 3.33, 5 and 6.66 g/kg body weight. The abnormal signs and symptoms of animals that observed were sedation and decrease in motor activity. The percent of death in 24 hours was used to determine LD_{50} value as shown in Fig. 13-15. The LD_{50} value was determined from a probit plot. The LD_{50} values, intraperitoneally route, were 3.275, 4.278 and 3.797 g/kg body weight for male, female and both sexes, respectively. As seen in figures, the dose of 6.66 g/kg body weight was not included. In our experiment, the dose caused 100% death. Thus, it is not convertable to probit value for plotting.

In acute toxicity study, the *M. alba* L. leaf extract was administered into mice and rats orally at doses as high as 5 g/kg.There was no death in all groups of animal. The abnormal sign and symptom of animals that observed were sedation and decrease in motor activity. Moreover, all animals were recovered within 15-30 minutes.

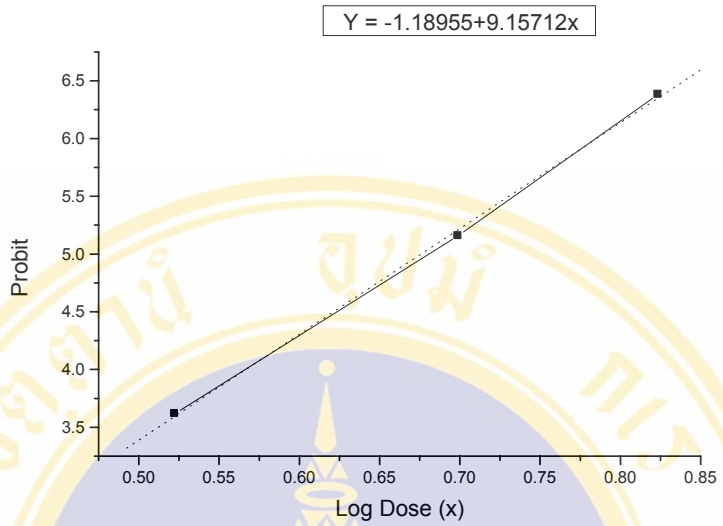


Fig. 10 Probit plot for the determination of the LD_{50} value of *Morus alba* L. leaf extract administered intraperitoneally to male rats. The LD_{50} value was 4.742 g/kg body weight.

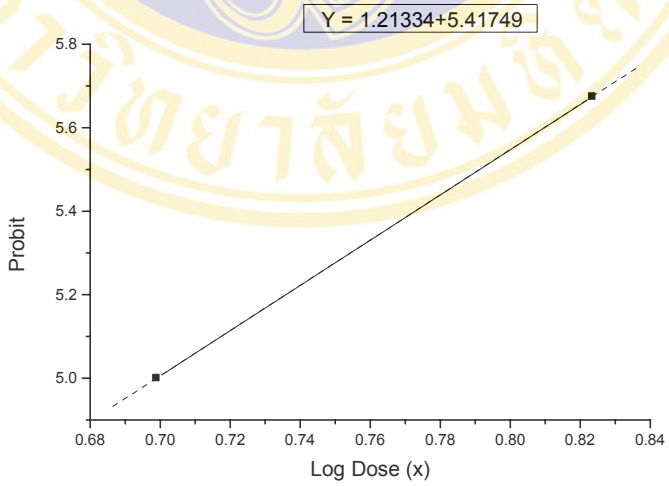


Fig. 11 Probit plot for the determination of the LD_{50} value of *Morus alba* L. leaf extract administered intraperitoneally to female rats. The LD_{50} value was 4.999 g/kg body weight. (The dose of 3.33 g/kg body weight was not included in the graph since 0% was not convertable to probit value)

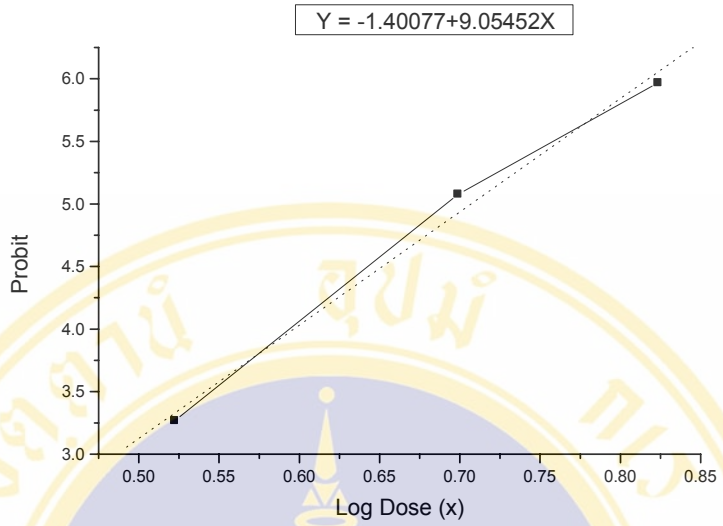


Fig. 12 Probit plot for the determination of the LD_{50} value of *Morus alba* L. leaf extract administered intraperitoneally to male and female rats. The LD_{50} value was 5.092 g/kg body weight.

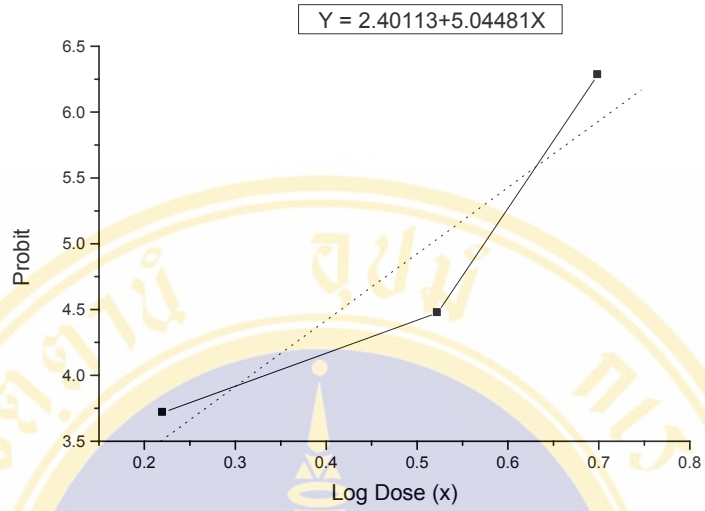


Fig. 13 Probit plot for the determination of the LD_{50} value of *Morus alba* L. leaf extract administered intraperitoneally to male mice. The LD_{50} value was 3.275 g/kg body weight.

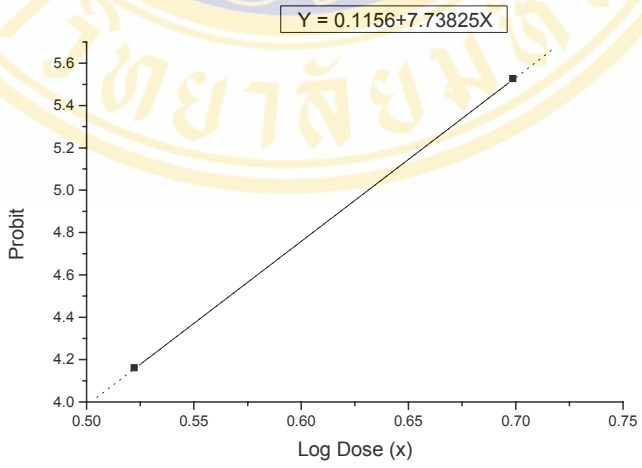


Fig. 14 Probit plot for the determination of the LD_{50} value of *Morus alba* L. leaf extract administered intraperitoneally to female mice. The LD_{50} value was 4.278 g/kg body weight. (The dose of 1.66 g/kg body weight was not included in the graph since 0% was not convertable to probit value)

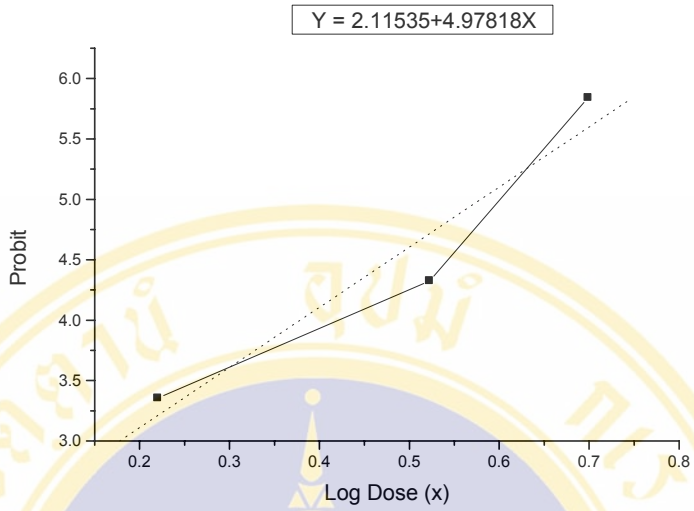


Fig. 15 Probit plot for the determination of the LD_{50} value of *Morus alba* L. leaf extract administered intraperitoneally to male and female mice. The LD_{50} value was 3.797 g/kg body weight.

2. Subchronic Toxicity Study in rats

2.1. Duration of subchronic toxicity study

The *M. alba* L. leaf extract was administered orally to rats of both sexes, once daily for 60 days, at doses of 1, 2 and 3 g/kg body weight/day.

2.2. Observation for abnormal signs and symptoms

The rats were observed daily. There was no death in all groups of rats during the treatment period. Moreover, the abnormal sign and symptom were not found throughout the period of study.

2.3. Body weight

The body weight of rats were monitored weekly. The growth curve was shown in table 1-2 and Fig 16-18. As shown in the growth curve, the body weight increased in normal rate. *M. alba* L. leaf extract did not affect weight gain significantly when compared to the non-treated animals.

Table 1 Weight of male rats treated with normal saline (control) and *M. alba* L. leaf extract for 60 days

Duration of treatment (week)	Weight of rats (g.)			
	Control (n = 10)	*M. alba* L. leaf extract 1 g/kg BW/day (n = 10)	*M. alba* L. leaf extract 2 g/kg BW/day (n = 10)	*M. alba* L. leaf extract 3 g/kg BW/day (n = 10)
0	149±4.19	157±4.66	160±4.21	160±2.44
1	217±4.84	222±6.39	223±5.36	212±2.73
2	255±8.33	258±6.20	268±6.68	253±4.82
3	282±11.11	294±7.35	306±7.79	287±4.52
4	323±11.63	330±8.06	336±9.73	315±6.13
5	353±13.24	344±9.31	350±10.69	338±6.80
6	371±14.16	363±11.65	371±11.49	346±7.35
7	390±16.17	380±12.73	391±12.65	361±7.51
8	409±14.94	391±13.28	398±12.85	371±6.77

All value are expressed as mean ± S.E.M.

Table 2 Weight of female rats treated with normal saline (control) and *M. alba* L. leaf extract for 60 days

Duration of treatment (week)	Weight of rats (g.)			
	Control (n = 10)	*M. alba* L. leaf extract 1 g/kg BW/day (n = 10)	*M. alba* L. leaf extract 2 g/kg BW/day (n = 10)	*M. alba* L. leaf extract 3 g/kg BW/day (n = 10)
0	137±2.59	141±2.25	141±2.17	143±4.14
1	176±2.72	171±3.87	171±2.14	175±3.69
2	193±3.89	190±3.94	188±2.62	193±4.67
3	213±4.66	205±3.71	204±2.92	208±5.54
4	224±5.56	217±3.50	213±3.01	220±4.70
5	237±6.87	231±4.30	225±3.63	218±5.18
6	247±6.90	241±4.65	236±3.92	235±4.92
7	251±6.27	250±5.10	241±4.51	244±5.21
8	256±7.26	248±3.64	245±4.36	244±5.68

All value are expressed as mean ± S.E.M.

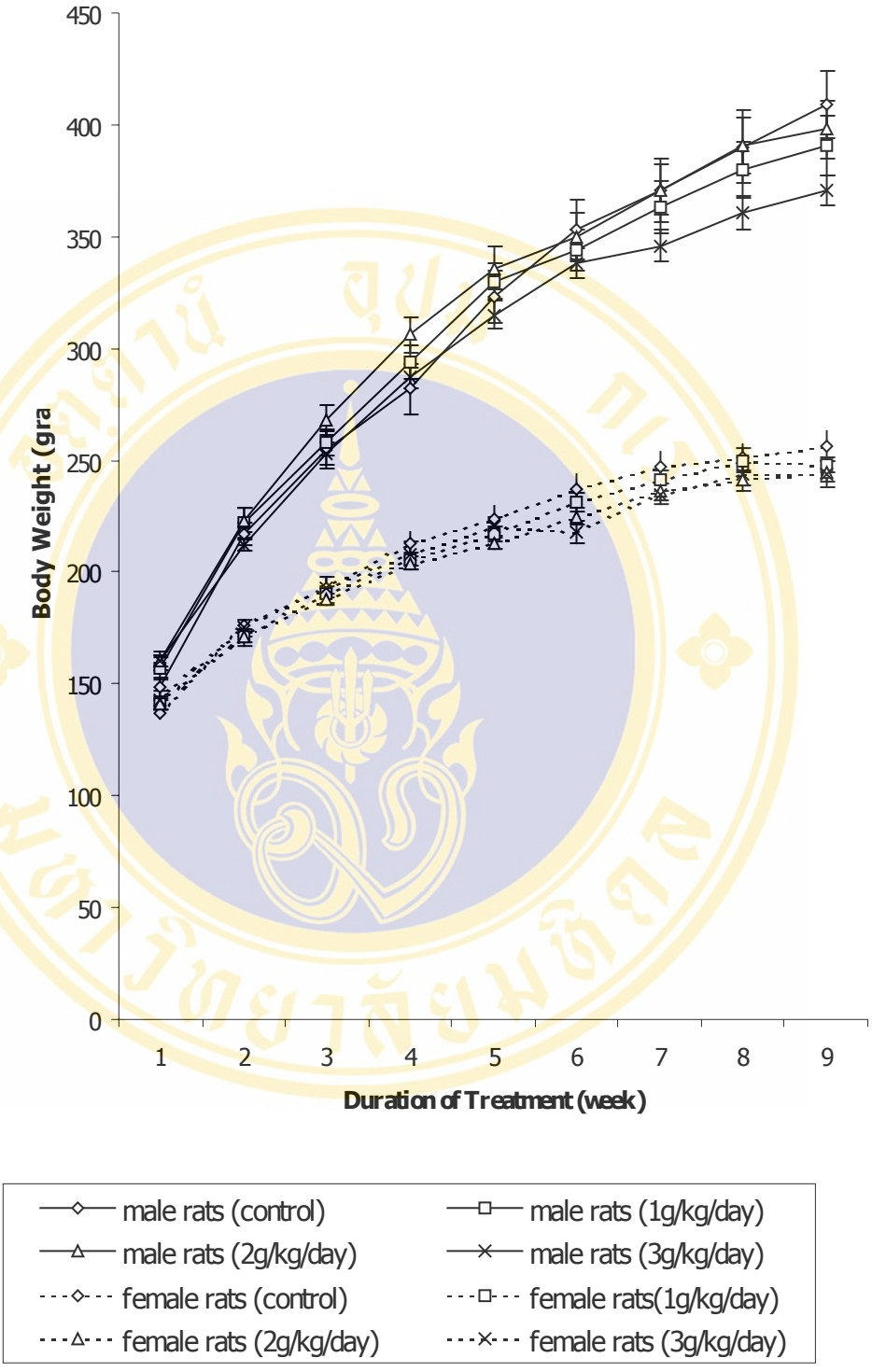


Fig. 16 Growth curve of male and female rats treated with *Morus alba* L. leaf extract , once daily for 60 days

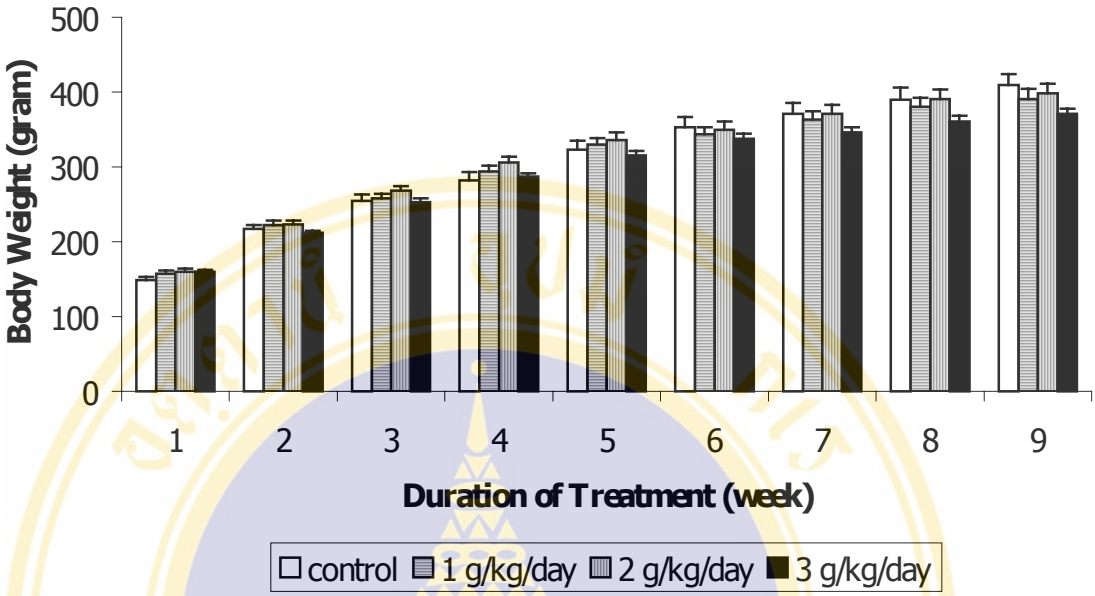


Fig. 17 Growth rate of male rats treated with *Morus alba* L. leaf extract , once daily for 60 days

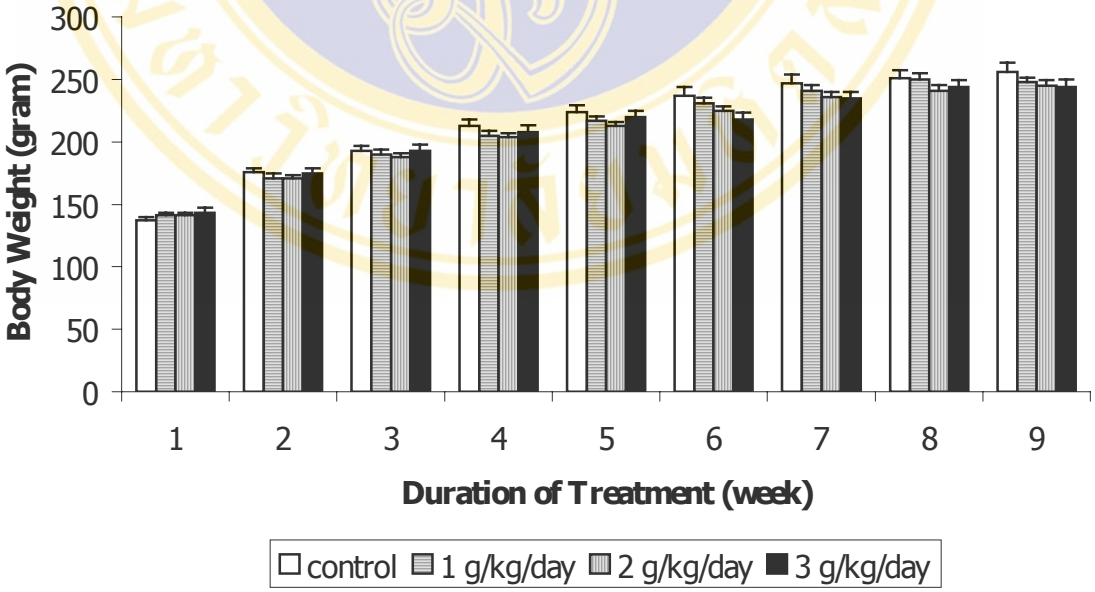


Fig. 18 Growth rate of female rats treated with *Morus alba* L. leaf extract , once daily for 60 days

2.4. Blood Examination

The blood samples were collected at the end of the treatment period by cardiac puncture. Animals were fasted overnight prior to blood collection.

2.4.1. Blood Glucose Content Assay

Blood glucose concentrations of the control animals and of *M. alba* L. leaf extract treated groups were shown in table 3 and fig. 19-20. Almost all doses of *M. alba* L. leaf extract did not significantly alter their blood glucose levels in both male and female rats, when compared to the control group. Only the dose of 3 g/kg body weight in female rats increased blood glucose level significantly.

Table 3 Blood glucose level (mg/dl) in male and female rats treated with normal saline (control) and *M. alba* L. leaf extract, once daily for 60 days

Treatment	Blood glucose level (mg/dl)	
	Male rats	Female rats
Control (normal saline) (n = 10)	138.00±9.90	107.90±5.39
M. alba L. leaf extracts 1 g/kg/day (n = 10)	148.90±7.20	114.30±7.01
M. alba L. leaf extracts 2 g/kg/day (n = 10)	161.70±8.86	98.20±2.70
M. alba L. leaf extracts 3 g/kg/day (n = 10)	143.60±6.99	138.00±13.25*

All value are expressed as mean ± S.E.M.

(*: significant differences from control; $p<0.05$)

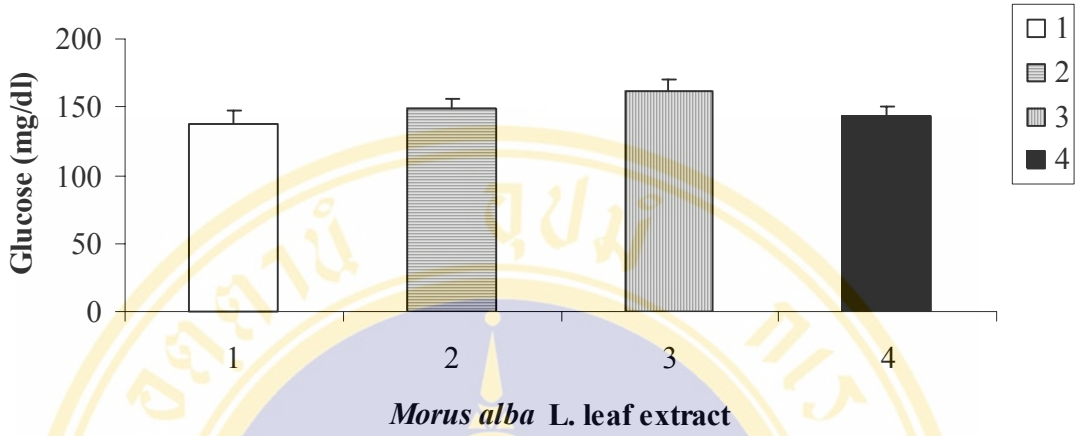


Fig 19 Blood glucose levels (mean ± S.E.M) in Wistar male rats administered with (1) normal saline (control), (2) *M. alba* L. leaf extract 1 g/kg body weight/day, (3) *M. alba* L. leaf extract 2 g/kg body weight /day, (4) *M. alba* L. leaf extract 3 g/kg body weight /day for 60 days.

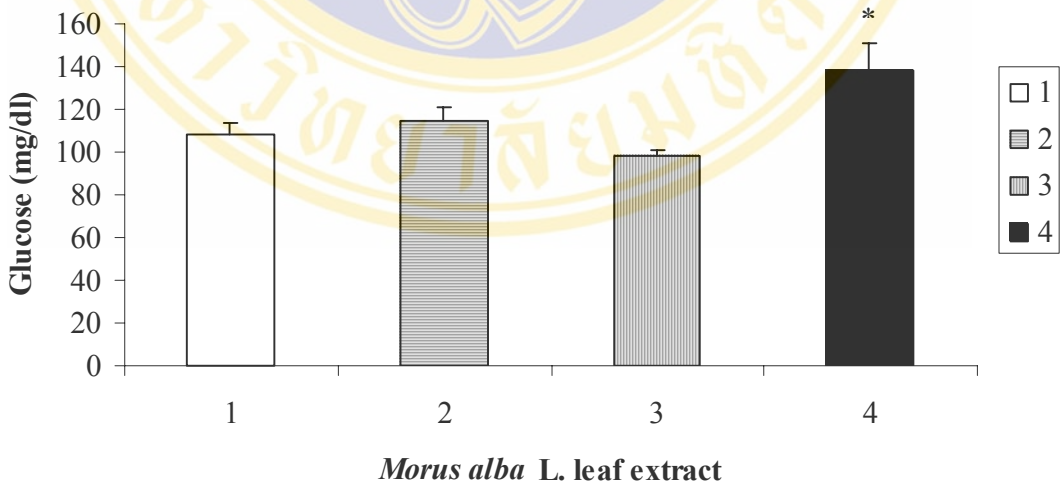


Fig 20 Blood glucose levels (mean ± S.E.M in Wistar female rats administered with (1) normal saline (control), (2) *M. alba* L. leaf extract 1 g/kg body weight/day, (3) *M. alba* L. leaf extract 2 g/kg body weight /day, (4) *M. alba* L. leaf extract 3 g/kg body weight /day for 60 days. (*: significant differences from control; $p<0.05$)

2.4.2. Blood Urea Nitrogen (BUN) Content Assay

Serum blood urea nitrogen (BUN) concentrations of the control animals and *M. alba* L. leaf extract treated groups were shown in table 4 and fig.21-22. All doses of *M. alba* L. leaf extract did not significantly alter the blood urea nitrogen (BUN) levels in both male and female rats, when compared to the control group.

Table 4 Blood Urea Nitrogen (BUN) level (mg/dl) in male and female rats treated with normal saline (control) and *M. alba* L. leaf extract, once daily for 60 days

Treatment	Blood Urea Nitrogen level (mg/dl)	
	Male rats	Female rats
Control (normal saline) (n = 10)	20.32±1.54	23.25±1.26
M. alba L. leaf extracts 1 g/kg/day (n = 10)	18.41±0.54	20.94±0.81
M. alba L. leaf extracts 2 g/kg/day (n = 10)	20.93±1.63	23.96±1.36
M. alba L. leaf extracts 3 g/kg/day (n = 10)	17.71±0.73	21.32±0.29

All value are expressed as mean ± S.E.M.

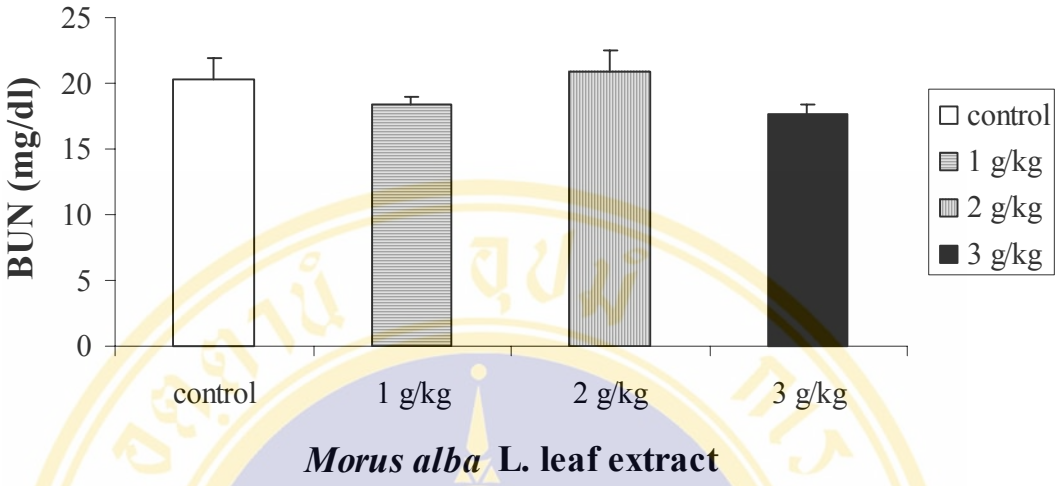


Fig. 21 Blood Urea Nitrogen (BUN) concentration (mean ± S.E.M) in Wistar male rats administered with (1) normal saline (control), (2) *M. alba* L. leaf extract 1 g/kg body weight/day, (3) *M. alba* L. leaf extract 2 g/kg body weight /day, (4) *M. alba* L. leaf extract 3 g/kg body weight /day for 60 days.

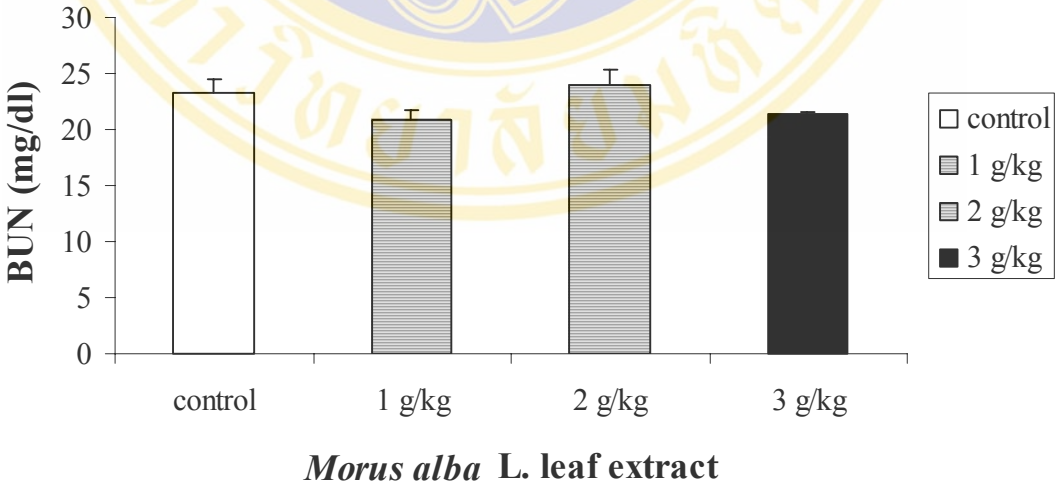


Fig. 22 Blood Urea Nitrogen (BUN) concentration (mean ± S.E.M) in Wistar female rats administered with (1) normal saline (control), (2) *M. alba* L. leaf extract 1 g/kg body weight/day, (3) *M. alba* L. leaf extract 2 g/kg body weight /day, (4) *M. alba* L. leaf extract 3 g/kg body weight /day for 60 days.

2.4.3. Serum Creatinine Content Assay

Serum creatinine concentrations of the control animals and *M. alba* L. leaf extract treated groups were shown in table 5 and fig. 23-24. All doses of *M. alba* L. leaf extract did not significantly alter the concentration of serum creatinine in both male and female rats, when compared to the control group.

Table 5 Serum creatinine level (mg/dl) in male and female rats treated with normal saline (control) and *M. alba* L. leaf extract, once daily for 60 days

Treatment	Serum creatinine level (mg/dl)	
	Male rats	Female rats
Control (normal saline) (n = 10)	0.68±0.03	0.67±0.03
M. alba L. leaf extracts 1 g/kg/day (n = 10)	0.66±0.03	0.65±0.02
M. alba L. leaf extracts 2 g/kg/day (n = 10)	0.67±0.03	0.66±0.03
M. alba L. leaf extracts 3 g/kg/day (n = 10)	0.67±0.03	0.63±0.02

All value are expressed as mean ± S.E.M.

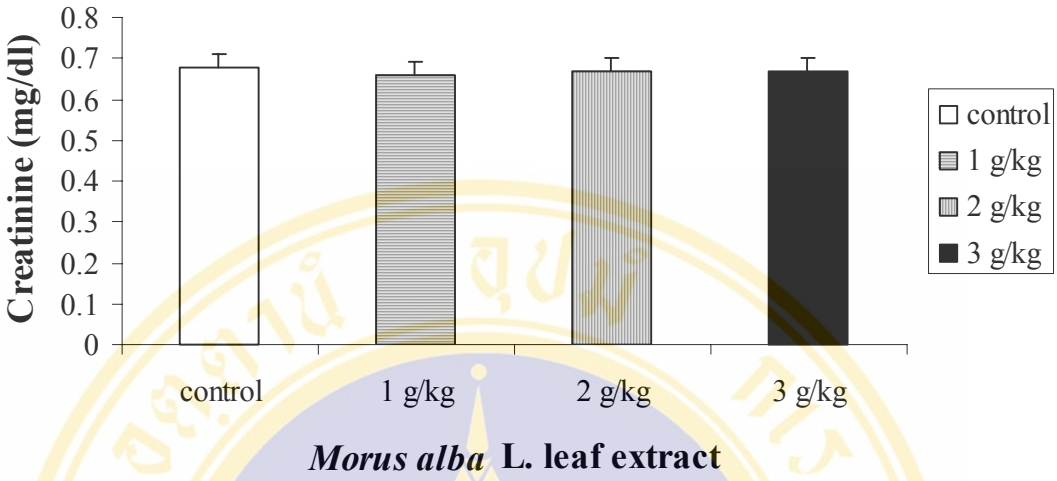


Fig. 23 Serum creatinine concentration (mean ± S.E.M) in Wistar male rats administered with (1) normal saline (control), (2) *M. alba* L. leaf extract 1 g/kg body weight/day, (3) *M. alba* L. leaf extract 2 g/kg body weight /day, (4)*M. alba* L. leaf extract 3 g/kg body weight /day for 60 days.

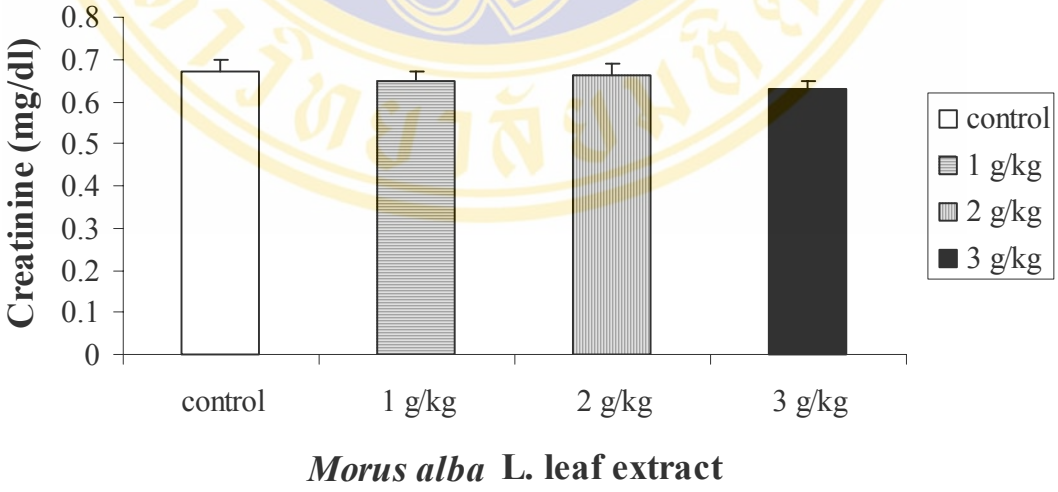


Fig. 24 Serum creatinine concentration (mean ± S.E.M) in Wistar female rats administered with (1) normal saline (control), (2) *M. alba* L. leaf extract 1 g/kg body weight/day, (3) *M. alba* L. leaf extract 2 g/kg body weight /day, (4) *M. alba* L. leaf extract 3 g/kg body weight/ day for 60 days.

2.4.4. Uric Acid Content Assay

Serum uric acid concentrations of the control animals and of *M.alba* L. leaf extract treated groups were shown in table 6 and fig 25-26.All doses of *M. alba* L. leaf extract did not significantly alter the uric acid levels in both male and female rats, when compared to the control group.

Table 6 Uric acid level (mg/dl) in male and female rats treated with normal saline (control) and *M. alba* L. leaf extract, once daily for 60 days

Treatment	Uric acid level (mg/dl)	
	Male rats	Female rats
Control (normal saline) (n = 10)	2.11±0.31	2.53±0.34
M. alba L. leaf extracts 1 g/kg/day (n = 10)	1.65±0.26	2.48±0.32
M. alba L. leaf extracts 2 g/kg/day (n = 10)	1.81±0.32	1.75±0.42
M. alba L. leaf extracts 3 g/kg/day (n = 10)	1.73±0.16	2.81±0.40

All value are expressed as mean ± S.E.M.

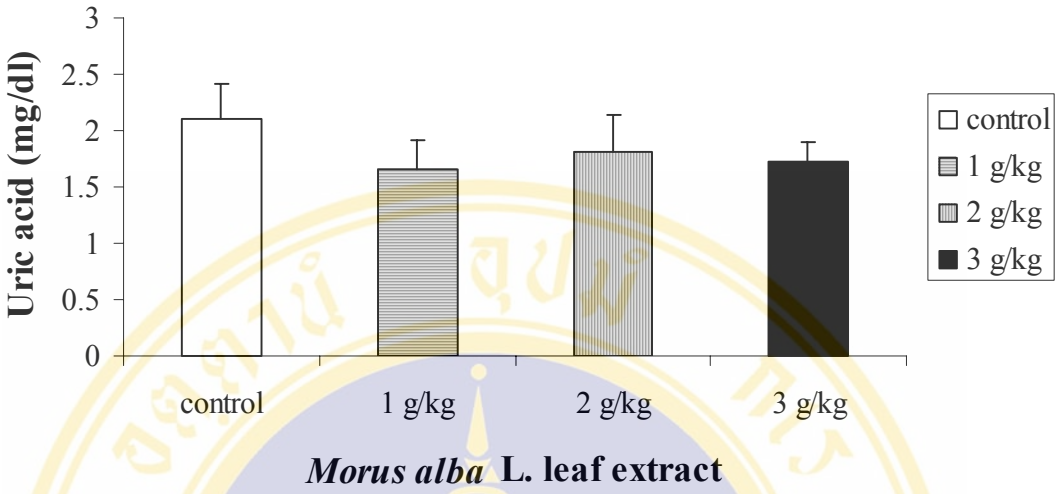


Fig. 25 Serum uric acid concentration (mean ± S.E.M) in Wistar male rats administered with (1) normal saline (control), (2) *M. alba* L. leaf extract 1 g/kg body weight/day, (3) *M. alba* L. leaf extract 2 g/kg body weight /day, (4) *M. alba* L. leaf extract 3 g/kg body weight /day for 60 days.

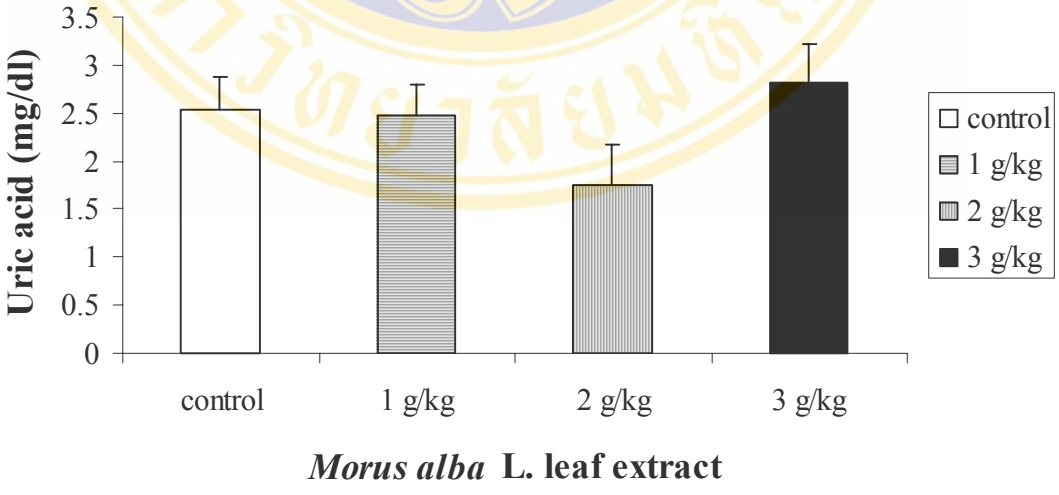


Fig. 26 Serum uric acid concentration (mean ± S.E.M) in Wistar female rats administered with (1) normal saline (control), (2) *M. alba* L. leaf extract 1 g/kg body weight/day, (3) *M. alba* L. leaf extract 2 g/kg body weight /day, (4) *M. alba* L. leaf extract 3 g/kg body weight/ day for 60 days.

2.4.5. Alkaline Phosphatase Activity Assay

Serum alkaline phosphatase of the control animals and of *M. alba* L. leaf extract treated groups were shown in table 7 and fig. 27-28. All doses of *M. alba* L. leaf extract did not significantly alter the activity of alkaline phosphatase in both male and female rats, when compared to the control group

Table 7 Alkaline phosphatase activity (U/l) in male and female rats treated with normal saline (control) and *M. alba* L. leaf extract, once daily for 60 days

Treatment	Alkaline phosphatase activity (U/l)	
	Male rats	Female rats
Control (normal saline) (n = 10)	90.40±4.00	42.00±2.36
M. alba L. leaf extracts 1 g/kg/day (n = 10)	80.80±2.90	46.70±2.12
M. alba L. leaf extracts 2 g/kg/day (n = 10)	83.00±4.52	40.10±3.72
M. alba L. leaf extracts 3 g/kg/day (n = 10)	79.70±3.79	50.50±4.81

All value are expressed as mean ± S.E.M.

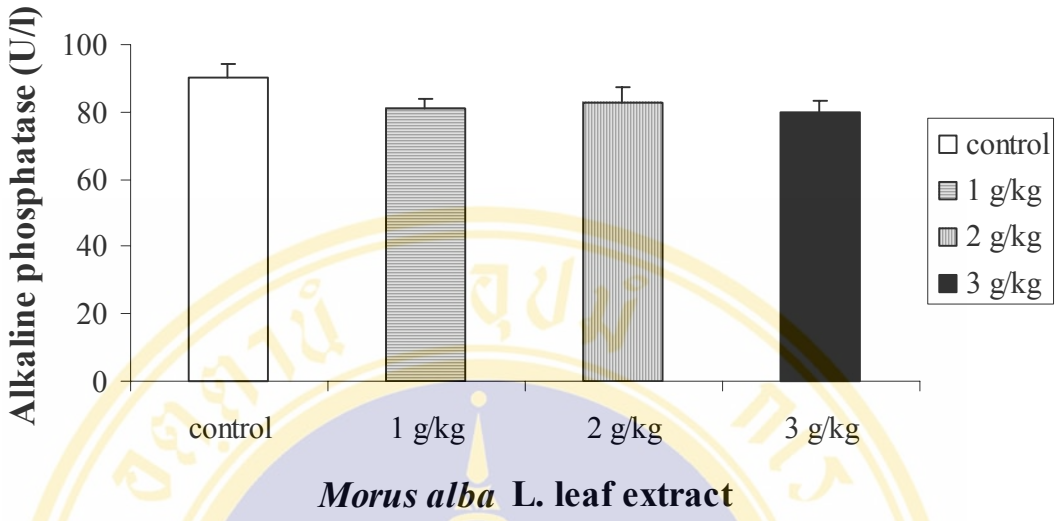


Fig. 27 Serum alkaline Phosphatase concentration (mean ± S.E.M) in Wistar male rats administered with (1) normal saline (control), (2) *M. alba* L. leaf extract 1 g/kg body weight/day, (3) *M. alba* L. leaf extract 2 g/kg body weight /day, (4) *M. alba* L. leaf extract 3 g/kg body weight /day for 60 days.

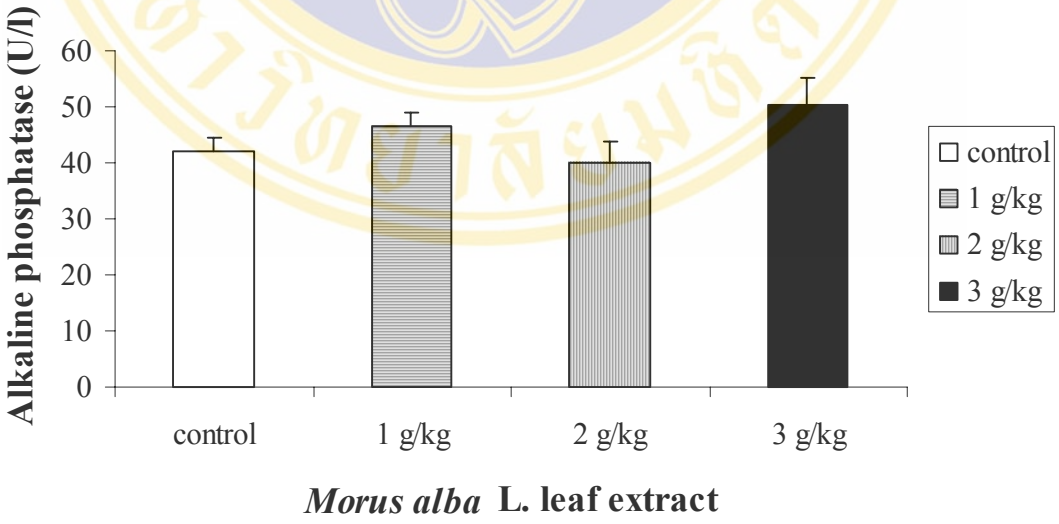


Fig. 28 Serum alkaline Phosphatase concentration (mean ± S.E.M) in Wistar female rats administered with (1) normal saline (control), (2) *M. alba* L. leaf extract 1 g/kg body weight/day, (3) *M. alba* L. leaf extract 2 g/kg body weight /day, (4) *M. alba* L. leaf extract 3 g/kg body weight /day for 60 days.

2.4.6. Aspartate Aminotransferase (AST) Activity Assay

Aspartate aminotransferase (AST) activity of the control animals and of *M. alba* L. leaf extract treated groups were shown in table 8 and fig. 29-30. All doses of *M.alba* L. leaf extract did not significantly alter the activity of aspartate aminotransferase (AST) in both male and female rats, when compared to the control group.

Table 8 Aspartate Aminotransferase (AST) activity (U/l) in male and female rats treated with normal saline (control) and *M. alba* L. leaf extract, once daily for 60 days

Treatment	Aspartate Aminotransferase (AST) activity (U/l)	
	Male rats	Female rats
Control (normal saline) (n = 10)	139.00±37.30	220.10±46.81
M. alba L. leaf extracts 1 g/kg/day (n = 10)	142.00±29.32	260.80±41.11
M. alba L. leaf extracts 2 g/kg/day (n = 10)	137.50±42.53	162.60±38.43
M. alba L. leaf extracts 3 g/kg/day (n = 10)	182.30±20.17	182.60±34.54

All value are expressed as mean ± S.E.M.

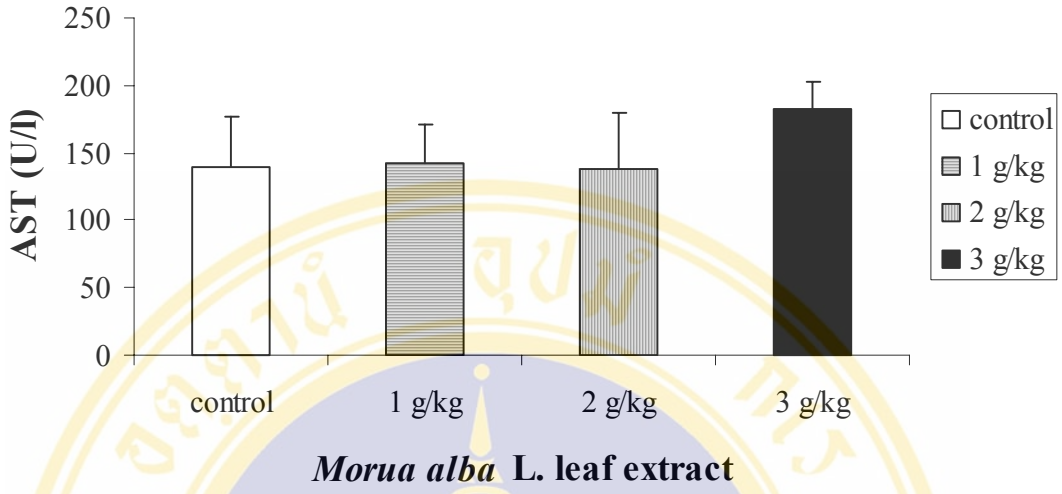


Fig. 29 Aspartate Aminotransferase (AST) activity (mean ± S.E.M) in Wistar male rats administered with (1) normal saline (control), (2) *M. alba* L. leaf extract 1 g/kg body weight/day, (3) *M. alba* L. leaf extract 2 g/kg body weight /day, (4) *M. alba* L. leaf extract 3 g/kg body weight /day for 60 days.

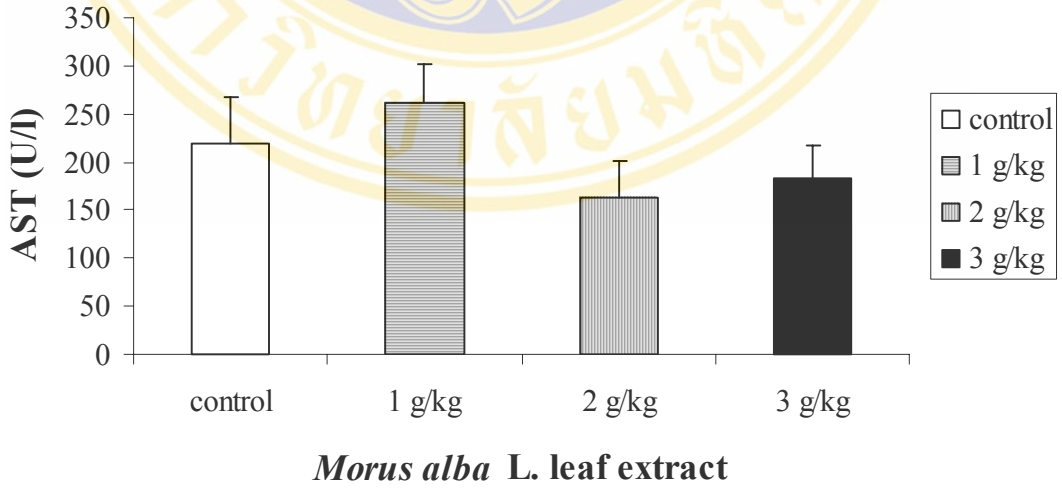


Fig. 30 Aspartate Aminotransferase (AST) activity (mean ± S.E.M in Wistar female rats administered with (1) normal saline (control), (2) *M. alba* L. leaf extract 1 g/kg body weight/day, (3) *M. alba* L. leaf extract 2 g/kg body weight /day, (4) *M. alba* L. leaf extract 3 g/kg body weight /day for 60 days.

2.4.7. Alanine Aminotransferase (ALT) Activity Assay

Alanine aminotransferase (ALT) activity of the control animals and of *M. alba* L. leaf extract treated groups were shown in table 9 and fig. 31-32. All doses of *M. alba* L. leaf extract did not significantly alter the activity of alanine aminotransferase (ALT) in both male and female rats, when compared to the control group.

Table 9 Alanine Aminotransferase (ALT) activity (U/l) in male and female rats treated with normal saline (control) and *M. alba* L. leaf extract, once daily for 60 days

Treatment	Alanine Aminotransferase (ALT) activity (U/l)	
	Male rats	Female rats
Control (normal saline) (n = 10)	36.70±6.60	38.90±8.22
M. alba L. leaf extracts 1 g/kg/day (n = 10)	33.00±4.15	31.90±4.53
M. alba L. leaf extracts 2 g/kg/day (n = 10)	35.00±5.87	33.70±6.84
M. alba L. leaf extracts 3 g/kg/day (n = 10)	34.40±2.23	41.10±6.51

All value are expressed as mean ± S.E.M.

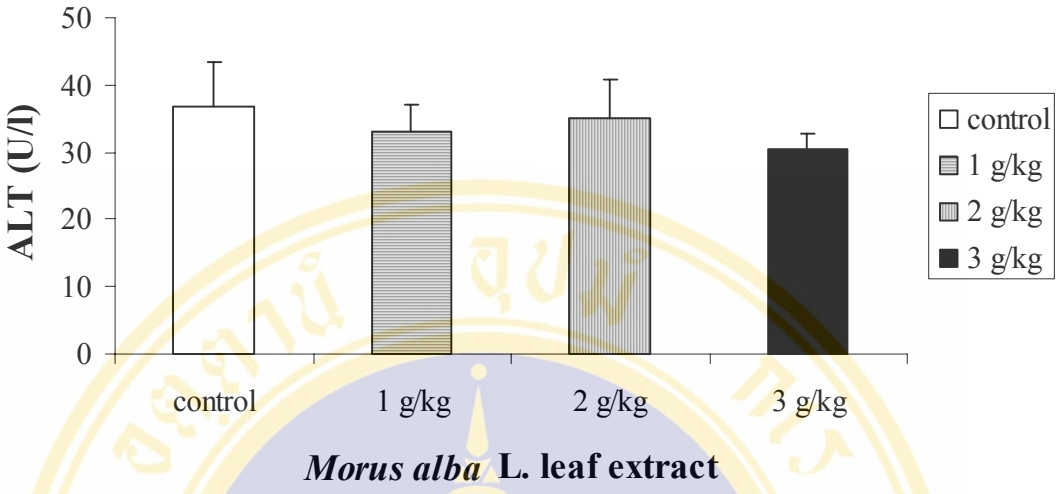


Fig. 31 Alanine Aminotransferase (ALT) activity (mean ± S.E.M) in Wistar male rats administered with (1) normal saline (control), (2) *M. alba* L. leaf extract 1 g/kg body weight/day, (3) *M. alba* L. leaf extract 2 g/kg body weight /day, (4) *M. alba* L. leaf extract 3 g/kg body weight /day for 60 days.

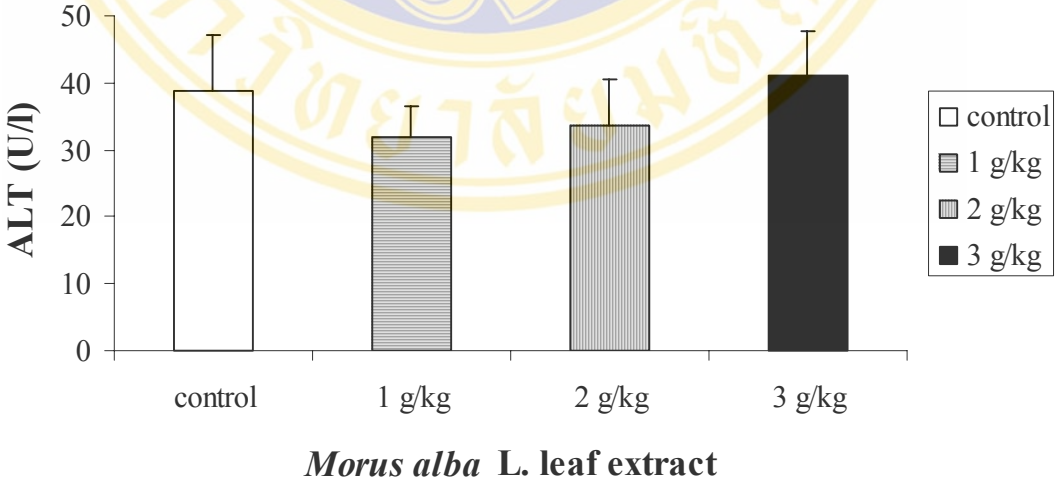


Fig. 32 Alanine Aminotransferase (ALT) activity (mean ± S.E.M) in Wistar female rats administered with (1) normal saline (control), (2) *M. alba* L. leaf extract 1 g/kg body weight/day, (3) *M. alba* L. leaf extract 2 g/kg body weight /day, (4) *M. alba* L. leaf extract 3 g/kg body weight /day for 60 days.

2.4.8. Serum Total Protein Content Assay

Serum total protein concentrations of the control animals and of *Morus alba* L. leaf extract treated groups were shown in table 10 and fig. 33-34. All doses of *M. alba* L. leaf extract did not significantly alter the total protein levels in both male and female rats, when compared to the control group

Table 10 Serum total protein level (g/dl) in male and female rats treated with normal saline (control) and *M. alba* L. leaf extract, once daily for 60 days

Treatment	Serum total protein level (g/dl)	
	Male rats	Female rats
Control (normal saline) (n = 10)	6.18±0.09	6.03±0.08
M. alba L. leaf extracts 1 g/kg/day (n = 10)	6.05±0.06	5.93±0.11
M. alba L. leaf extracts 2 g/kg/day (n = 10)	6.06±0.06	6.07±0.06
M. alba L. leaf extracts 3 g/kg/day (n = 10)	5.69±0.07	5.99±0.10

All value are expressed as mean ± S.E.M.

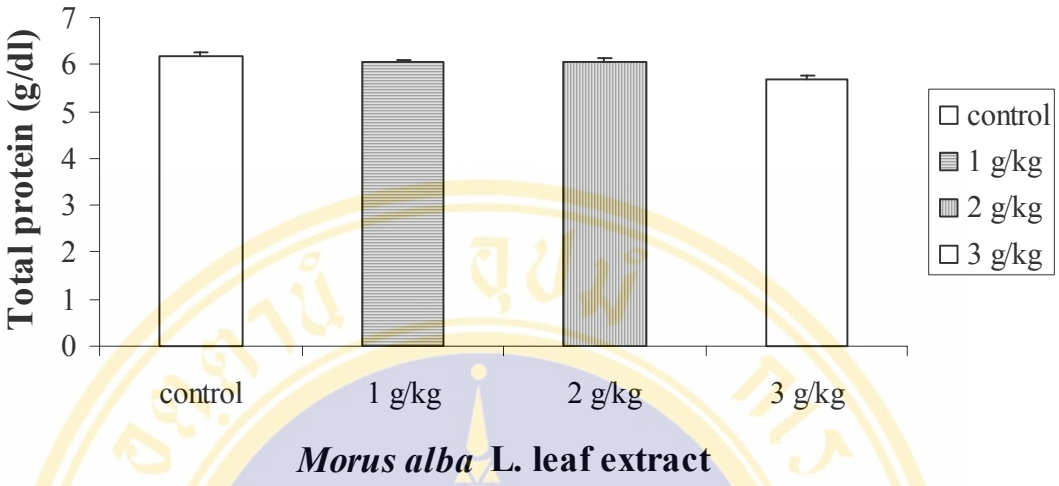


Fig. 33 Serum total protein concentration (mean ± S.E.M) in Wistar male rats administered with (1) normal saline (control), (2) *M. alba* L. leaf extract 1 g/kg body weight/day, (3) *M. alba* L. leaf extract 2 g/kg body weight /day, (4) *M. alba* L. leaf extract 3 g/kg body weight/ day for 60 days.

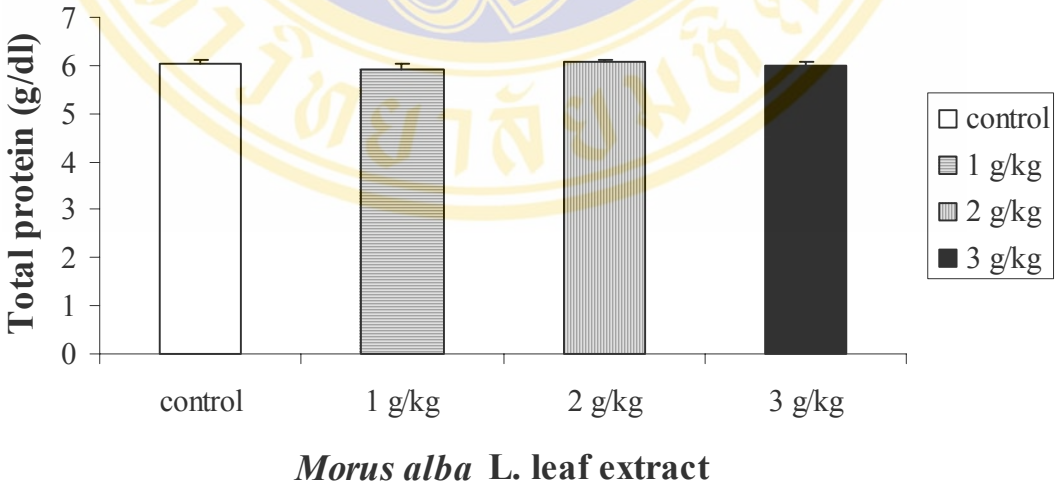


Fig. 34 Serum total serum protein concentration (mean ± S.E.M) in Wistar female rats administered with (1) normal saline (control), (2) *M. alba* L. leaf extract 1 g/kg body weight/day, (3) *M. alba* L. leaf extract 2 g/kg body weight /day, (4) *M. alba* L. leaf extract 3 g/kg body weight /day for 60 days.

2.4.9. Albumin Content Assay

Serum albumin concentrations of the control animals and of *M. alba* L. leaf extract treated groups were shown in table 11 and fig. 35-36. All doses of *M. alba* L. leaf extract did not significantly alter the albumin levels in both male and female rats, when compared to the control group

Table 11 Serum albumin level (g/dl) in male and female rats treated with normal saline (control) and *M. alba* L. leaf extract, once daily for 60 days

Treatment	Serum albumin Level (g/dl)	
	Male rats	Female rats
Control (normal saline) (n = 10)	3.80±0.06	3.75±0.08
M. alba L. leaf extracts 1 g/kg/day (n = 10)	3.74±0.04	3.69±0.07
M. alba L. leaf extracts 2 g/kg/day (n = 10)	3.73±0.04	3.70±0.07
M. alba L. leaf extracts 3 g/kg/day (n = 10)	3.67±0.07	3.70±0.05

All value are expressed as mean ± S.E.M.

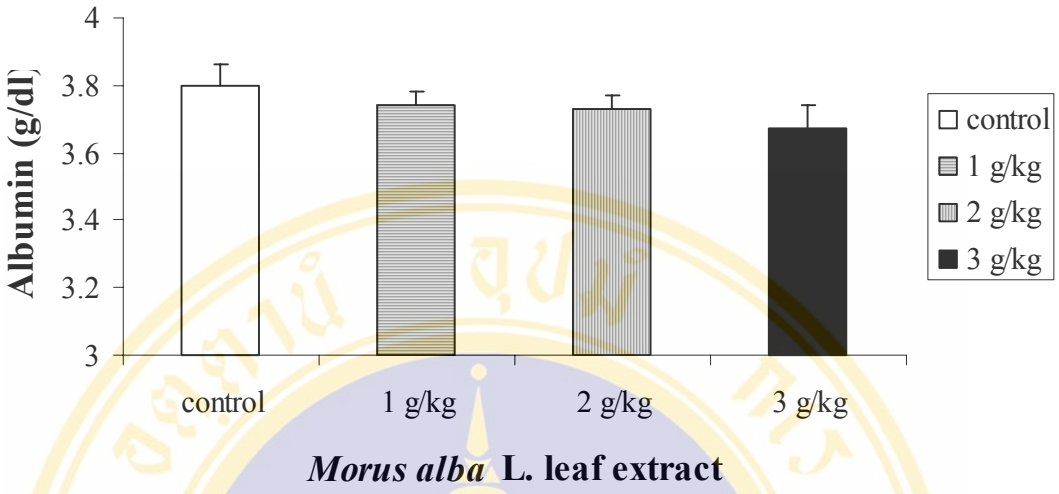


Fig. 35 Serum albumin concentration (mean ± S.E.M) in Wistar male rats administered with (1) normal saline (control), (2) *M. alba* L. leaf extract 1 g/kg body weight/day, (3) *M. alba* L. leaf extract 2 g/kg body weight /day, (4) *M. alba* L. leaf extract 3 g/kg body weight /day for 60 days.

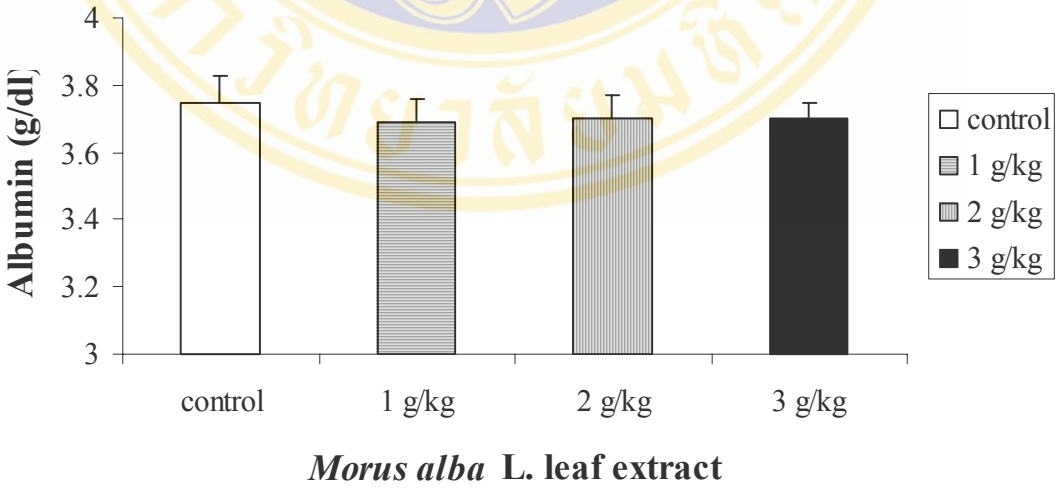


Fig. 36 Serum albumin concentration (mean ± S.E.M) in Wistar female rats administered with (1) normal saline (control), (2) *M. alba* L. leaf extract 1 g/kg body weight/day, (3) *M. alba* L. leaf extract 2 g/kg body weight /day, (4) *M. alba* L. leaf extract 3 g/kg body weight /day for 60 days.

2.4.10. Cholesterol Content Assay

Serum cholesterol concentrations of the control animals and of *M. alba* L. leaf extract treated groups were shown in table 12 and fig. 37-38. All doses of *M. alba* L. leaf extract did not significantly alter the cholesterol levels in both male and female rats, when compared to the control group

Table 12 Serum cholesterol level (mg/dl) in male and female rats treated with normal saline (control) and *M. alba* L. leaf extract, once daily for 60 days

Treatment	Serum cholesterol level (mg/dl)	
	Male rats	Female rats
Control (normal saline) (n = 10)	59.80±2.70	54.10±4.26
M. alba L. leaf extracts 1 g/kg/day (n = 10)	55.20±2.61	52.60±3.47
M. alba L. leaf extracts 2 g/kg/day (n = 10)	53.30±3.78	44.30±2.67
M. alba L. leaf extracts 3 g/kg/day (n = 10)	55.10±2.59	51.20±3.50

All value are expressed as mean ± S.E.M.

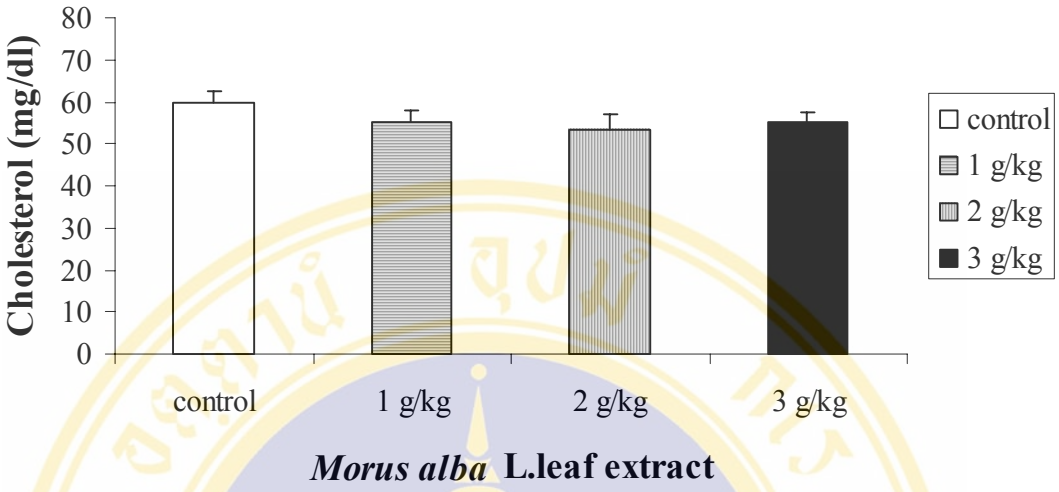


Fig. 37 Serum cholesterol concentration (mean ± S.E.M) in Wistar male rats administered with (1) normal saline (control), (2) *M. alba* L. leaf extract 1 g/kg body weight/day, (3) *M. alba* L. leaf extract 2 g/kg body weight /day, (4) *M. alba* L. leaf extract 3 g/kg body weight/ day for 60 days.

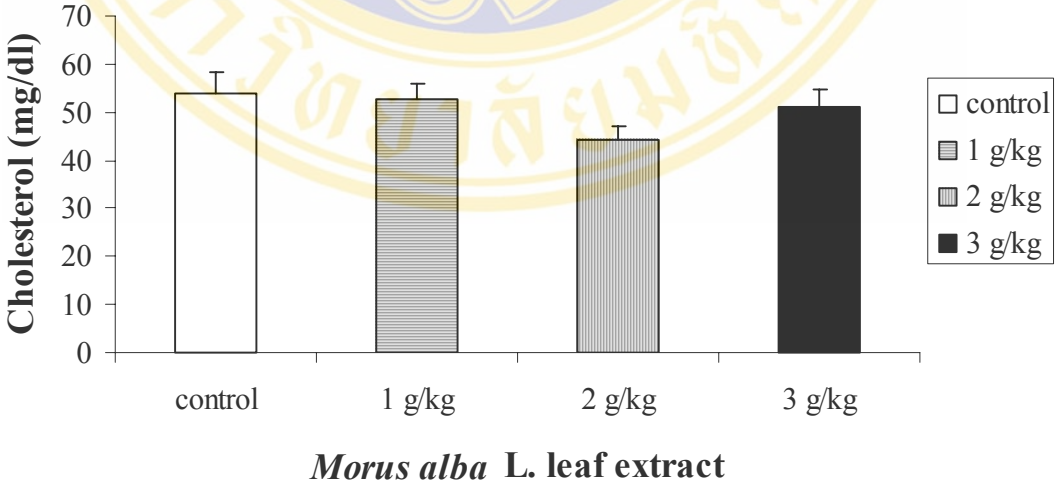


Fig. 38 Serum cholesterol concentration (mean ± S.E.M) in Wistar female rats administered with (1) normal saline (control), (2) *M. alba* L. leaf extract 1 g/kg body weight/day, (3) *M. alba* L. leaf extract 2 g/kg body weight /day, (4) *M. alba* L. leaf extract 3 g/kg body weight/ day for 60 days.

2.4.11. High-Density Lipoprotein (HDL) Content Assay

Serum high-density lipoprotein (HDL) concentrations of the control animals and of *M. alba* L. leaf extract treated groups were shown in table 13 and fig. 39-40. All doses of *M. alba* L. leaf extract did not significantly alter the high-density lipoprotein (HDL) levels in both male and female rats, when compared to the control group

Table 13 Serum HDL-cholesterol level (mg/dl) in male and female rats treated with normal saline (control) and *M. alba* L. leaf extract, once daily for 60 days

Treatment	Serum HDL-cholesterol level (mg/dl)	
	Male rats	Female rats
Control (normal saline) (n = 10)	34.10±1.68	27.90±1.86
M. alba L. leaf extracts 1 g/kg/day (n = 10)	29.90±1.25	27.90±1.64
M. alba L. leaf extracts 2 g/kg/day (n = 10)	29.67±1.55	24.30±2.00
M. alba L. leaf extracts 3 g/kg/day (n = 10)	29.90±1.27	27.60±1.45

All value are expressed as mean ± S.E.M.

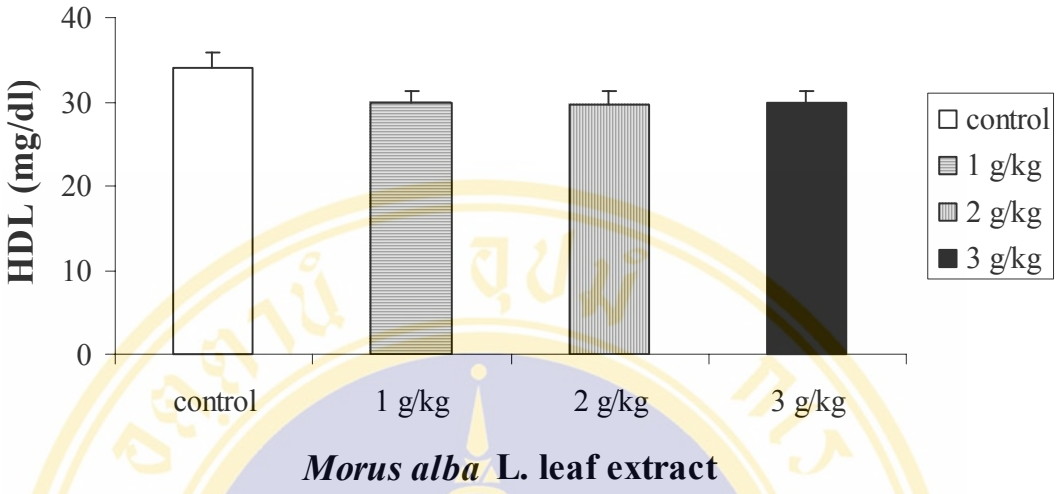


Fig. 39 Serum High-Density Lipoprotein (HDL) concentration (mean ± S.E.M) in Wistar male rats administered with (1) normal saline (control), (2) *M. alba* L. leaf extract 1 g/kg body weight/day, (3) *M. alba* L. leaf extract 2 g/kg body weight /day, (4) *M. alba* L. leaf extract 3 g/kg body weight /day for 60 days.

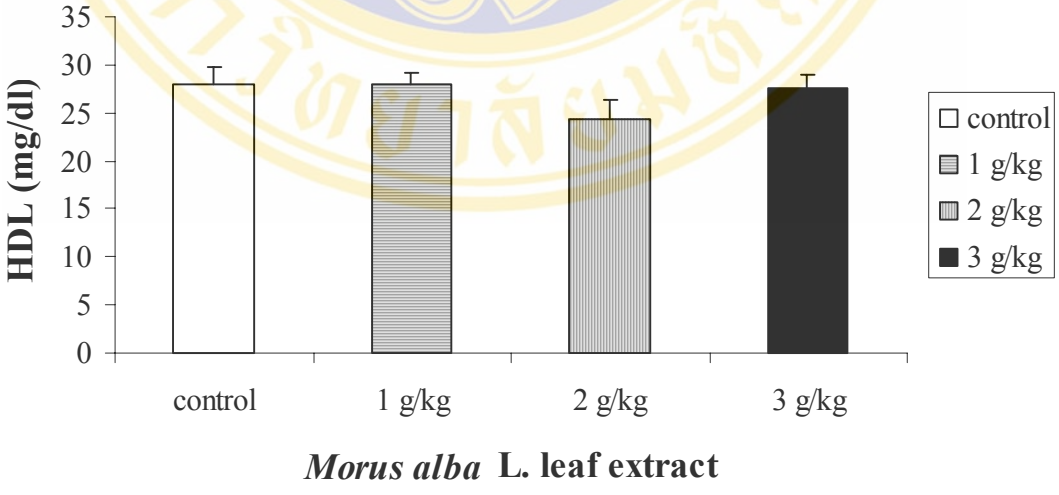


Fig. 40 Serum High-Density Lipoprotein (HDL) concentration (mean ± S.E.M) in Wistar female rats administered with (1) normal saline (control), (2) *M. alba* L. leaf extract 1 g/kg body weight/day, (3) *M. alba* L. leaf extract 2 g/kg body weight /day, (4) *M. alba* L. leaf extract 3 g/kg body weight /day for 60 days.

2.4.12. Low-Density Lipoprotein (LDL) Content Assay

Serum low-density lipoprotein (LDL) concentrations of the control animals and of *M. alba* L. leaf extract treated groups were shown in table 14 and fig. 41-42.All doses of *M. alba* L. leaf extract did not significantly alter the low-density lipoprotein (LDL) levels in both male and female rats, when compared to the control group

Table 14 Serum LDL-cholesterol level (mg/dl) in male and female rats treated with normal saline (control) and *M. alba* L. leaf extract, once daily for 60 days

Treatment	Serum LDL-cholesterol level (mg/dl)	
	Male rats	Female rats
Control (normal saline) (n = 10)	17.52±2.14	17.46±2.19
M. alba L. leaf extracts 1 g/kg/day (n = 10)	13.30±1.09	16.12±1.96
M. alba L. leaf extracts 2 g/kg/day (n = 10)	13.00±2.46	13.86±1.67
M. alba L. leaf extracts 3 g/kg/day (n = 10)	13.60±1.35	16.84±2.16

All value are expressed as mean ± S.E.M.

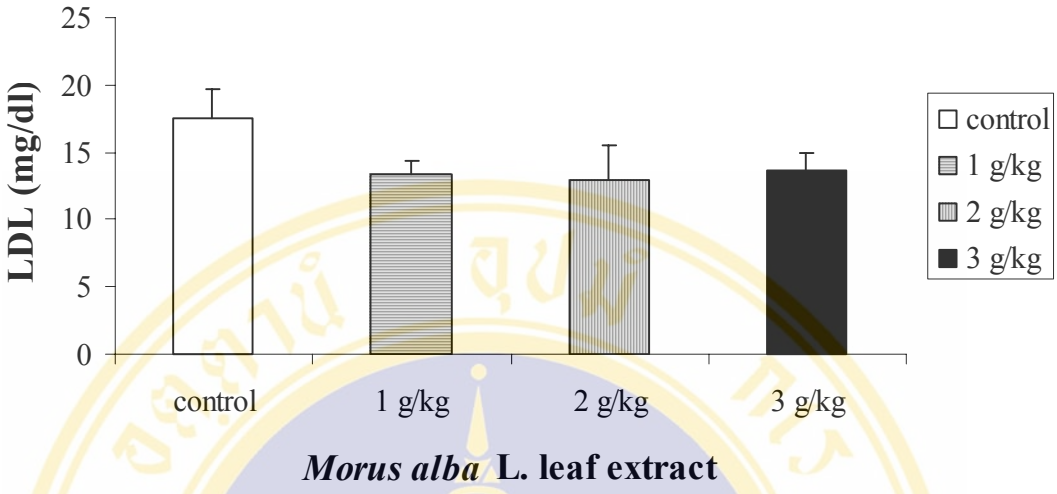


Fig. 41 Serum Low-Density Lipoprotein (LDL) concentration (mean ± S.E.M) in Wistar male rats administered with (1) normal saline (control), (2) *M. alba* L. leaf extract 1 g/kg body weight/day, (3) *M. alba* L. leaf extract 2 g/kg body weight /day, (4) *M. alba* L. leaf extract 3 g/kg body weight /day for 60 days.

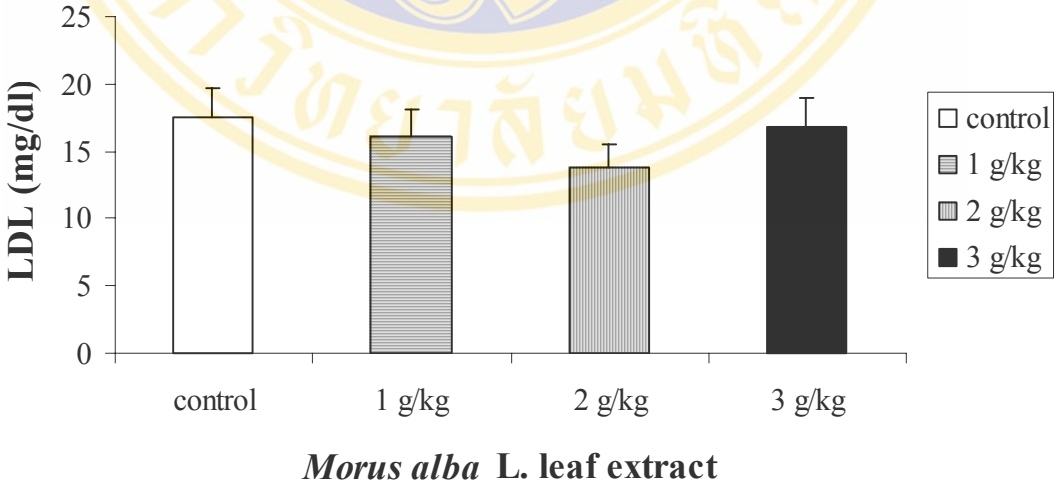


Fig. 42 Serum Low-Density Lipoprotein (LDL) concentration (mean ± S.E.M) in Wistar female rats administered with (1) normal saline (control), (2) *M. alba* L. leaf extract 1 g/kg body weight/day, (3) *M. alba* L. leaf extract 2 g/kg body weight /day, (4) *M. alba* L. leaf extract 3 g/kg body weight /day for 60 days.

2.4.13. Triglycerides Content Assay

Serum triglycerides concentrations of the control animals and of *M. alba* L. leaf extract treated groups were shown in table 15 and fig. 43-44. All doses of *M. alba* L. leaf extract did not significantly alter the triglyceride levels in both male and female rats, when compared to the control group

Table 15 Serum trigleceride level (mg/dl) in male and female rats treated with normal saline (control) and *M. alba* L. leaf extract, once daily for 60 days

Treatment	Serum triglyceride level (mg/dl)	
	Male rats	Female rats
Control (normal saline) (n = 10)	64.20±4.76	43.70±3.49
M. alba L. leaf extracts 1 g/kg/day (n = 10)	55.30±5.53	48.50±3.30
M. alba L. leaf extracts 2 g/kg/day (n = 10)	56.00±4.94	38.80±3.40
M. alba L. leaf extracts 3 g/kg/day (n = 10)	56.50±3.54	49.40±2.84

All value are expressed as mean ± S.E.M.

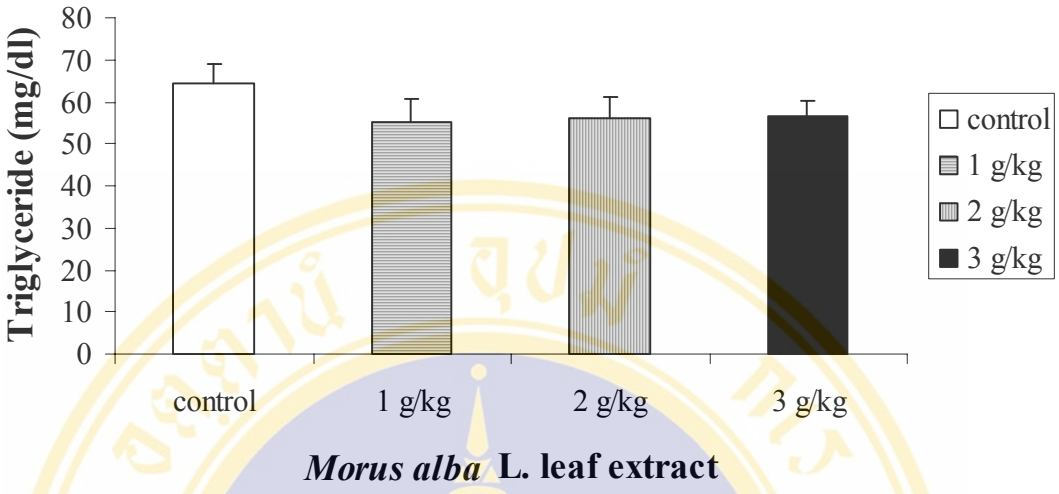


Fig. 43 Serum triglycerides concentration (mean ± S.E.M) in Wistar male rats administered with (1) normal saline (control), (2) *M. alba* L. leaf extract 1 g/kg body weight/day, (3) *M. alba* L. leaf extract 2 g/kg body weight /day, (4) *M. alba* L. leaf extract 3 g/kg body weight/ day for 60 days.

Fig. 44 Serum triglycerides concentration (mean ± S.E.M) in Wistar female rats administered with (1) normal saline (control), (2) *M. alba* L. leaf extract 1 g/kg body weight/day, (3) *M. alba* L. leaf extract 2 g/kg body weight /day, (4) *M. alba* L. leaf extract 3 g/kg body weight /day for 60 days.

2.5 Hematologic examination

The hematological parameters determined were red blood cell count, hemoglobin, hematocrit, white blood cell count and differential count. The hematological results were sown in table 16-19. All doses of the *M. alba* L. leaf extract did not significantly affect the hematological parameters in both male and female when compared to the control group.

2.6 Histopathologically examination

Specimens of brain, heart, lung, liver, spleen, kidney and bone marrow were examined. The histologic finding were shown as control group in Fig 45-53. All major organ specimens did not show any histopathological abnormality

Table 16 Hematologic response (R.B.C. , hemoglobin , hematocrit , W.B.C.) in male rats treated with normal saline (control) and *M. alba* L. leaf extract for 60 days

Treatment	R.B.C. (*10^6) (per µl)	Hemoglobin (g/dl)	Hematocrit (%)	W.B.C. (per µl)
Control (n = 10)	8.13±0.31	15.78±0.54	44.53±1.69	2720.00±513.34
M. alba L. leaf extracts 1 g / kg BW /day (n = 10)	7.86±0.20	14.76±0.26	42.23±0.83	3175.71±631.33
M. alba L. leaf extracts 2 g/ kg BW/day (n = 10)	7.94±1.41	15.31±0.36	42.87±0.89	4670.00±558.99
M. alba L. leaf extracts 3 g / kg BW/day (n = 10)	8.11±0.34	15.24±0.95	42.54±2.53	4045.00±597.67

All value are expressed as mean ± S.E.M.

Table 17 Hematologic response (differential white blood cell) in male rats treated with normal saline (control) and *M. alba* L. leaf extract for 60 days

Treatment	Differential White Blood Cell Count (%per µl)					
	Neutrophil (per µl)	Band (per µl)	Eosinophil (per µl)	Basophil (per µl)	Lymphocyte (per µl)	Monocyte (per µl)
Control (n = 10)	12.50±1.82	0	0.67±0.33	0	85.83±2.31	0
M. alba L. leaf extract 1 g/kg BW/day (n = 10)	15.13±3.14	0	0.75±0.31	0	83.87±3.07	0
M. alba L. leaf extract 2 g/kg BW/day (n = 10)	18.85±1.92	0	1.00±0.33	0	80.00±1.69	0
M. alba L. leaf extract 3 g/kg BW/day (n = 10)	13.29±2.04	0	1.33±0.33	0	84.42±2.22	0

All value are expressed as mean ± S.E.M.

Table 18 Hematologic response (R.B.C. , hemoglobin , hematocrit , W.B.C.) in female rats treated with normal saline (control) and *M. alba* L. leaf extract for 60 days

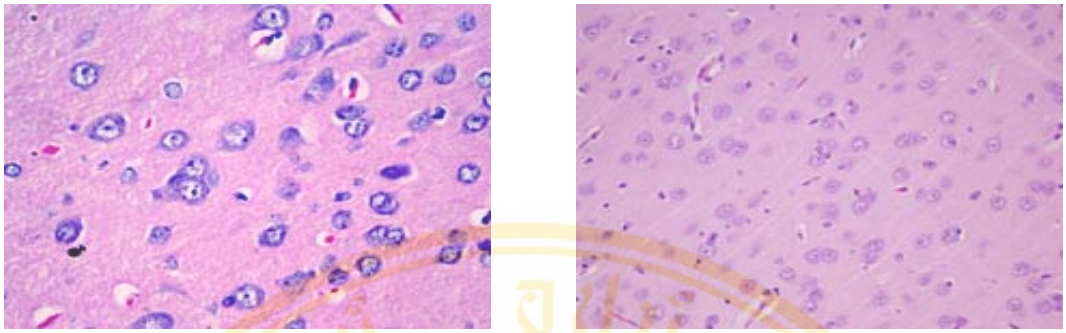
Treatment	R.B.C. (*10^6) (per µl)	Hemoglobin (g/dl)	Hematocrit (%)	W.B.C. (per µl)
Control (n = 10)	6.97±0.53	14.44±1.21	35.70±3.30	2318.33±440.71
M. alba L. leaf extracts 1 g / kg BW /day (n = 10)	7.20±0.30	14.52±0.64	40.30±1.86	2544.28±683.86
M. alba L. leaf extracts 2 g/ kg BW /day (n = 10)	7.18±0.22	14.85±0.45	41.77±1.44	3301.42±456.89
M. alba L. leaf extracts 3 g / kg BW /day (n = 10)	6.16±0.45	13.02±0.82	35.16±2.54	2358.33±364.88

All value are expressed as mean ± S.E.M.

Table 19 Hematologic response (differential white blood cell) in female rats treated with normal saline (control) and *M. alba* L. leaf extract for 60 days

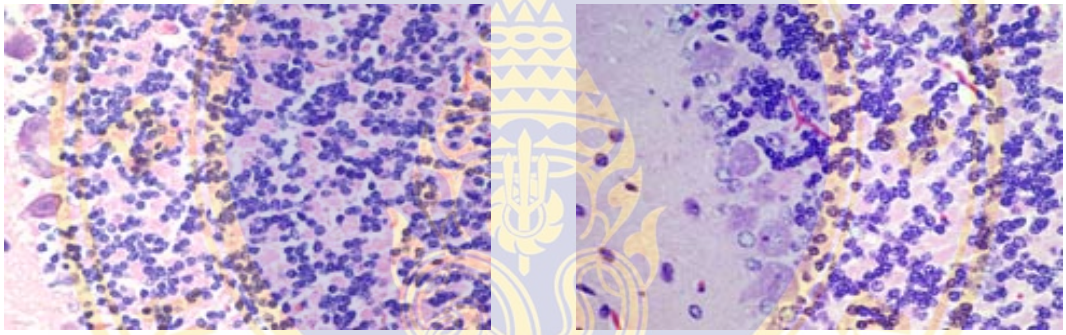
Treatment	Differential White Blood Cell Count (%per µl)					
	Neutrophil (per µl)	Band (per µl)	Eosinophil (per µl)	Basophil (per µl)	Lymphocyte (per µl)	Monocyte (per µl)
Control (n = 10)	12.80±2.52	0	0.83±0.48	0	87.83±2.93	0
M.alba L. leaf extract 1 g/kg BW/day (n = 10)	9.57±2.03	0	0.57±0.30	0	90.25±1.98	0
M. alba L. leaf extract 2 g/kg BW/day (n = 10)	13.86±2.55	0	0.87±0.23	0	82.75±3.00	0
M.alba L. leaf extract 3 g/kg BW/day (n = 10)	8.50±2.81	0	0.71±0.36	0	87.14±4.39	0

All value are expressed as mean ± S.E.M.



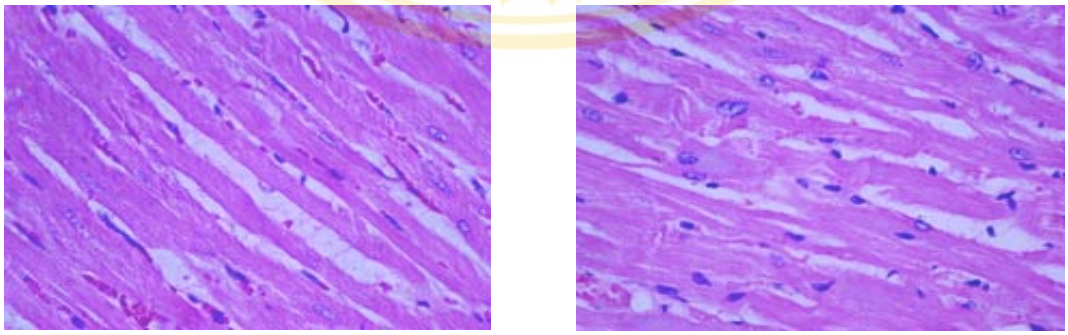
Brain (control group) Brain (treatment group)

Fig. 45 Cross section of cerebrum brain tissue (40X) in rats treated with normal saline (control) and *M. alba* L. leaf extract, once daily for 60 days



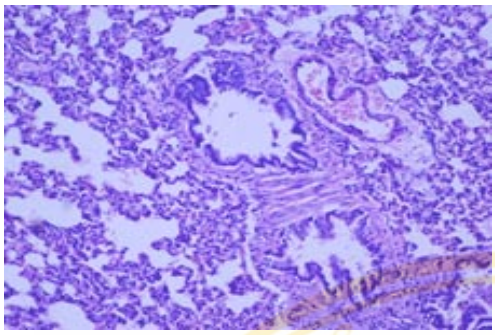
Brain (control group) Brain (treatment group)

Fig. 46 Cross section of cerebellum brain tissue (40X) in rats treated with normal saline (control) and *M. alba* L. leaf extract, once daily for 60 days

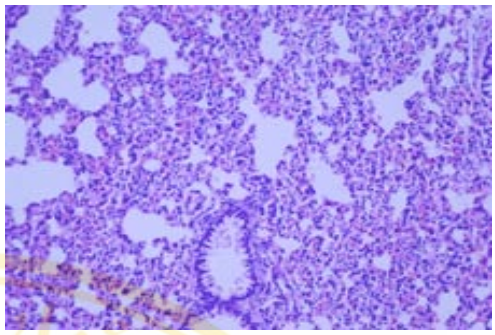


Heart (control group) Heart (treatment group)

Fig. 47 Cross section of heart tissue (40X) in rats treated with normal saline (control) and *M. alba* L. leaf extract, once daily for 60 days

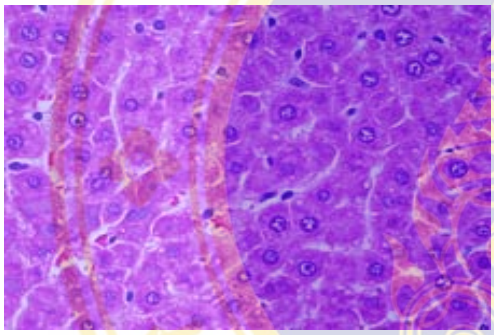


Lung (control group)

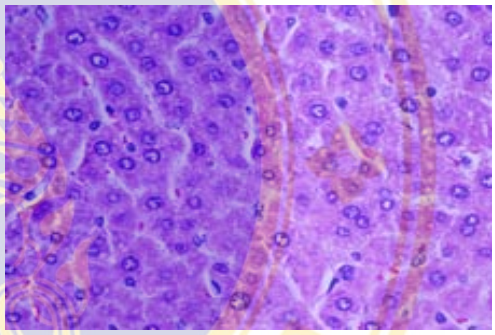


Lung (treatment group)

Fig. 48 Cross section of lung tissue (10X) in rats treated with normal saline (control) and *M. alba* L. leaf extract, once daily for 60 days

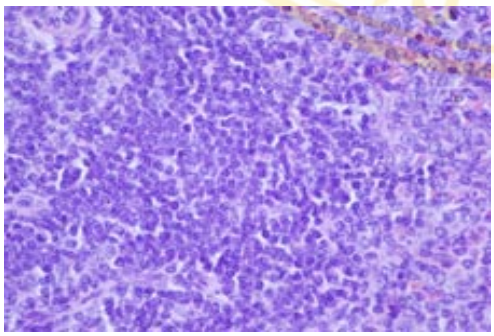


Liver (control group)

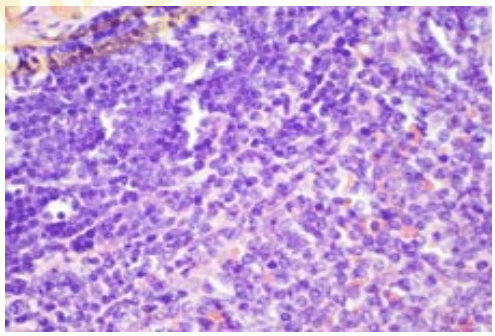


Liver (treatment group)

Fig. 49 Cross section of liver tissue (40X) in rats treated with normal saline (control) and *M. alba* L. leaf extract, once daily for 60 days

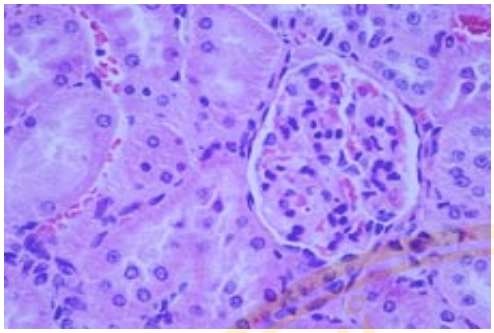


Spleen (control group)

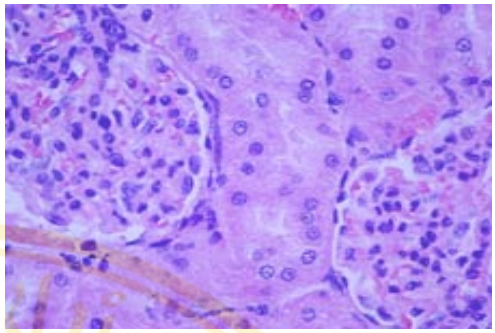


Spleen (treatment group)

Fig. 50 Cross section of spleen tissue (40X) in rats treated with normal saline (control) and *M. alba* L. leaf extract, once daily for 60 days



Kidney (control group)



Kidney (treatment group)

Fig. 51 Cross section of kidney tissue (40X) in rats treated with normal saline (control) and *M. alba* L. leaf extract, once daily for 60 days

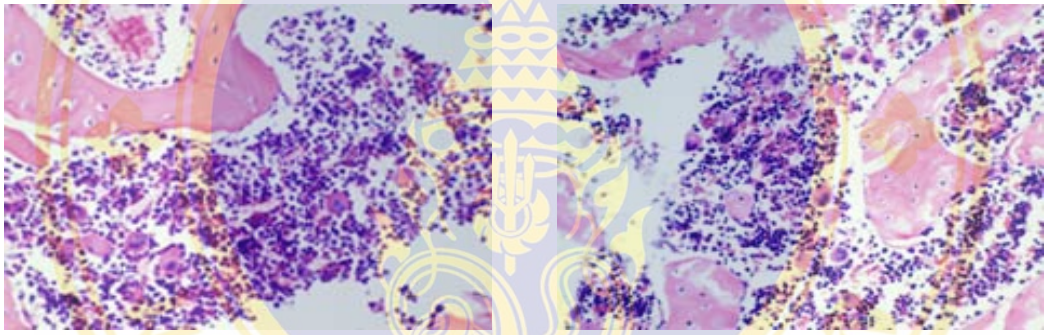


Fig. 52 Cross section of bone marrow (20X) in rats treated with normal saline (control) and *M. alba* L. leaf extract, once daily for 60 days

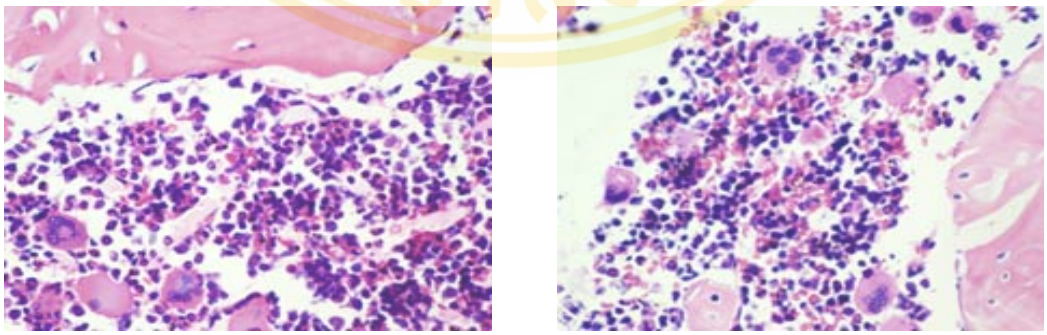


Fig. 53 Cross section of bone marrow (20X) in rats treated with normal saline (control) and *M. alba* L. leaf extract, once daily for 60 days

CHAPTER 5
DISCUSSION

Nowadays, diabetes mellitus is still a problem in many countries worldwide. Research and development of new anti-diabetic is necessary. Bionatural products that possess hypoglycemic activity are the leaf extracts of *M. alba* L. , the fruit, seeds, and tissue of *M. charantia,* the ethanol, petroleum ether, ethyl ether extract of *A. sativum* , the fractions of *A. cepa* Linn and others.

M. alba L. is a species widely cultivate in north and northeast regions of Thailand. In Thai traditional medicine , the leaf of *M. alba* L. have been used for the treatment of coughs , fever , relieve thirst and relieve sore throat. Among these pharmacological actions, it has been demonstrated to possess anti-hyperglycemic effect in streptozotocin-induced diabetic rats, especially the water extract of leaf. The degree of blood sugar reduction was comparable to glibenclamide. Therefore to ensure the safety of this plant in clinical use, the toxicological data of *M. alba* L. is necessary.

As shown in our results, the LD_{50} values of this extract in rats and mice were approximately 4.944 and 3.783 g/kg, respectively. A lethal dose of 0.5 to 5 g/kg has been classified as “slightly toxic” compound (142).Therefore, these extract was slightly toxic to rats and mice under our experimental conditions. In subchronic toxicity study ,1,2 and 3 g/kg body weight/day doses were administered orally into Wistar rats. The abnormal signs and symptom were not found throughout the period of study. Moreover, the increase in body weight which reflex animal growth rate indicated that all treated animals could maintained their normal growth rate throughout the time period of study. Thus, our subchronic toxicity data supported the result of acute toxicity study.

In addition , on the last day of subchronic treatment, blood samples were collected from all rats and were subjected for hematologic examination. The parameters assayed were blood glucose, blood urea nitrogen (BUN), creatinine, uric acid, alkaline phosphatase, aspartate aminotransferase (AST), alanine

aminotransferase (ALT), total protein, albumin, high-density lipoprotein (HDL), low-density lipoprotein (LDL), cholesterol and triglyceride

Serum uric acid, blood urea nitrogen (BUN) and serum creatinine were indicators for kidney function. Uric acid is an end-product of nucleoprotein metabolism found in the blood(143). It is derived from the breakdown of purines from the nucleic acids of cells and from xanthine (xanthine are purine compounds found in most body tissue). It is one means of quantifying the nuclear metabolic process. The physiological process of uric acid production is that of catabolism of purine nucleotides from organs having a high metabolic rate, such as the liver, bone marrow, and possibly muscle, as well as conditions in which excessive cellular breakdown takes place. Uric acid is totally filtered and excreted at the glomerulus and then total reabsorbed in the proximal tubule. The filtrate is then actively secreted in the distal tubule, which is responsible for total urinary urate(144). Increased serum uric acid concentrations can result either from a decrease in renal urate excretion or from excessive urate production. Low serum uric acid concentrations are inconsequential and usually are reflective of drugs that have hypouricemic activity(143).

Blood urea nitrogen is an end-product of protein metabolism. It is produced solely by the liver, is transported in the blood(143). Blood urea nitrogen is produced through a process of protein degradation. Protein is transformed from amino acids to ammonia (oxidated deamination) as an end product and then to urea (ornithine cycle) within the liver.(144) The concentration of blood urea nitrogen reflects renal function because the urea nitrogen in the blood is filtered completely at the glomerulus of the kidney, then reabsorbed (probably through diffusion) and tubularly secreted within nephrons(143). Only a fraction of all waste material contained in the glomerular filtrate is excreted, but because blood urea nitrogen is poorly reabsorbed by the tubules, little blood urea nitrogen is reabsorbed, a beneficial effect(144).

Creatinine is derived from creatine and phosphocreatine, a major constitutent of muscle(143). It is a sensitive indicator of renal function. It is an end-product of muscle metabolism that is liberated from the muscle and excreted in the urine at a virtually constant rate(144). Its rate of formation for a given individual is remarkably constant and is determined primarily by an individual’s muscle mass or lean body weight. Once creatinine is released from muscle into plasma. The glomerulus of the kidney filters

the creatinine, and it is not reabsorbed by the tubules. Creatinine is excreated unchanged in the urine because it is little modified in its passage through the nephron. It is more readily excreted by kidneys than is urea or uric acid(143).

In our experiment, all doses of *M. alba* L. leaf extract did not affect serum uric acid, blood urea nitrogen nor serum creatinine. Thus, the result indicated that *M. alba* L. leaf extract did not cause any renal toxicity.

Besides kidney effects, liver was another important target organ studied. aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase activities were monitored for evaluation of liver function. The aspartate aminotransferase (AST) enzyme , formerly called serum glutamate oxaloacetate transminase (SGOT) is widely distributed in the body tissues and is found in particularly high concentrations in those tissues with high metabolic activity(144). It is abundant in heart and liver tissue and moderately present in skeletal muscle, the kidney and the pancreas(143). When body cells containing AST are damaged or their activity impaired or destroyed because of deficient oxygen or glucose, the cell membrane become permeable or may rupture. The AST together with other cell contents, finds its way to the plasma , which increases the serum concentration of AST. The greater the intracellular concentration of the enzyme, the higher and the more rapid the rise in serum levels with cell damage(144). In cases of acute cellular injury to the heart or liver, the enzyme is released into the blood from the damaged cells and presumably is metabolized within the body. In clinical practice, AST determinations are used to evaluate myocardial injury and to diagnose and assess the prognosis of liver disease resulting from hepatocellular injury(143).

The alanine aminotransferase (ALT) enzyme, formerly called serum glutamate pyruvate transminase (SGPT) was another parameter monitored. This enzyme is found essentially in the same tissues that have high concentration of AST. In liver disease, ALT elevations parallel those of AST, although slightly more acute hepatocellular parenchymal damage must occur to produce abnormal values. The ALT is relatively more abundant in hepatic tissue versus cardiac tissue than AST. However, the liver still contains 3.5 times more AST than ALT. Although serum concentrations of both AST and ALT increase when disease processes affect liver cell structure, the ALT is a more liver-specific enzyme(143).

The alkaline phosphatase constitutes a large group of isoenzymes that plays important roles in the transport of sugar and phosphate (143). It is found in almost all physicochemical body tissues. It is manufactured by bone(40-75%), liver, intestine and placenta. It is called an enzyme of secretion (144). These isoenzymes of alkaline phosphatase have different physicochemical properties and are originated from different tissues(143). Alkaline phosphatase is necessary for hydrolysis of organic phosphates and is, therefore, important for digestion and absorption through the mucus membrane of the gastrointestinal tract. It is rapidly and constantly excreted into urine, bile and the gastrointestinal tract. The activity of alkaline phosphatase provided an important information about bone formation (osteoblastic activity). It is clinically useful as a parameter of liver function, being the most sensitive test of common bile duct obstruction. Alkaline phosphatase is frequently the first enzyme to be studied in hepatic disease and is used extensively in the differential diagnosis of jaundice(144).

In our experiment, all doses of *M. alba* L. leaf extract did not significantly altered AST, ALT and alkaline phosphatase activities. Thus, *M. alba* L. leaf extract did not induce any hepatotoxicity.

In addition to the liver enzymes, the effect of *M. alba* L. leaf extract on protein metabolism was also determined. Proteins are taken into the body via the diet. Almost all protein taken in food is fully digested to amino acids in the small intestine. Amino acids are transported to the liver by the bloodsteam where albumin, the alpha and beta globulins, prothrombin, and fibrinogen are synthesized exclusively. The plasma cell is the end-stage production and storage cell. Once synthesized, plasma proteins are released into the bloodstream. All body proteins form a large pool that can be drawn on by any tissue that needs protein. The protein that is filtered through the glomerulus is almost totally reabsorbed. Hypoproteinemia occurs slowly and insidiously in many unrelated disease states. It can occur secondary to an inadequate intake of protein and impaired absorption, inadequate protein synthesis within the body, an increased loss of protein that occurs with renal dysfunction or pathological states. Loss also occurs because of increased protein catabolism, increased levels of catabolic normones, or in gastrointestinal enteropathies. Hyperproteinemia usually occurs secondary to pathological loss of fluid or a marked decrease in water intake. The absolute amount

of serum proteins is unchanged, but there is an increase in concentrations resulting from the loss of solvent water(144).

In our experiment, all dose of *M. alba* L. leaf extract thus used did not significantly altered total serum protein levels. These data suggested that *M. alba* L. leaf extract did not affect protein metabolism.

Albumin is a plasma protein synthesized in the liver and the major constituent of the serum protein and contributes approximately 80% of serum colloid osmatic pressure. It can be directly measured. Alterations in serum albumin concentration parallel many alterations in the serum total protein. Albumin is the most important serum protein in maintaining intravascular oncotic pressure. It is considered the primary nutritional source for body tissue. Serum albamin is important in transport, being the major vehicle for calcium, magnesium, bilirubin and fatty acids transport as well as the transport of many drugs(144). Therefore, hypoalbuminemic states commonly are associated with edema and transudation of extracellular fluid. A lack of essential amino acids, from either malnutrition or malabsorption, or impaired synthesis by the liver can result in decreased serum albumin concentrations. Most form of hepatic insufficiency are associated with decreased synthesis of albumin. It can be lost directly from the blood because hemorrhage, burns, or exudates, or it may be lost directly into the urine because of nephrosis. Serum albumin concentrations seldom increase but may be noted in volume depletion, shock, or immediately after the administration of large amounts of intravenous albumin(143).

In our experiment, all animals treated with *M. Alba* L. leaf extract did not showed a significant alteration of serum albumin level. These data suggested that *M. Alba* L. leaf extract did not either reduce the production of albumin nor induce malnutrition and malabsorption.

In additon to protein metabolism, the effect of *M. alba* L. leaf extract on lipid metabolism was also determined. Serum cholesterol, triglyceride, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels were monitored to determine the effect of *Morus alba* L. leaf extract on lipid metabolism. Cholesterol is a fat-related chemical, a complex alcohol that can be synthesized by all body cells except the brain and ingested in the diet. This alcohol can form esters with fatty acid, and store in the form of cholesterol. Cholesterol is a primary constituted of the low-

density lipoproteins (LDL) but can be found in the high-density lipoproteins (HDL) as well as in the very low-density lipoproteins (VLDL). Cholesterol is the most important sterol ("a solid alcohol of animal or vegetable origin with properties like fats") in animal metabolism. It is found only in foods of animal origin. It is important in maintaining the permeability of cell membranes. In addition, it is the precursor for the synthesis of steroid hormone in adrenal cortex and ovary as well as bile acids. Cholesterol is synthesized by the body from small molecules in a long and complex series of condensation, transformation and ring closures. Of these, one vastly important reaction is that with acetyl coenzyme A (CoA) and acetoacetyl CoA because this pathway is shared with both carbohydrate and fatty metabolism. The major sites of cholesterol synthesis are the liver and the intestines in human. Cholesterol and all lipids are digested in the duodenum. After bile emulsification, cholesterol diffuses into the blood or lymph(144).

Triglecerides are the major form of fat found in nature, and their primary function is to provide energy for the cell. Because of their water insolubility, triglycerides are transported in the plasma in combination with other more polar lipids (phospholipids) and proteins, as well as with cholesterol and cholesteryl esters, in the complex lipoprotein macromolecules. Plasma triglycerides are derived from two sources, intestinal and liver. Intestinal triglycerides are synthesized from dietary fat. The source of the fatty acids present in the triglycerides entering the blood from the liver depends greatly on the individual's nutritional state. Thus in the fasting state, fatty acids derived from adipose cell triglycerides are taken up by the liver and a portion is reexcreted as VLDL. Following a meal, dietary carbohydrates are taken up by the liver and converted to triglycerides, which are secreted as lipoproteins. It is important to realize that, except during the absorption of dietary fat, the liver is the main contributor of triglyceride to the plasma. The size, triglyceride content, and particle density of the lipoprotein complexes formed by the intestines and liver varies according to the amount of triglyceride being released. Thus high rates of release result in large complexes with a higher triglyceride load and a correspondingly lower density. In fact, the lipoprotein complexes released from the liver under such condition may reach a size not much smaller than that of the intestinal chylomicrons, even

though they normally have a somewhat lower triglyceride content and therefore a higher density(145).

In our experiment, all animals which were fed with doses of *M. alba* L. leaf extract did not show any elevation or reduction in their serum cholesterol, triglyceride, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels. Therefore, the result indicated that *M. alba* L. leaf extract at doses studied were not capable of reducing serum lipid levels.

Coronary atherosclerosis is primarily resulted from the accumulation of fatty deposits in the walls of coronary arteries, which lead to the formation of fibrous tissue in the vessel wall. Coronary atherosclerosis is the most common type of heart disease and the leading cause of death in many countries. There are many factor associated with coronary atherosclerosis. Elevated total cholesterol (LDL cholesterol), decrease HDL cholesterol and elevated triglyceride (VLDL cholesterol , remnamt lipoproteins) are the major risk factor of coronary atherosclerosis (145).

Since serum cholesterol in the form of LDL as well as serum triglyceride transported as VLDL are both atherogenic. Elevation of these lipid parameters are a dangerous sign of disease. Thus, our result ensures the safety use of *M. alba* L. leaf extract in this aspect.

In addition to the metabolic effects mentioned above, serum glucose was monitored to illustrate the effect of this extract on glucose metabolism. Glucose concentration in the extracellular fluid is regulated closely by homeostatic mechanisms to provide body tissues with a ready source of energy. The plasma glucose concentration usually is measured in either the fasting or postprandial state, depending on the type of information desired. Generally, normal glucose values refer to the plasma glucose concentration in the fasting state(143).

In our experiment, almost all doses of *M. alba* L. leaf extract did not significantly altered serum glucose levels. Thus *M. alba* L. leaf extract might not affected glucose metabolism. But when the high dose of this leaf extract was fed to female rats, a significant evaluation of blood glucose was obtained. It should be noted that this dose was much higher than the therapeutic dose normally use. Chronic administration of a high dose of the water extract of *M. alba* L. might lead to the intake of a large amount

of sucrose contained in this leaf and resulted in some evaluation of serum gluocose level.

However, in Thailand nowadays, *M. alba* L. leaf extract is normally used in the form of tea for health promotion. One therapeutic benefit of drinking this tea that has been claimed is to lower blood sugar. But in our toxicological study, we did not obtain any lowering of serum glucose level in animals treated with lower dose of leaf extract. One possible reason is that our experimental animals were normal rats. *M. alba* L. has been demonstrated to be capable in lowering elevated blood glucose in streptozotocin and alloxan-induced diabetic rats. While in animals with normal blood glucose level, *M. alba* L. might not further reduce their blood glucose level. This phenomenon is considered to be a good property since hypoglycemia is considered as an adverse effect of anti-diabetic drugs. However, the high dose of leaf extract elevated plasma glucose. But, this was a dose much higher than administered dosage and might not affect the therapeutic use.

Regarding to hematologic examination, the parameters that examined include red blood cell count, hemoglobin, white blood cell count and differential white blood count .

Red blood cells or erythrocytes are produced in the bone marrow, released into the peripheral blood, circulate for approximately 120 days, and are cleared by the reticuloendothelial system. The primary function of red blood cells is to transport oxygen to tissues. The hematocrit is determined by centrifuging a capillary tube of whole blood and comparing the height of the settled red cells to the height of the column of whole blood. The percentage of red cells to the blood volume is the hematocrit value. A decrease in hematocrit may result from bleeding, bone marrow suppressant effect of drugs, chronic diseases, genetic alterations in red cell morphology or hemolysis. An increase in hematocrit may result from hemoconcentration, polycythemia vera, or polycythemia secondary to chronic hypoxia. Hemoglobin is the oxygen carrying compound contained in red blood cells. Therefore, the total hemoglobin concentration primarily depends on the number of red cells in the blood sample, although it also is slightly influenced by the amount of hemoglobin in each red cells. A hemoglobin determination is preferable to a red blood cell determination because it most directly reflects the oxygen transport capability of blood.

However, the hematocrit value is most commonly used clinically because it is technically simple to perform(143).

White blood cells have no physiologic function within the vascular system. The blood merely serves as a transportation network that allows white cells to move from their site of origin, the bone marrow, into various body tissues and cavities. Neutrophils are the most abundant of the circulating white blood cells, followed in order of frequency by lymphocytes, monocytes, eosinophils and basophils. The neutrophils, eosinophils, basophils, and monocytes are formed from stem cells in the bone marrow. Some lymphocytes are formed in the bone marrow, but most are formed in lymph nodes, thymus, and spleen(143).

The normal maturation sequence of the neutrophil in the bone marrow is as follows : the stem cell gives rise to the myeloblast, which then matures progessively into a promyelocyte, myelocyte, metamyelocyte, band neutrophil, and finally into a polymorphonuclear segmented neutrophil. The number of neutrophils commonly is increased during bacterial or fungal infections because these cells are essential in killing invading micro-organisms, and a shift to the left is commonly associated with a bacterial infection. However, neutrophils also are important in the pathogenesis of tissue damage in some noninfectious diseases. A decrease in neutrophils commonly causes in metastatic carcinoma, lymphoma, and chemotherapeutic agents(143).

Lymphocytes constitute the second most common white cell in circulating blood. These lymphocytes respond to foreign antigens by initiating the immune defense system. The vast majority of the lymphocytes are located in the spleen, lymph nodes, and other organized lymphatic tissue. Increased numbers of lymphocyte on a white differential count sometimes are accompanied with viral infections such as infectious mononucleosis, mumps, and rubella. A relative lymphocytosis sometimes is encountered when the total lymphocytes have remained constant despite a decline in the total neutrophils(143).

The precursors of macrophages are the monocytes, which are formed in the bone marrow and transported by the blood to tissues,where they mature. Monocytosis may be observed in subacute bacterial endocarditis, malaria, and tuberculosis, as well as during the recovery phase of some infections(143).

Eosinophils possess phagocytic activity, catalyze the oxidation of many substances, facilitate killing of micro-organisms, initiate mast cell secretion, protect against various parasites, and play some role in host defense. Eosinophilia probably is associated most commonly with allergic reactions to drugs, allergic disorders (e.g. hay fever, asthma, eczema), invasive parasitic infection (e.g. hookworm, schistosomiasis, trichinosis), collagen vascular diseases (e.g. rheumatoid arthritis, eosinophilic fascilis, eosinophilic-myalgia syndrome) and malignancies (e.g Hodgkin's disease)(143).

An increase in basophils commonly accompanies chronic myeloid leukemia, myelofibrosis, and polycythemia vera. A decrease in the number of basophils generally is not readily apparent because of the paucity of these cells in the blood(143).

In our experiment, all dose of *M. alba* L. leaf extract did not significantly affect all hematologic parameter studied. This indicated that *M. alba* L. leaf extract did not exhibit any toxic effect on hematoporesis as well as induce any pathologic associated with change in blood picture.

Regarding to histopathologically examination, all organ specimens obtained from treated animals did not show any histopathological abnormality.

Our result was in concord with the earlier experiment. In that study, the toxicity of mulberry leaf extract was explored in rats. The extract was administered orally at concentration of 0% (control group), 0.1%, 0.4% and 1% in basal diet for 90 days. No remarkable toxic effect in treated animals of both sexes was observed as determined by body weight gain or necropsy. Hematology and blood chemistry revealed no abnormalities. Pathological examination revealed no toxic change in major organs observed. Therefore, the authors suggested that dietary intake of 1% mulberry leaf extracts for 90 days (884.5 mg/kg/day for males and 995.7 mg/kg/day for females as mean daily intake) caused no toxicological change in rats (146).

The result of our toxicological study led to the conclusion that all doses of the water extracts of *M. alba* L. leaf used in our experiment were safe for subchronic administration since no serious side effect was detected.

CHAPTER 6
CONCLUSION

Results obtained in our study indicated that; in acute toxicity study, the LD_{50} of *M. alba* L. leaf extract in mice and rats was approximately 5 g/kg when administered intraperitoneally. The abnormal signs and symptoms found in rats were sedation, respiratory depression and decrease in motor activity. The abnormal signs and symptoms found in mice were sedation and decrease in motor activity. In orally, the only abnormal signs and symptoms in rats and mice were CNS depression and respiratory depression. Moreover, all animals were recovered within 15-30 minutes.

In subchronic toxicity study, the abnormal signs and symptom were not found throughout the period of study when administered orally for 60 days . In both male and female, almost all doses of the *M. alba* L. leaf extract did not significantly affect blood chemistry and hematological values when compared to the control group. Only the dose of 3 g/kg BW/day of female rats increased blood glucose level significantly. Moreover, Microscopic examination of the major organs indicated no significant histopathological abnormalities.

Finally, *M. alba* L. leaf extract is safe for used. Pharmacological dosage form of *M. alba* L. leaf extract might be a valuable drug in the future.

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APPENDIX

APPENDIX

Reagent Preparation

1. 10% Buffered neutral formalin solution

Formaldehyde, 37%-40%.....100.0 ml

Distilled water .....90.0 ml

Sodium phosphate, monobasic .....4.0 ml

Sodium phosphate, dibasic (anhydrous).....6.5 ml

Store in properly labeled container. Label as HAZARDOUS chemical.

2. 10% Ethylenediamine tetra-acetic acid disodium salt dihydrate (EDTA)

Ethylenediamine tetra-acetic acid disodium salt dihydrate .....100.0 g

Distilled water ....1000.0 ml

3. Mayer's hematoxylin stock solution

Ammonium or potassium alum .....50.0 ml

Distilled water .....1000.0 ml

Hematoxylin crystal .....1.0 g

Sodium iodate .....0.2 g

Citric acid .....1.0 g

Chloral hydrate .....50.0 g

4. Eosin stock solution

Eosin Y, water soluble .....1.0 g

Distilled water 100.0g

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