# Anti- Diabetogenic Impact of Mahogany (*Swietenia macrophylla)* and Bitter melon (*Momordica charantia)* on Alloxan Induced Diabetic Rabbit Model

***A Thesis***

***By***

**MAHABUBA BEGUM**
**Student ID. 1505018**
**Session: 2015-2016**
**Semester: January-June, 2016**

**MASTER OF SCIENCE (M.S.)**
**IN**
**PHARMACOLOGY**

**DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY**
**HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200, BANGLADESH**

**JUNE, 2016**

# Anti- Diabetogenic Impact of Mahogany (*Swietenia macrophylla)* and Bitter melon (*Momordica charantia)* on Alloxan Induced Diabetic Rabbit Model

*A Thesis*

*By*

**MAHABUBA BEGUM**
**Student ID. 1505018**
**Session: 2015-16**
**Semester: January-June, 2016**

***Submitted to the Department of Physiology and Pharmacology***
***Hajee Mohammad Danesh Science and Technology University,***
***Dinajpur-5200, Bangladesh***
***In fulfillment of the requirements for the degree of Master of Science***

**DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY**
**HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR0-5200, BANGLADESH**

**JUNE, 2016**

# Anti- Diabetogenic Impact of Mahogany (*Swietenia macrophylla)* and Bitter melon (*Momordica charantia)* on Alloxan Induced Diabetic Rabbit Model

***A Thesis***
***By***

**MAHABUBA BEGUM**
**Student ID. 1505018**
**Session: 2015-16**
**Semester: January-June, 2016**

***Approved as to the style and contents by***

| | |
|---|---|
| **(Dr. Fahima Binthe Aziz )** | **(Dr. Rakibul Islam)** |
| Supervisor | Co-supervisor |

**(Dr. Rakibul Islam)**
Chairman, Examination committee
Department of Physiology and Pharmacology

**DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY**
**HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR0-5200, BANGLADESH**

**JUNE, 2016**

DEDICATED

TO MY

BELOVED PARENTS

# ACKNOWLEDGEMENT

*At first, I gratefully wish to acknowledge Almighty Allah for His kind help to materialize my dream for the degree of Master of Science (MS) in Pharmacology.*

*I would like to express my heart-felt sense of gratitude and indebtedness to my research supervisor Dr. Fahima Binthe Aziz, Associate Professor, Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University, Dinajpur for her congenial, patience and practical cooperation, stimulating counsel, distinguishable scholastic guidance and sincere advice throughout the research work, giving valuable suggestions, fruitful discussion and timely instruction.*

*I would like to extend my gratitude and indebtness to my research co-supervisor Dr. Rakibul Islam, Associate Professor and head, Department of Physiology and Pharmacology, for his pleasant and realistic assistance, candid instruction during the research work, giving valuable suggestions, productive conversation to continue my research work.*

*I wish to express my deepest sense of gratitude to Dr. Md. Bazlar Rashid, Assistant Professor, Department of Physiology and Pharmacology and also to Dr. Md. Mahmudul Hasan, Assistant Professor and Dr. Misrat Masuma Parvez, Lecturer, Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University, Dinajpur for their generous help and extended co-operation during the research work.*

*I like to express profound gratitude and thanks to all reverend teachers of the Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur for their kind help, co-operation, encouragement and valuable suggestions*

*I also wish to memorize my family members and my closest friends Sumon, Boby, Roji, Ferdous, Nyon, Dip, Ambiara and others whom I cannot memorize at this moment for their honest advice, precious suggestion and continuous inspiration.*

*Finally, I express my cordial thanks to all Office staff of the Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University, Dinajpur for their sincere help and co-operation during the completion of this work.*

***The Author***

***June 2016***

# ABSTRACT

The present study was undertaken to investigate the anti-diabetogenic effect of bitter melon and mahogany on alloxan induced diabetes in experimental rabbits. The Newzeland white breed male rabbit at the age of 4 months were randomly assigned into five groups ($T_1$, $T_2$, $T_3$, $T_4$ and $T_5$) and each group was remind 4 rabbits. Group $T_1$ was kept for negative control or normal group. The rest of the group ($T_2$, $T_3$, $T_4$ and $T_5$) were injected with alloxan intramuscularly @ 75mg/kg body weight of rabbit. So group $T_2$ was positive control group for diabetes. Group $T_3$, $T_4$ and $T_5$ was considered for bitter melon, mahogany and both of them at a dose of 250 mg /kg body weight, 50 mg/kg body weight and at with combined with previous dose and was considered $T_3$, $T_4$ and $T_5$ respectively. After acclimatization, diabetes was induced in four groups of rabbits ($T_2$, $T_3$, $T_4$ and $T_5$) by administering alloxan injection in a dose of 75mg/kg body weight intramuscularly. The suspension of whole fruit was tested for its efficacy in alloxan induced diabetic rabbits. Over the course of the trial, observations were recorded for induction of diabetics, blood glucose level, body weight after 72 hours. Blood glucose level were increased significantly ($p<0.05$) in all treated groups compared to the control group and the highest induction was recorded in group $T_2$ treated with alloxan. Body weight was decreased significantly ($p<0.05$) in alloxan treated group and lowest was recorded in group $T_2$ which received alloxan treatment. There was significant decreased in in blood glucose level in all bitter melon and mahogany treated group ($T_3$, $T_4$, $T_5$) compared to the $T_2$ group and lowest glucose level was recorded in T5 group when treated with medicinal herbs and body weight was increased in all treated group $T_3$, $T_4$, $T_5$ compare to the $T_2$ group and highest was recorded in $T_5$ group while treated with those. The present study reveals that combined treatment with bitter melon and mahogany increase body weight and decrease glucose level without affecting health of rabbits. The results of this study show that chronic oral administration of a suspension of mahogany seed and bitter melon fruits, an appropriate dosage may be good alternative as anti-diabetic agent.

# CONTENTS

# CONTENTS (Contd.)

## CONTENTS (Contd.)

# LIST OF TABLES

# LIST OF FIGURES

# LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| B. wt. | : | Body weight |
| Conc. | : | Concentration |
| Cu. mm | : | Cubic millimeter |
| d.w. | : | Drinking water |
| DM | : | Diabetics mellitus |
| ESR | : | Erythrocyte Sedimentation Rate |
| *et al.* | : | Associates |
| Fig. | : | Figure |
| GDM | : | Gestational Diabetes Mellitus |
| GDP | : | Gross Domestic Product |
| Hb | : | Hemoglobin |
| ICD | : | International Classification of Diseases |
| IDDM | : | Insulin-Dependent Diabetes Mellitus |
| IDF | : | International Diabetes Federation |
| IND | : | International Nomenclature of Diseases |
| J. | : | Journal |
| LADA | : | Latent Autoimmune Diabetes of Adults |
| Lit | : | Liter |
| Ltd. | : | Limited |
| mg | : | Milligram |
| $mm^3$ | : | Cubic millimeter |
| MRDM | : | Malnutrition-Related Diabetes Mellitus |
| NIDDM | : | Non Insulin-Dependent Diabetes Mellitus |
| No. | : | Number |
| PBS | : | Phosphate Buffer Solution |
| PCV | : | Packed Cell Volume |
| PM | : | Population Mean |
| SE | : | Standard Error |
| SM | : | Sample Mean |
| STZ | : | Streptozotocin |
| TEC | : | Total Erythrocyte Count |
| Vol. | : | Volume |
| WHO | : | World Health Organization |

# CHAPTER 1

# INTRODUCTION

# CHAPTER 1

## INTRODUCTION

The usages of medicinal plants is traditionally rooted in Bangladesh and still an essential part of public healthcare. Recently, a dramatically increasing prevalence brought diabetes mellitus and its therapy to the focus of public health interests in Bangladesh. Traditional medicinal plants are commonly used in Bangladesh to treat diabetes. The available data regarding the anti-diabetic activity of the detected plants is not sufficient to adequately evaluate or recommend their use. Clinical intervention studies are required to provide evidence for a safe and effective use of the identified plants in the treatment of diabetes. Recent data suggest that the utilization of traditional medicine health services in Bangladesh is widespread and plays a crucial role in providing health care for poor people, people in rural areas and for tribal people.

In the context of using traditional medicinal plants for treating diabetes, extensive screening has been performed in many ethnomedical systems within the Indian subcontinent ( *Grover JK, Yadav S, Vats V: Mukherjee PK, Maiti K, Mukherjee K, Houghton PJ,2002,2006)* . However, in Bangladesh the traditional medicinal plants that are used for the treatment of diabetes have not yet been studied in great detail. Therefore, these herbal remedies are important objects of research, especially in context of the virtually exploding prevalence of diabetes mellitus in Bangladesh. Although diabetes is more prevalent in urban areas , in rural communities prevalence rates for diabetes rang from 2.3% to 6.8% in between 1999 to 2004.

A recent survey in Bangladesh demonstrated that in slum areas, 86% of female and 78% of male diabetic patients use either inadequate medical treatment or none ( *National*

*Institute of Population Research and Training (NIPORT),2006)* . In non-slum areas only 34% of female and male diabetic patients undergo adequate medical treatment raising the question, whether herbal remedies of the traditional Bangladeshi medicine may offer a safe, effective and reasonable alternative therapy for diabetes.

There are some synthetic drugs which are mostly used for the treatment of diabetics mellitus and also the treatment are cost effective. In case of synthetic drugs there are also some side effects and long term use may cause increase the risk of cholesterol in bile duct, gallstone, imsomnia, fat diposition and so on. In case of poor people it is not easy to use synthetic drug for long term.

On the other hand the use of herbal medicinal plant such as mahogany and bitter melon as the treatment for diabetics it is easy to use. In case of mahogany seed it contain saponin and flavonoids which have the great effect on lowering blood glucose level.

Its flavonoid content bergunauntuk blood circulation, specially to prevent the blockage of blood vessel, reduce cholesterol levels and accumulation of fat in the walls of blood vessels, helps reduce pain, bleeding and bruising as well as acting as antioxidants to eliminate free radicals.

Saponins are useful to prevent pestilence, reduce body fat, boost the immune system, improves blood sugar levels, and strengthen the liver function and slow the clotting process. Bitter melon also beneficial for health.

This plant has also some side effect. It is so bitter that not easy to consume. If the dose are higher may cause increased bile cholesterol, already feel sick in the gut. So as long as measure the dose flavonoids that help permeability of blood vessel flexibility, so it can be used for high blood pressure.

Diabetes mellitus (DM) is a heterogeneous group of metabolic disorders characterized by persistent hyperglycemia (Neelesh M, Sanjay J, Sapna M 2010 ) and derangement in the metabolism of carbohydrates, fats, and proteins as a result of defects in insulin secretion and/or insulin action . Diabetes mellitus, a metabolic disease with manifestation of hyperglycemia, is a fast growing health problem throughout the world. Recent data from the International Diabetes Federation (IDF) indicates that diabetes mellitus affects over 366 million people worldwide and this is likely to increase to 552 million or even more by the year 2030 (Whiting DR, Guariguata L, Weil C, Shaw J 2011) . In Africa, more than 14 million people have diabetes, accounting for about 4.3% of adults and is responsible for about 401276 deaths in 2012 in the region (International Diabetes Federation 2013). West Africa recorded the highest number of DM cases with Nigeria (3.2 million diabetics) and Côte d'Ivoire (421023 diabetics) occupying first and second positions, respectively. In Southern Africa, South Africa tops the list (2.0 million diabetics) followed by the Democratic Republic of Congo (737000 diabetics). Kenya was listed as the fifth country in Africa and the first from the eastern region of Africa (720730 diabetics), while Cameroon (517860 diabetics) recorded the highest figure from the central region. North Africa had the least number of diabetics among the African subregions with no single nation in the top ten list of African countries with DM . Generally, diabetes is classified into two main types: type-1 diabetes, a state of insulin deficiency because of defect in islet β-cell function and type-2 diabetes which mainly characterized by resistance to the actions of insulin. The overall prevalence of diabetes mellitus in the global population is approximately 6%, of which 90% is type 2 diabetes.

Diabetes mellitus is a multifarious group of symptoms characterized by hyperglycemia, abnormal lipid and protein metabolism, along with specific long-term complications affecting the retina, the kidney, and the nervous system mainly ( S. M. Setter, R. K.

Campbell, and C. J. Cahoon,2003). Consumption of calorie-rich diet, obesity, and sedentary life style have lead to tremendous increase in the number of diabetics worldwide especially in Asia. Many oral hypoglycaemic agents, such as sulfonylurea and biguanides, are available along with insulin for the treatment of diabetes mellitus, but these agents have significant side effects and some are ineffective in chronic diabetic patients (L. Pari and R. Saravanan, 2004). Thus, there is an increasing demand of new antidiabetic natural products especially neutraceuticals with lesser side effects and high antidiabetic potential.

In this context, worldwide efforts have been taken to improve plant-based therapies. WHO (World Health Organization, 1980) recommended for the assessment of traditional medicinal plant in connection with the management of diabetes mellitus (S. L. Badole, N. M. Patel, P. A. Thakurdesai,2008; J. H. Hsu, Y. C. Wu,2004). Currently, several hundred plants have been reported to have beneficial effects for the treatment of diabetes mellitus, and we have several reports in this line as well as of others (P. Ljubuncic, H. Azaizeh 2006). Research on phytomolecules as diabetic remedies is upraising gradually as these are with minimal or no side effects (O. Said, S. Fulder, K. Khalil 2008).

*Momordica charantia* (MC), traditional medicine for its glucose-lowering effects. It is a climber of the family Cucurbitaceae, and iswidely cultivated in Africa, Asia and South America both for food and for its medicinal use (Ooi et al., 2010). It is widely available in Bangladesh and also well-known as an agent with several anti-diabetic effects (*Shibib BA, Khan LA, Rahman R* 1993, *Chaturvedi P:2012)* . Numerous studies revealed anti-hyperglycemic effects for its fruits in experimental animal studies of induced diabetes, but also the leaves, stem and seeds were reported to be used for anti-diabetic treatment. Conflicting results were reported by small clinical trials; only modest hypoglycemic

effects less distinct than for metformin were shown in type 2 diabetes mellitus patients and no effect on the levels of plasma insulin and glucose was detectable in obese men , revealing the inconsistent outcomes for *Momordica charantia* regarding clinical trials. The parts used include the whole plant, fruit and seeds, which are bitter due to the presence of the chemical momordicin (Beloin et al., 2005). Preparations used include injectable extracts, juice extracts, and fried melon bits, among others. The glucose-lowering effect of its unripe fruit juice has been demonstrated in both experimental animal models (Welihinda et al., 1986) and human clinical trials (Srivastava et al., 1993). Active components of the fruit include charantin, vicine and insulin-like polypeptide (Lucy et al., 2002). Alcohol-extracted charantin from MC consists of mixed steroids and was reported to be more potent than tolbutamide (an oral glucose lowering drug) in an animal study (Sarkar et al., 1996). It has been shown to decrease blood glucose levels when injected subcutaneously into type 1 DM patients (Baldwa et al.,1977). Oral administration of bitter melon preparations also showed significant results when tried clinically in type 2 DM patients (Srivastava et al., 1993). Several mechanisms of action have been postulated including: enhanced insulin secretion, insulin-like action, tissue glucose uptake, liver muscle glycogen synthesis, glucose oxidation, and decreased hepatic gluconeogenesis. Hepatic portal inflammation and testicular lesions in dogs were reported in excessive administration of cerasee (acomponent of the wild variety of MC) (Dixit et al., 1978). It is furthermore contraindicated in pregnancy and when other glucose-lowering agents are being used (Basch et al., 2003).

Swietenia mahagoni (S. mahagoni), is under family Meliaceae, beautiful, lofty, evergreen, large native tree of tropical America, Mexico, South America, and India. The seeds of S. mahagoni have been reported for its anti-inflammatory, antimutagenecity, and antitumour activities. In Indonesia and in India, S. mahagoni seed used as folk

medicine to cure diabetes. The is no systematic work about the antidiabetic activity of S. mahagoni though there are very few informations of this plant inre this line (D.-D. Li, J.-H. Chen, Q. Chen *et al.,* 2005). The present study was therefore carried out to evaluate the traditional used of S. mahagoni as antidiabetic scientifically. Furthermore, the positive roles of natural products (neutraceuticals) for the correction of oxidative stress and hyperlipidaemia, which are diabetes-related complications, were also assessed.

The general objective of this study was to investigate the antidiabetogenic effect of bitter melon and mahogany on alloxan induced diabetic rabbits.

The specific objectives of this study are as follows:

- To evaluate the alloxan induced diabetics occurred in the experimental rabbits body weight of rabbits.
- To investigate the antidiabetic effect of *Momodica charantia* (Bitter melon) and *Swietenia macrophylla* (Mahogany) on blood glucose level in alloxan induced diabetic rabbit.
- To see the effects on body weight, blood glucose level and haematological parameters in experimental rabbits.

# CHAPTER 2

## REVIEW OF LITERATURE

# CHAPTER 2

# REVIEW OF LITERATURE

The purpose of this chapter is to provide a selective review of the research works accomplished in relation to the present study. Literatures anti-diabetogenic impact of *Momordica charantia* (Bitterr melon) and *Swietenia macrophylla* (Mahogany) on alloxane induced diabetic rabbit model which is related to this study has been reviewed under the following heading.

## 2.1 Diabetes

Diabetes mellitus is a multifarious group of symptoms characterized by hyperglycemia, abnormal lipid and protein metabolism, along with specific long-term complications affecting the retina, the kidney, and the nervous system mainly (S. M. Setter, R. K. Campbell, and C. J. Cahoon, 2003). Consumption of calorie-rich diet, obesity, and sedentary life style have lead to tremendous increase in the number of diabetics worldwide especially in Asia (G. Klein, J. Kim, K. Himmeldirk, 2007). Many oral hypoglycaemic agents, such as sulfonylurea and biguanides, are available along with insulin for the treatment of diabetes mellitus, but these agents have significant side effects (H. P. Rang, M. M. Dale, and J. M. Ritter, 1991), and some are ineffective in chronic diabetic patients (L. Pari and R. Saravanan, 2004). Thus, there is an increasing demand of new antidiabetic natural products especially neutraceuticals with lesser side effects and high antidiabetic potential.

In this context, worldwide efforts have been taken to improve plant-based therapies (P. Daisy, R. Jasmine, S. Ignacimuthu, 2009). WHO (WHO, Geneva, Switzerland, 1980) recommended for the assessment of traditional medicinal plant in connection with the

management of diabetes mellitus. (K. E. Innes and H. K. Vincent, 2007). Currently, several hundred plants have been reported to have beneficial effects for the treatment of diabetes mellitus, and we have several reports in this line (R. Maiti, U. K. Das, 2005) as well as of others. Research on phytomolecules as diabetic remedies is upraising gradually as these are with minimal or no side effects (O. Said, S. Fulder and Khalli, 2008).

### 2.1.1 History of diabetics

Diabetes is one of the first diseases described with an Egyptian manuscript from c. 1500 BCE mentioning “too great emptying of the urine. (Leonid Poretsky). The first described cases are believed to be of type 1 diabetes. (Leonid Poretsky, 2009). Indian physicians around the same time identified the disease and classified it as madhumeha or honey urine noting that the urine would attract ants. (Leonid poretsky, 2009). The term "diabetes" or "to pass through" was first used in 250 BCE by the Greek Apollonius of Memphis (Leonid Poretsky 2009)1 and type 2 diabetes were identified as separate conditions for the first time by the Indian physicians Sushruta and Charaka in 400-500 CE with type 1 associated with youth and type 2 with obesity. The term mellitus" or "from honey" was added by Thomas Willis in the late 1600s to separate the condition from diabetes insipidus which is also associated with frequent urination. (Leonid Poretsky, 2009).

### 2.1.2 Further history

The first complete clinical description of diabetes was given by the Ancient Greek physician Aretaeus of Cappadocia (fl. 1st century CE), who also noted the excessive amount of urine which passed through the kidneys.*”*(Dallas, John 2011).

Diabetes mellitus appears to have been a death sentence in the ancient era. Hippocrates makes no mention of it, which may indicate that he felt the disease was incurable. Aretaeus did attempt to treat it but could not give a good prognosis; he commented that "life (with diabetes) is short, disgusting and painful."(Medvei, Victor Cornelius 1993). The disease must have been rare during the time of the Roman empire with Galen commenting that he had only see two cases during his career. (Leonid Poretsky, 2009).

In medieval Persia, Avicenna (980–1037) provided a detailed account on diabetes mellitus in *The Canon of Medicine*, "describing the abnormal appetite and the collapse of sexual functions," and he documented the sweet taste of diabetic urine. He also described diabetic gangrene, and treated diabetes using a mixture of lupine, trigonella (fenugreek), and zedoary seed, which produces a considerable reduction in the excretion of sugar, a treatment which is still prescribed in modern times. The sweet urine symptom of diabetes is evident in the Chinese name for diabetes, *táng niǎo bìng* meaning "sugar urine disease". This name has also been borrowed into Korean and Japanese. In 1776 Matthew Dobson confirmed that the sweet taste comes from an excess of a kind of sugar in the urine and blood. (Dobson, M. 1776).

Although diabetes has been recognized since antiquity, and treatments of various efficacy have been known in various regions since the Middle Ages, and in legend for much longer, pathogenesis of diabetes has only been understood experimentally since about 1900 (Patlak M, December 2002). An effective treatment was only developed after the Canadians Frederick Banting and Charles Best first used insulin in 1921 and 1922. (Leonid Poretsky, 2009).

The discovery of a role for the pancreas in diabetes is generally described by Joseph von Mering and Oskar Minkowski, who in 1889 found that dogs whose pancreas was

removed developed all the signs and symptoms of diabetes and died shortly afterwards. (Von Mehring J, Minkowski O. 1890). In 1910, Sir Edward Albert Sharpey-Schafer suggested that people with diabetes were deficient in a single chemical that was normally produced by the pancreas—he proposed calling this substance insulin, from the Latin insula, meaning island, in reference to the insulin-producing islets of Langerhans in the pancreas. The endocrine role of the pancreas in metabolism, and indeed the existence of insulin, was further clarified in 1921, when Sir Frederick Grant Banting and Charles Herbert Best repeated the work of Von Mering and Minkowski, and went further to demonstrate they could reverse induced diabetes in dogs by giving them an extract from the pancreatic islets of Langerhans of healthy dogs. *(*Banting FG, Best CH, Collip 1991). The islets of Langerhans was discovered in 1869 by an anatomist named Paul Langerhans. He identified the keys cells in the pancreas which produce the main substance that controls glucose levels in the body. (Bryan, Jenny 2004).

Banting, Best, and colleagues (especially the chemist Collip) went on to purify the hormone insulin from bovine pancreases at the University of Toronto. This led to the availability of an effective treatment—insulin injections—and the first patient was treated in 1922. The first successful patient treated was a 14-year-old boy who weighed only 65 pounds. When he was given the extract on January 23, his ketonuria and glycosuria were almost eliminated. His blood sugar levels dropped as low as 77%. Six more patients were treated in February 1922 and quickly experienced an improved standard of life. A pharmaceutical firm named Eli Lilly and Company, with the University of Toronto, began the mass production of insulin by the fall of 1923, 25,000 patients were being treated in Canada and the United States. Insulin production and therapy rapidly spread around the world, largely as a result of this decision. The distinction between what is now known as type 1 diabetes and type 2 diabetes was first

clearly made by Sir Harold Percival (Harry) Himsworth, and published in January 1936. (Himsworth, 1936).

**2.2 Present status of diabetics**

As of 2014, an estimated 387 million people have diabetes worldwide (International Diabetes Federation 2014) with type 2 DM making up about 90% of the cases. (Shi, Yuankai; Hu, Frank B, 2014). This represents 8.3% of the adult population, with equal rates in both women and men *(*Vos T, Flaxman AD, Naghavi M 2012). From 2012 to 2014, diabetes is estimated to have resulted in 1.5 to 4.9 million deaths each year (*World Health Organization 2013)* · Diabetes at least doubles a person's risk of death (*WHO October 2013).* The number of people with diabetes is expected to rise to 592 million by 2035 (*International Diabetes Federation 2014).* The global economic cost of diabetes in 2014 was estimated to be $612 billion USD (International Diabetes Federation, 2013). In the United States, diabetes cost $245 billion in 2012 (American Diabetes, Association 2013).

**2.3 Epidemiology of diabetics**

As of 2013, 382 million people have diabetes worldwide (Shi, Yuankai; Hu, Frank B 2014). Type 2 makes up about 90% of the cases (Abdalla S, Aboyans V; et al. 2012). This is equal to 8.3% of the adult population (Shi, Yuankai; Hu, Frank B, 2014) with equal rates in both women and men (Salomon JA, Abdalla S, 2012).

**In 2014, the International Diabetes Federation (IDF)** estimated that diabetes resulted in 4.9 million deaths (International Diabetes Federation.2014). The World Health Organization (WHO) estimated that diabetes resulted in 1.5 million deaths in 2012, making it the 8th leading cause of death *(*World Health Organization 2013). The

discrepancy between the two estimates is due to the fact that cardiovascular diseases are often the cause of death for individuals with diabetes; the IDF uses modelling to estimate the amount of deaths that could be attributed to diabetes (International Diabetes Federation, 2013). More than 80% of diabetic deaths occur in low and middle-income countries (Mathers CD, Loncar D 2006).

Diabetes mellitus occurs throughout the world, but is more common (especially type 2) in more developed countries.

**Green A, in R and King H 2004**, stated that the greatest increase in rates was expected to occur in Asia and Africa, where most people with diabetes will probably live. The increase in rates in developing countries follows the trend of urbanization and lifestyle changes, including a "Western-style" diet. This has suggested an environmental (i.e., dietary) effect, but there is little understanding of the mechanism(s) at present (Wild S, Roglic G, Green A, 2004).

### 2.4 Classification of diabetics

Diabetes can be classified into the following general categories:

1. Type 1 diabetes (due to β-cell destruction, usually leading to absolute insulin deficiency)

2. Type 2 diabetes (due to a progressive insulin secretory defect on the background of insulin resistance)

3. Gestational diabetes mellitus (GDM) (diabetes diagnosed in the second or third trimester of pregnancy that is not clearly overt diabetes)

4. Specific types of diabetes due to other causes, e.g., monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young [MODY]), diseases of the exocrine pancreas (such as cystic fibrosis), and drug- or chemical-induced diabetes (such as in the treatment of HIV/AIDS or after organ transplantation)

Other classification of diabetics according to the American diabetic association

### 2.4.1 Earlier classifications

The first widely accepted classification of diabetes mellitus was published by WHO in 1980 (WHO, 1980.) and, in modified form, in 1985. The 1980 and 1985 classifications of diabetes mellitus and allied categories of glucose intolerance included clinical classes and two statistical risk classes. The 1980 Expert Committee proposed two major classes of diabetes mellitus and named them, IDDM or Type 1, and NIDDM or Type 2. In the 1985 Study Group Report the terms Type 1 and Type 2 were omitted, but the classes IDDM and NIDDM were retained, and a class of Malnutrition-related Diabetes Mellitus (MRDM) was introduced. In both the 1980 and 1985 reports other classes of diabetes included Other Types and Impaired Glucose Tolerance (IGT) as well as Gestational Diabetes Mellitus (GDM). These were reflected in the subsequent International Nomenclature of Diseases (IND) in 1991, and the tenth revision of the International Classification of Diseases (ICD-10) in 1992. The 1985 classification was widely accepted and is used internationally. It represented a compromise between clinical and etiological classification and allowed classification of individual subjects and patients in a clinically useful manner even when the specific cause or etiology was unknown. The recommended classification includes both staging of diabetes mellitus based on clinical descriptive criteria and a complementary etiological classification.

### 2.4.2 Revised classification

The classification encompasses both clinical stages and etiological types of diabetes mellitus and other categories of hyper–glycaemia (Kuzuya and Matsuda, 1997).

The clinical staging reflects that diabetes, regardless of its etiology, progresses through several clinical stages during its natural history. Persons who have, or who are developing, diabetes mellitus can be categorized by stage according to the clinical characteristics, even in the absence of information concerning the underlying etiology. The classification by etiological type results from improved understanding of the causes of diabetes mellitus.

## 2.5 Pathophysiology

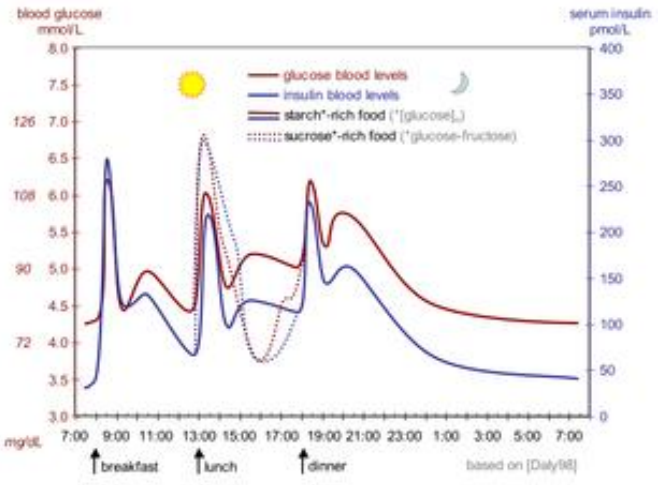
Insulin is the principal hormone that regulates the uptake of glucose from the blood into most cells of the body, especially liver, muscle, and adipose tissue. Therefore, deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus (American Diabetes Association.2014). The body obtains glucose from three main places: the intestinal absorption of food, the breakdown of glycogen, the storage form of glucose found in the liver, and gluconeogenesis, the generation of glucose from non-carbohydrate substrates in the body (David G. Gardner, Dollors 2011). Insulin plays a critical role in balancing glucose levels in the body. Insulin can inhibit the breakdown of glycogen or the process of gluconeogenesis, it can stimulate the transport of glucose into fat and muscle cells, and it can stimulate the storage of glucose in the form of glycogen (David G. Gardner, Dolores 2011).

Insulin is released into the blood by beta cells (β-cells), found in the islets of Langerhans in the pancreas, in response to rising levels of blood glucose, typically after eating.

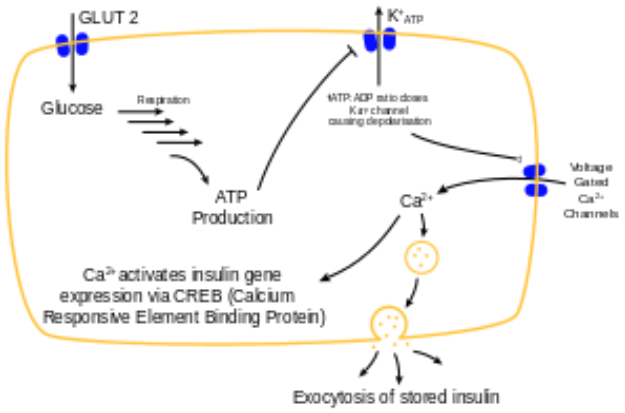
Insulin is used by about two-thirds of the body's cells to absorb glucose from the blood for use as fuel, for conversion to other needed molecules, or for storage. Lower glucose levels result in decreased insulin release from the beta cells and in the breakdown of glycogen to glucose. This process is mainly controlled by the hormone glucagon, which acts in the opposite manner to insulin (Kim E. Barrett, 2012).

If the amount of insulin available is insufficient, if cells respond poorly to the effects of insulin (insulin insensitivity or insulin resistance), or if the insulin itself is defective, then glucose will not be absorbed properly by the body cells that require it, and it will not be stored appropriately in the liver and muscles. The net effect is persistently high levels of blood glucose, poor protein synthesis, and other metabolic derangements, such as acidosis (David G. Gardner, Dolores 2011).

When the glucose concentration in the blood remains high over time, the kidneys will reach a threshold of reabsorption, and glucose will be excreted in the urine (Robert K. Murray 2012). This increases the osmotic pressure of the urine and inhibits reabsorption of water by the kidney, resulting in increased urine production (polyuria) and increased fluid loss. Lost blood volume will be replaced osmotically from water held in body cells and other body compartments, causing dehydration and increased thirst (polydipsia) (David G. Gardner, Dolores 2011).




The fluctuation of blood sugar (red) and the sugar-lowering hormone insulin (blue) in humans during the course of a day with three meals — one of the effects of a sugar-rich vs a starch-rich meal is highlited.




Mechanism of insulin release in normal pancreatic beta cells — insulin production is more or less constant within the beta cells. Its release is triggered by food, chiefly food containing absorbable glucose.

### 2.6 Signs and symptoms

The classic symptoms of untreated diabetes are weight loss, polyuria (increased urination), polydipsia (increased thirst), and polyphagia (increased hunger) (Cooke DW,

Plotnick L, 2008). Symptoms may develop rapidly (weeks or months) in type 1 DM, while they usually develop much more slowly and may be subtle or absent in type 2 DM.

Several other signs and symptoms can mark the onset of diabetes, although they are not specific to the disease. In addition to the known ones above, they include blurry vision, headache, fatigue, slow healing of cuts, and itchy skin. Prolonged high blood glucose can cause glucose absorption in the lens of the eye, which leads to changes in its shape, resulting in vision changes. A number of skin rashes that can occur in diabetes are collectively known as diabetic dermadromes.

**The most common sign of diabetics are:**

- Frequent urination
- Disproportionate thirst
- Intense hunger
- Unusual weight loss
- Increased fatigue
- Weight gain
- Irritability
- Blurred vision
- Itchy skin
- Gums are red and/or swollen –
- Frequent gum disease/infection
- Sexual dysfunction among men (International Diabetes Federation 2015)


Fig. 1: Symptoms of diabetes by Mikael Haggstrom

### 2.7 Complications

All forms of diabetes increase the risk of long-term complications. These typically develop after many years (10–20), but may be the first symptom in those who have otherwise not received a diagnosis before that time.

The major long-term complications relate to damage to blood vessels. Diabetes doubles the risk of cardiovascular disease (Sarwar N, Gao P, Seshasai SR, Gobin R 2010) and about 75% of deaths in diabetics are due to coronary artery disease (O'Gara PT, Kushner FG, Ascheim DD, 2013). Other "macrovascular" diseases are stroke, and peripheral vascular disease.

The primary complications of diabetes due to damage in small blood vessels include damage to the eyes, kidneys, and nerves (World Health Organization 2014). Damage to the eyes, known as diabetic retinopathy, is caused by damage to the blood vessels in the

retina of the eye, and can result in gradual vision loss and blindness (World Health Organization 2014). Damage to the kidneys, known as diabetic nephropathy, can lead to tissue scarring, urine protein loss, and eventually chronic kidney disease, sometimes requiring dialysis or kidney transplant(World Health Organization 2014). Damage to the nerves of the body, known as diabetic neuropathy, is the most common complication of diabetes (World Health Organization 2014). The symptoms can include numbness, tingling, pain, and altered pain sensation, which can lead to damage to the skin. Diabetes-related foot problems (such as diabetic foot ulcers) may occur, and can be difficult to treat, occasionally requiring amputation. Additionally, proximal diabetic neuropathy causes painful muscle wasting and weakness.

There is a link between cognitive deficit and diabetes. Compared to those without diabetes, those with the disease have a 1.2 to 1.5-fold greater rate of decline in cognitive function (Cukierman, T 2005).

**2.8 Diagnosis**

Diabetes mellitus is characterized by recurrent or persistent high blood sugar, and is diagnosed by demonstrating any one of the following: (World Health Organisation. 1999).

- Fasting plasma glucose level ≥ 7.0 mmol/l (126 mg/dl)
- Plasma glucose ≥ 11.1 mmol/l (200 mg/dl) two hours after a 75 g oral glucose load as in a glucose tolerance test
- Symptoms of high blood sugar and casual plasma glucose ≥ 11.1 mmol/l (200 mg/dl)

- Glycated hemoglobin ($HbA_{1C}$) ≥ 48 mmol/mol (≥ 6.5 DCCT %). (American Diabetes Association 2010)

Table 1: WHO diabetes diagnostic criteria (Vijan, S 2010)

| **Condition** | **2 hour glucose** | **Fasting glucose** | **$HbA_{1c}$** | |
|---|---|---|---|---|
| Unit | mmol/l(mg/dl) | mmol/l(mg/dl) | mmol/mol | DCCT % |
| Normal | <7.8 (<140) | <6.1 (<110) | <42 | <6.0 |
| Impaired fasting glycaemia | <7.8 (<140) | ≥6.1(≥110) & <7.0(<126) | 42-46 | 6.0–6.4 |
| Impaired glucose tolerance | ≥7.8 (≥140) | <7.0 (<126) | 42-46 | 6.0–6.4 |
| Diabetes mellitus | ≥11.1 (≥200) | ≥7.0 (≥126) | ≥48 | ≥6.5 |

A positive result, in the absence of unequivocal high blood sugar, should be confirmed by a repeat of any of the above methods on a different day. It is preferable to measure a fasting glucose level because of the ease of measurement and the considerable time commitment of formal glucose tolerance testing, which takes two hours to complete and offers no prognostic advantage over the fasting test (Varas C, Gause D, Brancati FL 2001). According to the current definition, two fasting glucose measurements above 126 mg/dl (7.0 mmol/l) is considered diagnostic for diabetes mellitus.

Per the World Health Organization people with fasting glucose levels from 6.1 to 6.9 mmol/l (110 to 125 mg/dl) are considered to have impaired fasting glucose (World Health Organization 2006). People with plasma glucose at or above 7.8 mmol/l (140 mg/dl), but not over 11.1 mmol/l (200 mg/dl), two hours after a 75 g oral glucose load are considered to have impaired glucose tolerance. Of these two prediabetic states, the latter in particular is a major risk factor for progression to full-blown diabetes

mellitus, as well as cardiovascular disease. (Santaguida PL, Balion C, Hunt D, 2008). The American Diabetes Association since 2003 uses a slightly different range for impaired fasting glucose of 5.6 to 6.9 mmol/l (100 to 125 mg/dl) (Bartoli E, Fra GP, Carnevale Schianca GP 2011).

Glycated hemoglobin is better than fasting glucose for determining risks of cardiovascular disease and death from any cause (Selvin E, Steffes MW, Zhu H, Matsushita K, 2010).

**2.9 Prevention**

There is no known preventive measure for type 1 diabetes (WHO. October 2013). Type 2 diabetes can often be prevented by a person being a normal body weight, physical exercise, and following a healthful diet (WHO. October 2013). Dietary changes known to be effective in helping to prevent diabetes include a diet rich in whole grains and fiber, and choosing good fats, such as polyunsaturated fats found in nuts, vegetable oils, and fish (Harvard School of Public Health 2014). Limiting sugary beverages and eating less red meat and other sources of saturated fat can also help in the prevention of diabetes (Harvard School of Public Health 2014). Active smoking is also associated with an increased risk of diabetes, so smoking cessation can be an important preventive measure as well (Willi C, Bodenmann P, Ghali WA, 2007).

**2.10 Management**

Diabetes mellitus is a chronic disease, for which there is no known cure except in very specific situations (WebMD website 2015). Management concentrates on keeping blood sugar levels as close to normal, without causing low blood sugar. This can usually be accomplished with a healthy diet, exercise, weight loss, and use of appropriate

medications (insulin in the case of type 1 diabetes; oral medications, as well as possibly insulin, in type 2 diabetes).

Learning about the disease and actively participating in the treatment is important, since complications are far less common and less severe in people who have well-managed blood sugar levels (Nathan DM, Cleary PA, Backlund JY, 2005). The goal of treatment is an $HbA_{1C}$ level of 6.5%, but should not be lower than that, and may be set higher (National Institute for Health and Clinical Excellence 2008). Attention is also paid to other health problems that may accelerate the negative effects of diabetes. These include smoking, elevated cholesterol levels, obesity, high blood pressure, and lack of regular exercise(National Institute for Health and Clinical Excellence 2008). Specialized footwear is widely used to reduce the risk of ulceration, or re-ulceration, in at-risk diabetic feet. Evidence for the efficacy of this remains equivocal, however (Cavanagh PR 2004).

### 2.11 Diabetogenic agent Alloxan

Commercial name: Alloxan monohydrate

Generic name: 2,4,5,6-pyrimidinetetrone

Others name: Mesoxalylurea

5-Oxobarbituric acid

#### 2.11.1 History

**Alloxan** (2, 4, 5, 6-pyrimidinetetrone) is an oxygenated pyrimidine derivative. It is present as alloxan hydrate in aqueous solution. Alloxan was originally prepared in 1818 by Brugnatelli (1761-1818) (Luigi Valentino Brugnatelli; Gaspare Brugnatelli 1818) and was named in 1838 by Wöhler and Liebig ( F. Wöhler und J. Liebig 1838) .The name

"Alloxan" emerged from an amalgamation of the words "allantoin" and "Oxalsäure" (oxalic acid).

### 2.11.2 Biological effects

Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas (that is beta cells) when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus (called "alloxan diabetes") in these animals, with characteristics similar to type 1 diabetes in humans. Alloxan is selectively toxic to insulin-producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the GLUT2 glucose transporter. Alloxan, in the presence of intracellular thiols, generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid. The beta cell toxic action of alloxan is initiated by free radicals formed in this redox reaction. One study suggests that alloxan does not cause diabetes in humans (Lenzen, S. 2008). Others found a significant difference in alloxan plasma levels in children with and without diabetes Type 1.

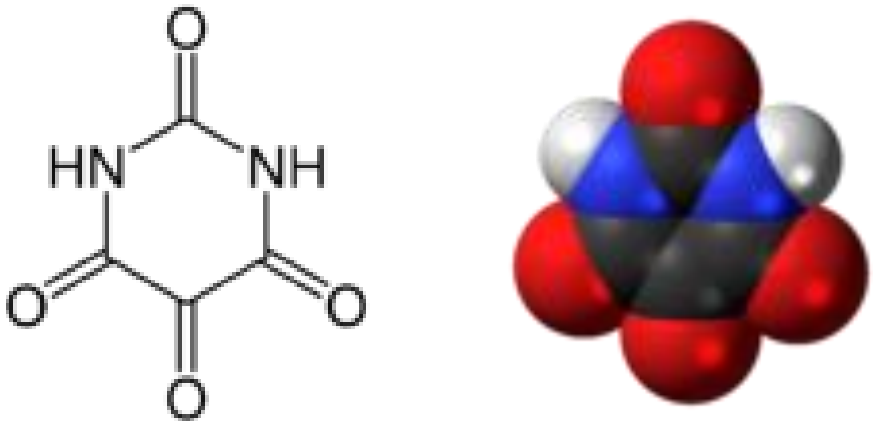
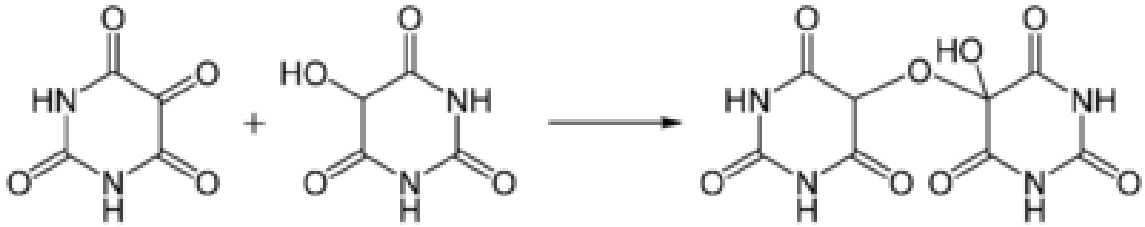



Fig. 2: Structure of alloxan

### 2.11.3 Synthesis

The original preparation for alloxan was by oxidation of uric acid by nitric acid. In another method the monohydrate is prepared by oxidation of barbituric acid by chromium trioxide (Holmgren, A. V.; Wenner, W. 1952).

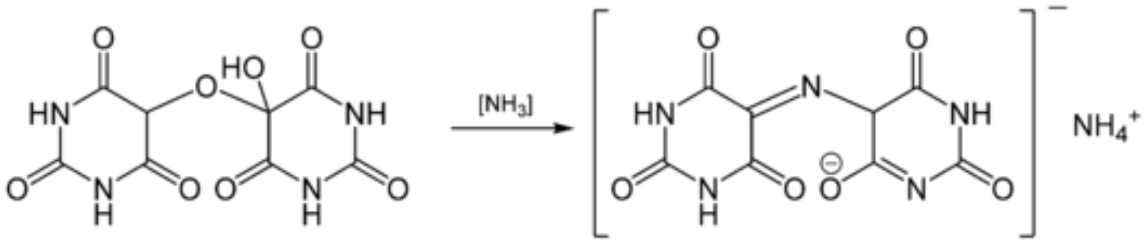
Alloxan is a strong oxidizing agent and it forms a hemiacetal with its reduced reaction product dialuric acid (in which a carbonyl group is reduced to a hydroxyl group) which is called alloxantin (Tipson, R. S. 1953).



Alloxane (left) with dialuric acid (center) and alloxantin (right)

### 2.11.4 Commercial use

Alloxan is a raw material for the production of the purple dye murexide. Carl Wilhelm Scheele discovered the dye in 1776. Murexide is the product of the complex *in-situ* multistep reaction of alloxantin and gaseous ammonia. Murexide results from the condensation of the unisolated intermediate uramil with alloxan, liberated during the course of the reaction.




Murexide dye (right) from reaction of alloxantin (left)

Scheele sourced uric acid from human calculi (such as kidney stones) and called the compound lithic acid. William Prout investigated the compound in 1818 and he used boa constrictor excrement with up to 90% ammonium acid urate.

Liebig and Wöhler in the nineteenth century coined the name *murexide* for the dye after the Murex trunculus snail, which is the source of the Tyrian purple of antiquity.

It is also formed as an unintended byproduct in the whitening of maida flour and other flour and may cause diabetes if consumed more.

### 2.11.5 Impact upon beta cells

Because it selectively kills the insulin-producing beta-cells found in the pancreas, alloxan is used to induce diabetes in laboratory animals (Danilova, I.G., Sarapultsev, P.A., Medvedeva, S.U., Gette, I.F., Bulavintceva 2014, Loreto D, Elina V. 2009). This occurs most likely because of selective uptake of the compound due to its structural similarity to glucose as well as the beta-cell's highly efficient uptake mechanism (GLUT2). In addition, alloxan has a high affinity to SH-containing cellular compounds and, as a result, reduces glutathione content. Furthermore, alloxan inhibits glucokinase, a SH-containing protein essential for insulin secretion induced by glucose (Szkudelski T. 2001).

Some studies have shown that alloxan is not toxic to the human beta-cell, even in very high doses, probably because of differing glucose uptake mechanisms in humans and rodents (*Tyrberg, B.; Andersson, A.; Borg, L. A. 2001, Eizirik, D. L.; Pipeleers, D. G.; Ling, Z.; Welsh, N.; Hellerström, 1994).*

Alloxan is, however, toxic to the liver and the kidneys in high doses.

### 2.11.6 Mechanism of action

Alloxan is known to induce diabetes in experimental animals through destruction of insulin-producing B-cells of pancreas. The mechanism of DNA damage induced by alloxan was investigated using 32P-labeled human DNA fragments. Cu(II)-dependent DNA damage increased with the concentration of alloxan and NADH. Alloxan induced DNA cleavage frequently at thymine and cytosine residues in the presence of NADH and Cu (II). Catalase and bathocuproine, a Cu (I)-specific chelator, almost completely inhibited DNA damage, suggesting the involvement of H2O2 and Cu (I). Alloxan induced Cu (II)-dependent production of 8-oxodG in calf thymus DNA in the presence of NADH. UV-visible and electron spin resonance (ESR) spectroscopic studies showed that superoxide anion radical and alloxan radical were generated by the reduction of alloxan by NADH, and also by the autoxidation of dialuric acid, the reduced form of alloxan. These results suggest that the copper-oxygen complex derived from the reaction of H2O2 with Cu (I) participates in Cu(II)-dependent DNA damage by alloxan plus NADH and dialuric acid. The mechanism of DNA damage is discussed in relation to diabetogenic action of alloxan. (Murata M et al; Free Radic Biol Med 1998).

### 2.11.7 Absorption, Distribution and Excretion

The uptake of [2-(14)C]alloxan by the pancreatic gland was investigated in control and streptozotocin-induced diabetic (STZ) rats, using both in vitro and in vivo techniques. Whether after 10 to 60 min incubation of pieces of pancreas in the presence of [2-(14) C]alloxan or 60 min to 24 h after intravenous injection of [2-(14)C]alloxan to control and insulin-treated STZ rats, the radioactive content of the pancreas (dpm/mg wet weight) only represented, in the STZ rats, about two thirds of the reference value found in control animals. These findings indicate that insulin-producing islet B-cells participate to a

sizeable extent to the overall uptake of [2-(14)C]- alloxan by the whole pancreatic gland, despite the fact that they account for no more than about one percent of the total pancreas mass. Hence, it should be possible to preferentially label the endocrine moiety of the pancreas, in the perspective of its imaging and quantification by a non-invasive procedure, by use of a suitable radiolabelled molecule selectively taken up by islet, as distinct from acinar, pancreatic cells (Malaisse WJ et al; Int J Mol Med 2001).

**2.12 Antidiabetogenic agent Bitter melon (*Momordica charantia* )**

**Krawinkel MB and Keding GB. in 2006, 2014,** describe that bitter gourd (*Momordica charantia*) is an important market vegetable in Southern and Eastern Asia and is widely spread throughout most of tropical Africa and. It is also referred to as bitter melon, balsam pear, bitter apple, and bitter African or wild cucumber (Krawinkel MB and Keding GB 2006) . Fruits and leaves of most wild Momordica species are consumed as vegetables and have a similar bitter taste and almost identical medical uses.

**Zhu Y, Dong Y, Qian X, Cui F,Guo Q, Zhou X,Wang Y, Zhang Y in 2012,** established that it has been used as a traditional antidiabetic remedy in eastern countries for many years. It is now commercially available as tea (from fruits or leaves), juice, extracts, and tablets. Although these products promise health benefits, most of the manufacturers do not provide scientific evidence on the effectiveness of bitter gourd or their products. However, in recent years researchers have focused on the antidiabetic effects of bitter gourd. The goals of these studies are to provide safe and clear preparation and dosage recommendations.

**Whereas Islam S, Jalaluddin C, M. Hettiarachchy NS. in 2011,** stated that fresh bitter gourd is used as a nourishing food. It contains: 93.8% water, 0.9% protein, 0.1% lipid, 3.3% dietary fiber, 20 kJ energy per 100 g, and a small quantity, 0.05%, of vitamin.

It is a good source of phenolic compounds (Islam S, Jalaluddin, M. Hettiarachchy NS 2011).

The immature fruits of bitter gourd can be prepared in many ways such as frying or cooking as curries. In addition, fruits can be dehydrated, pickled or canned (Krawinkel MB and Keding GB 2006). They are usually blanched or soaked in salt water before cooking to reduce the bitter taste. Study suggests that incorporating bitter foods in commonly consumed food dishes can mask the bitter taste of bitter gourd (Snee LS, Nerukar VR, Doolay DA, Efirt JT, Shovic AC, 2011, 2014).

**Zhu Y, Dong Y, Qian X, Cui F,Guo Q, Zhou X,Wang Y, Zhang Y in 2012**, identified the hypoglycemic potential components in bitter gourd have been as glycosides, saponins, alkaloids, triterpenes, polysaccharides, proteins, and steroids. Although several pure chemicals were isolated from bitter melon and applied for investigating their antidiabetic mechanisms, the mixture of these hypoglycemic chemicals such as saponins or charantins seemed to present a significantly higher bioactivity Zhu Y, Dong Y, Qian X, Cui F, Guo Q, Zhou X, Wang Y, Zhang Y 2012).

**Oishi Y, Sakamoto T, Udagawa H, Taniguchi H, Kobayashi- Hattori K, Ozawa Y, in 2007** discover that the main active component related to the anti-diabetic effect of Momordica charantia is present in the butanol fraction, and it may be saponin.

**Whereas Qixuan C, Laureen QC, Chen LY, Edmund TS, in 2003,** established that bitter gourd contains a high dosage of ‘plant insulin’ and lowers the blood-sugar levels effectively.

**Koona SJ, Kudipudi S, Sridhar GR, Rao SB, Apparao A. in 2010, identified** ‘Plant insulin’ which is a chemical substances similar to animal insulin existing in plants.

**Krawinkel MB and Keding GB. in 2006, 2014** found that Polypeptide-p is an unidentified insulin-like protein similar to bovine insulin found in *M. charantia* fruit, seed, and tissue culture.

**Oishi Y, Sakamoto T, Udagawa H, Taniguchi H, Kobayashi-Hattori K, Ozawa Y, in 2007** proved that bitter gourd reduces the amount of glucose that is released into the blood by inhibiting the enzymes that break down disaccharides into two monosaccharides. The blood glucose lowering effects of *Momordica charantia* were closely associated with its inhibitory activity against disaccharidase (Oishi Y, Sakamoto T, Udagawa H, Taniguchi H, Kobayashi-Hattori K, Ozawa Y, 2007). This effect is important for the treatment of both Type I and Type II diabetic patients and helps to prevent high blood sugar levels after meals. Bitter melon has shown to stimulate glycogen storage by liver and insulin secretion by islets of Langerhans (Zhu Y, Dong Y, Qian X, Cui F, Guo Q, Zhou X, Wang Y, Zhang Y, Xiong Z. 2012).

**Hamissou M, Smith AC, Carter RE, Triplett JK. in 2013,** indicated that bitter melon may decrease hepatic gluconeogenesis, increase hepatic glycogen synthesis, and increase peripheral glucose oxidation in erythrocytes and adipocytes.

A systematic review published in ***Diabetes Care*** **in 2003,** cited a handful of human studies that support bitter melon's role in lowering blood glucose.

**Whereas Yeh G, Eisenberg D, Kaptchuk T, Phillips R in 2003,** stated that two placebo-controlled, short-term metabolic studies reported bitter melon fruit juice's and extract's acute effects on lowering blood glucose. Two additional uncontrolled, open-label trials cited in the review reported positive effects on glycemic control after subjects used bitter melon for seven to 11 weeks ( Yeh G, Eisenberg D, Kaptchuk T, Phillips R 2003). Despite each of the four studies mentioned having less than 20 subjects, the

authors of the review highlighted bitter melon as one of the most promising supplements for diabetes management.

Only a handful of large human clinical trials have studied bitter melon's antidiabetic effects. The largest study evaluating the fruit's blood glucose-lowering effect was conducted in India and published in 1999 in the *Bangladesh Medical Research Council Bulletin*. Researchers used an aqueous suspension of bitter melon pulp in 100 patients with type 2 diabetes. They evaluated bitter melon's effect one hour after administration and then two hours later with a 75-g oral glucose tolerance test. After the two-hour glucose tolerance test, subjects' average blood glucose was 222 mg/dL. This was 14% lower than the previous day's value of 257 mg/dL. Regardless, researchers measured bitter melon's effects after only two hours; whether there is any long-term benefit to using bitter melon is unknown.

In a randomized, double-blind, placebo-controlled study conducted in **2004**, **Dans et al** aimed to determine whether the addition of bitter melon capsules to standard therapy could decrease hemoglobin A1c levels by 1% in three months. The 40 subjects were either newly diagnosed with type 2 diabetes or had poorly controlled type 2 diabetes with A1c levels between 7% and 9%. The researchers advised the subjects to self-administer two capsules of bitter melon extract three times per day. The results, published in the *Journal of Clinical Epidemiology* in 2007, revealed only a 0.217% decline in the experimental group.

**Suggested Actions of Bitter Gourd on Blood Glucose**

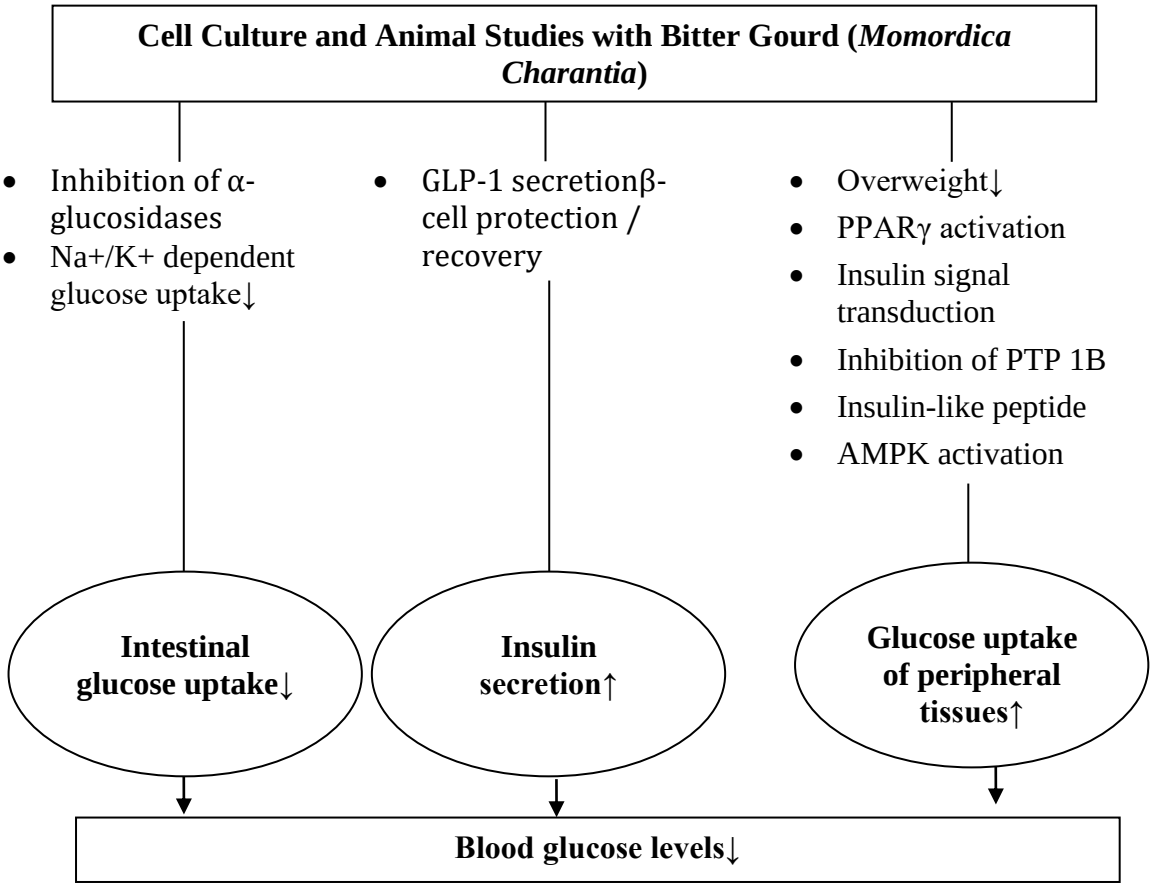



Fig. 3: Suggested Actions of Bitter Gourd on Blood Glucose

GLP-1 = glucagon-like peptide-1; PPARγ = peroxisome proliferator-activated receptor; PTP 1B = protein tyrosine phosphatase 1B; AMPK = AMP-activated protein kinase

**2.13 Antidiabetogenic agent Mahogany *(Swietenia macrophylla.)***

*Swietenia macrophylla* is a beautiful, lofty, evergreen large tree, native to tropical America, Mexico, and South America, usually 30 – 40 m in height, and 3 m in girth (Rastogi RP, Mehrotra BN 1990). The seeds of *S. macrophylla* have been reported for their anti-inflammatory, anti-mutagenic, and anti-tumor activities (Guevera AP, Apilado A, Sakarai H, Kozuka M, Tokunda H. 1996).The seeds of *S. macrophylla* are traditionally used by the local healers of Azhagar hills, Madurai, Tamil Nadu, India, for curing diabetes. Hence, the present study was undertaken to evaluate the antidiabetic

potential of the alcoholic seed extract of *S. macrophylla* experimentally in normal and alloxan-induced diabetic rats, to prove its use by the tribes in folk medicine.

**Chan KC, Tang TS, Toh HT in 1976,** stated that *Swietenia macrophylla* King (Meliaceae), commonly known as big leaf mahogany (vernacular) and 'skyfruit' (local), is used to treat diabetes and high blood pressure in Malaysia.

**Guevera AP, Apilado A, Sakarai H, Kozuka M, Tokunda H. in 1996,** found that *S. macrophylla* seeds have been reported to have anti-inflammatory, anti-mutagenic and anti-tumor activities and to be effective against diabetes in rats( Maiti A, Dewanjee S, Kundu M, Mandal SC.2007).

**In Chinese pharmacology and other traditional medicines 2013,** this plant has antipyretic, antifungal, and antihypertensive properties, pharmacological effects obtained from dried seeds, finely ground to powder.

Traditionally, raw seeds of *S. macrophylla* are chewed to treat diabetes. **Chan KC, Tang TS, Toh HT. in 1976,** stated these seeds are chewed or pounded and swallowed to treat high blood pressure and in India, they are used to treat diabetes and hypertension.

### 2.13.1 Ecological factor of the plant

S. mahagoni, one of several species referred to as mahogany, is indigenous to the southern region of Florida, the Bahamas, Cuba, Jamaica, and the island of Hispaniola. The species is now planted as an ornamental and timber tree outside its natural range in several Caribbean islands, Hawaii, India, Sri Lanka, and Fiji. The species is reportedly best adapted to areas with annual precipitation, ranging from 760 mm to 1 780 mm (Francis JK. 1991). The plant grows in variety of soil types but prefers habitats with

moist and deep soils. The species range is restricted by cool, moist conditions and low-pH soils, particularly soils with high clay content.

### 2.13.2 Medicinal history

The parts of the plant have been used locally to treat many human ailments such as malaria, diabetes, diarrhea and hypertension. The fruit of the plant is used as a powerful anti-hyperglycemic drug. In some African countries the seed oil is used as an alternative body ointment therapy for a range of skin cuts, itches and wounds to ameliorate the healing process. A decoction of the bark is used to increase appetite, and treat anemia, diarrhea, dysentery, fever and toothache; it is also used as an energizer in cases of tuberculosis. A decoction of the leaf is used to treat nerve disorders, while an infusion of the seed relieves chest pain (Bacsal K, Chavez L, Diaz I, Espina S, Javillo J, 1997). Mahogany seeds have potential in controlli ng amoebiasis, coughs and intestinal parasitism (Grandtner MM 2005).

### 2.13.3 Constituent phytochemicals

The total ash content of bark, analyzed by extraction with various solvents, is reported to be 22.0%, containing sulphated ash (14.5%), and water soluble ash (1.4%), and the total acid insoluble ash is 0.6% (Sanyal M, Datta PC 1986). The bark also contains tannin (15.0%) (Howes FN, Watt G, Watt JM, Breyer-Brandwijk MG, Krishnan Marg KS. 1953; 1889; 1976) with no presence of alkaloid principle.

Cyclomahogenol, a new tetrac. yclic triterpene, has been identified in the leaves of the plant( Chakraborty DP, Basak SP1971). The methanolic and water extract of seeds showed the presence of tannins, alkaloids, saponins and terpenoids as main phytoconstituents (Hajra S, Mehta A, Pandey P. 2011).

The crude methanolic extract of seeds contains alkaloids, terpenoids, anthraquinones, cardiac glycosides, saponins, and volatile oils (Sahgal G, Ramanathan S, Sasidharan S, Mordi MN, Ismail S, 2009). Two potent antimicrobial limonoids, swietenolide and 2-hydroxy-3-O-tigloylswietenolide , have been isolated from the methanolic extracts of the seed, and the structures of the compounds have been confirmed using spectroscopic analysis (Rahman AK, Chowdhury AK, Ali HA, Raihan SZ, Ali MS, Nahar 2009). When the ether extract of the seed was systematically separated, 28 tetranotriterpenoids related to swietenine and swietenolide were found. Among them, several new compounds, named swietemahonins A, D, E, and G and 3-O-acetyl-swietenolide and 6-O-acetyl-swietenolide were identified. These compounds have been shown to strongly inhibit platelet-activating factor (PAF)-induced platelet aggregation (Ekimoto H, Irie Y, Araki Y, Han GQ, Kadota S, Kikuchi 1991). In the seeds, two tetranotriterpenoids, mahonin and secomahoganin were isolated. The structures of these compounds were determined through 2D-nuclear magnetic resonance techniques, 1H-13C cosy, and 1H-13C long range cosy.

The possible biosynthetic pathways to the above compounds have also been proposed (Kadota S, Marpaung L, Kikuchi T, Ekimoto H. 1990). Also using spectroscopic methods, 11 new mexicanolide-type limonoids, swietmanins, 2-hydroxy-3-O-isobutyrylproceranolide, 2-hydroxy-3-O-benzoylproceranolide, and a new andirobin-type limonoid, swietmanin J, together with 19 known compounds have been isolated from the fruits and their structures (Lin BD, Yuan T, Zhang CR, Dong L, Zhang B, Wu Y, Yue JM 2009).

### 2.13.4 Antioxidant effect

**Sahga G, Ramanathan S, Sasidharan S, in 2009,** have been reported that the seed of *S. mahagoni* possesses antioxidant activity. The methanol extract of the seed was shown to be a potent antioxidant in various in vitro assays (i.e., xanthine oxidase assay, hydrogen peroxide-scavenging activity, ferric-reducing antioxidant power (FRAP) assay and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay). The methanol extract inhibited superoxide formation with a value of 47.2%, which was less than standard drug allopurinol (87.51%). Hydrogen peroxide-scavenging activity of the extract was found to be 49.5, which was comparable to standard drug ascorbic acid (51.1). The seed extract had FRAP activity of 0.728 mmol Fe++/g, which was higher than the FRAP activity of ascorbic acid (0.405). The extract showed a DPPH-scavenging activity of 23.29% at 1 mg/mL concentration, this value was less than that of ascorbic acid (Sahga G, Ramanathan S, Sasidharan S, 2009).

### 2.13.5 Antidiabetic effect

**De D, Chatterjee K, Ali KM, Bera TK, Ali KM**, **Bera TK, Ghosh D in 2011,** reported that the aqueous-methanol extract of *S. mahagoni* seed has been reported to exhibit hypoglycemic and antihyperlipidemic potency in streptozotocin-induced diabetic rats. Oral feeding of the extract to diabetic rats for 21 d lowered the blood glucose level and improved liver glycogen content. Furthermore, treatment with the seed extract normalized the levels of serum urea, uric acid, creatinine, cholesterol, triglyceride and lipoproteins. In addition, the extract increased the activity of antioxidant enzymes and reduced the oxidative stress in liver, kidney and skeletal muscles

**Whereas Hajra S, Mehta A, Pandey P, Vyas SP. in 2011,** found that the ethanolic extract of *S. mahagoni* seed inhibited α-amylase to an extent of 70.33% at a

concentration of 200 μg/mL (Seed extract acted as an antagonist to the peroxisome proliferator-activated receptor γ in both the yeast two-hybrid system and diabetic mice. The activity was comparable to that of standard drug rosiglitazone (Li DD, Chen JH, Chen Q, Li GW, Chen J, Yue JM 2005).

### 2.13.6 Anti-HIV effect

**Otake T, Mori H, Morimoto M, Ueba N, Sutardjo S, Kusumoto IT, Hattori M, Namba T. in 1995,** found that the Methanol extract of mahagany bark is reported to exhibit anti-HIV-1 activity by inhibiting a key enzyme, HIV protease, which is required by the virus to replicate in host cells. Bark extract also suppressed the formation of syncytia in co-cultures of human acute lymphoblastic leukemia cell line (MOLT-4) and MOLT-4/HIV-1 cell.

### 2.13.7 Immunomodulatory effect

**Whereas Hajra S, Mehta A, Pandey P in 2012,** established that the methanolic extract of seeds enhanced the immune efficiency, as assessed by neutrophil adhesion, phagocytic index by carbon clearance, hemagglutinating antibody titer and delayed type hypersensitivity responses in rat models. Oral administration of the extract significantly increased the neutrophil adhesion to nylon fiber, when compared to control group. In addition, it showed a significant increase in circulating antibody titer and phagocytic index in carbon clearance in a concentration-dependent manner. The effect may be mediated by significantly increasing circulating antibody titer.

S. mahagony has a potent antimicrobial activity on a variety of microorganisms, including pathogenic microorganisms. The various phytochemicals present in the plant are responsible for the observed antimicrobial activity. Most of the areal parts of the

plant have been shown to significantly inhibit the propagation of various microorganisms.

**Sahgal G Ramanathan S, Sasidharan S, Mordi MN, Ismail S in 2009,** found that the crude methanolic extract of the seed has been shown to inhibit growth of 5 Gram-positive and 9 Gram-negative bacteria. Further, the extract of the seed at a concentration of 1 mg/mL has been shown to inhibit growth of the fungus Candida albicans. The methylene chloride and methanol extracts of the seed have been assayed for their inhibitory action on ten microbial species, of which four are pathogenic bacteria (Escherichia coli, Staphylococcus aureus, Xanthomona campestris and Bacillus subtilis), one yeast, one fungi (Candida albicans) and five molds (Pythium ultimum, Rhizoctonia solani, Sclerotium rolfsii, Aspergillus fumigatus and A. pytiumparasitica). The methylene chloride extract inhibited growth of seven species, while the methanol extract was active only against Rhizoctonia solani (Goun E, Cunningham G, Chu D, Nguyen C, Miles D. 2003). The methanol extract of the seed also had an inhibitory effect on Candida albicans in both in vivo and in vitro assays. In vitro disc diffusion assays showed a minimum inhibitory concentration of the extract to be 12.5 mg/dL. The extract had a deleterious effect on cell structures of C. albicans, causing morphological change and death, as evidenced by electron microscope images of extract-treated fungi. Treatment of fungus-infected mice with seed extract reduced colony-forming units in the kidney and the blood when compared to positive control mice (Sahgal G, Ramanathan S, Sasidharan S, Mordi MN, Ismail S 2011). The oil extracted from the seed reduced growth rates of several diseases causing by bacterial (Shigellady senterial, Salmonella typhi, and Staphylococcuss aureus) and fungal (Macrophomina phascolma, Alternaria alternate and Curvularia lunata) pathogens (Shipar MAH, Chowdhury R, Majid MA, Uddin MH, Rahman IMM. 2004). The alcoholic and aqueous extracts of the leaf,

stem/bark and root have inhibitory effects on Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris and Bacillus subtilis. The efficiency of inhibition is comparable to benzyl penicillin and ampicillin (Sharma M, Mohan V, Abraham M, Joshy PJ, Reghuvaran DK, 2011).

### 2.13.8 Anti-inflammatory effect

**Ghosh S, Besra SE, Roy K, Gupta JK, Vedasiromoni JR in 2009,** reported that the methanolic extract of *S. mahagoni* seed has an ameliorating effect on paw edema induced by carrageenan and arachidonic acid, acetic acid-induced writhing, ear inflammation induced by croton oil, cotton pellet-induced granuloma and Freund's adjuvant-induced polyarthritis in rats. The extract significantly reduced the acetic acid-induced writhing in rats; the writhing reducing effect was superior to the standard ibuprofen. Carrageenan-induced paw edema was reduced by 56.8% and 68.0% in rats treated with doses of 50 and 100 mg/kg extract, respectively. Croton oil-induced ear inflammation was reduced by 7.35% at 50 mg/kg dose and 47.06% at 100 mg/kg. Polyarthritis induced by Freund's adjuvant was reduced by 53.79%, which was more than the positive control, ibuprofen. Cotton pellet-induced granuloma was reduced by 28.29% at 50 mg/kg and by 42.86% at a dose of 100 mg/kg; the effect of the extract was far more than the standard drug ibuprofen (14.29%). The extract also significantly increased the intraperitoneal count of white blood cells and macrophage.

Although there are many studies which indicate effective anti-inflammatory activity of *S. mahagoni* in animal models, the mechanism of action has yet to be explored. Future studies that measure the mRNA expression of inflammatory marker genes (e.g., cyclooxygenase and nitric oxide synthase) and expression of other inflammatory markers

(e.g., interleukin (IL)-1β, IL-6, monocyte chemotactic protein-1, tumor necrosis factors-α and C-reactive protein) will be helpful in elucidating the precise mechanism of action.

### 2.13.9 Hepatoprotective effect

**Whereas Haldar PK, Adhikari S, Bera S, Bhattacharya S, Panda SP, Kandar CC. in 2011,** reported that the petroleum and 80% aqueous methanol extracts of *S. mahagoni* bark showed a hepatoprotective effect against paracetamol-induced hepatic damage in male Wistar rats. Treatment with *S. mahagoni* bark extract significantly reduced the alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and serum bilirubin levels, suggesting the hepatoprotective effect. Histopathology revealed that the liver cells in the extract-treated group were indistinguishable from liver cells of healthy rats. The extract also significantly reduced the thiobarbituric acid-reactive substances, when compared to the paracetamol-treated group. Bark extract was also reported to increase the reduced glutathione level in the liver. The above findings indicate that the hepatoprotective efficacy of the bark extract may be mediated through the modulation of lipid peroxidation and the augmentation of endogenous enzymatic and non-enzymatic antioxidant defense systems. To determine safety for human use, acute toxicity tests were carried out on Swiss albino mice; the dose at which 50% of recipients died (LD50), for oraiiy administered bark extract, was 200 mg/kg.

### 2.13.10 Antidiarrheal activity

**Hajra S, Mehta A, Pandey P in 2012**, reported that the ethanolic, methanolic and aqueous extracts of *S. mahagoni* seed show antidiarrheal activity in castor oil-induced diarrhea as well as in charcoal-induced gastrointestinal motility in Wistar albino rats. The ethanolic, methanolic and aqueous extracts of seeds at various concentrations (50, 100, 200 and 300 mg/kg) were used in this study. Among the three solvent extracts, the

ethanolic extract showed the most potent antidiarrheal activity, as evidenced by reduction in the rate of defecation and improved consistency of faeces. Treatment with the extract produced a profound decrease in intestinal transit and significantly inhibited castor oil-induced enteropooling compared to standard drugs diphenoxylate (50 mg/kg) and atropine sulfate (2.5 mg/kg). The delayed onset of diarrhea, inhibition of castor oil-induced enteropooling and the suppressed propulsive movement all support the traditional claim that *S. mahagoni* functions as an antidiarrheal drug in the Indian system of medicine without any side effects.

### 2.13.11 Gastroprotective effect

**Alrdahe SS, Abdulla MA, Razak SA, Kadir FA in 2010,** found that the ethanol extract from the seed of the *S. mahagoni* has shown gastroprotective activity against ethanol-induced gastric mucosal injury in rats. Six groups of rats were orally fed with carboxymethyl cellulose, omeprazole (standard drug, 20 mg/kg) or seed extract (50, 100, 200 and 400 mg/kg) one hour before oral administration of absolute ethanol (to generate gastric mucosal injury). The carboxymethyl cellulose group exhibited severe mucosal injury, whereas pre-treatment with plant extract provided significant protection of gastric mucosa in rats. The carboxymethyl cellulose group showed severe damage to gastric mucosa (edema and leucocyte infiltration of sub-mucosa) compared to the plant extract-treated group, which showed gastric protection as evidenced by histological observations. The authors also carried out similar studies using ethanol extract of mahagany leaf. The leaf extract significantly reduced the mucosal injury and increased the mucus secretion when compared to the control group; the effect was similar to standard drug omeprazole. The extract, in addition, reduced the edema and leucocyte infiltration into the sub-mucosal layer

### 2.13.12 Depressant, anticonvulsant and neuropharmacological activity

**Panda SP, Bera S, Naskar S, Adhikary S**, **Kandar CC, Haldar PK in 2010,** reported that the methanol extract of *S. mahagoni* bark also showed depressant (sleep-potentiating) and anticonvulsant effects in male Swiss albino mice. The extract significantly increased pentobarbitone-induced shortened sleeping time in a dose-dependent manner. The anticonvulsant effect of the extract at the doses of 25 and 50 mg/kg was examined against seizures induced by pentylenetetrazole (80 mg/kg) and strychnine (2.5 mg/kg). In these experiments the extract significantly delayed the onset of seizures and also antagonized these seizures in a dose-dependent manner. The effects of the extract were comparable to the reference drug diazepam (2.0 mg/kg). The ethanol extract of *S. mahagoni* seed has shown antinociceptive potency and neurodepressive effect. Anti-nociceptive activity was tested using the model of acetic acid-induced writhing in mice at oral doses of 300 and 600 mg/kg. The extract showed a significant writhing inhibition in mice, which was comparable to the standard drug diclofenac sodium. Neurodepressive activity was studied using the pentobarbital-induced hypnosis effects on exploratory behavior, such as open field test, hole cross test and hole board test.

**Rahman MA, Akther P, Roy D, Das AK in 2010,** reported that treatment with leaf extract significantly increased pentobarbital-induced hypnosis and decreased the exploratory behavior of the mice, indicating its depressant activity on central nervous system.

In this context, worldwide efforts have been taken to improve plant-based therapies (P. Daisy, R. Jasmine, S. Ignacimuthu, and E. Murugan, 2009) WHO (World Health Organization 1980) recommended for the assessment of traditional medicinal plant in

connection with the management of diabetes mellitus (S. L. Badole, N. M. Patel, J. H. Hsu, Y. C. Wu, S. S. Liou, and I. M. Liu 2008, 2004). Currently, several hundred plants have been reported to have beneficial effects for the treatment of diabetes mellitus, and we have several reports in this line R. Maiti, U. K. Das, and D. Ghosh, 2005; C. Mallick, R. Maiti, and D. Ghosh, 2006) as well as of others P. Ljubuncic, H. Azaizeh, U. Cogan, and A. Bomzon 2006; A. Sharma, M. Vijayakumar, C. V. Rao, 2009). Research on phytomolecules as diabetic remedies is upraising gradually as these are with minimal or no side effects O. Said, S. Fulder, K. Khalil, H. Azaizeh, 2008; B. K. Rao, P. R. Sudarshan, M. D. Rajasekhar, 2003).

# CHAPTER 3

## MATERIALS AND METHODS

# CHAPTER 3

# MATERIALS AND METHODS

This research work is conducted at animal Laboratory in the Department of Physiology and Pharmacology, at Hajee Mohammad Danesh Science & Technology University, Dinajpur for a period of 45 days to evaluate the combined efficacy of bitter melon and mahogany seed on Alloxan induced diabetic rabbit.

## 3.1 Experimental site

The laboratory animal house at the Department of Physiology and Pharmacology was the Experimental Site.

## 3.2 Experimental flowchart

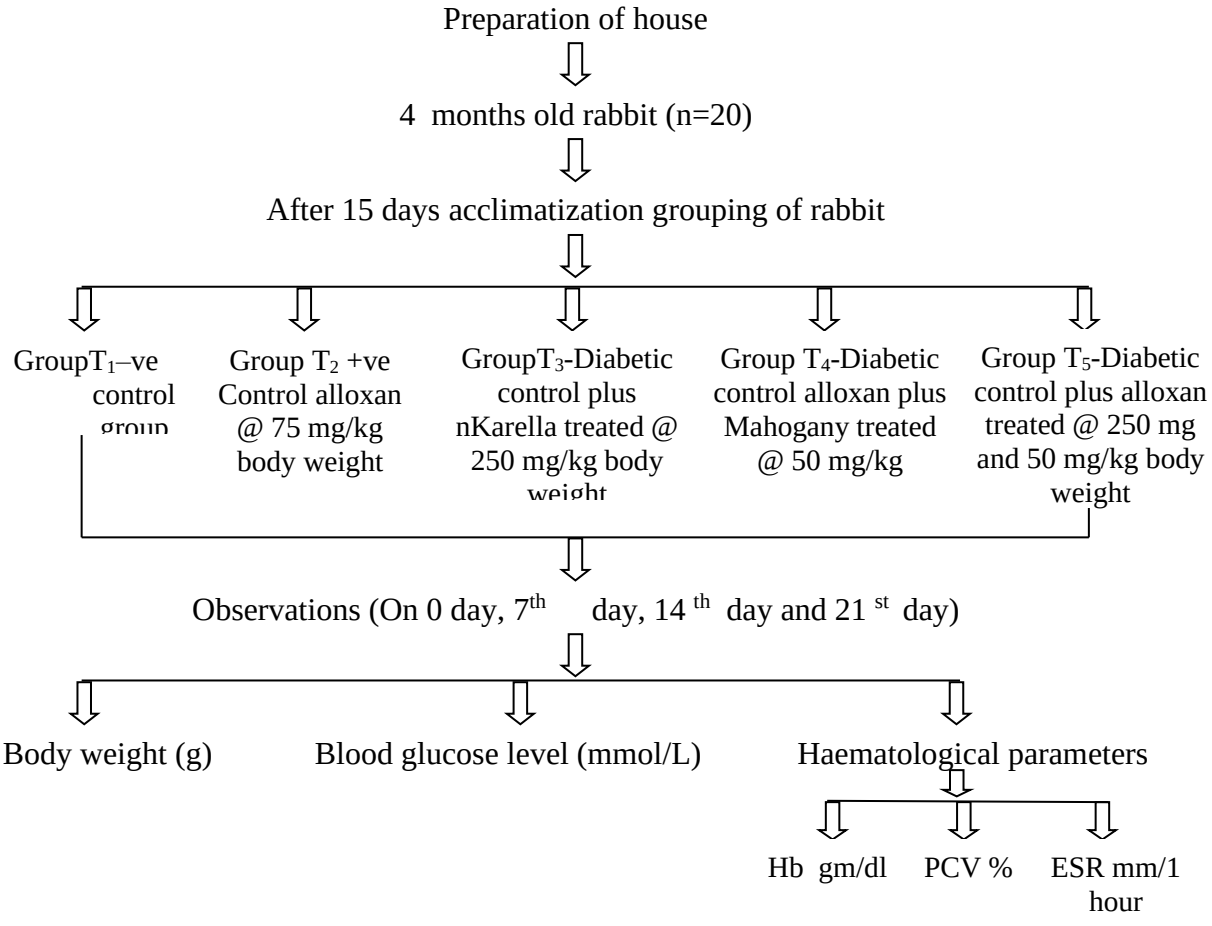



Fig. 4: Experimental Layout

### 3.3 Experimental animal:

Twenty Newzealand white rabbit aged between 4 months and weighting between 1000 to1200 g were collected from rabbit farm under the department of Physiology and Pharmacology, HSTU, Dinajpur.

### 3.4 Preparation of House

First the room as well as the wire cages were washed by sweeping and washing with tap water using hose pipe connected with the tap. The room was disinfected with a phenolic disinfectant (phenyl) and allowed to dry leaving the room unused with the electric fan and the bulb switched on. The room was properly ventilated.

### 3.5 Acclimatization of rabbit

All the rabbit were housed at screen bottomed wire cages arranged in rows and kept in the departmental (Dairy & Poultry Science, HSTU) animal house. The animals were fed with pellet at a recommended dose of100 g/kg daily as advised by ICDDRB. Drinking water was supplied adlibitum. The rabbit were maintained in this condition during the experimental period.

### 3.6 Experimental animal grouping:

Twenty rabbits were used to carry out this investigation. These rabbits were divided into five groups containing 4 rabbits in each group. The groups were designated and maintained as follows:

**Group $T_1$:** The rabbits were fed with pellet diet, vegetables etc. and given water *adlibitum* and then their body weight and blood glucose were recorded after acclimatization. This group of rabbits served as negative control. Body weights and blood glucose level were measured at the time when that of other groups was measured.

**Group $T_2$:** After acclimatization, body weights and blood glucose level were measured after 18 hours of starvation. Then Alloxan hydrochloride injection was given at a dose rabbits of 75 mg/1000 to1200 gm (Puri and Prabhu, 2002). through intramuscular route to each rabbits to induce diabetes. The rabbit were fed with pellet diet and given water ad libitum from Day 1-15 on 15th day blood glucose level and the body weight were again measured to ensure diabetic condition. Then all the rabbit of this group were kept for 14 days without any treatment. During that period on Day 0, 7, 14 & 21 the body weight and blood glucose level were measured. This group served as diabetic (positive) control group.

**Group $T_3$:** After acclimatization, body weights and blood glucose level were measured after 18 hours of starvation. Then Alloxan hydrochloride was injected in all rabbits of this group at a dose rabbits of 75 mg/kg through intramuscular route. The rabbits were fed normal diet and given water ad libitum for I5days. Then blood glucose level and body weight were measured on 15th day of Alloxan hydrochloride injection for confirming diabetic condition. After that suspension of bitter melon fed at a dose of 150 mg, 3mL water/1000 to 1200 gm b.w./day for 14 days. During treatment of bitter melon suspension body weight and blood glucose were recorded on Day 0 (Pre-treatment) and Day 7, 14 & 21 (during treatment). This group served as treatment group 1 to find the effect of suspension of bitter melon as antidiabetic drug.

**Group $T_4$:** After acclimatization, body weights and blood glucose level were measured after 18 hours of starvation. Then Alloxan hydrochloride was injected in all rabbits of this group at a dose rabbits of 75 mg/kg in intramuscular route. The rabbits were fed normal diet and given water ad libitum for I5days. Then blood glucose level and body weight were measured on 15th day of Alloxan hydrochloride injection for confirming diabetic condition. After that suspension of mahogany seed were fed at a dose of 50

mg/kg (Panda SP, Haldar PK, Bera S, Adhikary S, Kandar CC 2010). Body weight/day for 14 days. During treatment of suspension of mahogany seed body weight and blood glucose were recorded on Day 0 (Pre-treatment) and Day 7, 14 & 21 (during treatment). This group served as treatment group 2 to find the effect of suspension of mahogany seed as antidiabetic drug.

**Group $T_5$:** After acclimatization, body weights and blood glucose level were measured after 18 hours of starvation. Then Alloxan hydrochloride injection was given at a dose rabbits of 75 mg/1000 to1200 gm (Puri and Prabhu, 2002). In intramuscuiar route to each rabbits to induce diabetes. The rabbit were fed normal diet and given water ad libitum from Day 1-15 on 15th day blood glucose level and the body weight were again measured to ensure diabetic condition. After that suspension of bitter melon and mahogany seed were fed at a previous dose for 14 days. During combined treatment of suspension of bitter melon and mahogany seed body weight and blood glucose were recorded on Day 0 (Pre-treatment) and Day 7, 14 & 21 (during treatment). This group served as **treatment group 3** to find the combined effect of suspension of bitter melon and black cumin seed as antidiabetic drug.

Fig. 5: Experimental Animals

**Chemicals**

1. Alloxan monohydrate –. (NH-CO-NH-COCO.H2O). (Sigma Aldrich Chemical, Saint Louis, MO, USA), Dresden, Germany

2. Blood Glucose determination Kit – Glucolab Active blood glucose system (strip method)

**3.7 Preparation & administmiceion of Alloxan Solution**

**3.7.1 Materials:**

- Saline for injection
- Alloxan (sigma)
- Distilled water

**3.7.2 Procedure:**

- Alloxan was dissolved in normal saline .
- This solution was injected intravenously and intraperitoniously to rabbits and maintained fasting condition for 18 hours.
- After 18 hours diabetic condition could not found than this solution was injected intramuscularly to rabbits and maintained fasting condition for 18 hours.
- To induce diabetic condition in rabbits a dose of 75mg Alloxan per kg of body weight was chosen following the recommendation of works done previously. ((Puri and Prabhu, 2002).


Fig. 6: Preparation and administration of Alloxan solution

### 3.8 Symptoms following Administration of Alloxan in Rabbits

Induction of alloxan diabetes by beta cell necrosis of the islets of langerhans required few minutes to few hours to many days to be expressed. As it caused **B** cell necrosis there was a massive release of pre-formed insulin from the dying beta cells. A confirmation of the hypoglycemia was done by measuring RBS (found to be as low as17 mmol/L) of the animals just prior to their deaths. These seizures were found to occur within the first few minutes, a few hours or even upto two days, of injecting alloxan monohydrate.

### 3.9 Collection, Preparation, Preservation & Administration of suspension of bitter melon and mahogany seed

#### 3.9.1 Collection

Fresh bitter melon and mahogany seed were purchased from the local market at a reasonable price.

Fig. 7: Bitter Melon (*Momordica carantia*) and Mahogany (*Swietenia macrophulla*) seed

#### 3.9.2 Preparation of bitter melon suspension

#### 3.9.3 Materials Required

- Bitter melon
- Blender machine
- Pestle and mortar
- Distilled water
- Beaker
- Pipette
- Stirrer
- Sieve and other conventional laboratory instruments.

### 3.9.4 Procedure

Fresh bitter melon and mahogany seed are purchased from the local market at a reasonable price then these are measured separately by electronic balance and grinded with mortar and pestle than blended with blender machine. Finally, the extracts are mixed with 100 ml distilled water separately and stirred to make homogenous mixture and then filtered through silk cloth.


Fig. 8: Preparation and administration of bitter melon suspension


Fig. 9: Preparation of mahogany seed suspension

### 3.9.5 Preservation

**Working Instrument:** Refrigerator

### 3.9.6 Procedure

All above solution were preserved at 0°-4°c  in Laboratory.

### 3.9.7 Administration

#### 3.9.7.1 Working Instruments

- Micropipette
- Leather gloves
- Electronic balance

### 3.9.7.2 Procedure

Prepared suspension of bitter melon and mahogany seed were fed orally after the solution was made in distilled water to the experimental rabbit with the help of a micropipette.

The use of micropipette ensured the administration of requisite quantity, which was ascertained on the basis of body weigh of each individual rabbit.

## 3.10 Observation of rabbits

- Body weight and fasting blood glucose level of each rabbits were measured after 18 hours of fasting before Alloxan injection.
- Body weight and fasting blood glucose level of each rabbits were measured on 15th day of Alloxan injection.
- Body weight and fasting blood glucose level of each rabbit were measured on Day 0 (Pre-treatment) and Day 7 & 14 & 21 (during treatment) of different treatment.

## 3.11 Recording of Different Parameters

### 3.11.1 Recording of Blood Glucose

**Procedure**

#### 3.11.1.1 Collection of blood

**Materials Required**

- Leather gloves
- Pinching needle
- Blood
- Ethanol
- Cotton
- Glucolab (R),

- Active monitor,
- Glucolab test strip

**Procedure**

For time-to-time blood glucose level determination the blood samples were collected from the tip of the ear vein of each rabbit as a drop. The drop was then immediately placed on the strip of the Glucolab® Active monitor to find the glucose level quickly.

**3.11.1.2 Determination of Blood Glucose**

Blood samples were collected from ear vein at Day 0 (Pre-treatment) and Day 7,14&21 (during treatment) for estimation of blood glucose level. Estimation of blood glucose by Glucolab® Active monitor blood glucose system (strip method).

**Materials Required**

- Glucolab (R) active monitor
- Glucolab test strip

**Test Principle**

The test zone of the strip contains glucose dye oxidoreductase 0.7 U, bis- (2- Hydroxy ethyl)-(4-hydroximinocyclohexa-5-dienylidene) ammonium chloride 8.3 ug, 2, 1 8-phosphomolybdicacid 88pg, stabilizer 0.8mg per cm2. Glucose dye oxidoreductase mediator reaction. The enzyme glucose dehydrogenase converts the glucose in a blood sample to gluconolactone. This reaction liberates an electron that reacts with a coenzyme electron acceptor, the oxidized form of the mediator hexacyanoferrate (Ill), forming the reduced form of the mediator, hexacyanoferrate (III). The test strip employs the electrochemical principle of biamperometry. The meter applies a voltage between two identical electrodes, which causes the reduced mediator formed during the incubation

period to be reconverted to an oxidized mediator. This generates a small current that is read by the system. D' Costa et al. (1986); Mor and Guamaccia, (197 7) and Hauge (1964).

**Procedure**

A drop of blood was collected from the ear. At the same time the Glucolab (R) active monitor was started with a single small press. After the monitor showed the code number the strip was inserted into the monitor. A drop of the blood was poured on the test zone of the strip. Before using the test strip new coding chip was inserted by the side of the monitor. The values were expressed in m mol/L.


Fig. 10: Determination of blood glucose level

### 3.11.2 Recording of Body weight

#### 3.11.2.1 Determination of body weight

Body weight was taken on day 0(pretreatment), 7 ,14 and 21(during treatment).

#### 3.11.2.2 Materials Required

- Leather gloves
- Electric balance

#### 3.11.2.3 Procedure

- Body weight of all groups was recorded before treatment (on day 0), during treatment period of 7th and 14th and 21 day by the help of electric balance.

Fig. 11: Recording of body weight

### 3.11.3 Clinical Examination

The effect of the alloxan on rabbit, extract of bitter melon and mahogany seed, combined effect of bitter melon and mahogany seed, blood glucose level, body weight, and hematological parameters was recorded.

### 3.11.4 Hematological Test:

Blood samples were collected from ear vein of rabbit of both control and treated groups to study the effect of the serum extract of bitter melon and mahogany seed on diabetic rabbit and the following parameters were observed:

(a) Hemoglobin estimation (Hb)

(b) Packed Cell Volume (PCV)

(c) Erythrocyte Sedimentation Rate (ESR)

#### 3.11.4.1 Determination of Hemoglobin Concentrations (Hb):

The N/10 hydrochloric acid was taken in a graduated tube up to 2 marks with the help of a dropper. Well-homogenized blood sample was then drawn into the Sahli pipette up to 20 cm. mark. The tip of the pipette was wiped with sterile cotton and the blood of the pipette was immediately transferred into the graduated tube containing hydrochloric acid. This blood and acid were thoroughly mixed by stirring with a glass stirrer. There was a formation of acid hematinic mixture in the tube by hemolysing red blood cells by the action of hydrochloric acid (HCL). The tube containing acid hematin mixture was kept standing in the comparator for 5 minutes. After that distilled water was added drop by drop. The solution was mixed well with a glass stirrer until the color of the mixture resembled to the standard color of the comparator. The result was read in daylight by observing the height of the liquid in the tube considering the lower meniscus of the liquid column. The result was then expressed in gm %. The above procedure was matched by the Helligehemo meter method as described by Lamberg and Rothstein (1977).

#### 3.11.4.2 Determination of Packed Cell Volume (PCV):

The citrated well mixed blood sample was drawn into special loading pipette (Wintrobe pipette). The tip of the pipette was inserted up to the bottom of a clean, dry Wintrobe

hematocrit tube. Then the Wintrobe tube was filled from the bottom by pressing the rubber bulb of the pipette. As blood came out, the pipette was slowly withdrawn but pressure was continued on the rubber bulb of the pipette so as to exclude air bubbles. The tip of the pipette was tried to keep under the rising column of blood to avoid foaming and the tube was filled exactly to the 10 cm mark. Then the Wintrobe hematocrit tube was placed in the centrifuge machine and was centrifuged for 30 minutes at 3000 rpm. Then, the hematocrit or PCV was recorded by reading the graduation mark; the percent volume occupied by the hematocrit was calculated by using the following formula as described by Lamberg and Rothstein (1977).

$$\text{PCV\%} = \frac{\text{Height of the red cell volume in cm}}{\text{Height of total blood in cm}} \text{ X } 100$$

**3.11.4.3 Determination of Erythrocyte Sedimentation Rate (ESR):**

The fresh anticoagulant blood was taken into the Wintrobe hematocrit tube by using special loading pipette exactly up to 0 marks. Excess blood above the mark was wiped away by sterile cotton. The filled tube was placed vertically undisturbed on the wooden rack for one hour. After one hour the ESR was recorded from the top of the pipette. The result was expressed in mm in 1st hour.

**3.12 Data and Statistical analysis**

Data were analyzed using SPSS v.11 for Windows (SPSS Inc., Chicago, IL, USA). Statistically significant differences between group means were determined by analysis of variance (ANOVA). Mean values were considered significantly different at $P<0.05$. Data are expressed as mean ± SEM.

# CHAPTER 4

# RESULTS AND DISCUSSION

# CHAPTER 4

# RESULTS AND DISCUSSION

The experiment was conducted to determine the efficacy of alloxan to induce diabetics in rabbit. Attempts were also made to study the efficacy of Bitter melon fruit and Mahogany seed on blood glucose level and body weight in alloxan induced diabetic rabbit. And also compare the combined effect of bitter melon fruit and mahogany seed on blood glucose level and body weight in alloxan induced diabetic rabbit. To perform the experiment, twenty rabbits were randomly divided into five equal groups. Alloxan was injected (I/M) at the dose rate of 75mg/kg body weight to the groups of rabbits ($T_2$, $T_3$, $T_4$ and $T_5$) for induction of diabetic syndrome. Group $T_1$ rabbits were kept as non-diabetic (Normal) control without giving Alloxan and any other treatment. Group $T_2$ rabbits were kept as positive control without (giving any other treatment except Alloxan). Next two groups of rabbits ($T_3$ and $T_4$) were treated with suspension of bitter melon fruit at dose of 150 mg/kg and mahogany seed at a dose of 50mg/kg for consecutive 21 days respectively after 21 days of alloxan administration. Then the last group of rabbit ( $T_5$ ) were treated with both suspension of bitter melon and mahogany seed at the same dose. All the control and treated rabbits were closely observed 21 days of treatment period.

## 4.1 Blood glucose level (mmol/L)

### 4.1.1 Alloxan induced diabetics and Comparison with Control

Blood glucose level of different groups of rabbits are presented in Table - 1. The study was revealed that glucose level was the highest in group $T_2$, which was treated with alloxan compare to the $T_1$ group. This treatment significantly ($p \leq .0.05$) increase the

blood glucose level in treated rabbits. The present results are agreed with other results. Reddy et al. (2014); Yakaiah *et al.* (2013); Akhtar *et al.* (1982); Puri and Prabhu, (2002) suggested that alloxan treatment increased the blood glucose level in treated birds compared to the control rabbits. Alloxan induced experimental diabetes is also associated with marked reduction of anti-oxidant enzyme superoxide dismutase activity in islets cells. In antioxidant enzyme superoxide dismutase activity (Halliwell, 1989). Alloxan induced diabetes is also suggested to result from initial islet cell inflammation followed by activation of macrophages and lymphocytes might be the source of cytotoxic oxygen radicals (Trivedi *et al.,* 2004). Alloxan has been shown to inactivate a calcium and calmodulin dependent protein kinase which reduces insulin secretion (Katzung 1993). (Gillman *et al.,* 1990) claim that structural similarity between alloxan and glucose may be responsible for its affinity with the **B** receptor on the **B** cell.. Alloxan binds almost instantly to islets cell membranes and causes rapid in vitro or in vivo inhibition of the insulin secretory mechanism (Gordsky, 1982). According to (Burger, 1960) zinc removal from insulin in chelate form may be the reason for its diabetogenic effect.

### 4.1.2 Alloxan induced diabetics and Comparison with bitter melon fruit

Blood glucose level of different groups of rabbits are presented in Table - 1.The study was revealed that glucose level was the lowest in group $T_3$, which was treated with bitter melon compare to the $T_4$ group. The effect of fruit suspension at a dose of 150gm/kg body weight in lowering blood sugar level showed statistically significant Comparison with $T_2$ group. We have evaluated the suspension of the unripe fruit of the *Momordica charantia* (Bitter gourd) was assessed for its anti diabetic activity in alloxan-induced diabetic rabbits. The blood sugar levels were highly decreased of a treatment with high dose of extract. The blood sugar levels are almost comes to the Normal levels. The

present results are agreed with other results. Ying ZD et al 2012; Akhtar MS et al, 1981 and Biyani MK et al. (2003); Leung L *et al.,* 2009;

Yakaiah Vangoori, *et al.,* 2013**;** suggested that results of this study show that chronic oral administration of an extract of Momordica charantia fruit at an appropriate dosage may be good alternative anti diabetic agent in Alloxan induced diabetics.

### 4.1.3 Alloxan induced diabetics and Comparison with mahogany seed

Blood glucose level of different groups of rabbits are presented in Table -4.1.The study was revealed that glucose level was the low in group $T_4$, which was treated with mahogany seed compare to the $T_2$ group. The effect of seed suspension at a dose of 50mg/kg body weight in lowering blood sugar level showed statistically significant Comparison with $T_2$ group. We have evaluated the suspension of the seed of the *switaneia macrophylla* (mahogany) was assessed for its anti diabetic activity in Alloxan-induced diabetic rabbits. The blood sugar levels were highly decreased of a treatment with high dose of extract. The blood sugar levels are almost comes to the Normal levels. The present results are agreed with other results, Naveen YP , Divya Rupini G, Ahmed F, Urooj A et al 2014.; the results of this study also aggred with Panda SP, Haldar PK, Bera S, Adhikary S, Kandar CC, 2010. The result of this study indicate that a dose of 50 mg/ kg body weight of *Swietenia macrophylla* (mahogany seed) might be a beneficial adjuvant to oral hypoglycemic agents in Alloxan induced diabetics.

### 4.1.4 Alloxan induced diabetics and Comparison among different groups of rabbits

The fall in the blood sugar was compared among the groups of animals.

The study was reveled that blood glucose level was the lowest in group $T_5$ compare to the $T_3$ and $T_4$ group, which was treated with bitter melon fruit and mahogany seed. The effect of this combined treatment significantly ($p \leq .0.05$) affects the blood glucose level.

**Table 1:** Effects of bitter melon fruit and mahogany seed suspension and combined treatment on blood glucose (m mol/L, mean±SE) concentration in Alloxan induced diabetic rabbits (n=4).

| Group | Day 0 (Mean ± SE ) mmol/L | Day 7 (Mean ± SE ) mmol/L | Day 14 (Mean ± SE) mmol/L | Day 21 (Mean ± SE) mmol/L |
|---|---|---|---|---|
| $T_1$ | $7.550^{b} \pm 0.44$ | $7.725^{c} \pm 0.37$ | $7.425^{d} \pm 0.24$ | $7.875^{d} \pm 0.13$ |
| $T_2$ | $28.33^{a} \pm 0.69$ | $27.00^{a} \pm 1.15$ | $24.23^{a} \pm 0.60$ | $19.02^{a} \pm 0.70$ |
| $T_3$ | $27.95^{a} \pm 0.72$ | $23.45^{b} \pm 0.76$ | $18.27^{b} \pm 0.71$ | $12.98^{b} \pm 0.45$ |
| $T_4$ | $28.42^{a} \pm 0.82$ | $21.13^{b} \pm 0.48$ | $14.52^{c} \pm 0.48$ | $10.93^{c} \pm 0.15$ |
| $T_5$ | $29.05^{a} \pm 0.44$ | $21.58^{b} \pm 0.92$ | $14.52^{c} \pm 1.41$ | $10.15^{c} \pm 1.06$ |

Values with the different superscripts in the same column are statistically significant ($P<0.05$).

### 4.2 Body weight (gm)

The percent increased in body weight gain in normal control rabbits (Group $T_1$, n=4) was 1130 gm. On the contrary, in diabetic positive group (Group $T_2$, n=4), the percentage of body weight loss was 1000gm. The percent increased in body weight gain over 21 days in. Group $T_3$ (n=4), following oral administration of suspension of bitter melon @ 150 gm/kg was 1080 gm. In Group $T_4$ (n=4), following administration of mahogany seeds @ 50 mg/kg for 21 days the percentage of body weight gain was 1113 gm. In Group $T_5$ (n=4), following administration of bitter melon and mahogany seeds @ previous doses

for 21 days the percentage of body weight gain was 1083 gm comparison with $T_2$ group which is treated with alloxan.

**Table 2:** Effects of bitter melon fruit and mahogany seed suspension and combined treatment on body weight(gm) in Alloxan induced diabetic rabbits(n=4).

| Group | Day 0 (Mean ± SE) gm | Day 7 (Mean ± SE)gm | Day 14 (Mean ± SE )gm | Day 21 (Mean ± SE)gm |
|---|---|---|---|---|
| $T_1$ | 1056.0 $^{a}$ ± 21.34 | 1078.0 $^{a}$ ± 21.75 | 1108.0 $^{a}$ ± 21.75 | 1133.0 $^{a}$ ± 20.56 |
| $T_2$ | 1025.0 $^{a}$ ± 32.27 | 1020.0 $^{a}$ ± 31.09 | 1010.0 $^{b}$ ± 29.72 | 1000.0 $^{b}$ ± 32.40 |
| $T_3$ | 1056.0 $^{a}$ ± 21.35 | 1048.0 $^{a}$ ± 20.56 | 1065.0 $^{ab}$ ± 21.06 | 1080.0 $^{a}$ ± 20.82 |
| $T_4$ | 1069.0 $^{a}$ ± 11.97 | 1073.0 $^{a}$ ± 11.09 | 1083.0 $^{a}$ ± 14.36 | 1113.0 $^{a}$ ± 21.75 |
| $T_5$ | 1044.0 $^{a}$ ± 15.73 | 1053.0 $^{a}$ ± 18.87 | 1065.0 $^{ab}$ ± 16.58 | 1083.0 $^{a}$ ± 16.52 |

Values with the different superscripts in the same column are statistically significant ($P<0.05$).

### 4.3 Hematological parameter:

The results 0f various blood parameters are depicted n following table 3

**Table 3.** Effect of bitter melon and mahogany seed on diabetic rabbit and combined treatment on hematological parameters

### 3.1 Hb (Hemoglobin) g/dl

| Group | Day 0 (Mean ± SE )g/dl | Day 7 (Mean ± SE ) g/dl | Day 14 (Mean ± SE) g/dl | Day 21 (Mean ± SE ) g/dl |
|---|---|---|---|---|
| $T_1$ | 11.90 $^a$ ± 0.54 | 12.10 $^a$ ± 0.60 | 12.43 $^a$ ± 0.55 | 12.60 $^a$ ± 0.64 |
| $T_2$ | 12.15 $^a$ ± 0.57 | 12.10 $^a$ ± 0.43 | 11.82 $^a$ ± 0.48 | 12.15 $^a$ ± 0.25 |
| $T_3$ | 12.20 $^a$ ± 0.63 | 12.07 $^a$ ± 0.65 | 12.55 $^a$ ± 0.68 | 12.43 $^a$ ± 0.62 |
| $T_4$ | 11.25 $^a$ ± 0.45 | 11.45 $^a$ ± 0.49 | 11.27 $^a$ ± 0.58 | 11.70 $^a$ ± 0.47 |
| $T_5$ | 11.75 $^a$ ± 0.71 | 11.38 $^a$ ± 0.73 | 11.95 $^a$ ± 0.60 | 11.98 $^a$ ± 0.60 |

* = Significant at the 0.05% level

Hemoglobin content is presented in (Table 4.3.1). The values of Hb in all treated groups and control group were almost similar and the values were within the normal range. These values show a little fluctuation they were not statistically significant ($p>0.05$).

### 3.2 Packed Cell Volume (%)

| Group | Day 0 (Mean ± SE ) % | Day 7 (Mean ± SE) % | Day 14 (Mean ± SE ) % | Day 21 (Mean ± SE ) % |
|---|---|---|---|---|
| $T_1$ | 40.47 $^{a}$ ± 0.78 | 40.08 $^{ab}$ ± 0.37 | 40.70 $^{ab}$ ± 0.39 | 41.14 $^{bc}$ ± 0.37 |
| $T_2$ | 38.97 $^{a}$ ± 1.058 | 37.83 $^{c}$ ± 0.82 | 37.00 $^{c}$ ± 0.78 | 35.82 $^{d}$ ± 0.47 |
| $T_3$ | 38.17 $^{a}$ ± 0.81 | 38.75 $^{bc}$ ± 0.52 | 40.33 $^{b}$ ± 0.60 | 40.72 $^{c}$ ± 0.55 |
| $T_4$ | 39.83 $^{a}$ ± 0.55 | 40.60 $^{ab}$ ± 0.57 | 41.65 $^{ab}$ ± 0.46 | 42.67 $^{ab}$ ± 0.49 |
| $T_5$ | 39.65 $^{a}$ ± 0.95 | 41.03 $^{a}$ ± 0.62 | 42.38 $^{a}$ ± 0.44 | 43.00 $^{a}$ ± 0.70 |

Packed cell volume is presented in (Table 4.3.2). The values of PCV in all treated groups and control group were almost similar and the values were within the normal range. These values show a little fluctuation they were not statistically significant ($p>0.05$).

### 3.3 Erythrocyte Sedimentation Rate (mm/1$^{st}$ hour)

| Treatment group | Day 0 (Mean ± SE) mm/1$^{st}$ hour | Day 7 (Mean ± SE) mm/1$^{st}$ hour | Day 14 (Mean ± SE) mm/1$^{st}$ hour | Day 21 (Mean ± SE) mm/1$^{st}$ hour |
|---|---|---|---|---|
| $T_1$ | 1.875 $^{b}$ ± 0.27 | 1.700 $^{a}$ ± 0.22 | 1.900 $^{b}$ ± 0.23 | 1.950 $^{b}$ ± 0.22 |
| $T_2$ | 1.850 $^{b}$ ± 0.07 | 2.200 $^{a}$ ± 0.11 | 2.750 $^{a}$ ± 0.17 | 3.525 $^{a}$ ± 0.10 |
| $T_3$ | 2.550 $^{a}$ ± 0.32 | 2.300 $^{a}$ ± 0.19 | 2.075 $^{b}$ ± 0 .08 | 2.150 $^{b}$ ± 0.08 |
| $T_4$ | 2.250 $^{ab}$ ± 0.14 | 2.275 $^{a}$ ± 0.13 | 1.925 $^{b}$ ± 0.11 | 1.850 $^{bc}$ ± 0.06 |
| $T_5$ | 2.150 $^{ab}$ ± 0.06 | 1.925 $^{a}$ ± 0.29 | 1.625 $^{b}$ ± 0.24 | 1.450 $^{c}$ ± 0.20 |

Erythrocyte sedimentation rate content is presented in (Table 4.3.3). The values of ESR in all treated groups and control group were almost similar and the values were within the normal range. The highest ESR was recorded in Group $T_4$ and lowest in Group $T_1$. Although these values show a little fluctuation they were not statistically significant ($p>0.05$).

# CHAPTER 5

# CONCLUSION

# CHAPTER 5

# CONCLUSIONS

The study concluded that Mahogany showed better results in reducing blood glucose level in alloxan induced rabbits. Bitter melon showed also better results in reducing blood glucose level in alloxan induced rabbits. But combined both mahogany and bitter melon showed splendid results to reducing blood glucose level in alloxan induced rabbits.

We could consider Mahogany and Bitter melon for diabetic patients as a practical choice for reducing blood glucose.

# REFERENCES

# REFERENCES

**A .Verrotti, A. Scaparrotta, C. Olivieri and F. Chiarelli (2012).** Seizures and type 1 diabetes mellitus: current state of knowledge. European journal of endocrinology 167 (6): 749–58.

**A. Fuangchan, P. Sonthisombat, T. Seubnukarn, R. Chanouan, P. Chotchaisuwat, and Sirigulsati (2011).** Hypoglycemic effect of bitter melon compared with metformin in newly diagnosed type 2 diabetes patients. J Ethnopharmacol**,** 134**:**422–428.

**A. Sharma, M. Vijayakumar, C. V. Rao, M. K. Unnikrishnan, and G. D. Reddy, (2009).** Action of Portulaca oleracea against streptozotocin-induced oxidative stress in experimental diabetic rats, Journal of Complementary and Integrative Medicine, vol. 6, no. 1, article 1

**AE. Kitabchi, GE. Umpierrez, JM. Miles and JN. Fisher (2009).** Hyperglycemic crises in adult patients with diabetes. Diabetes Care **32** (7): 1335–43.

**AE. Kitabchi, GE. Umpierrez, JM. Miles, JN. Fisher, (2009).** Hyperglycemic crises in adult patients with diabetes. Diabetes Care **32** (7): 1335–43.

**American Diabetes Association (2013).** Economic costs of diabetes in the U.S. in 2012. Diabetes Care **36** (4): 1033–46.

**B. K. Rao, P. R. Sudarshan, M. D. Rajasekhar, N. Nagaraju, and C. A. Rao, (2003).** Antidiabetic activity of Terminalia pallida fruit in alloxan induced diabetic rats, Journal of Ethnopharmacology, 85(1): 169–172.

**B. Qin, M. Nagasaki, M. Ren, G. Bajotto, Y. Oshida, and Y. Sato, (2004).** Gosha-jinki-gan (a Herbal Complex) corrects abnormal insulin signaling, Evidence-Based Complementary and Alternative Medicine, 1: 269–276.

**B.A. Shibib, LA. Khan and R. Rahman (1993**). Hypoglycaemic activity of Coccinia indica and Momordica charantia in diabetic rats: depression of the hepatic gluconeogenic enzymes glucose-6-phosphatase and fructose-1,6-bisphosphatase and elevation of both liver and red-cell shunt enzyme glucose-6-phosphate dehydrogenase. Biochem J, **292**(Pt 1)**:** 267–270.

**BD. Lin, T. Yuan, CR. Zhang, L. Dong, B. Zhang, Y. Wu and JM. Yue. (2009).** Structurally diverse limonoids from the fruits of Swietenia mahagoni[J]. J Nat Prod, 72(12): 2084-2090.

**C. Kenny (2014).** When hypoglycemia is not obvious: diagnosing and treating under-recognized and undisclosed hypoglycemia. Primary care diabetes **8**(1): 3–11.

**C. Mallick, R. Maiti, and D. Ghosh, (2006).** Comparative study on antihyperglycemic and antihyperlipidemic effects of separate and composite extract of seed of Eugenia jambolana and root of Musa paradisiaca in streptozotocin-induced diabetic male albino rat," Iranian Journal of Pharmacology and Therapeutics, 5(1): 27–33,

**C. Mallick, R. Maiti, and D. Ghosh, (2006).** Antidiabetogenic effects of separate and composite extract of seed of Jamun (Eugenia jambolana) and root of Kadali (Musa paradisiaca) in streptozotocin-induced diabetic male albino rat: a comparative study, International Journal of Pharmacology, 2(5): 492–503.

**CP. Ooi, Z. Yassin and T-A. Hamid (2012).** Momordica charantia for type 2 diabetes mellitus. Cochrane Database Syst Rev 8**.**

**D.-D. Li, J.-H. Chen, Q. Chen. (2005).** Swietenia mahagony extract shows agonistic activity to PPARγ and gives ameliorative effects on diabetic db/db mice," Acta Pharmacologica Sinica, 26(2): 220–222.

**DR. Whiting, L. Guariguata**, **C. Weil and J. Shaw**. **(2011).** diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabet Res Clin Pract; 94: 311-321

**DW. Cooke, L. Plotnick (2008).** Type 1 diabetes mellitus in pediatrics. Pediatr Rev 29 (11): 374–84; quiz 385.

**E. Goun, G. Cunningham, D. Chu, C. Nguyen and D. Miles (2003).** Antibacterial and antifungal activity of Indonesian ethnomedical plants [J]. Fitoterapia, 76(4): 592-596.

**EA .Hu, A. Pan, V. Malik, Q. Sun (2012).** White rice consumption and risk of type 2 diabetes: meta-analysis and systematic review. BMJ (Clinical research ed.) 344: e1454.

**G. Sahgal, S. Ramanathan, S. Sasidharan, MN. Mordi, S. Ismail and SM. Mansor (2009).** Phytochemical and antimicrobial activity of Swietenia mahagoni crude methanolic seed extract[J]. Trop Biomed, 26(3): 274-279.

**G. Brugnatelli (1818).** Sopra i cangiamenti che avvengono nell'ossiurico (ac. urico) trattato coll'ossisettonoso (ac. nitroso). G Fis Chim 117–29, 1818.

**G. Klein, J. Kim, K. Himmeldirk, Y. Cao, and X. Chen, (2007).** Antidiabetes and anti-obesity activity of Lagerstroemia speciosa, Evidence-Based Complementary and Alternative Medicine, 4(4): 401–407.

**GA. P. Guevara, A. Apilado, H. Sakurai, M. Kozuka, and H. Tokuda,(1996 )"**Anti-inflammatory, antimutagenic and antitumor promoting activities of Mahogany seeds, Swietenia macrophylla (Meliaceae)," Philippine Journal of Science, vol. 125: 271–278.

**H. Ekimoto, Y. Irie, Y. Araki, GQ. Han, S. Kadota, and T. Kikuchi (1991).** Platelet aggregation inhibitors from the seeds of Swietenia mahagoni: inhibition of in vitro and in vivo platelet-activating factor-induced effects of tetranortriterpenoids related to swietenine and swietenolide. Planta Med, 57(1): 56-58.

**J. H. Hsu, Y. C. Wu, S. S. Liou, and I. M. Liu (2004)**, Mediation of endogenous β-endorphin by tetrandrine to lower plasma glucose in streptozotocin-induced diabetic rats, 1: 193–201.

**JK. Grover, S. Yadav and V. Vats (2002).** Medicinal plants of India with anti-diabetic potential. 81: 81–100.

**K. E. Innes and H. K. Vincent, (2007).** The influence of yoga-based programs on risk profiles in adults with type 2 diabetes mellitus: a systematic review," Evidence-Based Complementary and Alternative Medicine, 4(4): 469–486.

**K. Shigetoshi, M. Lamik, K. Tohru, and E. Hisao, (1990).** Constituents of the seeds of Swietenia mahagany Jacq, isolation, structure and H1 and C13 NMR signal assignments of new tetranor triterpenoids related to swietenin and swietenolide, Chemical & Pharmaceutical Bulletin, 38: 639–651

**L. Ali, AK. Khan, MI. Mamun, M. Mosihuzzaman, N. Nahar, M. Nur-e-Alam and B. Rokeya (1993).** Studies on hypoglycemic effects of fruit pulp, seed, and whole plant of Momordica charantia on normal and diabetic model rats Planta Med, **59:** 408–412**.**

**L. Pari and R. Saravanan, (2004).** Antidiabetic effect of diasulin, a herbal drug, on blood glucose, plasma insulin and hepatic enzymes of glucose metabolism hyperglycaemic rats, Diabetes, Obesity and Metabolism, 6(4): 286–292.

**M. Dorner, M. Pinget and JM. Brogard (1977).** Essential labile diabetes. MMW Munch Med Wochenschr (in German) 119 (19): 671–4.

**M. Neelesh, J. Sanjay, M. Sapna (2010)**. Antidiabetic potential of medicinal plants. Acta Pol Pharm; 67: 113-118.

**MF. Kadir, MS. Bin Sayeed, T. Shams and MMK. Mia (2012).** Ethnobotanical survey of medicinal plants used by Bangladeshi traditional health practitioners in the management of diabetes mellitus. J Ethnopharmacol, 144**:** 605–611

**N. Sarwar, P. Gao, SR. Seshasai, R. Gobin, S. Lewington, L. Pennells, A. Thompson, N. Sattar, IR. White, KK. Ray, J. Danesh (2010).** "Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: A collaborative meta-analysis of 102 prospective studies". The Lancet 375 (9733): 2215–22.

**O. Said, S. Fulder, K. Khalil, H. Azaizeh, E. Kassis, and B. Saad, (2008)**. Maintaining a physiological blood glucose level with ‘glucolevel’, a combination of four anti-diabetes plants used in the traditional Arab herbal medicine, Evidence-Based Complementary and Alternative Medicine, 5(4): 421–428.

**P. Chaturvedi (2012),** Antidiabetic potentials of Momordica charantia: multiple mechanisms behind the effects. J Med Food 15:101–107.

**P. Daisy, R. Jasmine, S. Ignacimuthu, and E. Murugan, (2009).** A novel Steroid from Elephantopus scaber L. an Ethnomedicinal plant with antidiabetic activity, 16(2-3): 252–257.

**P. Ljubuncic, H. Azaizeh, U. Cogan, and A. Bomzon (2006**). The effects of a decoction prepared from the leaves and unripe fruits of Crataegus aronia in streptozotocin-induced diabetic rats, Journal of Complementary and Integrative Medicine, 3(1), article 6.

**R. Maiti, U. K. Das, and D. Ghosh (2005).** Attenuation of hyperglycemia and hyperlipidemia in streptozotocin-induced diabetic rats by aqueous extract of seed of Tamarindus indica, Biological and Pharmaceutical Bulletin, 28(7): 1172–1176,

**S. Lenzen, (2008).** The Mechanisms of Alloxan- and Streptozotocin-induced Diabetes. Diabetologia 51(2): 216–226.

**S. Lenzen and U. Panten. (1988)** Alloxan: History and mechanism of action. Diabetologia 31:337–42,

**S .Kadota, L. Marpaung, T. Kikuchi and H. Ekimoto. (1990).** Constituents of the seeds of Swietenia mahagoni JACQ. III. structures of mahonin and secomahoganin [J]. Chem Pharm Bull, 38(6): 1495-1500.

**S. L. Badole, N. M. Patel, P. A. Thakurdesai and S. L. Bodhankar (2008**). Interaction of aqueous extract of Pleurotus pulmonarius (Fr.) Quel-Champ. with Glyburide in alloxan induced diabetic mice, 5(2): 159–164.

**S. M. Setter, R. K. Campbell, and C. J. Cahoon, (2003).** Biochemical pathways for microvascular complications of diabetes mellitus," Annals of Pharmacotherapy, 37(12): 1858–1866.

**T. Szkudelski (2001)**. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res 50: 536–546.

**T. Miura, C. Itoh, N. Iwamoto, M. Kato, M. Kawai, SR. Park and I. Suzuki (2001).** Hypoglycemic activity of the fruit of the Momordica charantia in type 2 diabetic mice. J Nutr Sci Vitaminol, 47:340–344.

**U. Risérus, WC. Willett and FB. Hu (2009).** Dietary fats and prevention of type 2 diabetes. Progress in Lipid Research 48(1): 44–51.

**VS. Malik, BM. Popkin, GA. Bray, JP. Després, WC. Willett and FB. Hu (2010).** Sugar-Sweetened Beverages and Risk of Metabolic Syndrome and Type 2 Diabetes: A meta-analysis. Diabetes Care 33(11): 2477–83.

**VS. Malik, BM. Popkin, GA. Bray, JP. Després and FB. Hu (2010).** Sugar Sweetened Beverages, Obesity, Type 2 Diabetes and Cardiovascular Disease risk. Circulation 121(11): 1356–64.

**World Health Organization (1980).** World Health Organization Expert Committee on Diabetes Mellitus: Second Report, Technical Report Series, no. 646.

**YY. Chen, XN. Wang, CQ. Fan, S. Yin and JM. Yue (2007).** Swiemahogins A and B, two novel limonoids from Swietenia mahogany[J]. Tetrahedron Lett, 48(42): 7480–7484.