ANTIDIABETOGENIC IMPACT OF BITTER MELON (*MOMORDICACHARANTIA*) AND GARLIC (*ALLIUM SATIVUM*) ON ALLOXAN INDUCED DIABETIC RABBIT MODEL

A Thesis

By

AMBIARA

Student ID. 1504014

Session: 2015-16

Semester: January-June, 2016

MASTER OF SCIENCE (MS)
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DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200, BANGLADESH

MAY, 2016

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

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
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Approved as to the style and contents by

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

_____

(Dr. Rakibul Islam)

Chairman
Examination committee
Department of Physiology and Pharmacology

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

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ABSTRACT

The present study was undertaken to investigate the antidiabetic effect of the bitter melon and garlic on alloxan induced diabetes in experimental rabbits. After acclimatization of the age of 4 months rabbits (New Zealand white) were randomly assigned into five groups (A, B, C, D and E) and each group was remained 4 rabbits. Group A was kept for negative control (without alloxan), Group B was kept for positive control (with alloxan) treated with alloxan intramuscullarly @75mg /kg body weight, Group C was treated with bitter melon @250mg/kg body weight orally along with alloxan, Group D was treated with garlic @750mg/kg body weight orally along with alloxan, Group E treated with combined at previous doses of bitter melon and garlic along with alloxan. Over the course of the trial, observations were recorded for induction of diabetics, blood glucose level, body weight, packed cell volume, ESR and Hb (Hemoglobin) level after 72 hours of injection. Blood glucose level were increased significantly ($p<0.05$) in all treated groups compared to the negative control group and the highest induction was recorded in group B treated with alloxan. Body weight was decreased significantly ($p<0.05$) in all treated groups and lowest was recorded in group B which received treatment Alloxan. There was significant decreased in blood glucose level in all treated group C, D, E compared to the B group and lowest glucose level was recorded in E groupwhen treated with combined medicinal herbs and body weight was increased in all treated group C, D, E compared to the B group and highest was recorded in D group. PCV level was the highest in group E which was treated with both garlic and bitter melon compare to the A group. The Hb (Hemoglobin) gm/dl concentration was the highest in group E which was treated with both garlic and bitter melon compare to the A group. The study was revealed that the Hb (Hemoglobin) gm/dl concentration was the highest in group B treated with alloxan.ESR was highest in group B treated with alloxan and lowest in group E. The present study reveals that combined

treatment with bitter melon and garlic 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 bitter melon and garlic is 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 for Social Science
STZ	:	Streptozotocin
Vol.	:	Volume
WHO	:	World Health Organization

CHAPTER I

INTRODUCTION

Medicinal plants continue to be an important therapeutic aid for alleviating ailments of humankind. Over the last 2500 years, there have been very strong traditional systems of medicine such as Chinese, Ayurvedic, and the Unani, born and practiced, more in the eastern continent. These traditions are still flourishing, since; approximately 80% of the people in the developing countries rely on these systems of medicine for their primary health care needs (Tsay and Agrawal, 2005). These plants contain substances that can be used for therapeutic purposes, of which are precursors for the synthesis of drugs (A Sofowora, 1984). A lot of research work has been carried out on some medicinal herbs and they have been found to have definite action on the nervous, circulatory, respiratory, digestive and urinary systems; as well as the sexual organs, the skin, vision, hearing and taste (Bailey *et al.,* 1989).

Diabetes mellitus is a wide spread disorder which has long been in the history of medicine. Despite continuous introduction of the modern drugs, diabetes and its related complication is still a global medical issue. Before the advent of synthetic insulin and oral hypoglycemic drugs, the major form of treatment involved the use of plants (Wadkar *et al.* 2008). Some of them are being used in traditional systems of medicines from hundreds of years in many countries across the world. The indigenous medicinal practices as an alternative to modern drugs have been uprising worldwide apparently due to their merits of efficacy, better patient tolerance, relatively less expensive and less frequent side-effects.

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. 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 was selected for induction of alloxan diabetes in rabbits.

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.

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

Allium sativum (Garlic) contains chemically active substances such as enzymes, amino acids, minerals and sulphur containing compounds such as alliin (S-allyl cysteine sulphoxide (SACS) and allicin (diallyl disulphide) which are responsible for garlic's pungent odour and many of its medicinal effects (Murrary and Pizzorno, 1999). Allicin which is associated with garlic action does not exist in garlic until it is crushed or cut where injury to the garlic bulb activates the enzyme allinase which metabolizes allin to allicin (Block, 1985). Garlic has been found to have antibacterial and antifungal activity, reduce aortic plaque deposits (Durak et al., 2002) inhibits vascular calcification in human patients with high blood cholesterol (Durak et al., 2004) reduce hyperlipidemia (Kojuri *et al.,* 2007; Mader, 1990) and hyperglycaemia (Ojo, 2012).The primary purpose of this research was to compare biochemical effects of *Allium sativum* and *allium cepa* extracts in alloxan induced diabetic rats.

In this study, the general objective is to see the antidiabetic potential of bitter melon and garlic suspension in alloxan induced diabetic rabbits.

Objectives

The specific objectives are as follows:

- To evaluate the alloxan induced diabetes occurred in experimental rabbits.
- To investigate antidiabetic effect of bitter melon and *g*arlic in alloxan induced diabetic rabbit.
- To see the effects of bitter melon and *g*arlic on body weight, blood glucose level and hematological parameters (PCV, Hb and ESR) in experimental rabbits.

CHAPTER II

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 of anti-diabetogenic impact of *Momordica charantia* (Bitter melon) and *Allium Sativum* (Garlic) 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 metabolic disorder characterized by hyperglycemia due to defect in insulin secretion, insulin action or both. Over the last century human life style and food habits have drastically changed which lead to various chronic diseases. Diabetes milletus is one such disease which is causing serious problems to human health(Kumar PJ and Clark M, 2002). Around 200 million people around the world are being diagnosed with diabetes.according to WHO statistics diabetes is the sixth leading cause of disease-related death in the world. On long standing it leads to many micro and macro vascular complications. The microvascular complications of diabetes includes nephropathy, retinopathy, and neuropathy. In type-1 diabetes the first signs of these complications may develop during adolescence, particularly if insulin is insufficient in the body. Similar complications may occur in the later life of patients with type-2 diabetes. They frequently occur during the time of diagnosis (Beverley B and Eschwège, 2003).

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 diabete*s* 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

2.3 Causes of Diabetes

Insufficient production of insulin (either absolutely or relative to the body's needs), production of defective insulin (which is uncommon), or the inability of cells to use insulin properly and efficiently leads to hyperglycemia and diabetes. This latter condition affects mostly the cells of muscle and fat tissues, and results in a condition known as "insulin resistance." This is the primary problem in type 2 diabetes. The absolute lack of insulin, usually secondary to a destructive process affecting the insulin producing beta cells in the pancreas, is the main disorder in type 1 diabetes. In type 2 diabetes, there also is a steady decline of beta cells that adds to the process of elevated blood sugars. Essentially, if someone is resistant to insulin, the body can, to some degree, increase production of insulin and overcome the level of resistance. After time, if production decreases and insulin cannot be released as vigorously, hyperglycemia develops.

Glucose is a simple sugar found in food. Glucose is an essential nutrient that provides energy for the proper functioning of the body cells. Carbohydrates are broken down in the small intestine and the glucose in digested food is then absorbed by the intestinal cells into the bloodstream, and is carried by the bloodstream to all the cells in the body where it is utilized. However, glucose cannot enter the cells alone and needs insulin to aid in its transport into the cells. Without insulin, the cells become starved of glucose energy despite the presence of abundant glucose in the bloodstream. In certain types of diabetes, the

cells' inability to utilize glucose gives rise to the ironic situation of "starvation in the midst of plenty". The abundant, unutilized glucose is wastefully excreted in the urine. Insulin is a hormone that is produced by specialized cells (beta cells) of the pancreas. (The pancreas is a deep-seated organ in the abdomen located behind the stomach.) In addition to helping glucose enter the cells, insulin is also important in tightly regulating the level of glucose in the blood. After a meal, the blood glucose level rises. In response to the increased glucose level, the pancreas normally releases more insulin into the bloodstream to help glucose enter the cells and lower blood glucose levels after a meal. When the blood glucose levels are lowered, the insulin release from the pancreas is turned down. It is important to note that even in the fasting state there is a low steady release of insulin than fluctuates a bit and helps to maintain a steady blood sugar level during fasting. In normal individuals, such a regulatory system helps to keep blood glucose levels in a tightly controlled range. As outlined above, in patients with diabetes, the insulin is either absent, relatively insufficient for the body's needs, or not used properly by the body. All of these factors cause elevated levels of blood glucose (hyperglycemia). (Medscape.com. Type 2 Diabetes Mellitus).

2.4 Pathophysiology of Diabetes

Type 1 Diabetes Mellitus (DM) is a catabolic disorder in which circulating insulin is very low or absent, plasma glucagon is elevated, and the pancreatic beta cells fail to respond to all insulin secretory stimuli. The pancreas shows lymphocytic infiltration and destruction of insulin-secreting cells of the islets of Langerhans, causing insulin deficiency. Patients need exogenous insulin to reverse this catabolic condition, prevent ketosis, decrease hyperglucagonemia, and normalize lipid and protein metabolism. One theory regarding the etiology of type 1 DM is that it results from damage to pancreatic beta cells from infectious

or environmental agents. In a genetically susceptible individual, the immune system is thereby triggered to develop an autoimmune response against altered pancreatic beta cell antigens or molecules in beta cells that resemble a viral protein. Approximately 85% of type 1 DM patients have circulating islet cell antibodies, and the majority also have detectable anti-insulin antibodies before receiving insulin therapy. Most islet cell antibodies are directed against glutamic acid decarboxylase (GAD) within pancreatic beta cells. Currently, autoimmunity is considered the major factor in the pathophysiology of type 1 DM. Prevalence is increased in patients with other autoimmune diseases, such as Graves disease, Hashimoto thyroiditis, and Addison disease. Approximately 95% of patients with type 1 DM have either human leukocyte antigen (HLA)-DR3 or HLA-DR4. HLA-DQs are considered specific markers of type 1 DM susceptibility. Amino acid metabolism also plays a key role in the pathogenesis of diabetes. Amino acid profiles could help assess risk of developing diabetes (Wang TJ et al 2011).It might help elucidate further how diabetes evolves. Recent evidence suggests a role for vitamin D in the pathogenesis and prevention of diabetes mellitus. Vitamin D deficiency is also an important independent predictor of development of coronary artery calcification in individuals with type 1 DM (Young K.A et al., 2011). Joergensen et al, 2011; determined that vitamin D deficiency in type 1 diabetes may predict all causes of mortality but not development of microvascular complications (Joergensen et al.,2011). The contribution of vitamin D deficiency to mortality must be mediated by nonvascular mechanisms. Type 2 diabetes is characterized by the combination of peripheral insulin resistance and inadequate insulin secretion by pancreatic beta cells. Insulin resistance, which has been attributed to elevated levels of free fatty acids in plasma, (Boden G,1996) leads to decreased glucose transport into muscle cells, elevated hepatic glucose production, and increased breakdown of fat. For type 2 Diabetes Mellitus to occur, both defects must exist. For

example, all overweight individuals have insulin resistance, but diabetes develops only in those who cannot increase insulin secretion sufficiently to compensate for their insulin resistance. Their insulin concentrations may be high, yet inappropriately low for the level of glycemia. Beta cell dysfunction is a major factor across the spectrum of pre-diabetes to diabetes. A study of obese adolescents by Bacha et al confirms what is increasingly being stressed in adults as well: Betacell function happens early in the pathological process and does not necessarily follow stage ofinsulin resistance. Singular focus on insulin resistance as the "be all and end all" is gradually shifting, and hopefully better treatment options that focus on the beta cell pathology will emerge to treat the disorder early. In the progression from normal glucose tolerance to abnormal glucose tolerance, postprandial blood glucose levels increase first; eventually, fasting hyperglycemia develops as suppression of hepatic gluconeogenesis fails. During the induction of insulin resistance, such as is seen after high-calorie diet, steroid administration, or physical inactivity, increased glucagon levels and increased glucose-dependent insulinotropic polypeptide (GIP) levels accompany glucose intolerance; however, postprandial glucagonlike peptide-1 (GLP-1) response is unaltered (Hansen K.B *et al.,* 2011).This has physiologic implications; for example, if the GLP-1 level is unaltered, GLP-1 may be a target of therapy in the states mentioned above.

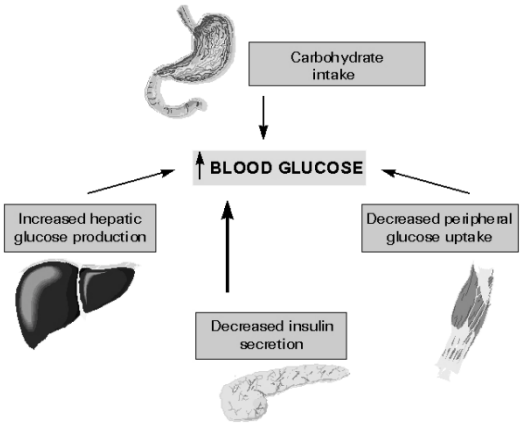


Fig: Pathophysiology of Diabetes

The high mobility group A1 (HMGA1) protein is a key regulator of the insulin receptor gene (*INSR*) (Chiefari E *et al.,* 2011).Functional variants of the *HMGA1* gene are associated with an increased risk of diabetes. These variants were shown to lead to reduction in protein content of both *HMGA1* and *INSR*. Although the path physiology of the disease differs between the types of diabetes, most of the complications, including microvascular, macrovascular, and neuropathic, are similar regardless of the type of diabetes. Hyperglycemia appears to be the determinant of microvascular and metabolic complications. Macrovascular disease, however, is much less related to glycemia. Insulin resistance with concomitant lipid abnormalities (ie, elevated levels of small dense low-density lipoprotein cholesterol [LDL-C] particles, low levels of high-density lipoprotein cholesterol [HDL-C], elevated levels of triglyceride-rich remnant lipoproteins) and thrombotic abnormalities (ie, elevated type-1 plasminogen activator inhibitor [PAI-1], elevated fibrinogen as well as conventional atherosclerotic risk factors (eg, family history, smoking, hypertension, elevated LDL-C, low HDL-C), determine cardiovascular risk. Unlike liver and smooth muscle, insulin resistance is not associated with increased myocardial lipid accumulation (Krssak M *et al.,* 2011) Increased cardiovascular risk appears to begin prior to the development of frank hyperglycemia, presumably because of the effects of insulin resistance. Stern in 1996 and Haffner and D'Agostino in 1999developed the "ticking clock" hypothesis of complications, asserting that the clock starts ticking for microvascular risk at the onset of hyperglycemia, while the clock starts ticking for macrovascular risk at some antecedent point, presumably with the onset of insulin resistance

2.5 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.

2.5.1 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.

2.5.2 Chemical Causes of Diabetes

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.

2.6 Alloxan

Diabetogenic agent Alloxan
Commercial name: Alloxan monohydrate
Generic name: 2,4,5,6-pyrimidinetetrone
Others name: Mesoxalylurea 5-Oxobarbituric acid

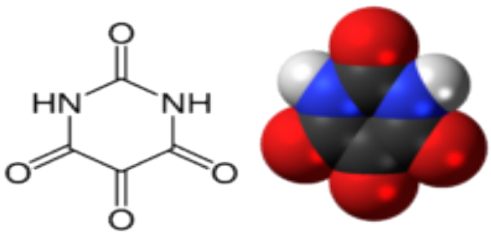


Fig 3: Structure of Alloxan

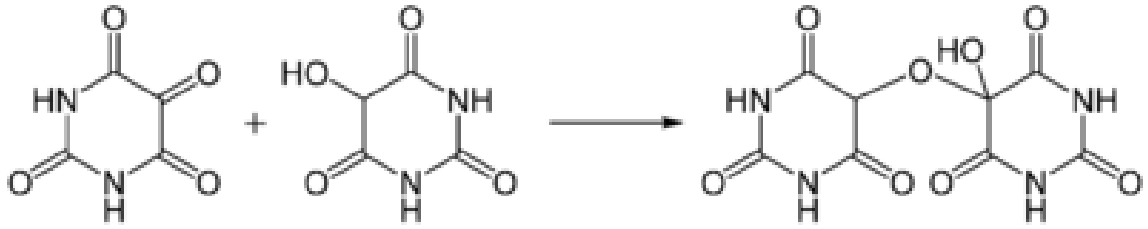
History

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.

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 and 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)

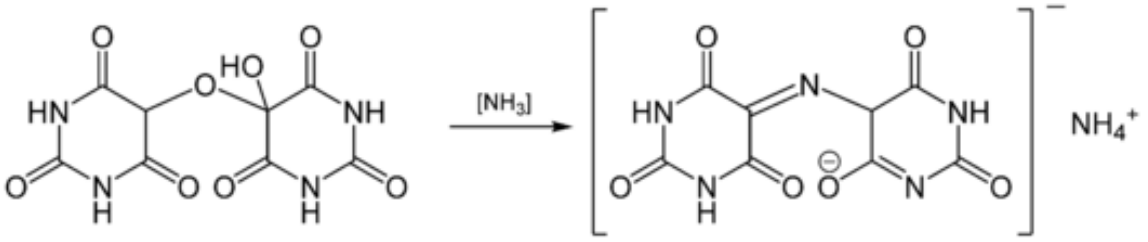
Biological effects

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.

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.

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 *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 (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.* 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 . 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

isadministered intraperitoneally or subcutaneously its effective dose must be higher (Antia et al., 2005).

2.7 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. It increases the mitosis of pancreatic cells and partially recover the destroyed cells. various medicinal properties are claimed for *Momordica charantia* namely antidiabetic, abortifacient, anthelmintic, contraceptive, antimalarial and laxative and also in galactogogue, jaundice, leprosy, pneumonia and rheumatism. Charantin (mixture of sterol glycosides), vicine (pyrimidine nucleotide) and p-insulin (polypeptide) are reported as the active ingredients. Additionally, alkaloids, glucoside, saponins and mucilage are the other reported contents.

Biological source

It is obtained from edible fruit of *Momordica charantia*, belonging to the family Cucurbitaceae.

Chemical constituents

The plant contains several biologically active compounds

a) Chiefly momordicin I & momordicin II, cucurbitacin B

b) Glycosides (momordin, charantin,

charantosides, goyaglycosides)

c) Terpenoid compounds- momordicinin,
momordicilin, momordol
d) Cytotoxic (ribosome inactivating) proteins
such as momorcharin & momordin.

Uses

Bitter melon is used as anti-diabetic. It contains lectin that has insulin like activity due to its nonprotein specific linking toghther to insulin receptors. This lectin lowers blood glucose level by acting on peripheral tissues. Lectin is a major contributor to hypoglycemic effect.

Scientific work done- Triterpenoids Isolated from *Bitter Melon* has showed antidiabetic activity

Dosage form

It is used as fresh juice, tincture, juice extract & powdered.

Dose

Fresh juice- 57-113 gm daily, Tincture- 1.3 ml/ twice/ daily, Juice extract- 300-600 mg, Powered leaf- 1-2 gm

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.

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.

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.

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.

Moreover, this vegetable is beneficial in curing liver diseases, skin ailments and other windy complaints (Satyavati*et al.,* 1987). Bitter gourd has been used in various Asian traditional medicine systems for a very

long time and traditionally it is being believed that bitter gourd stimulates digestion and also this can be helpful in people with sluggish digestion, dyspepsia, and constipation, it can sometimes make heartburn and ulcers worse(Leslie Taylor ,2002). The fact that bitter gourd is also a demulcent and at least a mild inflammation modulator, however means that it rarely does have these negative effects, based on clinical experience and traditional reports. Traditional medicine of Brazil believes that bitter gourd is used for treating tumors, wounds, rheumatism, malaria, leucorrhea, inflammation, menstrual problems, diabetes, colic, fevers, worms, and also used as an abortions inducer as well as an aphrodisiac agent (Maiti R et al., 2012). It is also employed topically for skin problems, vaginitis, hemorrhoids, scabies, itchy rashes, eczema, and leprosy. The bitter gourd is specifically used as a folk medicine for the management of diabetes. Studies have shown that it contains a hypoglycemic or insulin-like principle, designated as 'plant-insulin', which has been found highly beneficial in lowering the blood and urine sugar levels (Abascal K & Yarnell E, 2005). It should, therefore, be included liberally in the diet of the patients suffering from diabetes. For better results, these patients should take the juice of about four or five fruits every morning, on an empty stomach or alternatively the seeds of bitter gourd can be supplemented in food formulations in the powdered form. They can also use bitter gourd in the form of decoction by boiling the pieces in water or in the form of dry powder. The juice of fresh leaves of bitter gourd is valuable in treating piles problems. 3 teaspoonfuls of leaf juice mixed with a glassful of buttermilk should be taken every morning for about a month in this condition. A paste of the roots of bitter gourd plant can also be applied over piles with beneficial results. Bitter gourd is highly beneficial in the treatment of blood disorders like blood boils, scabies, itching, psoriasis, ringworm and other fungal diseases. A cupful of fresh juice of bitter gourd mixed with a teaspoonful of lime juice should be taken, sip by sip, on empty stomach daily for four to six months

in these conditions. Its regular use in endemic regions of leprosy acts as a preventive medicine. The roots of this plant were used in folk medicine against respiratory disorders from ancient times (Bhakru HK, 1990).

Anti-diabetic effect of *M. charantia*

To date, more than 100 *in vivo* studies have demonstrated the blood sugar-lowering effect of this bitter fruit. The fruit has also shown the ability to enhance cells' uptake of glucose, to promote insulin release, and to potentiate the effect of insulin. The bioactive compounds present in bitter gourd activate a protein called AMPK (AMP-activated protein kinase α), which is well known for regulating fuel metabolism and enabling glucose uptake processes which are impaired in patients with diabetes. A study by observed that extract or powder of fresh and dried whole fruit remarkably lowered the blood sugar in diabetic rats (Virdi *et al.,* 2003). Another study reported that bitter gourd extracts has anti-diabetic, hepato-renal protective and hypolipidemic effects in alloxan-induced diabetic rats (Batran *et al.,* 2006). Recent study reported that bitter melon appears to be exerting in reducing capillary permeability than the fenugreek extract. In general, the increase in capillary permeability is a sign of microvascular dysfunction at the arteriolar and capillary level, which is a common and severe complication of diabetes (Wehash *et al.,* 2012). More recently, a detailed antidiabetic mechanism has been reviewed (Joseph and Jini, 2013). Lectin of bitter gourd has insulin-like activity and which is due to its linking together of 2 insulin receptors. This lectin lowers blood glucose concentrations by acting on peripheral tissues and, similar to insulin's effects in the brain, suppressing appetite. Lectin is likely a major contributor to the hypoglycemic effect that develops after eating bitter gourd and it may be a way of managing adult-onset diabetes. Lectin binding is non-protein specific, and this is likely why bitter gourd has been credited with immunostimulatory activity - by linking receptors that modulate the

immune system, thereby stimulating said receptors. Another scientiest studied the changes in glycolconjugate metabolism during the development of diabetic complications and their modulation by feeding bitter gourd and spent turmeric as a fiber-rich source (Vijayalakshmi *et al.,* 2009). Treatment resulted in decreased level of total sugar content in liver, spleen, and brain while an increase in amount was observed in heart and lungs. Uronic acid content got decreased in liver, spleen and brain, and a marginal increase was also observed in testis. Amino sugar content decreased in liver, spleen, lungs and heart in patients with diabetes. Decrease in sulfation of glycoconjugates was observed in liver, spleen, lungs and heart during diabetes and this effect was significantly ameliorated by treatment with bitter gourd and spent turmeric, except in brain tissues. Protein content decreased in liver, while higher level was observed in brain. The studies clearly showed the ameliorative properties by slowing down the release of glucose from fiber in the gastrointestinal tract (GI) and short-chain fatty acid production from fiber by colon microbes. Effect of bitter gourd on streptozotocin-induced diabetic rats with particular emphasis on kidney heparin sulfate (HS) was studied by Sureshkumar et al. [49]. This Study showed a partial reversal of all the diabetes induced effects by bitter gourd. Increase in the components of glycol-conjugates during diabetes was significantly decreased by the feeding of bitter gourd. Diabetes associated elevation in the activities of enzymes involved in the synthesis and degradation of glycosaminoglycans (GAGs) were significantly lowered by bitter gourd supplementation. GAGs composition revealed decrease in amino sugar, and uronic acid contents during diabetes and bitter gourd feeding was effective in countering this reduction. Decrease in sulfate content in the GAGs during diabetes was also ameliorated by the intake of bitter gourd. HS treatment resulted in 43% reduction of diabetic rats whereas bitter gourd feeding to diabetic rats showed 27% reduction. These results clearly indicated the beneficial role of bitter gourd in controlling glyco-

conjugate and heparin sulfate related kidney complications during diabetes thus prolonging late complications of diabetes. A study in mice revealed that lipid and saponin extracts of melon are more effective in lowering glycated haemoglobin levels and excessive body weight gain than the hydrophilic extract or the whole fruit. White bitter gourd varieties were found to contain significantly lower saponin concentrations (0.25%) compared to green varieties (0.67%). The lipid extract contained higher amounts of conjugated linoleic and linolenic acids i.e up to 65.89%. Charantin is one of the hypoglycemic compounds consisting of a mixture of (1:1) sitosteryl glucoside (C35H60O6) and stigmasteryl glucoside (C35H58O6), belongs to steroidal saponins (Habicht D *et al.,* 2011). Another studied shown that charantin when taken either orally or intravenously in rabbits, it produces hypoglycemic effects (Lolitkar and Rao, 2010). Protein P- insulin is an another hypoglycemic agent of polypeptide in nature with the molecular weight of about 11,000 Da and consists of 166 amino acids. Clinical study revealed that the polypeptide-p- ZnCl2 produced blood sugar lowering effect. Another report that besides the fruits, p- insulin was also found in seeds and tissue cultures of bitter gourd Khanna and Mohan, 1973).

2.8 Garlic *(Allium Sativum)*

Garlic (*Allium sativum*) is one of the most popular herbs used worldwide to reduce various risk factors associated with cardiovascular diseases. Garlic, a member of the Liliaceae family, is a common food for flavour and spice and it is one of the herbs most commonly used in modern folkloric medicine. Garlic was an important medicine to the ancient Egyptians as listed in the medical text Codex Ebers (ca. 1550 BC) especially for the working class involved in heavy labour because it was an effective remedy for many aliments such as heart problems, headache, bites, worms and tumours. *Allium sativum* is members of the lily family, having blood glucose lowering, anti-oxidant, antihypertensive

and antihyperlipidemic effects (Sharma *et al.,* 1977; Sheela and Augusti, 1992). Volatile oils in raw garlic have been reported to lower fasting blood glucose level in both animal and human trials (Jain *et al.,* 1973). The active compounds are believed to be sulphur-containing compounds: allyl propyl disulfide (APDS) in onions and diallyl disulfide (allian) in garlic. These active compounds lower glucose levels by competing with insulin (a disulfide) for insulin-inactivating sites in the liver, resulting in increased levels of plasma insulin (Sheela and Augusti, 1992; Lucy *et al.,* 2002).

Garlic is stated to possess many therapeutic benefits. Garlic's strong odour is largely due to sulphur-containing compounds (e.g. S-allylcysteine sulphoxide), which are believed to account for most of its medicinal properties (Augusti KT,1996). Actually, garlic contains a variety of effective compounds that exhibit anticoagulant (anti-thrombotic), (Thomson M, 2002) antioxidant, (Anwar MM & Meki AR, 2003), antibiotic,(Bakri IM & Douglas CW, 2005). hypocholesterolaemic,(Ali M *et al.,* 2000) hypoglycaemic, (Augusti KT,1996) as well as hypotensive activities.

As mentioned above, although a large number of sulphur thiosulphinates are present in sufficient quantities at normal consumption levels (3-5 g per day). Allicin has been shown to be important in many health effects of garlic (Jamison JR, 2003). However, the anti-cancer effect of garlic might be shared between allicin and other unidentified compounds (Hassan HT, 2004). Garlic contains about 1% alliin, which is converted enzymatically by allicinase to allicin, and other sulphur-containing compounds (Block E *et al.,* 1986).

Garlic has been found to be effective in lowering serum glucose levels in STZ-induced as well as alloxan-induced diabetic rats and mice. Most of the studies showed that garlic can reduce blood glucose levels in diabetic

mice, rats and rabbits.14 Augusti and Sheela consistently showed that S-allyl cysteine sulphoxide, (allicin), a sulphur-containing amino acid in garlic (200 mg/kg body weight), had a potential to reduce the diabetic condition in rats almost to the same extent as did glibenclamide and insulin (Sheela CG *et al.,* 1995). Aged garlic extract was also effective in preventing adrenal hypertrophy, hyperglycaemia and elevation of corticosterone in mice made hyperglycaemic by immobilization stress (Kasuga S *et al.,* 1992). In addition, Liu and co-workers reported that both garlic oil and diallyl trisulphide improved glycaemic control in STZ-induced diabetic rats (Liu C-T *et al.,* 2005). Ingestion of garlic juice resulted in better utilization of glucose in glucose tolerance tests performed in rabbits, while allicin at a dose of 250 mg/kg was 60% as effective as tolbutamide in alloxan-induced diabetic rabbits (Mathew PT & Augusti KT, 1973). In contrast, garlic powder intake (6.25% by weight in diet) for 12 days reduced hyperphagia and polydipsia, but did not Thomson et al 109 alter either hyperglycaemia or hypoinsulinaemia in STZ- induced diabetic mice (Swanston-Flatt SK *et al.,* 1990). Similarly, Baluchnejadmojarad and Rohgani found no hypoglycaemic effect of an aqueous extract of garlic in rats with STZ-induced diabetes although they did observe a significant effect of garlic on vascular reactivity (Baluchnejadmojarad T & Rohgani M, 2003). Liu and co-workers have speculated that these inconsistent results are at least partly due to the use of different preparations or derivatives of garlic in the different studies.20Staba and coworkers have established that the chemicals present in a garlic product are largely dependent on the processing conditions, such as temperature, duration of preparation, and extraction solvents used (Staba EJ *et al.,* 2001).

Antihyperglycemic activity was observed in ethyl acetate, ethanol, and petroleum ether extract of alloxan induced rabbits. Allicin, apigenin, allicin, s- allyl cysteine sulfoxide is responsible for hypoglycemic activity. It has been found that ethyl acetate extract is most potent and active

principle producing maximum hypoglycemic activity due to increased insulin like activity of plasma (Kasuga S*et al.,* 1999). Garlic is stated to possess many therapeutic benefits as it contains varieties of effective compound that exhibits anticoagulant, antioxidant, antibiotics, hypocholesterolaemic, hypoglycemic as well as hypotensive activity (Ram PR & Bhola ND, 1990). Garlic contain chemical constituents such as alliin, allicin, volatile oils, vitamins and minerals (Sharma *et al.,* 1977).

CHAPTER III

MATERIALS AND METHODS

This research work was 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 garlic on alloxan induced diabetic rabbit.

3.1 Experimental Site

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

3.2 Experimental design

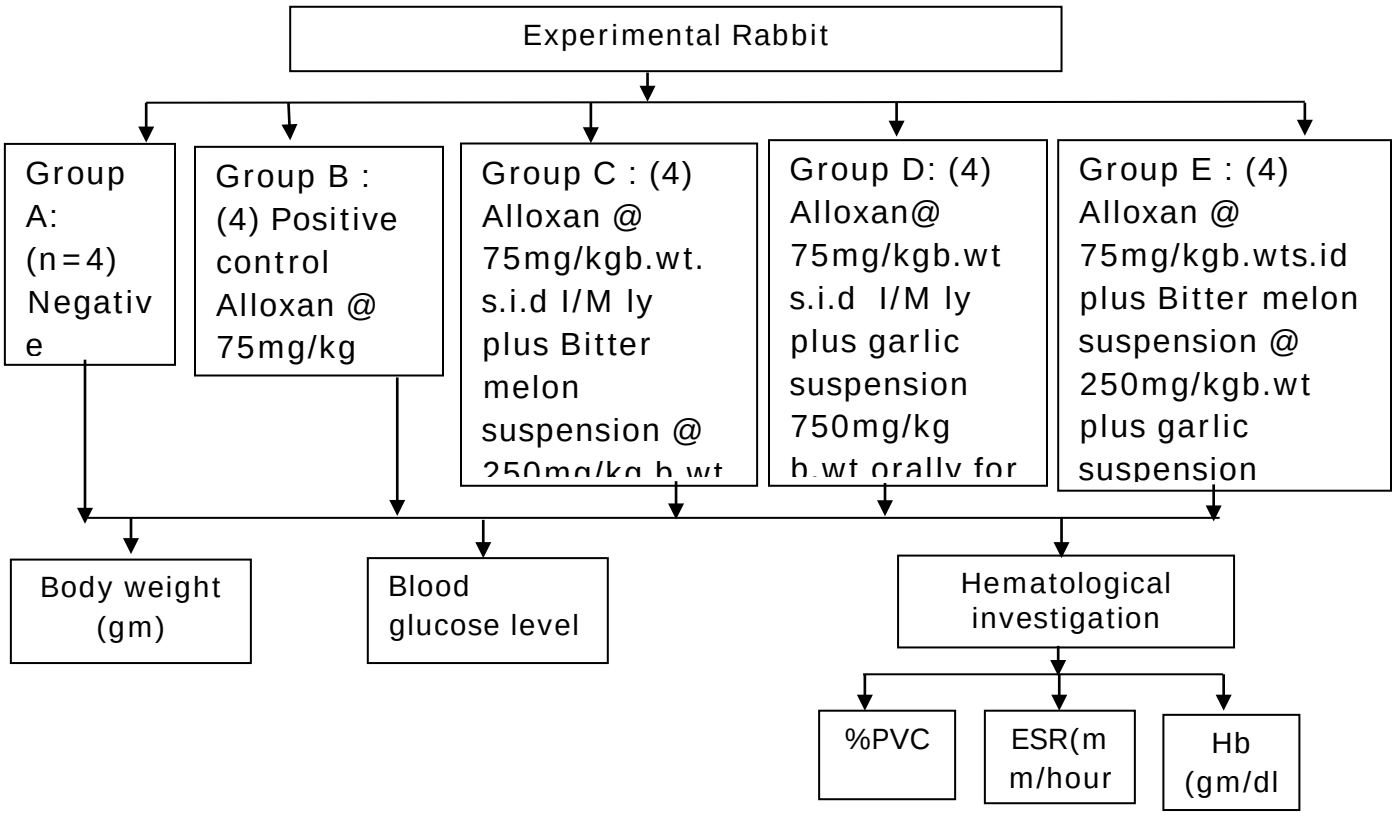


Figure 1. Layout of the experiment

3.3 Experimental Animal

Twenty white rabbit aged about 4 months and weighting between 1000 to1200g were collected from rabbit farm under the department of Animal Genetics and Breeding, HSTU, Dinajpur.

3.4 Preparation of House

At 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 were fed with pellet at a recommended dose of100 g/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

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

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

Group B: After acclimatization, body weights and blood glucose level were measured after 18 hours of starvation. Then Alloxan hydrochloride injection was given at a dose of 75 mg/1000 to1200gm (Puri and Prabhu, 2002) in intramuscular route to each rabbits to induce diabetes.

The rabbits were fed normal diet and given water adlibitum from Day 1-15 on 15th day blood glucose level and the body weight were again measured to ensure diabetic condition. Then all the rabbit of this group were kept for 14 days without any treatment. During that period on Day 0, 7 & 14 the body weight and blood glucose level were measured. This group served as diabetic positive control group.

Group C: After acclimatization, body weights and blood glucose level were measured after 18 hours of starvation. Then Alloxan hydrochloride was injected in all rabbits of this group at a dose rabbits of 75 mg/kg in intramuscular route. The rabbits were fed normal diet and given water adlibitum for 15days. Then blood glucose level and body weight were measured on 15th day of Alloxan hydrochloride injection for confirming diabetic condition. After that suspension of bitter melon fed at a dose of 250gm, 3mL water/1000 to 1200gmb.wt./day for 21 days. During treatment of bitter melon suspension body weight and blood glucose level were recorded on Day 0 (Pre-treatment) and Day 7, 14 & 21 (during treatment). This group served as treatment group 1to find the effect of suspension of bitter melon as antidiabetic drug.

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

750 mg/kg body weight/day for 14 days. During treatment of body weight and blood glucose were recorded on Day 0 (Pre-treatment) and Day 7, 14 & 21 (during treatment). This group served as treatment group 2to find the effect of suspension of garlic as antidiabetic drug.

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

Fig. 5: Experimental Animals

Chemicals

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

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

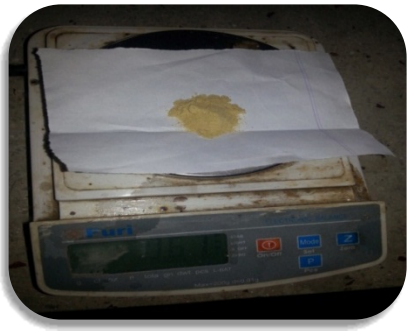
3.7 Preparation and Administration of Alloxan Solution

Materials

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

Procedure

- Alloxan was dissolved in normal saline.
- Before giving Alloxan, the normal blood glucose levelsof all rabbits were estimated. After 2 hours of Alloxan injection the Dextrose (5gm) mixed with water fed to the all-diabetic rabbits orally to prevent a hypoglycemic condition of rabbits.
- This solution was injected intraperitonealy and intravenously to rabbits and maintained fasting condition for 18 hours.
- After 18 hours of treatment, if diabetic condition could not found then this solution was injected intramuscularly to rabbits and maintained fasting condition for 18 hours.
- To induce diabetic condition in rabbits, a dose of 75mg Alloxan per kg of body weight was chosen for following the recommendation of works done previously. (Puri and Prabhu, 2002).



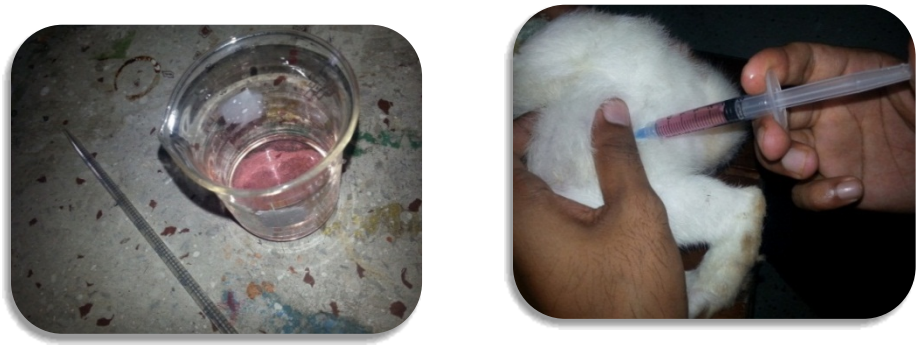


Fig. 6: Preparation and administration of Alloxan solution

3.8 Symptoms following Administration ofAlloxan in Rabbits

Alloxaninduced diabetes by β cell necrosis of the Islets of langerhans required few minutes to few hours or even many days to beexpressed the condition. As it caused βcell necrosis there was a massive release of pre-formed insulin from the dying beta cells. A confirmation ofthe 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 injecting Alloxan monohydrate.

3.9 Collection, Preparation, Preservation & Administration of Suspension of Bitter Melon and Garlic

3.9.1 Collection

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

Fig. 7: Bitter Melon (Karela) and Garlic

3.9.2 Preparation of Bitter Melon and garlic Suspension

Materials Required

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

Procedure

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

Fig. 8: Preparation and administration of bitter melon suspension

Fig. 9:Preparation and administration of garlic suspension

3.9.3 Preservation

All prepared solution were preserved in refrigerator at 0°-4°c under Physiology and Pharmacology laboratory.

3.9.4 Administration

Working Instruments:Micropipette, Leather gloves, Electronic balance.

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

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

3.9.5 Observation of Rabbits

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

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

• Body weight and fasting blood glucose level of each rabbits were measured Day 0 (Pre-treatment) and Day 7, 14& 21 (during treatment) of different treatment.

3.10 Recording of Different Parameters

3.10.1 Recording of Blood Glucose

Collection of blood

Materials Required : Leather gloves, Pinching needle, Ethanol, Cotton, Glucolab(R) active monitor, Glucolab test strip

Procedure

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

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

Materials Required

- Glucolab (R) active monitor
- Glucolab test strip

Test Principle

The test zone of the strip contains glucose dye oxidoreductase 0.7 U, bis-(2- Hydroxy ethyl)-(4-hydroximinocyclohexa-5-dienylidene) ammonium chloride 8.3 ug, 2, 1 8-phosphomolybdicacid 88pg, stabilizer 0.8mg per cm2. Glucose dye oxidoreductase mediator reaction. The enzyme glucose dehydrogenase converts the glucose in a blood sample to gluconolactone. This reaction liberates an electron that reacts with a coenzyme electron acceptor, the oxidized form of the mediator hexacyanoferrate (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

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

Fig. 10: Determination of blood glucose level

3.10.3 Recording of Body Weight

Determination of Body Weight

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

Materials Required

- Leather gloves
- Electric balance

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

Fig. 11: Recording of body weight

3.11 Statistical Analysis of Recorded Data

Data were analyzed by using SPSS v.20 for Windows (SPSS Inc., Chicago, IL, USA). Statistically significant differences between group means were determined by Analysis of Variance (ANOVA). Mean values were considered significantly different at $P<0.05$. Data are expressed as mean ± SEM.

CHAPTER IV

RESULTS AND DISCUSSION

The experiment was conducted to determine the efficacy of alloxan to induce diabetes in rabbits. Attempts were also made to study the antidiabetogenic efficacy of bitter melon fruits and garlic on blood glucose levels and body weights in alloxan induced diabetic rabbits and also to study the combined effect of bitter melon fruit (Karela) and garlic.To perform the experiment, twenty rabbits were randomly divided into five equal groups. Alloxan was injected (I/M) at the dose rate of 75mg/kg body weight to the groups of rabbits (B, C, D and E) for induction of diabetic syndrome. Group A rabbits were kept as negative control without giving alloxan and any other treatment. Group B rabbits were kept as diabetic positive 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 250 mg/kg and garlic at a dose of 750mg/kg for consecutive 21 days respectively after 21 days of alloxan administration. Group E for combined treatment. All the control and treated rabbits were closely observed 21 days of treatment period.

4.1 Blood Glucose Level (mmol/L)

4.1.1 Alloxan induced diabetics and comparison with control:

Blood glucose level of different groups of rabbits are presented in Table-1.The study was revealed that glucose level was the highest in alloxan treated group B,compared to the negative group A. This treatment significantly ($p \leq .0.05$) increase the blood glucose level in treated rabbits. The present results are agreed with others result of Lenzen S, 2008; Tasaka Y *et al.,* 1988; West E *et al.,* 1996 who suggested that alloxan treatment 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 was revealed that glucose level was the lowest in group C, which was treated with bitter melon compare to the group B . The effect of fruit suspension at a dose of 250gm/kg body weight in lowering blood sugar level showed statistically significant comparison with group B. We have evaluated 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 were highly decreased with a treatment of high dose of extract. The blood sugar levels are almost comes to the normal levels. The present results are agreed with other results. Sarkar S *et al.,* 1996; Miura T *et al.,* 2001; Leatherdale BA *et al.,* 1981 and Akhar MS*et al.,* 1981 who suggested that 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 garlic

Blood glucose level of different groups of rabbits are presented in Table - 1.The study was revealed that glucose level was the low in group D, which was treated with garlic compare to the B group. The effect of seed suspension at a dose of 750mg/kg body weight in lowering blood sugar level showed statistically significant comparison with group B. We have evaluated the use of garlic was assessed for its antidiabetic activity in alloxan induced diabetic rabbits. The blood sugar levels were highly decreased with a treatment of high dose of extract. The blood sugar levels are almost comes to the Normal levels. The present results are agreed with other results. (Sharma *et al.,* 1977; Sheela and Augusti, 1992; Jain *et al.,* 1973); the results of this study indicate that a dose of

750 mg/ kg body weight of *Allium Sativum* might be a beneficial oral hypoglycemic agents in Alloxan induced diabetes.

4.1.4 Alloxan induced diabetics and comparison between different groups of rabbits

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

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

Treatment group	Day 0 (Mean ± SE of Mean	Day 7 (Mean ± SE of Mean	Day 14 (Mean ± SE of Mean	Day 21 (Mean ± SE of Mean
A	7.55^{b} ±.44	7.73^{d} ±.37	7.43^{d} ±.25	7.88^{d} ±.13
B	28.33^{a} ±.69	27.00^{a} ±1.15	23.48^{a} ±.89	19.02^{a} ±.70
C	27.95^{a} ±.72	23.45^{c} ±.76	18.27^{c} ±.71	12.98^{b} ±.46
D	29.13^{a} ±.63	26.10^{ab} ±.76	21.27^{b} ±.81	14.02^{b} ±.46
E	28.27^{a} ±.83	23.88^{bc} ±.48	19.30^{bc} ±.80	11.25^{c} ±.34

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

4.2 Body weight (gm)

The percent increased in body weight gain in negative control rabbits (Group A, n=4) was 1133 gm. On the contrary, in diabetic positive control group (Group B, n=4), the percentage of body weight loss was 1000gm. The percent increased in body weight gain over 21 days in. Group C (n=4), following oral administration of suspensionof bitter melon@250 mg/kg was 1080 gm. In Group D (n=4), following administration of garlic suspension @750 mg/kg for 21 days the percentage of body weight gain was 1085 gm. In Group E (n=4), following administration of bitter melon and garlic suspension @ previous doses for 21 days the percentage of body weight gain was 1168 gm comparison with B group which is treated with alloxan (Table 2). Here we observed that the highest body weight gain was increased in garlic treatment group (D) than group A and B but little bit similar to the group C. The results of this study indicate that a dose of 750 mg/ kg body weight of *Allium Sativum* might be a beneficial for weight gain in alloxan induced diabetes.

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

Treatment group	Day 0 (Mean ± SE of Mean	Day 7 (Mean ± SE of Mean	Day 14 (Mean ± SE of Mean	Day 21 (Mean ± SE of Mean
A	1056.0^{a} ± 21.34	1078.0^{a} ± 21.74	1083.0^{ab} ± 26.88	1133.0^{a} ± 20.56
B	1025.0^{a} ± 32.27	1020.0 ^{a} ± 31.09	1010.0^{b} ± 29.72	1000.0 ^{b} ± 32.40
C	1056.0 ^{a} ± 21.34	1048.0^{a} ± 20.56	1090.0^{a} ± 28.57	1080.0^{a} ± 20.81
D	1063.0 ^{a} ± 16.13	1062.0^{a} ± 14.93	1078.0^{ab} ± 18.87	1085.0 ^{a} ± 21.01

E	1075.0[a] ± 14.43	1063[a] ± 10.30	1083.0[ab] ± 11.08	1068.0[ab] ± 13.15

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

4.3 % PCV (Packed Cell Volume)

The percentage of packed cell volume of different treatment groups were shown in Table 3. The study was revealed that % of PCV level was the highest in group E which was treated with both garlic and bitter melon compare to the group A. This treatment significantly ($p \leq .0.05$) increase the packed cell volume level in treated rabbits.

Table 3: % PCV (Packed Cell Volume)

Treatment group	Day 0 (Mean ± SE of Mean	Day 7 (Mean ± SE of Mean	Day 14 (Mean ± SE of Mean	Day 21 (Mean ± SE of Mean
A	40.47[ab] ± 0.77	40.08 [ab] ± 0.36	40.70[a] ± 0.39	41.25[bc] ± 0.38
B	38.97[ab] ± 1.05	37.83[c] ± 0.82	37.00[a] ± 0.77	35.82[d] ± 0.47
C	38.17 [b] ± 0.81	38.75[bc] ± 0.51	40.33[a] ± 0.60	40.72[c] ± 0.55

D	40.28^{ab} ± 0.43	40.42^{ab} ± 0.30	41.03^{a} ± 0.42	42.17^{ab} ± 0.26
E	41.33^{a} ± 0.60	41.70^{a} ± 0.53	42.20^{a} ± 0.53	42.95^{a} ± 0.55

4.4 Hb (Hemoglobin) gm/dl

The Hb (Hemoglobin) gm/dl concentration of different treatment groups were shown in Table 4. The study was revealed that the Hb (Hemoglobin) gm/dl concentration was the highest in group E which was treated with both garlic and bitter melon compare to the group A. This treatment significantly ($p \leq .0.05$) increase the Hb (Hemoglobin) gm/dl concentration in treated rabbits. Table 4: Hb (Hemoglobin) gm/dl

Treatment group	Day 0 (Mean ± SE of Mean	Day 7 (Mean ± SE of Mean	Day 14 (Mean ± SE of Mean	Day 21 (Mean ± SE of Mean
A	11.93^{a} ± 0.52	12.10^{a} ± 0.61	12.43^{a} ± 0.55	12.60^{a} ± 0.64
B	12.15^{a} ± 0.57	12.10^{a} ± 0.43	11.82^{a} ± 0.48	11.90^{a} ± 0.34
C	12.20^{a} ± 0.62	12.07^{a} ± 0.65	12.55^{a} ± 0.68	12.43^{a} ± 0.62
D	12.52^{a} ± 0.35	12.52^{a} ± 0.35	12.48^{a} ± 0.39	12.57^{a} ± 0.37
E	12.65^{a} ± 0.33	12.57^{a} ± 0.37	12.60^{a} ± 0.38	12.82^{a} ± 0.29

4.5 ESR (Erythrocyte Sedimentation Rate) mm in 1^{st} hour

The (Erythrocyte Sedimentation Rate) mm/h of different treatment groups were shown in Table 5. The study was revealed that ESR was highest in group B treated with alloxan and lowest in group E.

Table 5: ESR (Erythrocyte Sedimentation Rate) mm in 1st hour

Treatment group	Day 0 (Mean ± SE of Mean	Day 7 (Mean ± SE of Mean	Day 14 (Mean ± SE of Mean	Day 21 (Mean ± SE of Mean
A	$1.88^{ab} \pm 0.27$	$1.70^{b} \pm 0.22$	$1.90^{b} \pm 0.23$	$1.95^{bc} \pm 0.22$
B	$1.85^{b} \pm 0.06$	$2.20^{ab} \pm 0.11$	$2.75^{a} \pm 0.16$	$3.53^{a} \pm 0.10$
C	$2.55^{a} \pm 0.32$	$2.30^{a} \pm 0.19$	$2.08^{b} \pm 0.08$	$2.15^{b} \pm 0.08$
D	$2.15^{ab} \pm 0.12$	$2.00^{ab} \pm 0.04$	$1.78^{b} \pm 0.06$	$1.68^{cd} \pm 0.06$
E	$2.35^{ab} \pm 0.15$	$2.18^{ab} \pm 0.16$	$1.83^{b} \pm 0.11$	$1.53^{d} \pm 0.11$

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. Also A*llium Sativum* may be the beneficial for oral hypoglycemic agents in diabetic patients. Moreover combination of *Momordica charantia* and A*llium Sativum* will be used for the treatment of diabetic patients without any health hazzards.

However, due to some short comings only one trial was performed in short term basis and modern equipments are also not available. Before field application as the hypoglycemic agents in case of diabetic patients further trial on a large scale basis is needed and also to make the findings more acurate and effective further study is essential to see any adverse effect in relation to histopathology before making a definite conclusion.

CHAPTER V

CONCLUSIONS

This study concludes that:

Garlic showed better results in reducing blood glucose level in alloxan induced rabbits.

Bitter melon showed also better results in reducing blood glucose level in alloxan induced rabbits.

But combined both garlic and bitter melon showed splendid results to reducing blood glucose level in alloxan induced rabbits.

Clinician could consider garlic and bitter melon for diabetic patient as a practical choice for reducing blood glucose level.

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