ANTIDIABETOGENIC IMPACT OF BITTER MELON (*MOMORDICA CHARANTIA*) AND BLACK CUMIN (*NIGELLA SATIVA*) ON ALLOXAN INDUCED DIABETIC RABBIT MODEL

A Thesis

By

KISHOR KUMAR ROY

Student ID. 1405187

Session: 2014-15

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

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JUNE, 2016

DEDICATED
TO MY
BELOVED PARENTS
AND
WELL WISHERS

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The Author

ABSTRACT

The present study was undertaken to demonstrate and investigate the antidiabetic effect of the *Momordica charantia* (Bitter melon) and *Nigella sativa* (Black cumin) on Alloxan induced diabetes in experimental rabbits. . At the age of 2 to 3 months , rabbits were randomly assigned into five groups (A, B, C, D and E) and each group was contained 4 rabbits. After acclimatization, diabetes was induced in four groups of rabbits (B, C, D and E) by administering Alloxan injection in a dose of 75mg/ 1000 gm body weight (b.wt.) intramuscularlly. Group A was kept for normal control, Group B was kept for diabetic positive control,Group C was treated with bitter melon 150gm/kg body weight orally, Group D was treated with black cumin 250mg/kg body weight orally, Group E treated with both of them at previous doses. Over the course of trial, blood glucose level and body weight were recorded from all group of rabbit to know the antidiabetogenic effect of both bitter melon and black cumin.. Blood glucose level were increased significantly ($p<0.05$) in all alloxan treated groups compared to the control group A .Simultaneously body weight was decreased significantly ($p<0.05$) in all alloxan treated groups compared to the control group A. After treatment with the medicinal herbs in group C,D,E , there was significant decrease in blood glucose level in all treated groups C, D, E compared to the group B and body weight was increased in all treated group C, D, E compared to the group B.The present study also reveals that combined treatment with bitter melon and black cumin increases body weight and decreases glucose level without affecting health of rabbits. The results of this study show that chronic oral administration of a suspension of *Momordica charantia* fruit and *Nigella sativa* seed, an appropriate dosage may be good alternative as antidiabetic agent.

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LIST OF ABBREVIATIONS

µg	:	Microgram
ADA	:	American Diabetes Association
ALX	:	Alloxan
B. wt.	:	Body weight
Conc.	:	Concentration
Cu. mm	:	Cubic millimeter
d.w.	:	Drinking water
DM	:	Diabetics Mellitus
et al.	:	Associates
Fig.	:	Figure
GDM	:	Gestational diabetes mellitus
ICD	:	International Classification of Diseases
IDDM	:	Insulin-Dependent Diabetes Mellitus
IDF	:	International Diabetes Federation
IND	:	International Nomenclature of Diseases
J.	:	Journal
L	:	Liter
LADA	:	Latent Autoimmune Diabetes of Adults
mg	:	Milligram
mL	:	Mili Liter
mm^3	:	cubic millimeter
mmol	:	Milimole
MRDM	:	Malnutrition-related Diabetes Mellitus
NIDDM	:	Non Insulin-Dependent Diabetes Mellitus
No.	:	Number
PM	:	Population Mean
SE	:	Standard Error
SM	:	Sample Mean
SPSS	:	Statistical Package of Social Science
STZ	:	Streptozotocin
Vol.	:	Volume
WHO	:	World Health Organization

CHAPTER 1

INTRODUCTION

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INTRODUCTION

Diabetes mellitus is the major endocrine disease with deranged carbohydrate, fat and protein metabolism. This is also called a metabolic disease with manifestation of hyperglycemia, is a fast growing health problem through out the world usually due to a combination of hereditary and environmental causes. Being a major degenerative disease, diabetes is found in all parts of the world and it is becoming the third most lethal disease of mankind and increasing rapidly. World Health Organization (WHO) estimates that 346 million people suffer from diabetes worldwide. It is the most common endocrine disorder, affecting 16 million individuals in the United States and as many as 200 million individuals worldwide. Without urgent action, this number is likely to double by 2030. Statistical projection suggests that the number of diabetics will rise from 15 million in the year 1995 to 57 million in 2025 in India. 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 is 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. Pharmacological agents, including sulfonylureas, biguanides, alpha-glucosidase inhibitors, thiazolidinediones, and meglitinide, are also used; however, long-term complications of type 2 diabetes mellitus are unaltered with these agents (De Fronzo RA,1999).

Diabetes mellitus can be chemically or surgically induced in different animal species. Chemical induction of diabetes can be achieved by injecting uric acid, dialuric acid, dehydroascorbic acid, quinoline and magnesium. However, the most commonly used means of chemical induction of diabetes has been either Alloxan or Streptozotocin, as their diabetogenic dose is usually 4 to 5 times less than their lethal dose. However Guinea pigs are totally insensitive to Alloxan (Gordsky *et al.*, 1982). Alloxan (mesoxal urea) was the first chemical used to induce experimental diabetes. It was found by Leibig in mucus excreted during dysentery (Merck Index, 1976). The diabetogenic dose of Alloxan vary considerably amongst species, age and metabolic state of the animal. Nephrotoxicity is also a side effect (Bonar 1980). Alloxan diabetes can be prevented by sulphhydryl containing compounds such as glutathione, cystine and dimercaprol prior to Alloxan administration. It's monohydrate form as Alloxan monohydrate is less toxic than

its tetrahydrate form. Therefore Alloxan monohydrate is selected for induction of diabetes in rabbits.

Metformin is currently being used in type 2 diabetes as the first-choice oral agent, along with appropriate diet control and lifestyle advice. Metformin acts primarily by reducing the hepatic glucose output and improving insulin sensitivity in the liver and muscle. Metformin has pleiotropic vascular effects that act on endothelial imbalance, probably increasing nitric oxide bioavailability, decreasing atheroma plaque growth, improving the atherogenic lipid profile, and inhibiting lipid incorporation into vessel walls, thereby inhibiting vascular smooth muscle cell proliferation (Lima LM, *et al.*, 2009). The American Diabetic Association (ADA) has recommended metformin as a first line agent for the treatment of type 2 diabetes, as metformin helps in weight loss and lowers fasting plasma insulin concentrations, total and low-density lipoprotein cholesterol, and free fatty acids (American Diabetes Association, 2011) however, long-term complications are not altered with metformin therapy. Moreover, the hypoglycemic drugs lead to some unpleasant side effects such as lactic acidosis, peripheral edema, severe hypoglycemia, and abdominal discomfort (Lorenzati B, *et al*., 2010).

The antidiabetic drugs are mainly used to replace the insulin deficiency or to enhance the action of insulin and/or decrease the insulin resistance. Although many drugs and interventions are available to manage diabetes, these are expensive for the large diabetic population of developing countries. Complementary and alternative medicines involve the use of herbs and other dietary supplements as alternatives to main stream western medical treatment. A recent study has estimated that up to 30% of patients with diabetes mellitus use complementary and alternative medicine. Therefore, the search for new antidiabetic agents preferably medicinal herbal product are to be the main challenge in the modern world to protect this silent killer type of metabolic disease without creating health hazzard. On the other hand the WHO Expert Committee on diabetes has recommended that traditional medicinal herbs can be further investigated for the treatment of diabetes. On this regard, choice to solve the problem is the main challenge for the betterment of the mankind. Medicinal plants and its products continue to be an important therapeutic aid for alleviating the ailments of mankind. The World Health Organization (WHO) has listed 21000 plants, which are used for medicinal purposes around the world. Among them, 150 species are used commercially on a fairly large scale.

Momordica charantia (*M. charantia*), also known as bitter melon, karela, balsam pear, or bitter gourd, is a popular plant used for treating of diabetes related conditions amongst the indigenous populations of Asia, South America, India, the Caribbean and East Africa. Its fruit has a distinguishing bitter taste, which is more pronounced as it ripens, hence the name bitter melon or bitter gourd. Biochemical and animal model experiments have produced abundant data and hypotheses accounting for the antidiabetic effects of *M. charantia*. In this study, the antidiabetic potential of this unripe fruit extract of *Momordica charantia* (Karela) is screened on laboratory animal model.

Black Cumin (*Nigella sativa*), a small, annual herbaceous plant of the Ranunculaceae family. Black Cumin is a popular spice all over the world, especially in Latin America, North Africa, and all over Asia. It has known to the Egyptians 5,000 years ago and formed an important medicine of ancient Egypt. Seeds are the medicinal part of cumin, has numerous uses such as drug and spicy for about a thousand years ago. Black Cumin is used as an antioxidant, stimulant, antispasmodic, carminative particularly in veterinary practice and antimicrobial agent. It is used widely in traditional medicine to treat flatulence, digestive disorders and diarrhoea and in the treatment of wounds, Fungitoxic, also help to ease and increase secretion of milk in lactating women, also uses for cold and fever. Black Cumin contains volatile oils, vitamins like vitamin C, and A, also contain amino acids, proteins, minerals, starch tannin and fibers, in addition to flavones, and Glucosides. Black Cumin is used for the treatment of diabetes, recent researches record the activity of extraction of black cumin decreases the level of glucose in normal and in Alloxan induced diabetic rabbits and decreases lipid profile. In addition it decreases the levels of uric acid and total proteins and decreases level of creatinin. The present study is conducted to evaluate the effect of Black Cumin on diabetes mellitus in rabbits induced by Alloxan.

General Objectives

The overall goal is the search for new antidiabetic agents preferably medicinal herbal products are to be the main challenge in the modern world to protect this silent killer type of metabolic disease without creating health hazzard. On the other hand the WHO Expert Committee on diabetes has recommended that traditional medicinal herbs can be further investigated for the treatment of diabetes. On this regard choice to solve the problem is the main challenge for the betterment of the mankind.

Detailed Specific Research Objectives

For that research or study is on made evaluating or observing the following specific objectives:

- To evaluate the Alloxan induced diabetes occurred in experimental rabbits.
- To investigate the effect of *Momordica charantia* (Bitter melon) and *Nigella sativa* (Black cumin) in Alloxan induced diabetic rabbit.
- To demonstrate the combined effect of *Momordica charantia* (Bitter melon) and *Nigella sativa* (Black cumin) in Alloxan induced diabetic rabbit.
- To study the effects of plants suspension on body weight, blood sugar, 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* (Bitter melon) and *Nigella sativa* (Black Cumin) on Alloxan 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, *et al.*, 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, *et al.*, 1991), and some are ineffective in chronic diabetic patients (L. Pari, *et al.*, 2004). Thus, there is an increasing demand of new antidiabetic natural products especially neutraceuticals with lesser side effects and high antidiabetic potential.

2.2 History of diabetes

Diabetes was one of the first diseases described, with an Egyptian manuscript from c. 1500 BCE mentioning "too great emptying of the urine". The first described cases are believed to be of type 1 diabetes. Indian physicians around the same time identified the disease and classified it as madhumeha or "honey urine", noting the urine would attract ants. The term "diabetes" or "to pass through" was first used in 230 BCE by the Greek Apollonius of Memphis. The disease was considered rare during the time of the Roman empire, with Galen commenting he had only seen two cases during his career. This is possibly due to the diet and life-style of the ancient people, or because the clinical symptoms were observed during the advanced stage of the disease. Galen named the disease "diarrhea of the urine" (diarrhoea urinosa). The earliest surviving work with a

detailed reference to diabetes was that of Aretaeus of Cappadocia (2nd or early 3rd century CE). He described the symptoms and the course of the disease, which he attributed to the moisture and coldness, reflecting the beliefs of the "Pneumatic School". He hypothesized a correlation of diabetes with other diseases and he discussed differential diagnosis from the snakebite which also provokes excessive thirst. His work remained unknown in the West until the middle of the 16th century when, in 1552, the first Latin edition was published in Venice. Type 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 being overweight. The term "mellitus" or "from honey" was added by the Briton John Rolle in the late 1700s to separate the condition from diabetes insipidus, which is also associated with frequent urination. Effective treatment was not developed until the early part of the 20th century, when Canadians Frederick Banting and Charles Herbert Best isolated and purified insulin in 1921 and 1922. This was followed by the development of the long-acting insulin NPH in the 1940s.

2.2.1 Etymology

The word diabetes comes from Latin word *diabetes*, which in turn comes from Ancient Greek word *diabetes* which literally means "a passer through; a siphon." Ancient Greek physician Aretaeus of Cappadocia (fl. 1st century CE) used that word, with the intended meaning "excessive discharge of urine", as the name for the disease. Ultimately, the word comes from Greek word diabainein meaning "to pass through," which is composed of dia meaning "through" and bainein, meaning "to go". The word "diabetes" is first recorded in English, in the form diabete.

The word *mellitus* comes from the classical Latin word *mellitus*, meaning "mellite" (i.e. sweetened with honey; honey-sweet). The Latin word comes from *mell-*, which comes from *mel*, meaning "honey"; sweetness; pleasant thing, and the suffix *-itus*, whose meaning is the same as that of the English suffix "-ite". It was Thomas Willis who in 1675 added "mellitus" to the word "diabetes" as a designation for the disease, when he noticed the urine of a diabetic had a sweet taste (glycosuria). This sweet taste had been noticed in urine by the ancient Greeks, Chinese, Egyptians, Indians, and Persians.

2.2.2 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, *et al.,* 2014). This represents 8.3% of the adult population, with equal rates in both women and men (Vos T, *et al.,* 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, *et al.,* 2012). This is equal to 8.3% of the adult population (Shi, *et al.,* 2014) with equal rates in both women and men (Salomon JA, *et al.,* 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, *et al.,* 2006). Diabetes mellitus occurs throughout the world, but is more common (especially type 2) in more developed countries. The greatest increase in rates was expected to occur in Asia and Africa, where most people with diabetes will probably live in 2030 (Green A, *et al.,* 2004). 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, *et al.,* 2004).

2.4 Classification of diabetes

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 , 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 was 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 included 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 Diabetes mellitus

Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period. Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger. If left untreated, diabetes can cause many complications. Acute complications include diabetic ketoacidosis and nonketotic hyperosmolar coma. Serious long-term complications include cardiovascular disease, stroke, chronic kidney failure, foot ulcers, and damage to the eyes. Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced. There are three main types of diabetes mellitus:

- Type 1 DM results from the pancreas's failure to produce enough insulin. This form is previously referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes". The cause is unknown.
- Type 2 DM begins with insulin resistance, a condition in which cells fail to respond to insulin properly. As the disease progresses a lack of insulin may also develop. This form is previously referred to as "non insulin-dependent diabetes mellitus" (NIDDM) or "adult-onset diabetes". The primary cause is excessive body weight and not enough exercise.
- Gestational diabetes, is the third main form and occurs when pregnant women without a previous history of diabetes develop a high blood-sugar level.

2.5.1 Diabetes mellitus type 1

Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas, leading to insulin deficiency. This type can be further classified as immune-mediated or idiopathic. The majority of type 1 diabetes is of the immune-mediated nature, in which a T-cell-mediated autoimmune attack leads to the loss of beta cells and thus insulin. It causes approximately 10% of diabetes mellitus cases in North America and Europe. Most affected people are otherwise healthy and of a healthy weight when onset occurs. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes can affect children or adults, but was traditionally termed "juvenile diabetes" because a majority of these diabetes cases were in children. "Brittle" diabetes, also known as unstable diabetes or labile diabetes, is a term that was traditionally used to describe the dramatic and recurrent swings in glucose levels, often occurring for no apparent reason in insulin-dependent diabetes. This term, however, has no biologic basis and should not be used. Still, type 1 diabetes can be accompanied by irregular and unpredictable high blood sugar levels, frequently with ketosis, and sometimes with serious low blood sugar levels. Other complications include an impaired counter regulatory response to low blood sugar, infection, gastroparesis (which leads to erratic absorption of dietary carbohydrates), and endocrinopathies (e.g., Addison's disease). These phenomena are believed to occur no more frequently than in 1% to 2% of persons with type 1diabetes. Type 1diabetes is partly inherited, with multiple genes, including certain HLA genotypes, known to influence the risk of diabetes. In genetically susceptible people, the onset of diabetes can be triggered by one or more environmental factors, such as a viral infection or diet. There is some evidence that suggests an association between type 1 DM and Coxsackie B4 virus. Unlike type 2 DM, the onset of type 1 diabetes is unrelated to lifestyle. Type 1 diabetes is an autoimmune disease leading to the destruction of the insulin-producing pancreatic beta cells in the islets of Langerhans. Type 1 diabetes is most commonly diagnosed in children and young adults, and by the time of diagnosis, patients have very little endogenous insulin production. Insulin therefore has to be replaced by regular subcutaneous injections, and blood glucose levels must be frequently monitored to manage the risk of hypoglycaemia. Concordance of the disease in identical twins is around 27% (Hyttinen *et al*., 2003), indicating that although there is a genetic influence, environmental factors play a role in disease development. Indeed, most newly diagnosed

patients have no first-degree relatives with the disease. Incidence of type 1 diabetes ranges up to 100-fold, depending on the country and is estimated to be approximately 15–20 per 100 000 in the United Kingdom (Patterson *et al*., 2009). The incidence in Europe is increasing, with a predicted doubling of children under 5 developing the disease by 2020 (Patterson *et al*., 2009).

2.5.2 Diabetes mellitus type 2

Type 2 DM is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion. The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor. However, the specific defects are not known. Diabetes mellitus cases due to a known defect are classified separately. Type 2 DM is the most common type of diabetes mellitus. In the early stage of type 2, the predominant abnormality is reduced insulin sensitivity. At this stage, high blood sugar can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce the liver's glucose production. Type 2 DM is due primarily to lifestyle factors and genetics. A number of lifestyle factors are known to be important to the development of type 2 DM, including obesity (defined by a body mass index of greater than 30), lack of physical activity, poor diet, stress, and urbanization. Excess body fat is associated with 30% of cases in those of Chinese and Japanese descent, 60–80% of cases in those of European and African descent, and 100% of Pima Indians and Pacific Islanders. Even those who are not obese often have a high waist–hip ratio. Dietary factors also influence the risk of developing type 2 DM. Consumption of sugar-sweetened drinks in excess is associated with an increased risk. The type of fats in the diet is also important, with saturated fats and trans fatty acids increasing the risk and polyunsaturated and monounsaturated fat decreasing the risk. Eating lots of white rice also may increase the risk of diabetes. A lack of exercise is believed to cause 7% of cases. Type 2 diabetes is the most common type of diabetes with prevalence in the United Kingdom of around 4%. It is most commonly diagnosed in middle-aged adults, although more recently the age of onset is decreasing with increasing levels of obesity (Pinhas-Hamiel and Zeitler, 2005). Indeed, although development of the disease shows high hereditability, the risk increases proportionally with body mass index (Lehtovirta *et al*., 2010). Type 2 diabetes is associated with insulin resistance, and a lack of appropriate compensation by the beta cells leads to a relative insulin deficiency. Insulin resistance can be improved by weight reduction and exercise (Solomon *et al*., 2008). If lifestyle intervention fails, there are a

variety of drugs available to treat type 2 diabetes (Krentz *et al*., 2008), which can be divided into five main classes: drugs that stimulate insulin production from the beta cells (e.g. sulphonylureas), drugs that reduce hepatic glucose production (e.g. biguanides), drugs that delay carbohydrate uptake in the gut (e.g. α-glucosidase inhibitors), drugs that improve insulin action (e.g. thiazolidinediones) or drugs targeting the GLP-1 axis (e.g. GLP-1 receptor agonists or DPP-4 inhibitors).

2.5.3 Gestational diabetes

Gestational diabetes mellitus (GDM) resembles type 2 DM in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2–10% of all pregnancies and may improve or disappear after delivery. However, after pregnancy approximately 5–10% of women with gestational diabetes are found to have diabetes mellitus, most commonly type 2. Gestational diabetes is fully treatable, but requires careful medical supervision throughout the pregnancy. Management may include dietary changes, blood glucose monitoring, and in some cases insulin may be required.

Though it may be transient, untreated gestational diabetes can damage the health of the fetus or mother. Risks to the baby include macrosomia (high birth weight), congenital heart and central nervous system abnormalities, and skeletal muscle malformations. Increased levels of insulin in a fetus's blood may inhibit fetal surfactant production and cause respiratory distress syndrome. A high blood bilirubin level may result from red blood cell destruction. In severe cases, perinatal death may occur, most commonly as a result of poor placental perfusion due to vascular impairment. Labor induction may be indicated with decreased placental function. A Caesarean section may be performed if there is marked fetal distress or an increased risk of injury associated with macrosomia, such as shoulder dystocia.

2.5.4 Other types

Prediabetes indicates a condition that occurs when a person's blood glucose levels are higher than normal but not high enough for a diagnosis of type 2 DM. Many people destined to develop type 2 DM spend many years in a state of prediabetes.

Latent autoimmune diabetes of adults (LADA) is a condition in which type 1 DM develops in adults. Adults with LADA are frequently initially misdiagnosed as having type 2 DM, based on age rather than etiology.

Some cases of diabetes are caused by the body's tissue receptors not responding to insulin (even when insulin levels are normal, which is what separates it from type 2 diabetes); this form is very uncommon. Genetic mutations (autosomal or mitochondrial) can lead to defects in beta cell function. Abnormal insulin action may also have been genetically determined in some cases. Any disease that causes extensive damage to the pancreas may lead to diabetes (for example, chronic pancreatitis and cystic fibrosis). Diseases associated with excessive secretion of insulin-antagonistic hormones can cause diabetes (which is typically resolved once the hormone excess is removed). Many drugs impair insulin secretion and some toxins damage pancreatic beta cells. The ICD-10 (1992) diagnostic entity, Malnutrition-Related Diabetes Mellitus (MRDM or MMDM, ICD-10 code E12), was deprecated by the World Health Organization when the current taxonomy was introduced in 1999.

Other forms of diabetes mellitus include congenital diabetes, which is due to genetic defects of insulin secretion, cystic fibrosis-related diabetes, steroid diabetes induced by high doses of glucocorticoids, and several forms of monogenic diabetes.

"Type 3 diabetes" has been suggested as a term for Alzheimer's disease as the underlying processes may involve insulin resistance by the brain.

2.5.5 Pathophysiology

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 highlighted. 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. 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.

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. 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. 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. 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. 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 (glycosuria). 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).

2.5.6 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)

2.5.7 Diagnosis

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

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, *et al.*, 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, *et al.*, 2011). Glycated hemoglobin is better than fasting glucose for determining risks of cardiovascular disease and death from any cause (Selvin E, *et al.*, 2010).

2.6 Different Models Used To Induce Diabetes

For more study about diabetes, rodents such as rat, mouse, hamster, guinea pigs and the rabbits are suitable models. They are used for natural development of study. At present time best and quickest way to induce diabetes is with use of chemicals (Alloxan, streptozotocin, dithizone, monosodium glutamates etc.), viruses and genetically diabetic rabbits. In recent years, scientists and technologists have worked toward refining techniques that have led to the discovery of chemical agents that physiologically alter the function of the pancreas. The main advantage of using such chemicals is that body changes during and after the induction of diabetes can be observed. The five major diabetogenic agents are chemicals, biological agents, peptides, potentiators, and steroids but most commonly used chemicals agents are Alloxan and Streptozotocin**.**

2.7 Diabetes in rabbits

True diabetes is a rare to inexistent condition in rabbits, and is barely described in the literature, with the exception of obese rabbits and experimentally drug-induced diabetes

(e.g., Alloxan or Streptozotin induced diabetes). Both type 1, and type 2 have been observed; the symptoms of the latter being more common in obese rabbits. During the onset phase of the disease, rabbits were able to compensate for the lack of insulin production in the pancreas. This led to conclude that insulin may play a less important role in rabbits and herbivores sugar metabolism, than in carnivores. Many plants have furthermore hypoglycemic properties and, when ingested, may help the rabbit adjust its glucose level. A corrected diet, with a great variety of fresh vegetables and hay would help correct true diabetes or diabetes-like symptoms in a rabbit, without a need to inject insulin on a daily basis. In New-Zealand rabbits suffering from type 1 diabetes, endocrine cells of the Langerhans islets were affected and hyper granulation was observed, unlike healthy non-diabetic rabbits. In other animals, there is usually degranulation. The lack of insulin production was accompanied by glycosylation of the hemoglobin (attachment of glucose molecules to hemoglobin, the protein that is involved in oxygen transport in the red blood cells). When the diabetes was left untreated, diabetic rabbits suffered the same ill effects than humans: mineralization of the kidneys, eye trouble and blood vessel problems, independently from a corrected diet.

2.8 Chemical Causes of Diabetes

2.8.1 Alloxan

Alloxan is most prominent chemical compound used in diabetogenic research. In research it is used for induction of Type 1 diabetes. Alloxan is a urea derivative which causes selective necrosis of the β- cells of pancreatic islets. It has been widely used to induce experimental diabetes in animals such as rabbits, rats, mice and dogs with different grades of disease severity by varying the dose of Alloxan used.

Chemical Properties

- The chemical name of Alloxan is 2, 4, 5, 6 tetraoxypyrimidine; 2, 4, 5, 6-pyrimidinetetrone, which is an oxygenated pyrimidine derivative which is present as Alloxan hydrate in aqueous solution.
- Alloxan is prepared by the oxidation of uric acid by nitric acid and the monohydrate form is simultaneously prepared by oxidation of barbituric acid by chromium trioxide. The drug has been noted to its diabetogenic action when administered parenterally, i.e., intravenously, intraperitoneally or subcutaneously.

The dose of Alloxan required for inducing diabetes depends on the animal species and route of administration. Moreover, Alloxan has been demonstrated to be non-toxic to the human beta-cells, even in very high doses, because humans have different glucose uptake mechanisms as compared to rodents.

Phases of Diabetes Induction

Alloxan induces triphasic blood glucose response when injected into experimental animals. The first phase that comes within the first minutes after Alloxan administration is transient hypoglycemic phase that lasts maximally for 30 minutes. In this little phase hypoglycemic response has been noted to be result of stimulation of insulin secretion that increases the concentration of insulin in plasma. The mechanism behind the first phase of this hyperinsulinemia may be a temporary increase in ATP availability due to inhibition of glucose phosphorylation through glucokinase inhibition.

The second phase appears after 1 hour of administration of Alloxan and leads to rise in blood glucose concentration. Moreover, the plasma insulin concentration decreases at the same time. This is the first hyperglycemic phase for 2-4 hours, after the first contact of the pancreatic beta cells with the toxin. This hyperglycemic phase is result of inhibition of insulin secretion from the pancreatic beta cells, due to their beta cell toxicity.

The third phase is again a hypoglycemic phase i.e. for 4-8 hours after the Alloxan injection, which lasts for several hours. Changes occur during this phase are irreversible.

Mechanism of Action

Alloxan treatment evokes a sudden rise in insulin secretion in the presence or absence of glucose and this insulin release occurs for short duration followed by the complete suppression of the islet response to glucose even when high concentrations of glucose were used**.** Further, important feature of Alloxan action in pancreas is preceded by its rapid uptake by pancreatic beta cells. Moreover, in pancreatic beta cells, the reduction process occurs in the presence of reducing agents like reduced glutathione (GSH), cysteine, ascorbate and protein-bound sulfhydryl (-SH) groups. Alloxan reacts with two -SH groups in the sugar binding site of glucokinase and results in inactivation of the enzyme. As a result dialuric acid is formed which is then re-oxidized back to Alloxan establishing a redox cycle and generates reactive oxygen species (ROS) and superoxide radicals. The superoxide radicals liberate ferric ions from ferritin and reduce them to

ferrous and ferric ions and also undergo dismutation to yield hydrogen peroxide (H_2O_2). As a result, highly reactive hydroxyl radicals are formed in the presence of ferrous and H_2O_2. Another mechanism that has been reported is the effect of ROS on the DNA of pancreatic islets. In the beta cells Alloxan causes DNA fragmentation and damage. Antioxidants like superoxide dismutase, catalase and the non enzymatic scavengers of hydroxyl radicals have been found to protect against Alloxan toxicity. In addition cytosolic free elevated Ca^{2+} has also been reported to constitute an important step in the diabetogenic action of Alloxan. The calcium influx results from the ability of Alloxan to open voltage dependent calcium channels and enhances calcium entry into pancreatic cells. The increased concentration of Ca^{2+} ion further contributes to supraphysiological insulin release that along with ROS eventually causes damage of beta cells of pancreatic islets.

2.8.2 Streptozotocin (STZ)

Streptozotocin is naturally occurring chemical; used to produce Type- 1 diabetes in animal model and Type- 2 diabetes with multiple low doses. It is also used in medicine for treating metastatic cancer of islets of Langerhans.

Chemical Properties

- Streptozotocin is a monofunctional nitrosourea derivative.
- First isolated from *Streptomyces achromogenes***.**
- It has been used alone or in combination with other chemotherapeutic drugs (vincristine, 5-fluorouracil, methyl-CCNU, procarbazine and 6-thioguanine) for the treatment of colorectal carcinomas and other gastrointestinal cancers, but severe toxicity and myelosuppression were observed in most of the patients.
- Streptozotocin has broad spectrum antibiotic activity.

Mechanism of Action

Streptozotocin prevents DNA (Deoxyribonucleic acid) synthesis in mammalian and bacterial cells, in the bacterial cells; it renders special reaction with cytosine groups, resulting in degeneration and destruction of DNA. The streptozotocin enters the pancreatic cell via a glucose transporter-GLUT2 (Glucose transporter 2) and causes alkylation of DNA. Further STZ induces activation of poly adenosine diphoshate

ribosylation and nitric oxide release, as a result of STZ action, pancreatic -cells are destroyed by necrosis and finally induced insulin dependent diabetes.

2.8.3 Dithizone

Dithizone induce the symptoms of diabetes in cats, rabbits, golden hamsters and in mice. In dithizonised diabetic animals, the levels of serum zinc, iron, and potassium were found to be higher than normal but copper and magnesium levels were unchanged. After treatment with insulin, most of these serum levels were normal, except for serum potassium and magnesium.

Chemical Properties

- Chemical name of dithizone is 8-(p- toluene- sulfonylamino)– quinoline (8-TSQ).
- Dithizone is an organosulfur compound that acts as a chelating agent and forms complexes with lead, zinc and mercury.
- It is used to assess the purity of human pancreatic islet preparations used for transplantation into patients with type 1diabetes.

Mechanism of Action

Zinc-chelating agent such as dithizone is causes diabetes in laboratory animals. Dithizone has abilities to permeate membranes and to complex zinc inside liposomes with the release of protons, that can enhance diabetogenicity. When such complexing agents are added to lipid vesicles at pH 6 containing entrapped zinc ions, they acidify the contents of these vesicles. Such proton release occurs within the zinc-containing insulin storage granules of pancreatic beta-cells; solubilisation of insulin would be induced which leads to osmotic stress and eventually the granule rupture and finally diabetes is induced.

2.8.4 Gold Thioglucose

Gold thioglucose is diabetogenic compound, which is induced hyperphagia and severe obesity induced Type -2 diabetes.

Chemical Properties

- It is derivative of sugar glucose.
- Gold thioglucose is precipitated with methanol and recrystallized with water and methanol.

Mechanism of Action

Gold thioglucose developed obesity induces diabetes in genetically normal mouse strains. Gold thioglucose treated DBA/2 (Dilute Brown Non- Agouti), C57BLKs, and BDF1 mice gained weight rapidly and significantly increase non fasting plasma glucose level within 8-12 weeks. These mice showed impaired insulin secretion, mainly in early phase after glucose load and reduced insulin content in pancreatic islets**.**

2.8.5 Monosodium Glutamate

Monosodium glutamate induces Type -2 diabetes without polyphagia.

Chemical Properties

- It is most abundant naturally occurring non- essential amino acid.
- Freely soluble in water.

Mechanism of Action

Monosodium glutamate causes a very large insulin response after ingestion. It is developed glycosuria in both male and female mice but not induced polyphagia. Within 29 weeks level of glucose concentration in blood, total cholesterol and triglyceride were higher.

2.8.6 Virus Induced Diabetes

Juvenile- onset diabetes mellitus may be due to virus infections and beta- cell specific autoimmunity. In 1960s Gamble and co – workers reported newly diagnosed juvenile-onset diabetes (Type- 1) due to viral infections. At present time two viruses are reported first is D- variant of encephalomyocarditis (EMC-D) and another is Coxsackie virus.

D- Variant Encephalomyocarditis

EMC- D virus can infect and destroy pancreatic beta cells in certain inbred strains of mice and produce insulin dependent hyperglycemia. Pre-treatment with a potent immunosuppressive drug, cyclosporine-A increases severity and incidence of diabetes in ICR Swiss mice. In 1992 Utsugi et al demonstrated the clone of EMC-D virus known as NDK25. Intraperitoneal injection of NDK25 develops non- insulin dependent diabetes mellitus.

Coxsackie Viruses

Coxsackie viruses are also a possible cause of diabetes in mice; it can infect and destroy pancreatic acinar cells while leaving the adjacent islets of Langerhans intact. Coxsackie B4 virus is strongly associated with the development of insulin-dependent diabetes mellitus in humans. Diabetes induced by Coxsackie virus infection is a direct result of local infection leading to inflammation, tissue damage, and the release of sequestered islet antigen resulting in the re-stimulation of resting auto reactive T cells, further indicating that the islet antigen sensitization is an indirect consequence of the viral infection.

2.8.7 Hormone Induced Diabetes

Growth hormone induced diabetes

Growth hormone has long distinguished history in diabetes, with possible participation in the development of renal complications. Repeated administration of growth hormone in cats and adult dogs induces diabetes with all symptoms of diabetes including severe ketonuria and ketonemia. More prolonged administration of growth hormone produced permanent diabetes, there was loss of pancreatic islets tissues and of beta cells and only traces of insulin could be extracted from pancreas.

Corticosteroid induced diabetes

Corticosteroid used to reduce inflammation can lead to diabetes, which is called steroid diabetes. The most common glucocorticoids which cause steroid diabetes are prednisolone and dexamathasone. Glucocorticoids oppose insulin action and stimulate gluconeogenesis, especially in the liver, resulting in a net increase in hepatic glucose output and induce insulin resistance, hyperglycemia, and hyperlipidemia.

2.9 Alloxan

Diabetogenic agent Alloxan

Commercial name: Alloxan monohydrate

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

Others name: Mesoxalylurea 5-Oxobarbituric acid

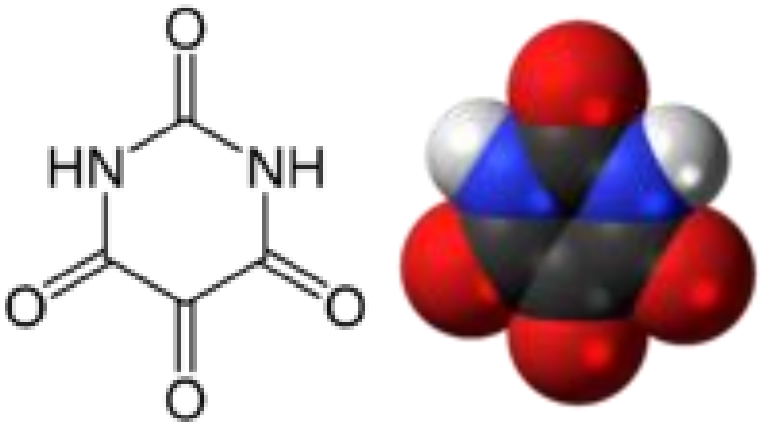


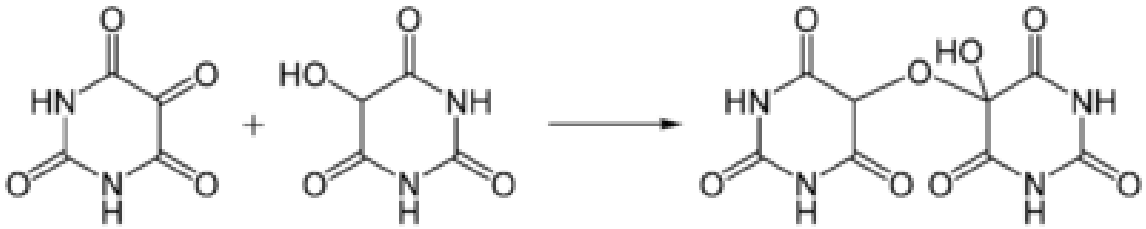
Fig: Structure of Alloxan

2.9.1 History

Alloxan was originally isolated in 1818 by Brugnatelli and was named in 1838 by Wöhler and Liebig. The name "Alloxan" emerged from an amalgamation of the words "allantoin" and "Oxalsäure" (oxalic acid). Alloxan (2, 4, 5, 6-tetraoxypyrimidine; 5, 6-dioxyuracil) is the next most commonly used chemical for induction of diabetes mellitus. It is a well-known diabetogenic agent widely used to induce T2Ds in animals. It used to produce experimental diabetes in animals such as rabbits, rats, mice and dogs. The diabetic effect of ALX is mainly attributed to rapid uptake by the β-cells and formation of free radicals, for which β-cells have poor defense mechanismsand there after highly reactive hydroxyl radicals that cause fragmentation of β-cell DNA. ALX is also taken up by the liver, but it has better protection to reactive oxygen species. Other mechanisms of β-cell damage by ALX include oxidation of essential–SH groups, especially that of glucokinase and disturbances in intracellular calcium homeostasis. A dose of 100 mg/kg has used to create a long-term diabetes models in rabbits. It should be noted that ALX has a narrow diabetogenic dose, and even light overdosing can cause general toxicity, especially to the kidney.

2.9.2 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).*



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

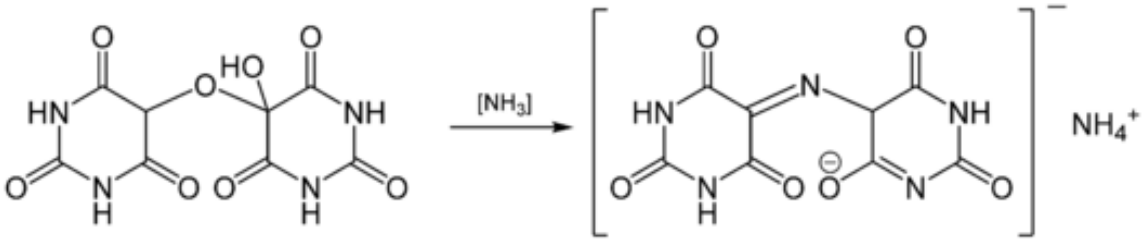
2.9.3 Biological effects

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. Others found a significant difference in Alloxan plasma levels in children with and without diabetes Type 1. The Alloxan model of diabetes was first described in rabbits by Dunn, Sheehan and McLetchie in 1943.

2.9.4 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.

2.9.5 Alloxan Induced Diabetes in Rabbits

Alloxan monohydrate is a common drug used for developing experimental diabetes in Animals. Alloxan (mesoxal urea) was the first chemical used to induce experimental diabetes.It was found by Leibig in mucus excreted during dysentery (Merck Index, 1976). The diabetogenic dose of Alloxan vary considerably amongst species, age and metabolic state of the animal. Nephrotoxicity is also a side effect (Bonar 1980). Alloxan diabetes can be prevented by sulphhydryl containing compounds such as glutathione, cystine and dimercaprol prior to Alloxan administration. It's monohydrate form as Alloxan monohydrate is less toxic than its tetrahydrate form. Therefore Alloxan monohydrate was selected for induction of Alloxan diabetes in rabbits (Saadia Shahzad Alam *et al*, 2005). The diabetic effect of Alloxan (2,4,5,6-tetraoxypyrimidine; 5,6-dioxyuracil) is mainly attributed to rapid uptake by the beta cells and the formation of free radicals, which beta cells have poor defense mechanisms to (Nerup *et al*., 1994). Alloxan is reduced to dialuric acid and then re-oxidized back to alloxan, creating a redox cycle for the generation of superoxide radicals that undergo dismutation to form hydrogen peroxide and thereafter highly reactive hydroxyl radicals that cause

fragmentation of beta cell DNA (Szkudelski, 2001). Alloxan is also taken up by the liver, but it has better protection to reactive oxygen species (Malaisse *et al*., 1982; Mathews and Leiter, 1999) and therefore is not as susceptible to damage. Other mechanisms of beta cell damage by Alloxan include oxidation of essential –SH groups, especially that of glucokinase (im Walde *et al*., 2002) and disturbances in intracellular calcium homeostasis (Kim *et al*., 1994). Doses in mice range from 50 to 200 $mg \cdot kg^{-1}$ and in rats from 40 to 200 $mg \cdot kg^{-1}$, depending on the strain and the route of administration with i.p and s.c. administration requiring up to three times as high a dose as the i.v. route (Szkudelski, 2001). A dose of 100 $mg \cdot kg^{-1}$ has been used to create a long-term diabetes models in rabbits (Wang *et al*., 2010). It should be noted that Alloxan has a narrow diabetogenic dose, and even light overdosing can cause general toxicity, especially to the kidney (Szkudelski, 2001). Alloxan is the next most commonly used chemical for induction of diabetes mellitus. It is a well- known diabetogenic agent widely used to induce Type 1 diabetes in animals (Viana *et al.,* 2004). Alloxan is a urea derivative which causes selective necrosis of the pancreatic islet β-cells. It is used to produce experimental diabetes in animals such as rabbits, rats, mice and dogs. With this agent, it is possible to produce different grades of severity of the disease by varying the dose of Alloxan used: these may be classified by measuring fasting blood sugar (FES) levels: e. g. in rabbits moderate diabetes has been defined as an FBS level of 180 – 250 mg/dl, and severe diabetes as an FBS level of above 250mg/dl (Huralikuppi, 1991). The severe diabetes produced by Alloxan results in blood sugar levels equivalent to a total pancreatectomy, hence sulphonylureas such as tolbutamide, which act mainly by stimulating insulin release from β-cells, show only a small hypoglycaemic effect in this instance. Therefore a test plant extract producing a significant hypoglycaemia (in a severely Alloxan induced diabetic animal) must be operating through a different mechanism. Moderate diabetic animals are recommended for use in testing drugs for use in Non insulin dependent diabetes mellitus (Williamson *et al*., 1996). For all animals a single dose of Alloxan, 140 – 180 mg/kg (usually 150 mg/kg) is administered as a 5% w/v in distilled water injected intravenously into the marginal ear vein of rabbit or intraperitoneally in case of mice and rats. A rest period of seven days for rabbits, 12 days for rats and mice is allowed during which the animals have free access to food and water. Alloxan and its reduction product dialuric acid establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. There after, highly reactive hydroxyl radicals are formed by fenton reaction. The action of

reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of *beta* cells (Szkudelski, 2001). Thus Alloxan induced diabetes mellitus served as a pathological biomodel for testing a substance with supposed antioxidant activities *in vivo* (Bartosikova *et al.,* 2003). The increase in oxygen free radicals in diabetic conditions is mainly because of the effect of the diabetogenic agent Alloxan. With this method (Macedo *et al.,* 2005) induced diabetes mellitus in experimental rats. The animals were deprived of food for 48 hours, and then weighed and anaesthesized with chloroform in a glass dome. A solution of 2% Alloxan (40mg/kg) diluted in 0.9% normal saline was administered to the animals through the iliac vein. The animals were allowed to resume feeding and drinking 30 minutes after the drug administration. In order to asses the effect of Alloxan and to chemically establish the diabetic condition, an incision was done in any of the four veins in the tail of the rat with a 15 scapel blade 10 days after induction a blood glucose level determination using a portable glucose analyszer was determined, a serum glucose level of 200 mg/dl was considered hyperglycemic. The most frequently used intravenous dose of Alloxan in rats is 65mg/kg, but when it is administered intraperitoneally or subcutaneously its effective dose must be higher (Federiuk *et al.,* 2004). Alloxan monohydrate 150mg/kg body weight was dissolved in normal saline and injected intraperitoneally after 18 hours fasting to induce hyperglycemia in experimental rats (Yanarday and Colak, 1998). In a separate study, the experimental animals were fasted for 18 hours before Alloxan injection and the blood glucose level (BGL) was monitored after alloxanization in blood samples collected by tail tipping method using a Glucometer. Rats with blood glucose level of greater than 150mg/dl were considered diabetic and were selected for study (WHO, 1985). The simplistic argument often made against the use of Alloxan to induce type II diabetes mellitus is that, Alloxan administration produces *beta* cells damage and thus leading to type I rather than type II diabetes mellitus. Studies conducted by Etuk and Mohammed (Unpublished) in 2009 showed that, there was no differential response to hypoglycaemic agents by Alloxan and glucose loading hyperglycaemic rats. Alloxan administration in experimental animals has been reported to produce pancreatic lesion which is proportional to the dose of the drug administered. And the size of the lesion also correlates with the pancreatic insulin content (McNeill, 1990). This perhaps explains why the drug at a low or medium dose does not produce absolute but insufficient insulin deficiency in experimental animals. Therefore the experimental dose of the drug must be carefully selected in order to avoid excessive pancreatic tissue damage. The most

frequently used intravenous dose of Alloxan in rats is 65mg/kg, but when it is administered intraperitoneally or subcutaneously its effective dose must be higher (Antia et al., 2005).

2.10 Antidiabetogenic agent Bitter melon (*Momordica charantia*)

M. Charantia (bitter melon or bitter gourd) is a flowering vine in the family Cucurbitaceae. It is a tropical plant that is widely cultivated in Asia, India, East Africa, and South America for its intensely bitter fruits that are commonly used in cooking and as a natural remedy for treating diabetes. It is a climbing perennial that usually grows up to 5 m, and bears elongated fruits with a knobbly surface. It is a useful medicinal and vegetable plant for human health and one of the most promising plants for diabetes.

2.10.1 Plant description

***Momordica. charantia* plant**

2.10.2 Nutrient profile

Bitter melon is a powerful nutrient-dense plant composed of a complex array of beneficial compounds. These include bioactive chemicals, vitamins, minerals and antioxidants which all contribute to its remarkable versatility in treating a wide range of illnesses. The fruits contain high amounts of vitamin C, vitamin A, vitamin E, vitamins B1, B2 and B3, as well as vitamin B9 (folate). The caloric values for leaf, fruit and seed were 213.26, 241.66 and 176.61 Kcal/100 g respectively. The fruit is also rich in minerals including potassium, calcium, zinc, magnesium, phosphorus and iron, and is a good source of dietary fiber (bitter melon "monograph", 2008). Medicinal value of bitter melon has been attributed to its high antioxidant properties due in part to phenols, flavonoids, isoflavones, terpenes, anthroquinones, and glucosinolates, all of which confer a bitter taste.

2.10.3 Phytochemistry

The main constituents of bitter melon which are responsible for the antidiabetic effects are triterpene, proteid, steroid, alkaloid, inorganic, lipid, and phenolic compounds. Several glycosides have been isolated from the *M. charantia* stem and fruit and are grouped under the genera of cucurbitane-type triterpenoids. In particular, four triterpenoids have AMP-activated protein kinase activity which is a plausible

hypoglycaemic mechanism of *M. charantia*. *M. charantia* fruits consist of glycosides, saponins, alkaloids, reducing sugars, resins, phenolic constituents, fixed oil and free acids. *M. charantia* consists of the following chemical constituents including alkaloids, charantin, charine, cryptoxanthin, cucurbitins, cucurbitacins, cucurbitanes, cycloartenols, diosgenin, elaeostearic acids, erythrodiol, galacturonic acids, gentisic acid, goyaglycosides, goyasaponins, guanylate cyclase inhibitors, gypsogenin, hydroxytryptamines, karounidiols, lanosterol, lauric acid, linoleic acid, linolenic acid, momorcharasides, momorcharins, momordenol, momordicilin, momordicin, momordicinin, momordicosides, momordin, momordolo, multiflorenol, myristic acid, nerolidol, oleanolic acid, oleic acid, oxalic acid, pentadecans, peptides, petroselinic acid, polypeptides, proteins, ribosome-inactivating proteins, rosmarinic acid, rubixanthin, spinasterol, steroidal glycosides, stigmasta-diols, stigmasterol, taraxerol, trehalose, trypsin inhibitors, uracil, vacine, v-insulin, verbascoside, vicine, zeatin, zeatin riboside, zeaxanthin, zeinoxanthin amino acids-aspartic acid, serine, glutamic acid, thscinne, alanine, g-amino butyric acid and pipecolic acid, ascorbigen, b-sitosterol-d-glucoside, citrulline, elasterol, flavochrome, lutein, lycopene, pipecolic acid. The fruit pulp has soluble pectin but no free pectic acid. Research has found that the leaves are nutritious sources of calcium, magnesium, potassium, phosphorus and iron; both the edible fruit and the leaves are great sources of the B vitamins.

2.10.4 Bioactive compounds

Based on the multitude of medical conditions that bitter melon can treat, scientists are more and more interested in studying its bioactive compounds and their actions on the body. However, as many studies report, there has been substantial emphasis on the anti-diabetic compounds and their hypoglycemic properties. A number of reported clinical studies have shown that bitter melon extract from the fruit, seeds, and leaves contain several bioactive compounds that have hypoglycemic activity in both diabetic animals and humans. Momordicine II and 3-hydroxycucurbita-5, 24-dien-19-al-7, 23- di-O-β-glucopyranoside, were isolated as saponins from *M. charantia*. Both compounds showed significant insulin releasing activity in MIN6 β-cells at concentration of 10 and 25 µg/mL. The major compounds that have been isolated from bitter melon and identified as hypoglycemic agents include charantin, polypeptide-p and vicine.

Charantin

Charantin is a typical cucurbitane-type triterpenoid in *M. charantia* and is a potential substance with antidiabetic properties. Pitiphanpong *et al.* demonstrated that charantin could be used to treat diabetes and can potentially replace treatment. It is a mixture of two compounds, namely, sitosteryl glucoside and stigmasteryl glucoside. Chen *et al.* isolated 14 cucurbitane triterpenoids, kuguacins, including two pentanorcucurbitacins, one octanorcucurbitacin, and two trinorcucurbitacins, along with six known analogues from the vines and leaves of *M. charantia*. The charantin from bitter melon fruit was extracted and estimated by high performance thin layer chromatographic method. Studies have reported that the compound is more effective than the oral hypoglycemic agent tolbutamide. In a study, two aglycones of charantin were isolated and identified as sitosterol and stigmastadienol glycosides, however, when tested separately for their hypoglycemic effects *in vivo*, these two constituents did not produce any notable changes in blood glucose levels. This is an indication that charantin may contain other specific components, yet to be identified, that are responsible for the hypoglycemic activity observed in diabetics.

Polypeptide-p

Bitter melon is one of the most commonly used vegetable that contains polypeptide-p and is used to control diabetes naturally. Polypeptide-p or p-insulin is an insulin-like hypoglycemic protein, shown to lower blood glucose levels in gerbils, langurs and humans when injected subcutaneously. The p-insulin works by mimicking the action of human insulin in the body and thus may be used as plant-based insulin replacement in patients with type-1 diabetes. Recently, Wang *et al.* have cloned and expressed the 498 bp gene sequence coding for the *M. charantia* polypeptide p gene and have also proved the hypoglycemic effect of the recombinant polypeptide in Alloxan induced diabetic rabbits. The oral intake of the extract from bitter melon seeds does produce hypoglycemic effects in streptozotocin (STZ) induced type-1 diabetic rabbits. This indicates that compounds in bitter melon seeds other than p-insulin may also be effective in the treatment of type-1 diabetes.

Vicine

The other major compound that has been isolated from the seeds of bitter melon is a glycol alkaloid known as vicine. This pyrimidine nucleoside has been shown to induce hypoglycemia in non-diabetic fasting rabbits by intraperitoneal or intravenous administration. However, vicine found in fava bean has been shown to induce favism, an acute disease characterized by hemolytic anemia, in individuals with a hereditary loss of the enzyme glucose-6-phosphatedehydrogenase. Although there have been no reports on favism induced by bitter melon, individuals susceptible to the disease should avoid eating the fruit. Further studies are required to ensure the safety and efficacy of using vicine to treat hyperglycemia.

2.10.5 Other components

Many other bitter melon constituents have been identified and isolated by various extraction techniques. The first study to show the *in vivo* hypoglycemic activity of the major compounds of bitter melon was done by a group of Japanese scientists. They isolated 11 compounds by fractionation of a methanol extract from dried bitter melon fruits. The structure of three cucurbitane triterpenoids were determined, as well as two other major compounds that were tested and shown to significantly lower blood glucose levels in diabetic rabbits. Four compounds that may be responsible for the bitter taste of the plant were isolated and identified as momordicosides K and L, and momordicines I and II. The last two compounds isolated were identified as sitosterol and stigmastadienol, the aglycones of charantin.

2.10.6 Medicinal properties of *M. charantia*

Bitter melon is traditionally known for its medicinal properties such as antidiabetic, anticancer, anti-inflammation, antivirus, and cholesterol lowering effects. It contains many phenolic compounds that may have the potential as antioxidant and antimutagen. The fruit, stems, leaves and roots of bitter melon have all been used in traditional medicine to help treat ailments such as hyperlipidemia, digestive disorders, microbial infections and menstrual problems. Bitter melon has been shown to possess powerful antiviral properties that can stimulate the immune system and activate the body's natural killer cells to help fight off viruses such as white spot syndrome virus and human immunodeficiency virus. Studies have also shown that bitter melon has anti-carcinogenic

properties and can be used as a cytotoxic agent against many types of cancer. Ray *et al.* showed that the extract of bitter melon modulates signal transduction pathways for inhibition of breast cancer cell growth and can be used as a dietary supplement for prevention of breast cancer. Bitter melon extract can also be used as a broad-spectrum antibacterial agent to fight off infections caused by *Escherichia coli*, *Salmonella, Staphylococcus aureus, Staphylococcus, Pseudomonas*, and *Streptobaccilus*. In addition, the plant possesses anti-helmintic properties, which are effective in the treatment of malaria. Traditionally, bitter melon has also been used as an abortifacient agent used to induce abortions. Therefore, pregnant women are advised to avoid consumption of the plant. The extract of the seed also have antispermatogenic effect.

2.10.7 Anti-diabetic effect of *M. charantia*

There are many traditional herbal remedies that have been used to treat diabetes in Asia and other developing countries. *M. charantia* is one of the plants that has been investigated thoroughly for the treatment of diabetes. With the traditional use supported by modern scientific evidence of the beneficial function of *M. charantia*, it is one of the most promising plants for diabetes today. Investigation of the traditional uses of *M. charantia* in India revealed that it is one of the most important plant for lowering blood glucose levels in patients with diabetes.

2.10.8 Possible modes of action of M. charantia and its extract

M. charantia and its various extracts and components are believed to exert their hypoglycemic effects via different physiological, pharmacological and biochemical modes. The possible modes of the hypoglycemic actions of *M. charantia* and its various extracts and compounds are its hypoglycemic effect, stimulation of peripheral and skeletal muscle glucose utilisation, inhibition of intestinal glucose uptake, inhibition of adipocyte differentiation, suppression of key gluconeogenic enzymes, stimulation of key enzyme of HMP pathway, and preservation of islet β cells and their functions. Today, over 140 different studies worldwide have investigated anti-hyperglycemic and hypoglycemic effects of the different extracts and ingredients of *M. charantia* in both human and animal models. According to Kim and Kim, *M. charantia* extract suppressed the activation of mitogen-activated protein kinases (MAPKs) including stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK), p38, and p44/42, and the activity of NF-κB. The findings suggest that *M. charantia* protects pancreatic β-cells through

down-regulation of MAPKs and NF-κB in MIN6N8 cells. A similar study suggest that *M. charantia* improves the serum and liver lipid profiles and serum glucose levels by modulating PPAR-γ gene expression. According to Ragasa *et al.*, clerosterol and 5α-stigmasta-7-en-3β-ol were isolated as sterols from *M. charantia* having significant hypoglycemic effects. *M. charantia* was identified to possess a potent neuroprotective activity against global cerebral ischemia-reperfusion induced neuronal injury and consequent neurological deficits in diabetic mice. Protein tyrosine phosphatase 1B (PTP1B), a negative regulator of insulin signaling, has served as a potential drug target for the treatment of type 2 diabetes. *M. charantia*, its extracts and isolated components are believed to exert their hypoglycaemic effects via different physiological and biochemical processes. These include insulin secretagogue like effect, stimulation of skeletal muscle and peripheral cell glucose utilization, inhibition of intestinal glucose uptake, inhibition of adipocyte differentiation, suppression of key gluconeogenic enzymes, stimulation of key enzymes, HMP pathway and preservation of pancreatic islet cells and their functions.

2.10.9 Preservation of pancreatic *β* cells and insulin secretion

It was previously demonstrated by Jeewathayaparan *et al.* that oral administration of *M. charantia* could lead to the secretion of insulin from endocrine pancreatic β cells. This observation was further confirmed by Ahmed *et al.* who investigated the effect of daily oral administration of *M. charantia* fruit juice and the distribution of α, β and δ cells in the pancreas of STZ-induced diabetic rabbits using immunohistochemical methods. The feeding of alcoholic extract from *M. charantia* showed definite improvement in the islets of Langerhans. Physiological experiments have also shown that *M. charantia* can stimulate insulin secretion from the endocrine pancreas and elicit glucose uptake in the liver. Current evidence therefore indicates that the recovery and subsequent increase in the number of insulin producing cells followed by the release of insulin may be part of the several pathways by which *M. charantia* exerts its hypoglycemic effects. In addition to the properties mentioned above, *M. charantia* and its extracts may possess cell-like proliferation and growth-like properties similar to that of insulin. Nevertheless, further experiment are required, at least at the molecular level, to determine the precise mechanisms whereby *M. charantia* can either repair damaged β cells or prevent their death.

2.10.10 *M. charantia* and glucose metabolism

Insulin plays a major biochemical role in stimulating the uptake of glucose by different cells of the body for the production of energy. Since *M. charantia* and its various extracts and components have been reported to exert hypoglycemic effects, and then it is important to understand whether *M. charantia* may have a direct effect in inducing a reduction in blood glucose level. Previous studies have shown that both the aqueous and alcoholic extracts of the fruit of *M. charantia* can inhibit the activities of fructose 1, 6-diphosphatase and glucose-6-phosphatase and at the same time stimulating the action of glucose-6-phosphatase dehydrogenase. It is previously reported that *M. charantia* and its various extracts can stimulate peripheral cell glucose uptake. A number of studies have investigated the effect of the powder and chloroform extract of *M. charantia* in comparison with insulin on glucose and amino acid uptakes by skeletal L6 myotubes and Na^+ and K^+ glucose uptakes by jejunum brush border membrane vesicles in both age-matched control and STZ-induced diabetic rabbits. The results show that either the lyophilized fruit juice or chloroform extract at 5-10 µg/mL can stimulate ^{3}H-deoxyglucose and ^{14}C-Me AIB (N-methyl-amino-α-isobutyric acid) uptakes by L6 myotubes. These effects were similar in magnitude to the effects obtained with 100 nmol/L insulin. Incubation of either insulin or *M. charantia* juice in the presence of wortmannin (a phosphatidylinositol 3-kinase inhibitor) resulted in a marked inhibition of ^{3}H-deoxyglucose uptake by L-6 myotubes. Together, the results have clearly demonstrated that *M. charantia* contains insulin like properties, similar to one phytochemical component of *M. charantia* called V-insulin. In addition to its insulin-like effects on skeletal muscle cells, daily oral intake of *M. charantia* fruit juice over a period of 10 weeks significantly reduced the amount of Na^+ and K^+-dependent ^{14}C-D-glucose absorbed by rabbit jejunum brush border membrane vesicle compared to vesicles obtained from STZ-induced diabetic rabbits. Taken together, these results clearly demonstrated that *M. charantia* and its extracts can directly regulate blood glucose via two mechanisms. Firstly, it can regulate how much glucose is absorbed by the gut into the blood following a meal and secondly, it can stimulate glucose uptake into skeletal muscle cells just like insulin. Moreover, it seems to exert its effect via the same intracellular signaling pathways as insulin in regulating glucose metabolism in the body.

2.10.11 Animal studies of *M. charantia*

Various animal studies have repeatedly shown hypoglycaemic effects of the seeds, fruit pulp, leaves and whole plant of *M. charantia* in normal animals. In particular, *M. charantia* improves glucose tolerance and suppresses postprandial hyperglycaemia in rabbits, and *M. charantia* extract can enhance insulin sensitivity and lipolysis. Some studies also claimed that the hypoglycaemic effect of *M. charantia* was comparable with oral medications such as tolbutamide, chlorpropamide and glibenclamide. Abundant biochemical data have shed light upon possible mechanisms of the anti-diabetic actions of *M. charantia* with the recurring theme being activation of the AMP-activated protein kinase system. Other studies suggested a role of the a- and g-peroxisome proliferator-activated receptors (PPARa and PPARg) which are pivotal in lipid and glucose haemostasis and may mitigate insulin resistance. The alcoholic extract of *M. charantia* was quite effective in lowering blood sugar levels and islet histopathology also showed improvement. The lowered blood sugar and improvement in islet histology remained as such even after discontinuation of extract feeding for 15 days. The acetone extract of whole fruit powder of *M. charantia* in doses 0.25, 0.50 and 0.75 mg/kg body weight lowered the blood glucose from 13.3% to 50.0% after 8 to 30-day treatment in Alloxan diabetic albino rats, confirming anti hyperglycemic effect of this plant in diabetic animals and humans. Bitter gourd (*Momordica charantia*) is a vegetable with antidiabetic properties such as charantin, vicine, and polypeptide-p, as well as other unspecific bioactive components such as antioxidants (Krawinkel MB, Keding GB). These results clearly provided experimental evidence that dried bitter gourd powder in the diet at 10% level improved diabetic status signifying its beneficial effect during diabetes (Shetty AK, 2005). Bitter melon is an alternative therapy that has primarily been used for lowering blood glucose levels in patients with diabetes mellitus (Basch E, 2003). *Momordica charantia* (bitter melon) is a popular fruit used for the treatment of diabetes and related conditions amongst the indigenous populations of Asia, South America, India and East Africa (Leung L, *et al*., 2009). Although they have not been definitively determined, research indicates the primary constituents responsible for the hypoglycemic properties of Momordica are charantin, insulin-like peptide (plant (p)-insulin), cucurbutanoids, momordicin, and oleanolic acids. P-insulin is structurally and pharmacologically similar to bovine insulin and is composed of two polypeptide chains held together by disulfide bonds (Nagy M.A., *et al.*, 2012). This study reported that bitter

melon extract was homologous to insulin taken from animal pancreas, and it showed that the average reduction in blood glucose level was statistically significant. The extract was considered safe to use as no hypersensitivity reaction was reported. Another study evaluated the effect of bitter melon juice on glucose tolerance in patients with type 2 diabetes, and it has been shown that glucose tolerance was improved in 73% of the participants (Heba Abduo, *et al.,* 2006). The following table summarizes the important aspects that the healthcare professional should know about bitter melon and its use for diabetes and related conditions.

Table 1: Summary of the important aspects that the healthcare professional should know about bitter melon and its use for diabetes and related conditions:

Scientific Name	Momordica charantia, Momordica muricata
Family	Cucurbitaceae
Active Constituents	• Insulin-like polypeptide called polypeptide-P. • Plant insulin or p-insulin. • Charantin: mixture of two steroid glycosides and vicine. • Flavonoids. • Alpha- and beta-momocharin. • Proteins: MAP30 and MRK29. • Others (e.g. Vitamins, elemental compounds, and fatty acids).
Mechanism of Action	Preliminary evidence suggests that bitter melon : • Increases pancreatic insulin secretion • Increases insulin sensitivity • Preserves β cells in the pancreatic islets • Depresses hepatic gluconeogenesis • Increases hepatic glycogen synthesis • Increases peripheral glucose utilization • Increases liver and muscle glycogen storage • Decreases glucose absorption • Polypeptide-P has insulin-like properties and similar pharmacokinetics of bovine insulin. Its onset is 30-60 minutes and peek effect is 4 hours. • Charantin has a hypoglycemic effect. • Flavonoids have a cholesterol lowering effect.

Adverse Reactions	An oral dose of 1 g of bitter melon three times a day is considered well-tolerated. However, some patients may experience diarrhea, abdominal pain, and epigastric pain. Seeds consumption was associated with headache. Also, hypoglycemic coma and seizures were reported in two children after consuming bitter melon tea. Another case report showed that intake of the crushed bitter melon fruit resulted in atrial fibrillation. The effect of bitter melon on liver function is not known in the clinical setting, despite the fact that an increase in liver function tests was reported experi-mentally in animal studies.
Toxicity	The red arils around the seeds of bitter melon are reported to be toxic to chil-dren. One case resulted in vomiting, diarrhea, and eventually death.
Interactions with Drugs	*Hypoglycemic drugs:* Risk of hypoglycemia might increase by concomitant use of hypoglycemic drugs such as insulin, glimepiride, metformin, and rosiglitazone. Therefore, patients with diabetes who take hypoglycemic drugs should be counseled about the increased risk of hypoglycemia when eating foods that contain bitter melon concomitantly. It is worthy to men-tion that bitter melon is included widely in traditional Asian and Indian cui-sine. Furthermore, a decrease in blood glucose level after eating food containing bitter melon by a patient taking chlorpropamide was reported. One experiment showed that bitter melon had minor effects on cytochrome P450 enzymes and glutathione S-transferase.
Interactions with Herbs/Supplements	*Hypoglycemic herbs and supplements:* Risk of hypoglycemia might be in-creased by concomitant use of hypoglycemic herbs and supplements such as fenugreek, alpha-lipoic acid, and chromium.

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. Two placebo-controlled, short-term metabolic studies reported bitter melon fruit juice's and extract's acute effects on lowering blood glucose.3 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.3 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 a level 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. The mean difference in the placebo group vs. the experimental group was not statistically significant. Also, the authors relied on self-reported intake of the supplements and did not disclose the actual amount of bitter melon the subjects took. The authors concluded that bitter melon's effectiveness is uncertain; however, the results could be used to estimate the sample size for larger trials. The pharmacology, clinical efficacy, adverse effects, drug interactions, and place in therapy of bitter melon are described. Bitter melon (Momordica charantia) is an alternative therapy that has primarily been used for lowering blood glucose levels in patients with diabetes mellitus. Components of bitter melon extract appear to have structural similarities to animal insulin Basch E *et al.,* 2003. Dried bitter gourd powder in the diet at 10% level improved diabetic status signifying its beneficial effect during diabetes Shetty AK, *et al.,* 2005. The hypoglycemic potential components in bitter gourd have been identified as glycosides, saponins, alkaloids, triterpenes, polysaccharides, proteins, and steroids (Zhu Y, *et al.,* 2012). 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, *et al.,* 2012). The main active component related to the anti-diabetic effect of Momordica charantia is present in the butanol fraction, and it may be saponin (Oishi Y, *et al.,* 2007). Bitter gourd also contains a high dosage of 'plant insulin' and lowers the blood-sugar levels effectively (Qixuan C, *et al.,* 2003). 'Plant insulin' refers to the chemical substances similar to animal insulin existing in plants (Koona SJ, *et al.,* 2010). Polypeptide-p is an unidentified insulin-like protein similar to bovine insulin found in *M. charantia* fruit, seed, and tissue culture (Krawinkel MB and Keding GB. 2006, 2014). The antidiabetic mechanisms of bitter melon also have been proposed. Bitter gourd reduces the amount of glucose that is released into the blood by inhibiting the enzymes that break down disaccharides into two monosaccharides (Oishi Y, *et al.,* 2007). The blood glucose lowering effects of *Momordica charantia* were closely associated with its inhibitory activity against disaccharidase (Oishi Y, *et al.,* 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, *et al.,* 2012). Evidence indicates that bitter melon may decrease hepatic gluconeogenesis, increase hepatic glycogen synthesis, and increase peripheral glucose oxidation in erythrocytes and adipocytes (Hamissou M, Smith AC, Carter RE, Triplett JK. 2013).

2.11 Antidiabetogenic Agent *Nigella sativa or* Black cumin

2.11.1 Etymology

Black cumin can refer to the seeds of either of two quite different plants, both of which are used as spices:

- *Bunium bulbocastanum*, black cumin is considered similar to caraway, but they are two distinctly different plants. The seeds differ dramatically in shape, color and size.
- *Nigella sativa*, black caraway is also called kalonji or nigella, and more common in the Far East, Mideast, India and Africa.

Scientific classification

Kingdom: Plantae

Order: Ranunculales

Family: Ranunculaceae

Genus: *Nigella*

Species: *N. sativa*

Nigella sativa (black-caraway, also known as nigella or *kalonji*), often called black cumin, is an annual flowering plant in the family Ranunculaceae, native to south and southwest Asia. *Nigella sativa* grows to 20–30 cm (7.9–11.8 in) tall, with finely divided, linear (but not thread-like) leaves. The flowers are delicate, and usually colored pale blue and white, with five to ten petals. The black caraway fruit is a large and inflated capsule composed of three to seven united follicles, each containing numerous seeds which are used as spice, sometimes as a replacement for black cumin (*Bunium bulbocastanum*).

Nigella sativa and its seed are variously called black-caraway, black-cumin, fennel-flower, nigella, nutmeg-flower, Roman-coriander, and ***kalonji*** (from Hindi). Synonymously, it may be referred to as thymoquinone after its principal extract under preliminary research for several possible effects in humans. Blackseed and black caraway may also refer to *Bunium persicum*.

2.11.2 Culinary uses

Nigella sativa seeds

The seeds of *Nigella sativa* are used as a spice in Indian and Middle Eastern cuisines. The black seeds taste like a combination of onions, black pepper and oregano. They have

a pungent bitter taste and smell. The dry-roasted black cumin seeds flavor curries, vegetables and pulses. It can be used as a "pepper" in recipes with pod fruit, vegetables, salads and poultry. In some cultures, the black seeds are used to flavor bread products. It is also used as part of the spice mixture panch phoron (meaning a mixture of five spices) and by itself in many recipes in Bengali cuisine and most recognizably in naan bread. *Nigella* is also used in Armenian string cheese, a braided string cheese called majdouleh or majdouli in the Middle East.

2.11.3 History

According to Zohary and Hopf, archaeological evidence about the earliest cultivation of *N. sativa* "is still scanty", but they report supposed *N. sativa* seeds have been found in several sites from ancient Egypt, including Tutankhamun's tomb. Although its exact role in Egyptian culture is unknown, it is known that items entombed with a pharaoh were carefully selected to assist him in the afterlife. The earliest written reference to *N. sativa* is thought to be in the book of Isaiah in the Old Testament, where the reaping of nigella and wheat is contrasted (Isaiah 28: 25, 27). Easton's Bible dictionary states the Hebrew word ketsah refers to *N. sativa* without doubt (although not all translations are in agreement). According to Zohary and Hopf, *N. sativa* was another traditional condiment of the Old World during classical times, and its black seeds were extensively used to flavor food. Seeds were found in a Hittite flask in Turkey from the second millennium BCE.

2.11.4 Chemistry

Nigella sativa oil contains conjugated linoleic (18:2) acid, thymoquinone, nigellone (dithymoquinone), melanthin, nigilline, and trans-anethole. In 2010, Nestlé filed a patent application for use of extracted thymoquinone from *N. sativa* as a food allergy treatment. Nestlé states that the patent would cover "the specific way that thymoquinone - a compound that can be extracted from the seed of the fennel flower - interacts with opioid receptors in the body and helps to reduce allergic reactions to food". More than 1000 different plants have been described for traditional treatment of diabetes (Marles and Fransworth, 1994). *Nigella sativa* belongs to the *Ranunculaceae* family (Goreja, 2003). The seeds of *N. sativa* (black seed), have long been used in folk medicine for a wide range of illnesses (Schleicher and Saleh, 1998). Black seeds contain over 100 valuable components and about 30% w/w of a fixed oil and 0.4-0.45% w/w of a volatile oil (El-

Tahir *et al*., 1993a). Analysis of its volatile oil revealed that the main active ingredients are thymoquinone, dithymquinone. The volatile oil has been shown to contain 18.4-24% thymoquinone and 46% monotrepenes (El-Tahir *et al*., 1993a). The fixed oil is composed mainly of unsaturated fatty acid**s** (Houghton *et al*., 1995). *Nigella sativa* seeds have been reported to possess antimicrobial (El-Alfy *et al*., 1975), antibacterial (El-Kamali *et al*., 1998), antioxidant (Burits and Bucar, 2000; Al-Enazi, 2007), anti-inflammatory (Houghton *et al*., 1995), antitumoral (Worthen *et al*., 1998), antihypertensive (El-Tahir *et al*., 1993b) and hypoglycemic properties (Al-Hader *et al*., 1993; El-Dakhakhny *et al*., 2002; Houcher *et al*., 2007). Chronic supplementation of the nigella seed oil decrease the progression of hyperglycemia and decrese the glycemic enzyme activity (Shafiee-Nick R *et al*., 2012). Similar findings was found in Salama RH. 2011 studies this study suggest that Nigella significantly decrease the elevated blood glucose level in diabetic condition and at the same time also increase the insulin level from Pancreatic cell. *Nigella sativa* have recently demonstrated an improvement in glycemic control and insulin resistance in type 2 diabetic patients induced by NS treatment (Bamosa AO *et al.*, 2010). Evidence also suggests that NS treatment exerts a therapeutic protective effect in diabetes by decreasing oxidative stress and preserving pancreatic beta-cell integrity (Kanter M *et al*., 2004). It also protect pancreas by significantly decreased the diabetes-induced increases in tissue melondialdehyde and serum glucose and significantly increased serum insulin and tissue superoxide dismutase (N.E. Abdelmeguid *et al.*, 2011, Al Wafai RJ. 2013). The hypolipidemic effect of NS has been demonstrated, earlier, in experimental animals. These studies reported that NS has a favorable effect on TG and lipoprotein pattern in normal rats (Dahri AH *et al*., 2005, Ahmad Najmi *et al*., 2008). Similar findings were encountered by the administration of thymoquinone, the active ingredient of *Nigella sativa*, to rabbits fed on cholesterol-enriched diet (Nader MA *et al.*, 2010) and to hypercholesterolemic rats (Ismail *et al.*, 2010). *Nigella sativa* and TQ treatment also suppressed pancreatic tissue lipid peroxidation malondialdehyde levels and increased the level of superoxide dismutase antioxidant enzyme correlated with the decrease in COX-2 mRNA expression. Results obtained in this study supported a potential role for *N. sativa* and TQ in ameliorating inflammation during diabetes and preserving β cells (Al Wafai RJ., 2013). Streptozotocin caused an approximately 3-fold increase in fasting blood sugar level after 2 days. *Nigella sativa* the inhibited the progression of hyperglycemia and decreased serum lipids and hepatic enzyme activity in diabetic rats. Therefore, it has the potential to be used as a natural product for the management of diabetes (Shafiee-

Nick R, *et al.,* 2012). *Nigella sativa* seeds were used as an adjuvant therapy in patients with diabetes mellitus type 2 added to their anti-diabetic medications. Nigella sativa at a dose of 2 gm/day caused significant reductions in FBG, 2hPG, and HbA1 without significant change in body weight. In Conclusion: the results of this study indicate that a dose of 2 gm/ day of *Nigella sativa* might be a beneficial adjuvant to oral hypoglycemic agents in type 2 diabetic patients (Bamosa AO, *et al.,* 2010). Combination of α-LA, L-carnitine and *N. sativa* will contribute significantly in improvement of the carbohydrate metabolism and to less extent lipid metabolism in diabetic rats, thus increasing the rate of success in management of DM (Ragaa Hamdy Mohamed Salama, 2011).

Al-Awadi and Gumma (1987) have reported the use of a plant mixture containing N. sativa, myrrh, gum, asafoetida and aloe by diabetics in Kuwait. They studied the effect of these drugs for their glucose lowering effect in rats and found it to be effective. Further studies on the plant mixture containing *N. sativ*a revealed that the blood glucose lowering effect was due to the inhibition of hepatic gluconeogenesis and the plant extract mixture may prove to be useful therapeutic agent in the treatment of non-insulin dependent diabetes mellitus (Al-Awadi *et al*., 1991; Mohamed *et al.,* 2009). The volatile oil of *N. sativa* alone also produced a significant hypoglycemic effect on normal and Alloxan induced diabetic rabbits without changes in insulin levels (Al-Hader *et al.,* 1993).

In a more recent study, the seed extract when given orally decreased the elevated glucose levels in Alloxan induced diabetic rabbits after 2 months of treatment. Another study was designed to investigate the possible insulinotropic properties of *Nigella sativa* oil in streptozotocin plus nicotinamide induced diabetes mellitus in hamsters. After four weeks of treatment with *N. sativa* oil significant decrease in blood glucose level together with significant increase in serum albumin level were observed (Farah *et al.,* 2002). The study was also confirmed for it protective effects in diabetes for crude extract and n-Hexane extract of *N. sativa* seed (Matira *et al.,* 2008). The clinical study of N. sativa on 60 diabetic patients demonstrates significant improvement with reference to total cholesterol, low density lipoprotein cholesterol (LDL- C), and fasting blood glucose indicating effective as an add-on therapy in patients of insulin resistance syndrome (Najmi *et al*., 2008).

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 black cumin on Alloxan induced diabetic rabbit.

3.1 Experimental Site

Experimental site was at the Department of Physiology and Pharmacology ,HSTU, Dinajpur .

3.2 Experimental Layout

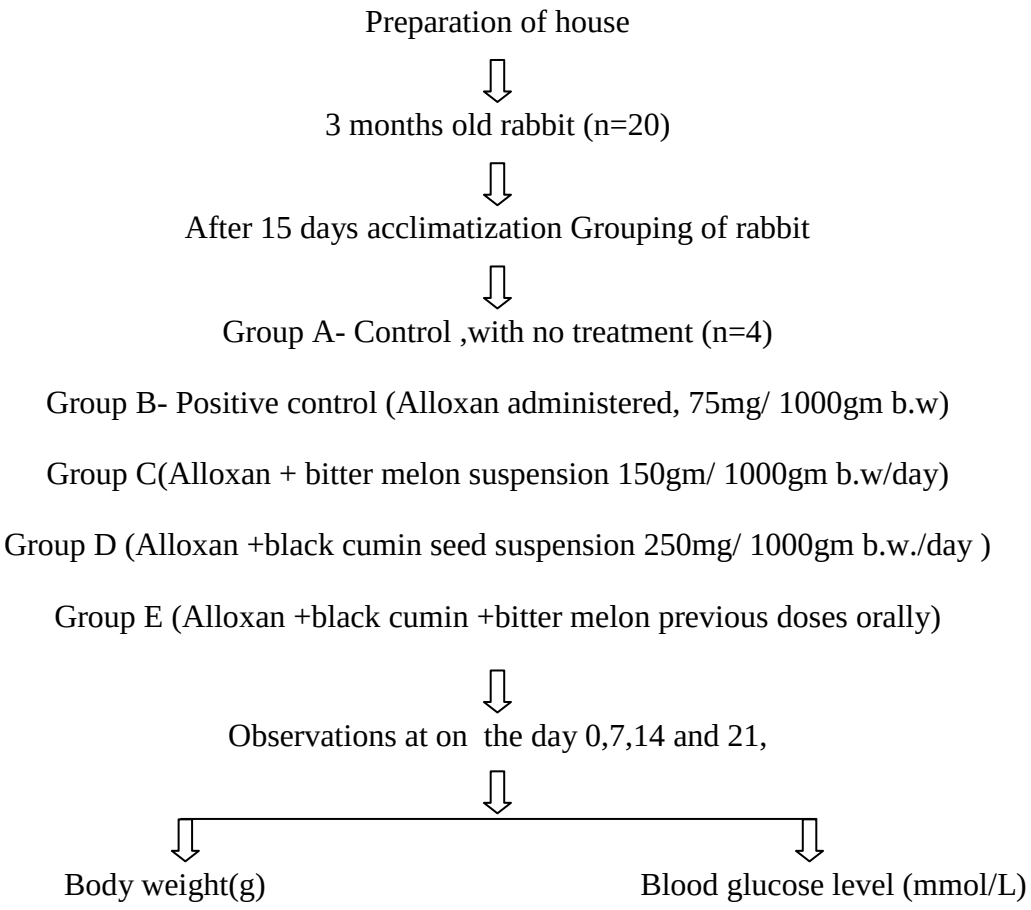


Fig. 1: Layout of Experiment

3.3 Experimental Animal

Twenty white rabbit aged between 3 months and weighting between 1000 to1200g were collected from the rabbit farm at the Department of Animal Genetics and Breeding, 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 rabbits were housed at screen bottomed wire cages arranged in rows and kept in the departmental (Physiology and Pharmacology, HSTU) animal house. The animals are fed with pellet at a recommended dose of 100 gm/kg as advised by ICDDRB. Drinking water was supplied adlibitum. The rabbit were maintained in this condition for a period of two weeks to acclimatize them prior to experimental uses.

3.6 Experimental Animal Grouping

The number of twenty rabbits were used to carry out the experiment. These rabbits were divided into five groups containing 4 rabbits in each group. The groups were designated and maintained as follows:

Group A: Four rabbits were kept as control and were not treated. After acclimatization, body weight and blood glucose level were measured at 0, 7, 14 and 21 days of experiment.

Group B: Four rabbits were treated with Alloxan at a dose of 75 mg /1000 gm body weigh intramuscularly for confirming diabetic condition.

Group C: Four rabbits were treated with Alloxan at a dose of 75 mg /1000 gm body weight intramuscularly for confirming diabetic condition. After confirming diabetic condition (within 72 hours) the suspension of bitter melon fed orally at a dose of 150gm/1000 gm b.w./day for 21 days . During treatment of suspension of bitter melon body weight and blood glucose level were measured at 0, 7, 14 and 21 days of

experiment. This group served as treatment group 1 to find the effect of suspension of bitter melon as antidiabetic drug.

Group D: Four rabbits were treated with Alloxan at a dose of 75 mg /1000 gm intramuscularly for confirming diabetic condition. After confirming diabetic condition (within 72 hours) the suspension of black cumin seed were fed orally at a dose of 250 mg/1000 gm body weight/day for 21 days. During treatment of suspension of black cumin seed body weight and blood glucose were measured at 0, 7, 14 & 21 days of experiment .This group served as treatment group 2 to find the effect of suspension of black cumin seed as antidiabetic drug.

Group E: Four rabbits were treated with Alloxan at a dose of 75 mg /1000 gm intramuscularly for confirming diabetic condition. After confirming diabetic condition (within 72 hours) the suspension of bitter melon and black cumin seed were fed orally at a previous dose for 21 days. During combined treatment of suspension of bitter melon and black cumin seed, body weight and blood glucose were recorded on Day 0 ,7, 14 & 21 days of experiment. This group served as treatment group 3 to find the combined effect of suspension of bitter melon and black cumin seed as antidiabetic drug.

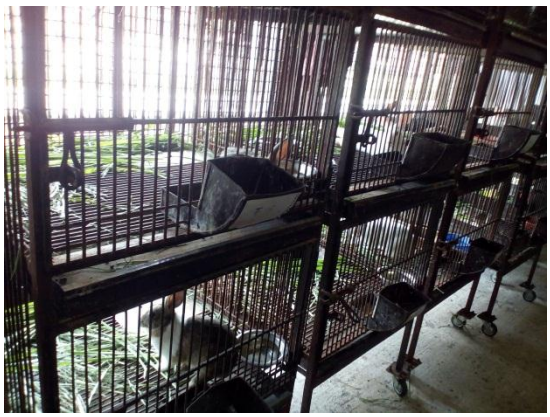


Fig. 2: Experimental Animals

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 and Administration of Alloxan Solution

Materials

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

Procedure

- Alloxan was dissolved in normal saline.
- To induce diabetic condition in rabbits, a dose of 75mg Alloxan per 1000 gm of body weight was chosen for following the recommendation of works done previously. (Puri and Prabhu, 2002).
- Before giving Alloxan, the normal blood glucose levels of all rabbits were estimated. After 2 hours of Alloxan injection the Dextrose (5gm) mixed with water was fed to the all-diabetic rabbits orally to prevent a hypoglycemic condition of rabbits.
- This solution was injected intramuscularly to rabbits and maintained fasting condition for 18 hours.

Fig. 3: Preparation and administration of Alloxan solution

3.8 Symptoms following Administration ofAlloxan in Rabbits

Alloxan induced diabetes by β cell necrosis of the islets of langerhans required few minutes to few hours to many days to be expressed. As it caused β 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 as 17 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 Alloxan injection.

3.9 Collection, Preparation, Preservation & Administration of Suspension of Bitter Melon and Black Cumin Seed

3.9.1 Collection

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

Fig. 4: Bitter Melon (Karela) and Black cumin (Kalajira)

3.9.2 Preparation of Bitter Melon and Black Cumin Suspension

Materials Required

- Bitter melon
- Blender machine
- Pestle and mortar

Filter paper

- Distilled water
- Beaker
- Pipette
- Stirrer
- Sieve and other conventional laboratory instruments.

Procedure

Fresh bitter melon and black cumin seed are measured separately by electronic balance and grinded with mortar and pestle than blended with blender machine. Finally, the suspension are mixed with 100 ml distilled water separately and stirred to make homogenous mixture and then filtered through filter paper.

Fig. 5: Preparation and administration of bitter melon suspension

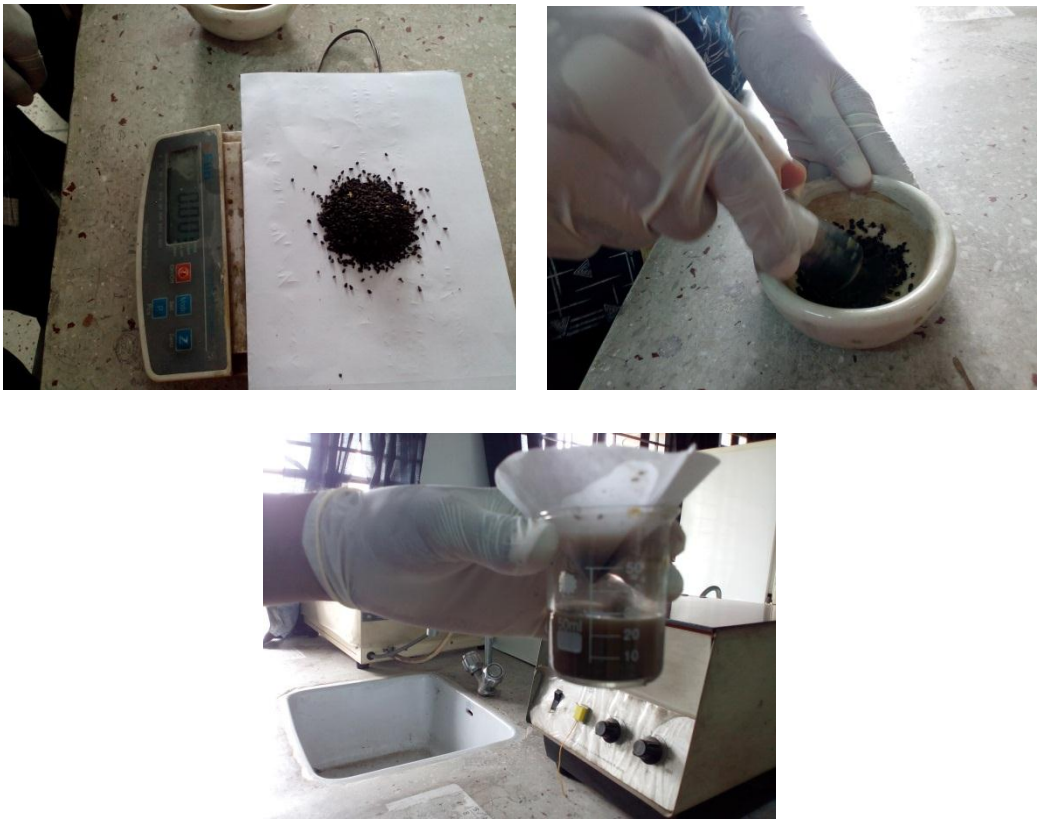


Fig. 6: Preparation and administration of black cumin suspension

3.9.3 Preservation

All solutions were preserved in a refrigerator at - 4°c.

3.9.4 Administration

Working Instruments

- Micropipette
- Gloves
- Electronic balance

Procedure

Prepared suspension of bitter melon and black cumin were fed orally at a dose of 150gm and 250 mg per 1000 gm body weight per day, respectively, 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 weight of each individual rabbit.

3.9.5 Observation of Rabbits

• Body weight and fasting blood glucose level of each rabbit were measured after 18 hours of fasting before Alloxan injection.

• Body weight and fasting blood glucose level of each rabbit were measured on after 72 hours of after Alloxan injection.

• Body weight and fasting blood glucose level of each rabbit were measured Day 0 7, 14& 21 of different treatment.

3.10 Measurement of Different Parameters

3.10.1Estimation of Blood Glucose

Materials Required

- Gloves
- Pinching needle
- Blood

- Ethanol
- Cotton
- 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 (III), 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, (1977) and Hauge (1964).

Procedure

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

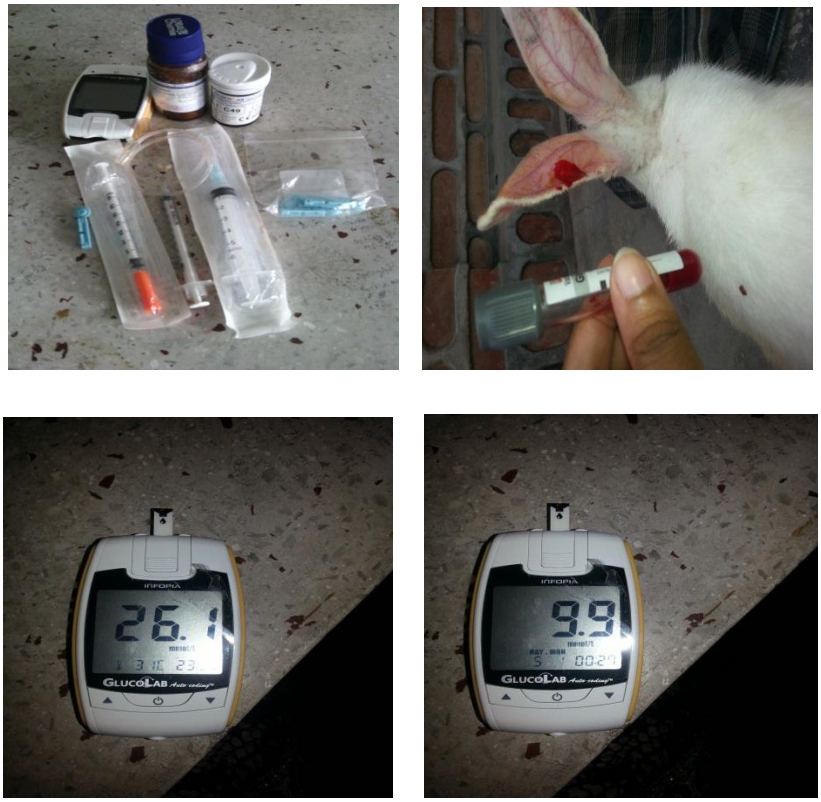


Fig. 7: Determination of blood glucose level

3.10.2 Recording of Body Weight

Determination of Body Weight

Body weight was taken on day 0, 7, 14 and 21 days of experiment.

Materials Required

- Gloves
- Electric balance

Procedure

Body weight of all groups was recorded at the day 0, 7 ,14 and 21 day of experiment by the help of electric balance.

Fig. 8: Recording of body weight

3.11 Statistical Analysis

Data were analyzed by analysis of variance using Completely Randomized Design with factorial arrangement of time and treatments (Steel and Torrie, 1986). All analyses were performed by SPSS program.

CHAPTER 4

RESULTS AND DISCUSSION

CHAPTER 4

RESULTS AND DISCUSSION

The experiment was conducted to determine the antidiabetogenic efficacy of Bitter melon fruits and Black cumin seeds on blood glucose levels and body weights in Alloxan induced diabetic rabbits. To perform the experiment, twenty rabbits were randomly divided into five equal groups. Alloxan was injected (I/M) at the dose rate of 75 mg/1000gm body weight to the groups of rabbits (B, C, D and E) for induction of diabetic syndrome. Group A rabbits were kept as non-diabetic (Normal) control without giving Alloxan and any other treatment. Group B rabbits were kept as diabetic control without (giving any other treatment except Alloxan). Next two groups of rabbits (C and D) were treated with suspension of bitter melon fruit at dose of 150 gm/1000 gm and black cumin seed ata dose of 250 mg/1000 gm for consecutive 21 days, respectively after confirming diabetic condition. All the control and treated rabbits were closely observed during 21 days of treatment period.

4.1 Blood Glucose Level (mmol/L)

4.1.1 Alloxan induced diabetics and comparison with negative control:

Blood glucose level of different groups of rabbits are presented in Table 1.The study was revealed that higher glucose level were found in group B, C, D, E which was treated with Alloxan compare to the animals in group A. This treatment showed significantly ($p \leq .0.05$) increased blood glucose level in treated rabbits. The present results are in agreement with other results. Reddy, *et al.* (2014); Yakaiah, *et al.* (2013); Akhtar *et al.* (1982); Puri and Prabhu, (2002**)** who reported that treatment with Alloxan increased the blood glucose level in treated rabbits compared to the control rabbits.

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 revealed that glucose level was low in group C, which was treated with bitter melon compare to the B group. The effect of fruit suspension at a dose of 150 gm/1000 gm body weight in lowering blood sugar level showed statistically significant Comparison with B group. The suspension of the unripe fruit of the *Momordica charantia* (Bitter

melon) was assessed for its antidiabetic activity in Alloxan induced diabetic rabbits. The blood sugar levels are almost comes to the Normal levels. The present results are in agreement with the results of Ying *et al.,* 2012; Akhtar *et al.,* 1981 and Biyani *et al.* (2003); Leung *et al*, 2009; Yakaiah *et al.,* 2013; suggested that results of this study showed that chronic oral administration of an extract of *Momordica charantia* fruit at an appropriate dosage may be good alternative antidiabetic agent in Alloxan induced diabetics.

4.1.3 Alloxan induced diabetics and Comparison with black cumin seed

Blood glucose level of different groups of rabbits are presented in Table 1. The results of this study revealed that glucose level was the low in group D, which was treated with black cumin compare to the B group. The effect of seed suspension at a dose of 250mg/1000 gm body weight in lowering blood sugar level showed statistically significant Comparison with B group. The suspension of the seed of the *Nigella sativa* (Black cumin) was assessed for its antidiabetic activity in Alloxan induced diabetic rabbits. The blood sugar levels of animals come down to about normal levels. The present results are in agreement with other results of Al-Hader *et al.,* 1993; El-Dakhakhny *et al.*, 2002; Houcher *et al.,* 2007; Kanter M*et al.,* 2004); the results of this study indicated that a dose of 250 mg/ kg body weight of *Nigella sativa* might be a beneficial adjuvant to oral hypoglycemic agents in Alloxan induced diabetes.

4.1.4 Alloxan induced diabetics and Comparison between different groups of rabbits

The decrease in the blood sugar was compared among the groups of animals. The results of this study revealed that blood glucose level was the lowest in group E compare to the C and D group, which was treated with bitter melon fruit and black cumin seed. The effect of this combined treatment significantly ($p \leq .0.05$) affect the blood glucose level.

Table 4.1: Effects of bitter melon and black cumin and combined treatment on blood glucose (mmol/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
A	$7.550^{b} \pm 0.44$	$7.725^{c} \pm 0.37$	$7.425^{d} \pm 0.25$	$7.875^{d} \pm 0.13$
B	$28.33^{a} \pm 0.69$	$27.00^{a} \pm 1.15$	$24.23^{a} \pm 0.60$	$19.02^{a} \pm 0.70$
C	$27.98^{a} \pm 0.73$	$23.45^{b} \pm 0.76$	$18.27^{bc} \pm 0.71$	$12.98^{b} \pm 0.45$
D	$28.98^{a} \pm 0.52$	$24.55^{b} \pm 0.34$	$19.52^{b} \pm 0.52$	$12.70^{b} \pm 0.44$
E	$28.65^{a} \pm 0.81$	$24.80^{b} \pm 0.58$	$16.83^{c} \pm 0.86$	$10.93^{c} \pm 0.29$

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

4.2 Body weight (gm)

The body weight in normal control rabbits (Group A, n=4) was increased On the contrary, in diabetic control group (Group B, n=4), the amount of body weight was decreased in. Group C (n=4), following oral administration of suspension of bitter melon the body weight of rabbits was increased compared to the B group. In Group D (n=4), following oral administration of black cumin seeds the body weight of rabbits was increasd compared to the B group. In Group E (n=4), following administration of bitter melon and black cumin seeds @ previous doses for 21 days the amount of body weight gain was increased comparison with other treated group.

Table 4.1: Effects of bitter melon and black cumin 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
A	$1056.0^{a} \pm 21.34$	$1078.0^{ab} \pm 21.75$	$1083.0^{a} \pm 26.89$	$1133.0^{a} \pm 20.56$
B	$1025.0^{a} \pm 32.27$	$1020.0^{b} \pm 31.092$	$1010.0^{b} \pm 29.72$	$1000.0^{b} \pm 32.40$
C	$1081.0^{a} \pm 11.97$	$1048.0^{ab} \pm 20.56$	$1065.0^{ab} \pm 21.02$	$1080.0^{a} \pm 20.82$
D	$812.5^{a} \pm 237.71$	$1044.0^{ab} \pm 14.63$	$1060.0^{ab} \pm 14.72$	$1078.0^{a} \pm 17.50$
E	$1088.0^{a} \pm 7.50$	$1095.0^{a} \pm 11.90$	$1113.0^{a} \pm 11.09$	$1131.0^{a} \pm 5.15$

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

CHAPTER 5

CONCLUSION

CHAPTER 5

CONCLUSION

This research work was conducted to study the effects of antidiabetogenic efficacy of Bitter melon fruits and Black cumin seeds on blood glucose levels and body weights in Alloxan induced diabetic rabbits. All the 20 rabbits randomly divided into 5 groups (n=4) to carry out this research work. Keeping one group as normal control group (A) and others four groups (B, C, D and E) as treated group. The rabbits of B, C, D and E were treated with alloxan 75mg per 1000 gm bd wt for confirmation of diabetic condition. After confirmation of diabetic condition, rabbits of B group was kept in diabetic positive control group and group C, D, E were treated with bitter melon and black cumin at a dose of 150 gm and 250 mg per 1000gm body weight orally respectively. Close observation was needed and data were recorded during the experimental period. Rabbits in group C, D and E statistically showed significantly ($p<0.05$) increase in body weight and decrease blood glucose level than that of diabetic control group B. These findings also indicated the oral administration for 21 days of *Momordica charantia* (Karela) and *Nigella sativa* (Black cumin) seed suspension significantly lower the blood glucose levels. Feeding of these herbal preparation also causes the body weight gain. The results of this experiment supports the traditional usage of the herbal preparation by Ayurvedic physicians for the control of diabetes. *Momordica charantia* has the potentiality to be used as an adjuvant in the treatment of Diabetes but it requires further study. *Nigella sativa* may be the beneficial adjuvant to oral hypoglycemic agents in diabetic patients. Moreover combination of *Momordica charantia* and *Nigella sativa* could be used for the treatment of diabetic patients without any health hazards. However, due to some short comings only one trial was performed in short term basis and modern equipments was also not available. Before field application as the hypoglycemic agents in case of diabetic patients further trial on a large scale basis is needed to make the findings more accurate and effective further study is essential to see any adverse effect in relation to histopathology before making a definite conclusion.

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