Evaluation of anti-diabetic activity of Gliclazide formulation of Bangladesh on alloxan induced diabetes in mice

B. Pharm (Honors' Project Report)

A dissertation submitted to the Department of Pharmacy, Daffodil International University for the partial fulfillment of Bachelor of Pharmacy Degree

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This Project, "Evaluation of anti-diabetic activity of Gliclazide formulation of Bangladesh on alloxan induced diabetes in mice's" submitted by Shathi Akter, Department of Pharmacy, Daffodil International University, has been accepted as satisfactory for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy and approved as to its style and contents.

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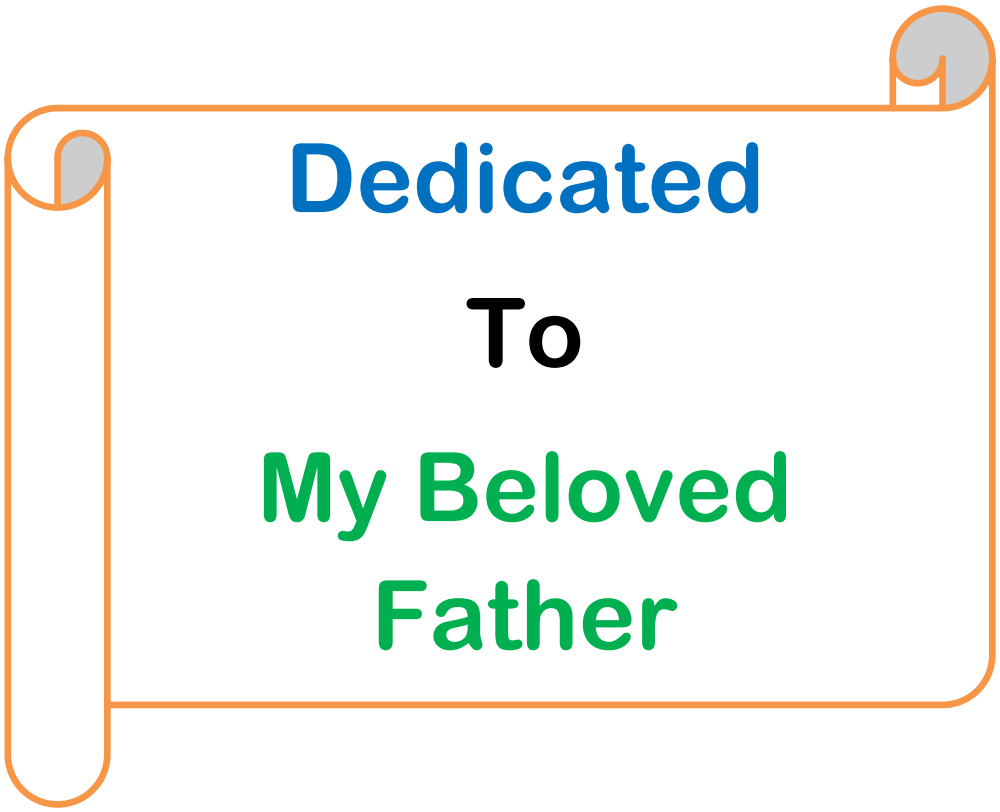
Dedicated
To
My Beloved
Father

DECLIARATION

I hereby declare that, this project report is done by me under the supervision of **Md. Al-Faruk, Senior Lecturer**, Department of Pharmacy, Daffodil International University, impartial fulfillment of the requirements for the degree of Bachelor of Pharmacy. I am declaring that this project is my original work. I am also declaring that neither this project nor any part thereof has been submitted elsewhere for the award of Bachelor or any degree.

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ABSTRACT

Objective: Four marketed oral anti-diabetic gliclazide tablets in Bangladesh have been studied for their drug content, release profile and glucose lowering capacities. This sort of study is a good indicator for in vivo evaluation of the quality of an oral anti-diabetic preparation gliclazide.

Method: Marketed preparations of gliclazide from different manufacturers were randomly chosen for this study. And the alloxan is used for the induction of diabetic in mice's. Glucometer in used to peak blood from tail vein of mice's for measuring glucose level.

Result: The drug content was within the United State Pharmacopoeia (USP) specified limit (73-87%) in all cases. The blood glucose levels were investigated after 7 days in alloxan-induced diabetic (150mg/kg b.w) in mice's (AIDMs); significant ($P<0.05$). After 14 days at 5hr of single dose (110 mg/kg body weight) treatment of the products; strongly significantly ($p<0.01$) reduced blood glucose level 54.75%, 61.93%, 56.5% and 61%respectively; which were consistent with anti-hyperglycemic effects of standard gliclazide (70%).

Conclusion: All the products were found to be qualified in very significantly lowering blood glucose level. It may be inferred that of the gliclazide tablets of Bangladeshi manufacturers complies with the standard specifications for drug contents, dissolution and anti-hyperglycemic properties.

Key words: Gliclazide; Oral hypoglycemic agent; Alloxan-induced diabetes in mice.

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ABBREVIATIONS

g	: Gram
L	: Litre
mg	: miligram
ml	: milliliter
mg/dL	: milligram per decilitre
mmol/L	: milimole per litre
D	: Diabetic
B.W	: Body weight
i.p.	: Intraperitoneal
WHO	: World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Overview

Diabetes is one of the oldest diseases affecting millions of people all over the world. [1] Diabetes mellitus is now a major public health problem in the developed as well as developing countries now-a-days. It ranked seventh among the leading causes of death, and third when it's fatal complications are taken into account [2]**.** Although numerous oral hypoglycemic drugs exist alongside insulin, still there is no promising therapy to cure diabetes [**3**]**.** The number of cases of non-insulin dependent diabetes mellitus (Type-2) has increased dramatically due to the changes in lifestyle, increasing prevalence of obesity, and ageing of populations. In the year 2000, the number of diabetic patients was 151 million and is estimated to rise to 300 million by 2025 [4].The commonly encountered acute and late diabetic complications are already responsible for major causes of morbidity, disability and premature deaths in Asian countries. [1] Bangladesh is full of medicinal plants, which are used by the people for the treatment of various diseases even at this modern era. In Bangladesh about 5 million people are affected with diabetes for various reasons, in recent years the popularity of complementary medicines has increased. Pharmaceutical sector in Bangladesh is a second promising sector in recent era [5]. Due to modern technical advances (compliance with several international standards), Bangladesh is now exporting medicines including anti-cancer, anti-diabetic, vaccines for viral diseases, and hormones to US, Europe and Asian countries (around 70 countries) after meeting its local demands. [6] It is extremely important to know the compatibility of the drug and its excipients in formulation which may impair the efficacy of the drugs [7]. Their analytical as well as pharmacological assay is essential for identifying them as quality product. [8]

1.2 Prevalence of diabetes mellitus

Quantifying the prevalence of diabetes and the number of people affected by diabetes, now and in the future, is important to allow rational planning and allocation of resources. The number of people with diabetes is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity. The IDF has subsequently released estimates of the numbers of people with diabetes for 2003 and forecasts for 2025 of 194 million and 334 million, respectively. Even if the prevalence of obesity remains stable until 2030, which seems unlikely, it is anticipated that the number of people with diabetes will more than double as a consequence of population aging and urbanization. In the light of the observed increase in prevalence of obesity in many countries of the world and the importance of obesity as a risk factor for diabetes, the number of cases of diabetes in 2030 may be considerably higher than stated here [10]. The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The most important demographic change to diabetes prevalence across the world appears to be the increase in the proportion of people 65 years of age. The prevalence of diabetes is higher in men than women, but there are more women with diabetes than men. The urban population in developing countries is projected to double between 2000 and 2030. [11] The overall rise was predicted to be much higher in developing countries than those of the developed counterpart. In developing countries, the majority of people with diabetes are in the 45 to 64 years age range, similar to the finding reported previously. [12] From the report IDF (International Diabetic Federation 2014) showed in figure1.1 [22]:

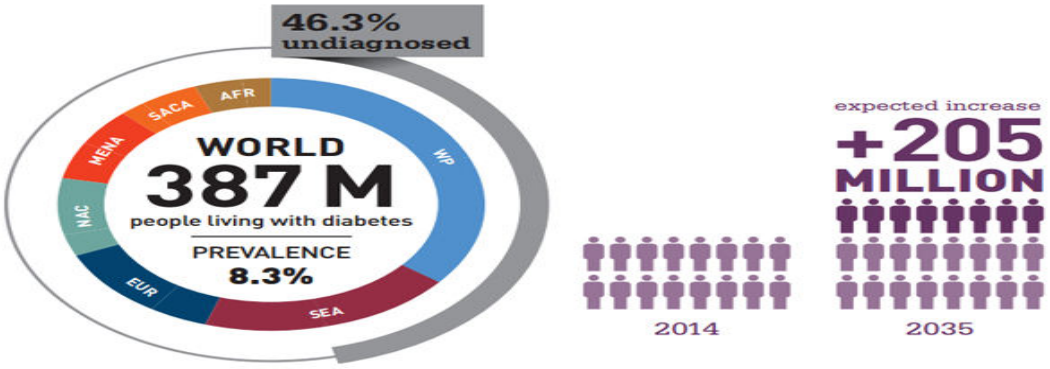

Figure-1.1: World prevalence of diabetic mellitus

Table 1.1 —List of countries with the highest numbers of estimated cases of diabetes for 2000 and 2030

Ranking	2000		2030	
	Country	Diabetic people (millions)	Country	Diabetic people (millions)
1	India	31.7	India	79.4
2	China	20.8	China	42.3
3	U.S	17.7	U.S	30.3
4	Indonesia	8.4	Indonesia	21.3
5	Japan	6.8	Pakistan	13.9
6	Pakistan	5.2	Brazil	11.3
7	Russian	4.6	Bangladesh	11.1
8	Brazil	4.6	Japan	8.9
9	Italy	4.3	Phippines	7.8
10	Bangladesh	3.2	Egypt	6.7

1.3 Prevalence of diabetic mellitus in Bangladesh

Diabetes is more prevalent in urban areas, in rural community's prevalence rates for diabetes rose from 2.3% to 6.8% in between 1999 to 2004 [9]. The prevalence of diabetes in the urban population has increased alarmingly in recent years. The prevalence of T2DM and impaired fasting glucose (IFG) and their risk factors in the urban population of Bangladesh exceeding 11.2 and 5.9%, respectively. [10]

1.4 Classification of diabetic mellitus

Diabetes mellitus (DM) is a chronic disease caused by inherited and/or acquired deficiency in the production of insulin by the pancreas, or by the ineffectiveness of the insulin that is produced1 Such a deficiency results in increased concentrations of glucose in the blood, which in turn damages many of the body's systems, in particular the blood vessels and nerves1 DM is a metabolic disorder and abnormally high blood glucose levels (hyperglycemia) [13]. The most widely accepted classification of diabetes is the etiological classification of disorders of glycaemia [14].

A. Type 1 Diabetes Mellitus.

B. Type 2 Diabetes Mellitus.

C. Gestational diabetes.

D. Secondary Diabetes Mellitus.

E. Other Specific Types of Diabetes.

1.4.1 Type 1 Diabetes Mellitus (β-cell destruction, usually leading to absolute insulin deficiency)

This form of diabetes, which accounts for only 5–10% of those with diabetes, previously encompassed by the terms insulin-dependent diabetes, type I diabetes, or juvenile-onset diabetes, results from a cellular-mediated autoimmune destruction of the β-cells of the pancreas. Markers of the immune destruction of the β-cell include islet cell autoantibodies, autoantibodies to insulin, autoantibodies to glutamic acid decarboxylase (GAD65), and autoantibodies to the tyrosine phosphatases IA-2 and IA-2β.

1.4.2 Type 2 Diabetes Mellitus

This form of diabetes, which accounts for ~90–95% of those with diabetes, previously referred to as non-insulin-dependent diabetes, type II diabetes, or adult-onset diabetes, encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency. At least initially, and often throughout their lifetime, these 5 individuals do not need insulin treatment to survive. There are probably many different causes of this form of diabetes. Although the specific etiologies are not known, autoimmune destruction of β-cells does not occur, and patients do not have any of the other causes of diabetes listed above or below. Most patients with this form of diabetes are obese, and obesity itself causes some degree of insulin resistance. Patients who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region.

1.4.2.1 Lifestyle in type 2 diabetic

A number of lifestyle factors are known to be important to the development of T2DM. People who had high levels of physical activity, a healthy diet, did not smoke, and consumed alcohol in moderation had an 82% lower rate of diabetes [15]. Obesity has been found to contribute to approximately 55% T2DM and decreasing consumption of saturated fats and trans fatty acids while replacing them with unsaturated fats may decrease the risk [16].

1.4.2.2 Medical Conditions

There are many medical conditions which can potentially give rise to or exacerbate T2DM. These include obesity, hypertension, elevated cholesterol (combined hyperlipidemia), and with the condition often termed metabolic syndrome (it is also known as Syndrome X, Reavan's syndrome). Other causes include acromegaly, Cushing's syndrome, thyrotoxicosis, pheochromocytoma, chronic pancreatitis, cancer and drugs, additional factor founds to increase the risk of T2DM includes aging [17].

1.4.2.3 Genetics

There is a strong inheritable genetic connection in T2DM: having relatives (especially first degree) with type 2 increases risks of developing T2DM very substantially. In addition, there is also a mutation to the Islet Amyloid Polypeptide gene at results in an earlier onset, more severe form of diabetes [15]. Genes significantly associated with developing T2 DM: include *TCF7L2, PPARG, FTO, NOTCH2, WFS1, CDKAL1, IGF2BP2, SLC30A8, JAZF1,* and HHEX [18].

1.4.2.4 Medications

Drugs used for any of several conditions, can interfere with the insulin regulation system, possibly producing drug induced hyperglycemia. Some examples follow, giving the biochemical mechanism in each case:

a) Atypical Antipsychotics - Alter receptor binding characteristics, leading to increased insulin resistance.

b) Beta-blockers - Inhibit insulin secretion [19].

c) Calcium Channel Blockers - Inhibits secretion of insulin by interfering with cytosolic calcium release.

d) Corticosteroids - Cause peripheral insulin resistance and gluconeogensis.

e) Fluoroquinolones- Inhibits insulin secretion by blocking ATP sensitive potassium channels.

f) Niacin - Causes increased insulin resistance due to increased free fatty acid mobilization.

g) Phenothiazines - Inhibit insulin secretion.

h) Protease Inhibitors -Inhibit the conversion of pro insulin to insulin.

i) Somatropin - May decrease sensitivity to insulin, especially in those susceptible.

j) Thiazide Diuretics - Inhibit insulin secretion due to hypokalernia. They also cause increased insulin resistance due to increased free fatty acid mobilization [18].

1.4.3 Gestational Diabetes

Gestational diabetes is a condition in which women without previously diagnosed diabetes exhibit high blood glucose levels during pregnancy. The precise mechanisms underlying gestational diabetes remain unknown. Pregnancy hormones and other factors are thought interface with the action of insulin as it binds to the insulin receptor. The interference probably occurs at the level of the cell signaling pathway behind the insulin receptor. Since insulin promotes the entry of glucose into most cells, insulin resistance prevents glucose from entering the cells properly. As a result, glucose remains in the bloodstream, where glucose levels rise. More insulin is needed to overcome this resistance [19].

Placental hormones and to a lesser extent increased fat deposits during pregnancy, seem to mediate insulin resistance during pregnancy. Early in pregnancy, maternal estrogen and progesterone increase and promote pancreatic cell hyperplasia and increase insulin release [20]. Increase in peripheral glucose utilization and glycogen storage with a concomitant reduction in hepatic glucose production, result in lower maternal fasting glucose levels [20].

1.4.4 Other specific types of diabetes

1.4.4.1 Genetic defects of the β-cell

Several forms of diabetes are associated with monogenetic defects in β-cell function. These forms of diabetes are frequently characterized by onset of hyperglycemia at an early age (generally before age 25 years). They are referred to as maturity-onset diabetes of the young and are characterized by impaired insulin secretion with minimal or no defects in insulin action. They are inherited in an autosomal dominant pattern. [20]

1.4.4.2 Genetic defects in insulin action

There are unusual causes of diabetes that result from genetically determined abnormalities of insulin action. The metabolic abnormalities associated with mutations of the insulin receptor may range from hyperinsulinemia and modest hyperglycemia to severe diabetes. [13]

1.4.4.3 Diseases of the exocrine pancreas

Any process that diffusely injures the pancreas can cause diabetes. Acquired processes include pancreatitis, trauma, infection, pancreatectomy, and pancreatic carcinoma. With the exception of that caused by cancer, damage to the pancreas must be extensive for diabetes to occur; adrenocarcinomas that involve only a small portion of the pancreas have been associated with diabetes. This implies a mechanism other than simple reduction in β-cell mass. Fibrocalculouspancreatopathy may be accompanied by abdominal pain radiating to the back and pancreatic calcifications identified on X-ray examination. Pancreatic fibrosis and calcium stones in the exocrine ducts have been found at autopsy. [11]

1.4.4.4 Endocrinopathics

Several hormones (e.g., growth hormone, cortisol, glucagon, and epinephrine) antagonize insulin action. Excess amounts of these hormones (e.g., acromegaly, Cushing's syndrome, glucagonoma, pheochromocytoma, respectively) can cause diabetes. This generally occurs in individuals with preexisting defects in insulin secretion, and hyperglycemia typically resolves when the hormone excess is resolved. [21]

1.4.4.5 Drug- or chemical-induced diabetes

Many drugs can impair insulin secretion. These drugs may not cause diabetes by themselves, but they may precipitate diabetes in individuals with insulin resistance. In such cases, the classification is unclear because the sequence or relative importance of β-cell dysfunction and insulin resistance is unknown. Certain toxins such as Vacor (a rat poison) and intravenous pentamidine can permanently destroy pancreatic β-cells. Such drug reactions fortunately are rare. There are also many drugs and hormones that can impair insulin action. Examples include nicotinic acid and glucocorticoids. Patients receiving α-interferon have been reported to develop diabetes associated with islet cell antibodies and, in certain instances, severe insulin deficiency. [11]

1.4.4.6 Infections

Certain viruses have been associated with β-cell destruction. Diabetes occurs in patients with congenital rubella, although most of these patients have HLA and immune markers characteristic of type 1 diabetes. In addition, coxsackievirus B, cytomegalovirus, adenovirus, and mumps have been implicated in inducing certain cases of the disease.[14]

1.4.4.7 Uncommon forms of immune-mediated diabetes

In this category, there are two known conditions, and others are likely to occur. The stiff-man syndrome is an autoimmune disorder of the central nervous system characterized by stiffness of the axial muscles with painful spasms. Patients usually have high titers of the GAD autoantibodies, and approximately one-third will develop diabetes.[13]

Anti–insulin receptor antibodies can cause diabetes by binding to the insulin receptor, thereby blocking the binding of insulin to its receptor in target tissues. [11]

1.4.4.8 Other genetic syndromes sometimes associated with diabetes

Many genetic syndromes are accompanied by an increased incidence of diabetes mellitus. These include the chromosomal abnormalities of Down's syndrome, Klinefelter's syndrome, and Turner's syndrome. Wolfram's syndrome is an autosomal recessive disorder characterized by insulin-deficient diabetes and the absence of β-cells at autopsy. Additional manifestations include diabetes insipidus, hypogonadism, optic atrophy, and neural deafness.

Clinical aspect of diabetes mellitus generally includes, sign and symptom, complication and diagnosis. [21]

1.5.1 Symptoms of Diabetes Mellitus

The classical symptoms of diabetes are polyuria (frequent urination), polydipsia (increased polyphagia (increased hunger), emaciation (unusual weight loss), extreme fatigue or lack of energy, blurred vision, frequent or recurring infections, cuts and braises (Figure1.2) that are slow to heal, tingling or numbness in hands and/or feet.

Symptoms may develop rapidly (weeks or months) in type 1 diabetes while in type 2 diabetes they usually develop much more slowly and may be subtle or absent [23].

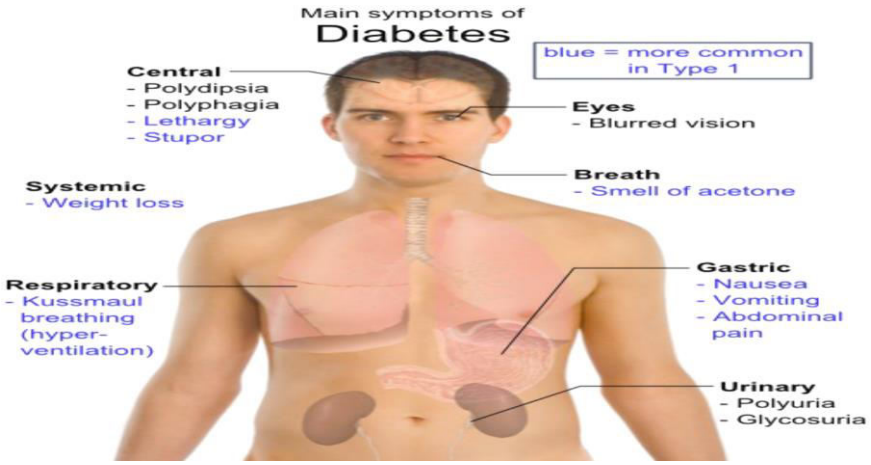

Figure 1.2 Overview of most significant symptoms of diabetes

1.5.2 Complications of Diabetes Mellitus

Diabetic complications can be classified broadly as acute glycemic (short-term) and chronic (long-term) complications. Other complications of diabetes include infections, metabolic difficulties, impotence, autonomic neuropathy and pregnancy problems [24].

1.5.2.1 Acute Glycemic Complications

Acute complication mainly include: Diabetic Ketoacidosis, Hyperglycemic Hyperosmolar Nonketotic Coma, and Hypoglycemia [24]. [10]

1.5.2.1.1 Diabetic Ketoacidosis (DKA)

Insulin deficiency causes the body to metabolize triglycerides and muscle instead of glucose for energy. Serum levels of glycerol and free fatty acids (FFAs) rise because of unstrained lipolysis, as does alanine from muscle catabolism. Glycerol and alanine provide substrate for hepatic gluconeogenesis, which is stimulated by the excess of glucagon that accompanies insulin deficiency. Glucagon also stimulates mitochondrial conversion of FFAs into ketones (figure1.3). Insulin normally blocks ketogenesis by inhibiting the transport of FFA derivatives into the mitochondrial matrix, but ketogenesis proceeds in the absence of insulin. The major ketoacids produced: acetoacetic acid and f3- hydroxybutyric acid are strong

organic acids that create metabolic acidosis. Acetone derived from the metabolism of acetoacetic acid accumulates in serum and is slowly disposed of by respiration [25]. Vomiting, dehydration, deep gasping breathing, confusion and occasionally coma are typical symptoms of DKA [26].

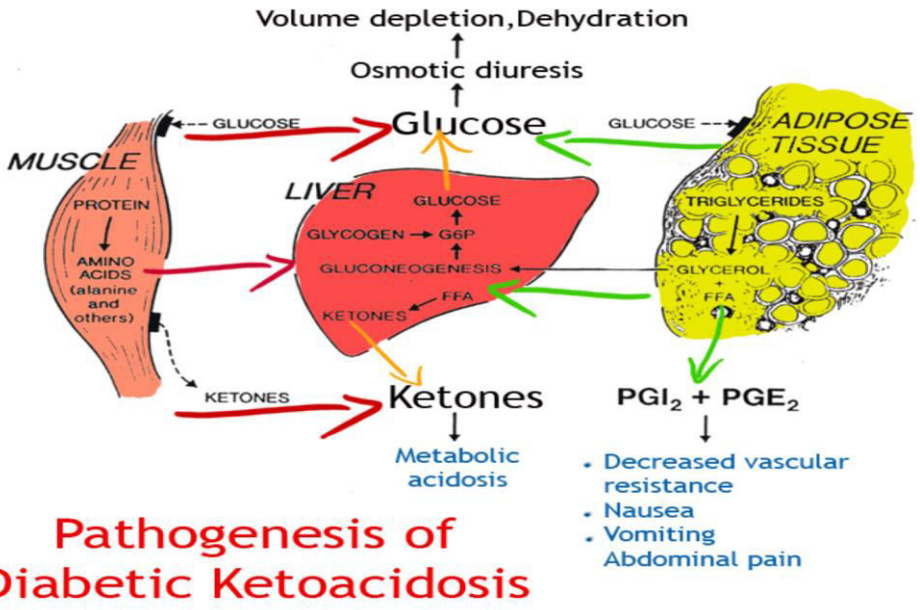

Figure 1.3: Pathogenesis of Diabetic Keratocidosis

1.5.2.1.2 Hyperglycemic hyperosmolar non-ketotic coma (HHNKC)

Hyperglycemic hyperosmolar non-ketotic coma (HHNKC) is characterized by severe hyperglycemia (glucose level typically greater than 600 to 800 mg/dL), dehydration, and altered mental status due to the absence of ketosis. It usually develops after a period of symptomatic hyperglycemia in which fluid intake is inadequate to prevent extreme dehydration from the hyperglycemia-induced osmotic diuresis. In this case, focal central system deficits may occur [27].

1.5.2.1.3 Hypoglycemia

Any person with diabetes who takes an oral hypoglycemic agent or insulin may experience low blood glucose. The mechanism is depicted in figure1.4. Severe hypoglycemia occurs when the patient inappropriately treats, ignores or does not recognize the early warning signs or when glucose counter regulation fails to return at the normal blood glucose level [28].

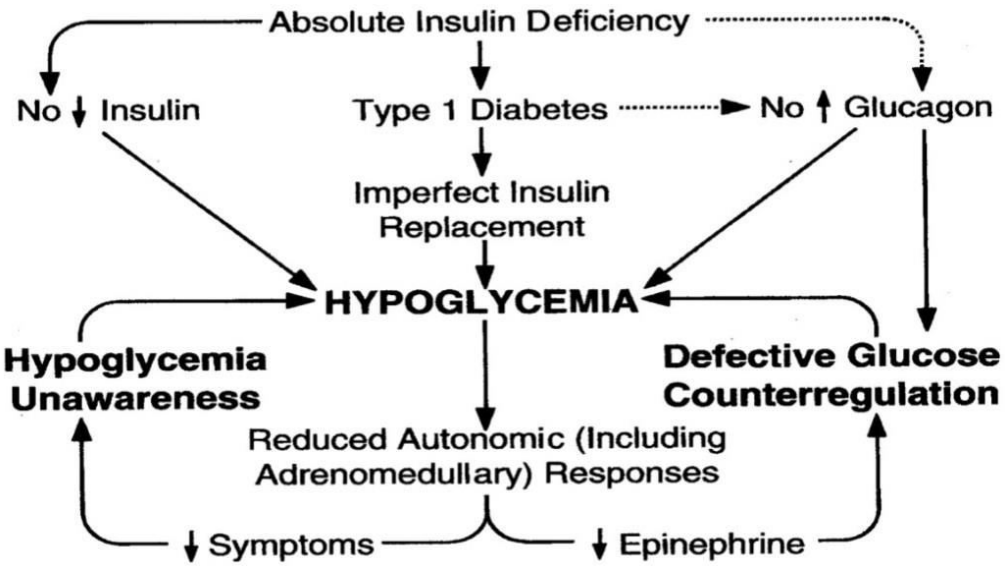

Figure 1.4: Hypoglycemia-Associated Autonomic Failures

1.5.2.2 Chronic Complications

Chronic complications generally develop after 12-15 years of diabetes. These complications may lead to organ dysfunction through micro vascular and macro vascular damage. Micro vascular complications include neuropathy (nerve damage), nephropathy (kidney disease) and vision disorders (e.g.; retinopathy, glaucoma, cataract and cornea! disease). Macro vascular complications include heart disease, stroke and peripheral vascular disease (which can lead to ulcers, gangrene and amputation) [29].

1.5.2.2.1 Diabetic Neuropathy

Increased levels of glucose cause an increase in intracellular diacylglycerol, which activates PKC. Moreover, excessive activation of the polyol pathway leads to increased levels of sorbitol and reactive oxygen molecules and decreased levels of nitric oxide and glutathione, as well as increased osmotic stresses on the cell membrane. Any one of these elements alone can promote neuronal cell damage [30].

1.5.2.2.2 Diabetic Nephropathy

In kidney, thickening of basement membrane interferes with the normal filtration properties of the capillaries hi the glomerulus, resulting in increased renal failure characterized by adecreased glomerular filtration rate. As the severity increases, the filtration become so poor that toxic end products accumulate hi the blood. Finally, total kidney failure occurs, which is known as nephropathy [29].

1.5.2.2.3 Macrovascular Disease

Macrovascular disease accounts for more than 70% of deaths hi people with diabetes, mostly from atherosclerosis (myocardial infarction), angina pectoris, congestive cardiac failure, stroke and peripheral arterial disease [30].

1.5.2.2.4 Psychological Complication

Diabetes itself does not cause changes hi personality or psychiatric illness, but particular subgroups of the diabetic population appear to be at risk for developing psychosocial problems. Young people with insulin- dependent diabetes mellitus (IDDM) may have a higher prevalence of eating disorders, such as anorexia nervosa and bulimia. Adults with longstanding diabetes may have a higher prevalence of symptoms of depression and anxiety [31].

1.6 Diagnosis of Diabetes Mellitus

Diagnostic interpretations of the fasting and 2-hr post load concentration in non-pregnant subjects are as follows:

1.6.1 Fasting Plasma Glucose (FPG) Test

FPG test measures blood glucose in a person who has not eaten anything for at least 8 hours. This test is used to detect diabetes and pre-diabetes [11]

Table: 1.2 FPG (Fasting Plasma Glucose)Test

Plasma Glucose Result	**Diagnosis**
3.89-6.1 1 mmol/L (70-1 10 mg/dl)	Normal
>6.1 K7.0 mmol/L (>100<126 mg/dl)	Pre-diabetes (impaired)
>7.78mmol/L (>140 mg/dl)	Diabetes

1.7 Therapy for Diabetes Mellitus

The goal of diabetes management is to keep blood glucose levels as close to normal as safely possible. People with diabetes must take responsibility for their day-to-day care. This includes monitoring blood glucose levels, dietary management, maintaining physical activity, keeping weight and stress under control, monitoring oral medications and if required, insulin use via injections or pump.[31]

1.7.1 Dietary Management and Physical Activity

In people with diabetes, food is an important part of treatment and diet has long been considered as the cornerstone in the management of diabetes. The word diet control which is generally used in this treatment does not mean eating less or sacrificing favorite foods in life. It means a planned regulated diet that will meet the nutritional needs of the body. The nutritional needs of a diabetic patient will remain same as before the diabetic was detected.

1.7.2 Anti-diabetic Drugs

For treating T2DM subjects, when patients fail to maintain normoglycemia by maintaining diet and exercise alone, the first line drugs are the oral hypoglycemic agents (OHAs).

The table below shows the sites of actions of drugs used for blood glucose control in T2DM, other than insulin [32].

Class	Action	Mechanism of action	Example
Sulphonylureas	Increase insulin secretion	Binds to sulphonylurea receptor on p-cell, leading to closure of ATP-sensitive potassium channels.	*Glibendamid, Gliclazide Glimepiride, Tolazamide*
Meglitinide Analogue			*RepaglinideNateglinide*
Thiazolidinediones	Reduce insulin resistance	PPARY agonist	*Pioglitazone Rosiglitazone*
Biguanides	Reduce insulin resistance, reduce hepatic glucose output	Not known	*Metformin*
a-Glucosidase inhibitors	Delay absorption of carbohydrate	Inhibits a-glucosidase Increase fibre in diet	*Acarbose Guar gum*
Agents that reduce fat absorption	Reduce weight	Inhibits pancreatic lipase	*Orlistat*
Centrally -acting agents		Serotonin and norepinephrine reuptake inhibitor	Sibutramine

Table 1.3 Oral agents used in the treatment of type 2 diabetes mellitus

1.7.3 Insulin Therapy

Insulin therapy is often an important part of diabetes treatment. People with type 1 diabetes require supplemental insulin because their bodies can no longer produce insulin themselves. However, T2DM is different. Less than one-third of patients with T2DM take insulin [37].

There are a number of different types of insulin available to fit everyone's lifestyle. Although there are several variations, the main types of insulin are:

i. **Rapid-acting**: Starts to work in about 5 minutes, reaches the peak of effectiveness in about one hour and continues working for up to four hours [38].

ii. **Regular or Short-acting**: This type of insulin begins to work in about 30 minutes, reaches the peak of effectiveness anywhere between two and three hours and continues working up to six hours [38].

iii. **Intermediate-acting**: Usually begins to work in two to four hours, reaches the peak of effectiveness anywhere between two and three hours and continues working up to six hours [37].

iv. **Long-acting**: Usually begins to work in six to ten hours and continues working up to 24 hours [38].

1.8 Approaches on Therapeutic Research on Diabetes Mellitus

Considering the limitation of existing therapies in restoring the quality of life to normal as well as reducing the risk of chronic diabetic complications by maintaining normal blood glucose level, search for alternating sources of oral hypoglycemic agents is a requirement. Hence, the inability of current therapies to control all the metabolic defects of diabetes and their pathological consequences and the great expense of modern therapy, there is a clear need for the development of alternative straregies for diabetes therapy [39].

1.8.1 Regeneration Therapy for Diabetes Mellitus

Regeneration therapy can be classified into three categories. The first category, in vitro regeneration therapy, makes use of transplanted cultured cells including embryonic stem (ES) cells, pancreatic precursor cells and beta-cell lines, in conjunction with immunosuppressive therapy or immune isolation for the treatment of patients with Type 1 diabetes. In the second type of regeneration therapy, ex vivo regeneration therapy, a patient's own cells such as bone marrow stem cells are transiently removed and induced to differentiates into beta-cells in vitro. However, at the present time, insulin-producing cells cannot be generated from bone marrow stem cells. In vivo regeneration therapy, the third type of regeneration therapy, enables impaired tissue to regenerate from a patient's own cells in vivo. Beta-Cell neogenesis from non-beta-cells and beta-cell proliferation in vivo has been considered in particular as regeneration therapies for patients with T2DM. Regeneration therapy for pancreatic beta-cells can be combined with various other therapeutic strategies, including islet transplantation, cell-based therapy, gene therapy and drug therapy to promote beta-cell proliferation and neogenesis. It is hoped that these strategies will in the future provide a cure for diabetes [40].

1.8.2 Restoration Therapy

The development of transgenic mice which express a protein called hepatocyte growth factor (HGF) holds another possibility as a treatment for diabetes. By encouraging the growth of pancreatic islet cells, this may allow beta cell proliferation, an increase in the total mass of islet cells and ultimately an increase in the production of insulin. To date, the development of HGF has only worked in vitro, but the findings on the transgenic mice in vivo showed a significantly decreased blood glucose level, increased pancreatic insulin levels as compared to controls and an increase hi the volume of islet cells. Probably the most important treatment option on the horizon is the use of stem cells for the treatment of diabetes [41].

1.9 Diabetes and Hyperlipidemia

The most common cardiovascular disease associated with hyperlipidemia is atherosclerosis. Atherosclerosis is the condition in which an artery wall thickens as the result of a build-up of fatty materials such as cholesterol. It is a syndrome affecting arterial blood vessels by a chronic inflammatory response in the walls of arteries. It is commonly referred to as a hardening or furring of the arteries. It is caused by the formation of multiple plaques within the arteries with eventual obstruction of blood flow. Diabetes is one of the risk factors usually thought to cause the formation of multiple plaques through endothelial damage of injury. Atherosclerosis events such as MI, cerebrovascular accident and gangrene of the legs ate most frequent concomitants of diabetes mellitus [42].

1.10 Aim of the work

The pathogenesis of diabetes mellitus is managed by insulin and oral administration of hypoglycemic drugs such as sulfonylureas and biguanides [46]. The discovery of the widely used sulfonylurea as anti-diabetic drug is Gliclazide N-(4-methylbenzene sulfonyl). Now-a-days although numerous natural remedies are being investigated [47-49] to alleviate the diabetic complications; still its regular control largely depends on chemical approach. Gliclazide given alone or in combination [51] with biguanides improves glycemic control and lipid concentration in patients who respond poorly to diet [50].

Pharmaceutical sector in Bangladesh is a second promising sector in recent era [52]. Due to modern technical advances (compliance with several international standards), Bangladesh is now exporting medicines including anti-cancer, anti-diabetic, vaccines for viral diseases, and hormones to US, Europe and Asian countries (around 70 countries) after meeting its local demands [53]. For exporting the products, they must comply several specifications settled by the local supervisory authority and internationals standard organizations (ISO), world health organizations (WHO) and so on. Our current study is a part of series bioequivalence assessment of several kinds of medicine checking their stability, dissolution characteristics and therapeutic efficacy which are the prerequisite for being a quality medicine in local as well as international pharma market. Gliclazide is manufactured by several pharmaceutical companies of Bangladesh in different brand names [53], it is extremely important to know the compatibility of the drug and its excipients in formulation which may impair the efficacy of the drugs [54]. Their analytical as well as pharmacological assay is essential for identifying them as quality product. This type of study [55] is a good marker for *in vivo* evaluation of a quality product. In the present work we use simple techniques for measuring the content of active drug, dissolution characteristics and pharmacological efficacy of these formulations.

1.11 General description of Gliclazide

Gliclazide is N-(4-methylbenzene sulfonyl) -N'-(3-azabicyclo [3.3.0] oct-3-yl) urea or 1-(3-azabicyclo[3.3.0] oct-3-yl) -3-p-tolylsulfonyl[42], Molecular weight 323.4, is a white or almost white crystalline powder, odorless, tasteless, m.p., 165-170^{o} C [44]. It is official in British Pharmacopoeia 2007[44]. Gliclazide (Glz) is a second-generation sulphonylurea oral hypoglycemic agent used in the treatment of non-insulin dependent diabetes mellitus (T2DM). It stimulates insulin secretion by pancreatic beta cells. In the long-term, it reduces hepatic gluconeogenesis, and increases insulin effects by acting at receptor or post-receptor sites. It also inhibits platelet aggregation and increases fibrinolysis [33, 34]. A survey of literature has revealed few U V spectrophotometric methods for simultaneous estimations of gliclazide in pharmaceutical formulation [34, 35] and for estimation of gliclazide and metformin in combined tablet dosage form [36].

1.11.1 Dosage & Administration of Gliclazide

Determination of the proper dosage for Gliclazide MR (modified release) for each patient should be made on the basis of frequent determinations of blood glucose during dose titration and throughout maintenance.

The daily dose of Gliclazide MR may vary from 30 to 120 mg once daily (i.e., one half tablet to 2 tablets of Gliclazide MR 60 mg, or 1 to 4 tablets of Gliclazide MR 30 mg).[56]

Recommended Dose and Dosage Adjustment

The recommended starting dose of Gliclazide MR is 30 mg daily, i.e. one half tablet of Gliclazide MR 60 mg or one tablet of Gliclazide MR 30 mg, even in elderly patients (over 65 years old). A single daily dose provides effective blood glucose control. The single

daily dose may be between 30 mg and 90 mg, or even 120 mg. The daily dose should not exceed 120 mg. [56]

Dose adjustment should be carried out in steps of 30 mg, according to the blood glucose response. Each step should last for at least two weeks.

For Gliclazide 80mg plain formulation: Initially, 40-80 mg daily gradually increased to 320 mg daily if necessary. Doses >160 mg daily may be given in 2 divided doses. [56]

Administration

It is recommended that the medication be taken at breakfast time. The 30 mg tablets cannot be split in half and should be swallowed whole. The 60 mg tablets (when available) can be halved. Both the 30 mg and 60 mg tablets must not be chewed or crushed. [56]

- Previously untreated patients should commence with a dose of 30 mg and will benefit from dose adjustment until the appropriate dose is reached.

- One Gliclazide MR 60 mg tablet is equivalent to two Gliclazide MR 30 mg tablets. The breakability of the Gliclazide modified-release 60 mg tablet allows the use of a dose of 30 mg as a half tablet and of 90 mg as one and a half tablets.

- Half a tablet of Gliclazide MR 60 mg or one tablet of Gliclazide MR 30 mg corresponds to one tablet of Gliclazide 80 mg.

- Gliclazide MR can replace an anti-diabetic treatment without any transitional period. If a patient is switched from a hypoglycemic sulfonylurea with a prolonged half-life (i.e. chlorpropamide) he/she should be carefully monitored (for 1 to 2 weeks) in order to avoid hypoglycemia due to possible residual effects of the previous therapy. [56]

Geriatrics

No significant differences in efficacy and tolerance were observed between patients over 65 years of age and younger patients, however greater sensitivity of some older individuals cannot be ruled out. Patients over 65 years of age should be started with Gliclazide MR 30 mg with dosage adjustments being made cautiously. [57]

Hepatic or Renal Impairment

Patients with renal or hepatic impairment should be started with Gliclazide MR 30 mg with dosage adjustments being made cautiously. [57]

Patients receiving Insulin

Maturity onset diabetics with no ketoacidosis or history of metabolic decompensation and whose insulin requirements are less than 40 units per day may be considered for Gliclazide MR therapy after cessation of insulin.

If a change from insulin to Gliclazide MR is contemplated in such a patient, discontinue insulin for a period of 2 or 3 days to determine whether any therapy other than dietary regulation and exercise is needed. During this insulin-free interval, test the patient's urine at least 3 times daily for glucose and ketone bodies and monitor the results carefully. The appearance of significant ketonuria accompanied by glucosuria within 12 to 24 hours after the withdrawal of insulin, strongly suggests that the patient is ketosis prone, and precludes the change from insulin to sulfonylurea therapy. [56]

CHAPTER TWO

LITERATURE REVIEW

2.1 Gliclazide

Gliclazideis a 'second generation' oral hypoglycemic agent. The particular interest with this drug is that it has shown certain effects on the blood for which it is hoped there may be some clinical benefit in diabetic angiopathies. Initial trials have suggested that gliclazide therapy may reverse or at least slow down the progression of diabetic retinopathy. [56]

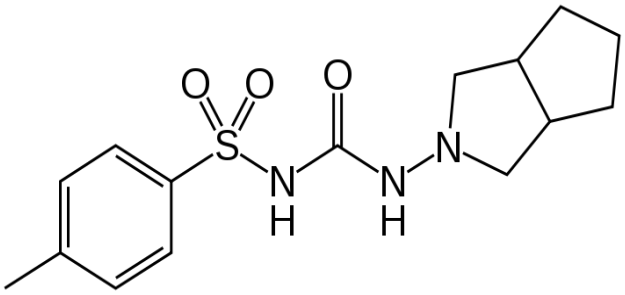

Figure 2.1: Structure of Gliclazide

Both newly diagnosed maturity onset diabetics as well as those previously treated with sulphonylureas respond well to gliclazide therapy. In the small comparative studies which have been reported, gliclazide was of comparable efficacy to other oral hypoglycaemic agents.

Gliclazide has been well tolerated by most patients, the most frequently reported side effects being gastrointestinal in nature which occurred in less than 2% of patients. [57]

2.1.1 Clinical pharmacology of Gliclazide

Gliclazide is a sulphonylurea drug with an intermediate half-life of around 11 hours. It is extensively metabolised, and renal clearance accounts for only 4% of total drug clearance. The molecule contains an azabicyclo-octyl group which confers special properties on the basic sulphonylurea moiety. Gliclazide stimulates insulin secretion through the β cell sulphonylurea receptor, and possibly through a direct effect on intracellular calcium

transport. It specifically improves the abnormal first phase insulin release in type 2 diabetes, and also has an effect on the second phase. This pattern of insulin release is thought toexplain the lower incidence of hypoglycaemic episodes and weight gain compared with some other sulphonylureas. There is also a reduction in hepatic glucose production and improvement in glucose clearance, without changes in insulin receptors. This suggests a possible post-receptor effect on insulin action, perhaps by stimulation of hepatic fructose-2,6-bisphosphatase and muscle glycogen synthase. Gliclazide reduces platelet adhesion, aggregation and hyperactivity and increases fibrinolysis (Golay et al., 1984). These actions, thought to be independent of its hypoglycaemic activity, may make gliclazide useful in halting the progression of diabetic microangiopathy.

2.1.2 Mechanism of Gliclazide

Gliclazide binds to the β cell sulfonyl urea receptor (SUR1). This binding subsequently blocks the ATP sensitive potassium channels. The binding results in closure of the channels and leads to a resulting decrease in potassium efflux leads to depolarization of the β cells. This opens voltage-dependent calcium channels in the β cell resulting in calmodulin activation, which in turn leads to exocytosis of insulin containing secretory granules. [58]

2.1.3 Absorption, Distribution, Metabolism and Fate of Gliclazide

Rapidly and well absorbed but may have wide inter- and intra-individual variability. Peak plasma concentrations occur within 4-6 hours of oral administration. Gliclazide is distributed in the extracellular fluid, leading to high concentrations in the liver, kidneys, skin, lungs, skeletal muscle, intestinal and cardiac tissue when administered to animals. Extensively metabolized in the liver. Less than 1% of the orally administered dose appears unchanged in the urine. Metabolites include oxidized and hydroxylated derivate, as well as glucuronic acid conjugates. Metabolites and conjugates are eliminated primarily by the kidneys (60-70%) and also in the feces (10-20%). [60]

2.1.4 Adverse effects of Gliclazide

The most notable effects are hypoglycaemia; gastrointestinal disturbances such as constipation, nausea, epigastric discomfort and heartburn; dermatological reactions such as rash and transient itching; and biochemical abnormalities such as elevated serum creatinine, increased serum alkaline phosphatase, raised serum AST, elevated BUN and raised serum bilirubin. Headache, slight disulfiram-like reactions and lassitude have been reported. Although very rare, severe hypoglycaemia may occur in patients receiving gliclazide. [58]

2.2 Alloxan

Alloxan is the most commonly used chemical for induction of diabetes mellitus. It is a well-known diabetogenic agent widely used to induce Type-II diabetes in animals. Alloxan is a urea derivative which causes selective necrosis of the pancreatic islet β-cells. [59]

Alloxan is a toxic glucose analogue that preferentially accumulates in pancreatic beta cells via the GLUT2 glucose transporter. In the presence of intracellular thiols, especially glutathione, alloxan generates reactive oxygen species (ROS) in a cyclic redox reaction with

its reduction product, dialuric acid. Autoxidation of dialuric acid generates superoxide radicals, hydrogen peroxide and, in a final iron-catalyzed reaction step, hydroxyl radicals. These hydroxyl radicals are ultimately responsible for the death of the beta cells, which have a particularly low antioxidative defense capacity, and the ensuing state of insulin-dependent "alloxan diabetes". As a thiol reagent, alloxan also selectively inhibits glucose-induced insulin secretion through its ability to inhibit the β cell glucose sensor glucokinase.

In 1838, Wolver and Liebig [61] synthesized a pyrimidine derivative, which they later called alloxan.

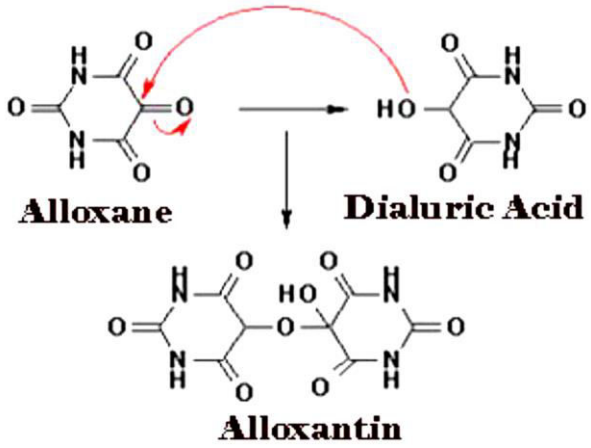

Figure-2.2: Structural formulas of alloxan and dialuric acid

In 1943, alloxan became of interest in diabetes research when Dunn and McLetchie reported that it could induce diabetes in animals as a result of the specific necrosis of the pancreatic beta cells [62] [61] [60]. The resulting insulinopenia causes a state of experimental diabetes mellitus called "alloxan diabetes" [63] [64] [65]. The reduction product of alloxan, dialuric acid has also been shown to be diabetogenic in animals and to cause ultra-structural changes identical to those observed in response to alloxan.

Alloxan selectively inhibits glucose-induced insulin secretion through specific inhibition of glucokinase, the glucose sensor of the β cell, and it causes a state of insulin-dependent

diabetes through its ability to induce ROS formation resulting in the selective necrosis of betacells. It can generate reactive oxygen species (ROS) in a cyclic reaction with the reduction product dialuric acid, as depicted in the following figure.

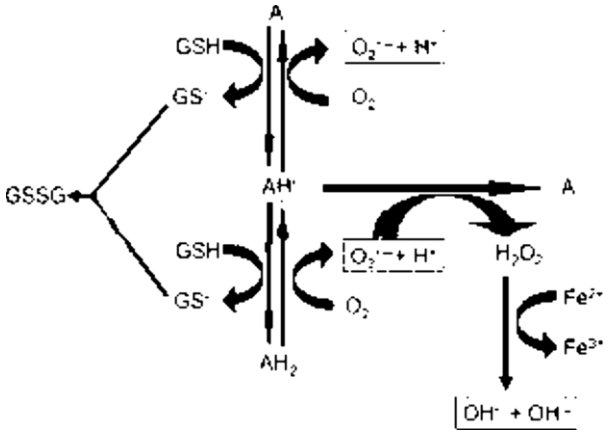

Figure-2.3: Redox cycling reactions between alloxan and dialuric acid

2.2.1 Alloxan induced diabetes

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 [60].

Moderate diabetic animals are recommended for use in testing drugs for use in Non-insulin dependent diabetes mellitus. For all animals a single dose of alloxan, 140–180 mg/kg (usually 150 mg/kg) is administered as a5% w/v in distilled water injected intravenously in to the marginal ear vein of rabbit or intraperitoneally in case of mice and rats. [59]

A rest period of seven days for rabbits, 14 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. Thereafter, 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 [59].

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 anaesthetized 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 assess the effect of alloxan and to chemically establish the diabetic condition, an incision was done in any of the fore veins in the tail of the rat with a 15 scapel blade 10days 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 [59].

CHAPTER THREE

METHOD AND MATERIALS

3.1 Materials

Four Gliclazide tablets of different Bangladeshi manufacturers were purchased from local market of Dhaka, Bangladesh. They were randomly marked as F-1 to F-4 and stored. The labels of all the products were claimed to contain 80 mg of active ingredient per tablet. Standard Gliclazide was given from technology laboratory of department of Pharmacy, Daffodil International University, Bangladesh. Alloxan was purchased from Supertech Co Ltd. All other chemicals were of reagent grade.

3.2 Instruments

USP Type-II (Paddle) Dissolution Tester (USP), UV spectrophotometer, Glucose meter Cera-chek 1070 (model: G 300, Korea), Electronic balance (Shemadzu, ATY224), pH meter HI 2210 (HANNA instruments)

3.3 Assay method

Twenty tablets were weighed and powdered. Powder equivalent to 100 mg of Gliclazide was weighed accurately and transferred into a 100 mL volumetric flask were shaken for 15 minutes with 100 ml of phosphate buffer and filtered using a Whatman No. 102 filter paper discarding the first 20 ml. 10 ml of the resulting solution were diluted to 100 ml and further 1 ml and 5 ml of the resulting solution were diluted to 100 ml with phosphate buffer. A standard solution of active gliclazide was also prepared to compare the drug content. Absorbance of the resulting solutions was measured at the maximum 232 nm and the content of gliclazide was calculated.

3.4 Dissolution method

A USP type-II six-vessel Dissolution Tester (Basket stirrer) with variable rotation speed was used for dissolution test. The basket rotation was set at 100 rpm and the temperature was controlled at $37^{o}C \pm 0.5^{o}C$ using 900 ml phosphate buffer of pH 6.8 as chamber volume [67]. When the dissolution medium attained the bath temperature, one tablet was placed in each vessel. Four tablets were thus tested simultaneously from each of the products of gliclazide. Samples were collected after 15, 30, 45 minutes and filtered. The absorbance was determined by UV spectrophotometer at 232 nm (UV-VIS spectrophotometer). A standard solution of the active ingredient was also prepared to compare the drug release.

3.5 Experimental animals

A total number of 25 Swiss albino mice's (5-6 weeks age, 24-42 gm body weight) female was purchased from animal house of International Centre for Diarrheal Disease Research, Bangladesh (ICDDR, B). Prior to the commencement of the experiment, all the mice were acclimatized to the new environmental condition for a period of one week. During the experimental period, mice were kept in a well-ventilated animal house at an ambient temperature ($25^{o}C$). They were supplied with standard pellets purchased by ICDDR, B (International Centre for Diarrheal Disease Research, Bangladesh) and fresh drinking water *ad libitum*. All animals were maintained with natural one weak light and dark cycle. The animals were randomly divided into groups for experiments. Animal handling and all experimental procedures were performed according to the Guidelines for Animal Experiments.

3.6 Experimental studies

The mice's was randomly assigned for grouping. The whole experiment was done by dividing mice's into seven groups and each having G-I (1), G-II and GII-G-VII each having 4 in numbers.

Table 3.1 Experimental design of the studies

Group-I	Normal Control or non-diabetic control
Group-II	Diabetic control (Alloxan induced)
Group-III	Diabetic + Gliclazide (standard control)
Group-IV	Diabetic+ Formulation-1
Group-V	Diabetic+ Formulation-2
Group-VI	Diabetic+ Formulation-3
Group-VII	Diabetic+ Formulation-4

3.7 Experimental design for Anti-diabetic Effect of Gliclazide

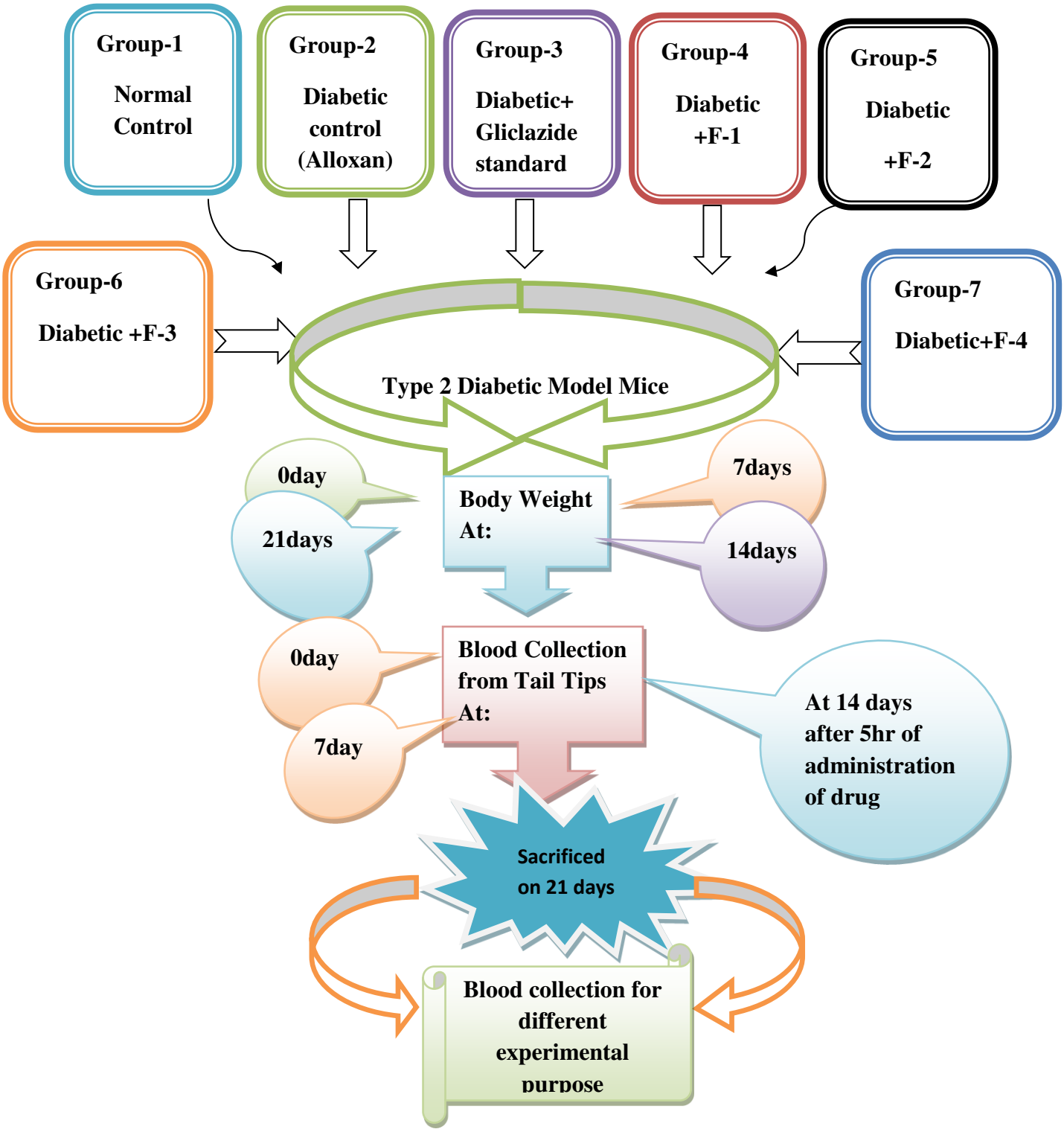

Figure 3.2 Schematic diagram indicate experimental design for anti-diabetic effect of Gliclazide.

3.8 Experimental induction of diabetes

Mice of group II-VII were rendered diabetic by intraperitoneal injection of freshly prepared solution of alloxan (150 mg/kg) in normal saline [68]. After 7 days of treatment, blood glucose content was measured by using glucometer from the tail vein of the mice. Mice with blood glucose levels above 181.2 mg/dl or 10.05mmol/L were considered as diabetic animal model for the study. Before induction of diabetes, baseline blood glucose level was also measured.

3.9 Preparation of dosage

Ten tablets of each of the four products were weighed and powdered. Doses were prepared according to the body weight of the mice and were administered orally as 110 mg/kg body weight. Powder containing equivalent amount of drug for each mice were weighed accurately and dissolved in small amount of phosphate buffer and then administered. Standard gliclazide dose was prepared by weighing required amount of gliclazide powder directly and dissolving in phosphate buffer (pH 6.8).

3.10 Drug administration on diabetic mice's

Group I served as a non-diabetic control or normal control while group II to VII were rendered diabetic. Group II served as diabetic control (Alloxan induced). Standard gliclazide was administered orally to Group III mice's. Product F1 was administered to group IV and F2, F3 and F4 were administered to group V, VI and VII mice, respectively.

3.11 Test of blood glucose level

After 5hours from the time of drug administration blood glucose levels were tested for every mice of each group. The tail vein of mice was pricked with a needle and withdrawing blood was tested with the help of glucometer (Cera-chek 1070, Korea).After oral administration of drug, mice were observed for 21 days and were sacrificed. Small amount of blood was collected directly from heart by syringes, centrifuged at 6000 rpm for 30 minutes and the serum was preserved for different experimental purposes.

3.12 Statistical analysis

Data were analyzed by ANOVA followed by Scheffe's post-hoc tests. All statistical data were expressed as mean ± standard error of the mean (SEM) and were tested using analysis of variance followed by students paired or unpaired t-test when needed. Differences were considered to be significant for values of $p< 0.05$.

CHAPTER FOUR

RESULT AND DISCUSSION

4.1 Result

The parameters of fasting blood glucose (FGL) level were measured to determine the effect of the gliclazide formulation on alloxan induced diabetic in mice's (AIDM) using gliclazide as standard anti-diabetic agents.

Time course experiment was performed for alloxan. The results are shown in the Figure 4.1 the maximum blood glucose concentration was observed between 7-14 days. Thus to check the anti-diabetic effect 7days alloxan induction was used for every experiment.

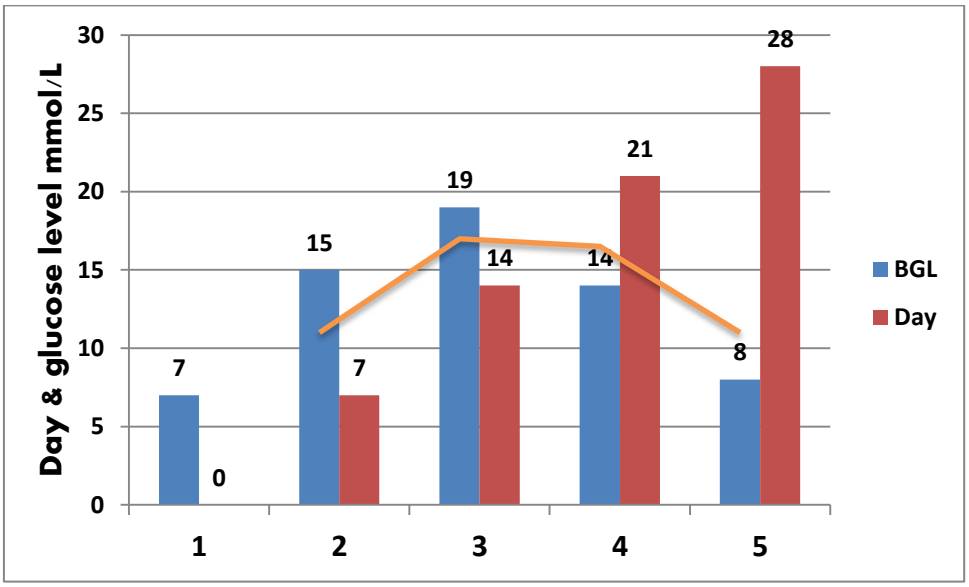

Figure 4.1 Time course after alloxan induction

4.2 All tested products comply the content and dissolution specification of USP

The average content of gliclazide and dissolution test results of all of the six products is given in Table 4.1. All samples contain drug within the USP specified limit (73-87%). The final dissolution data showed that all products comply with the USP specification (78-89%).

Table 4.1. Contents of gliclazide and dissolution results of commercial gliclazide tablets

Sample code	Content (%)	Release of drug within 45 minutes (%)
F-1	76.09	71.24
F-2	73.72	81.02
F-3	81.54	87.24
F-4	88.73	79.95

4.3 Blood glucose level after 7 days alloxan induced diabetes in mice's

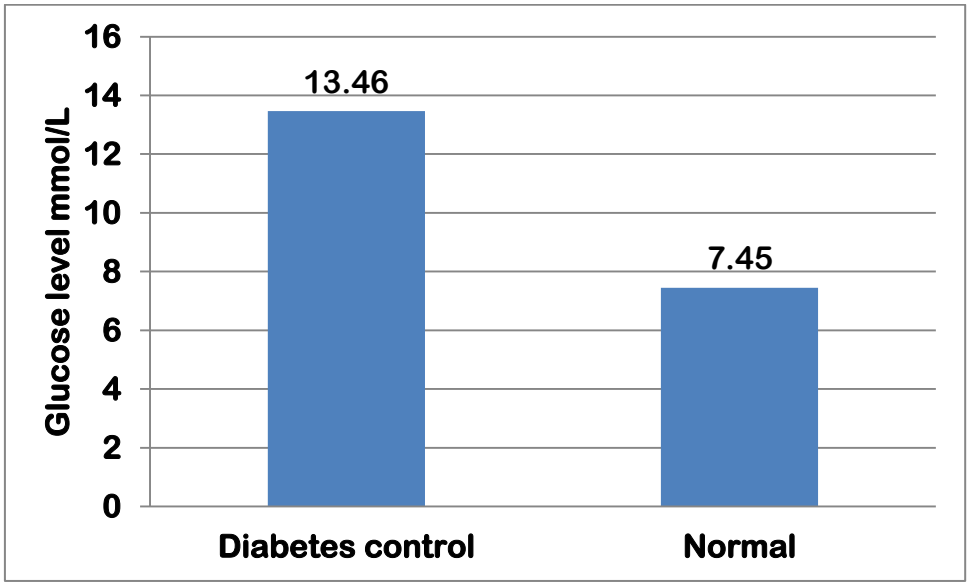

Figure 4.2: Blood glucose level in G-I (Normal control) and G-II (Diabetes control)

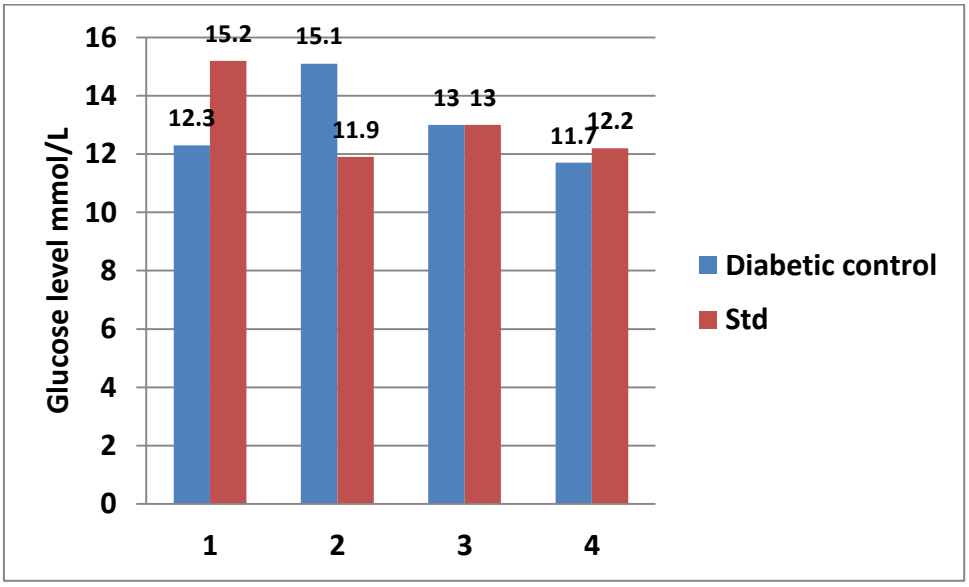

Figure 4.3: Blood glucose level in G-II (Diabetes control) and G-III (Gliclazide Std)

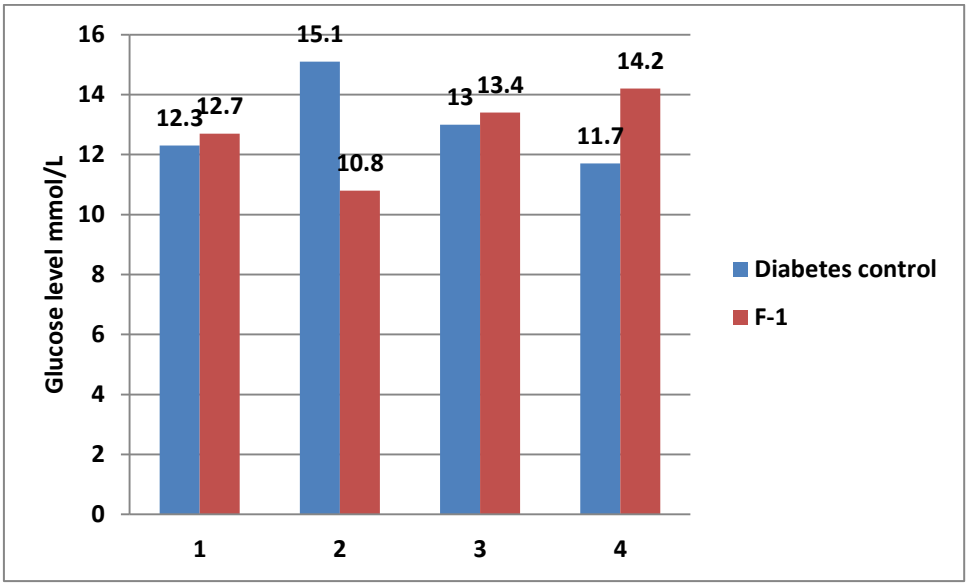

Figure 4.4: Blood glucose level in G-II (Diabetes control) and G-IV (Formulation-1)

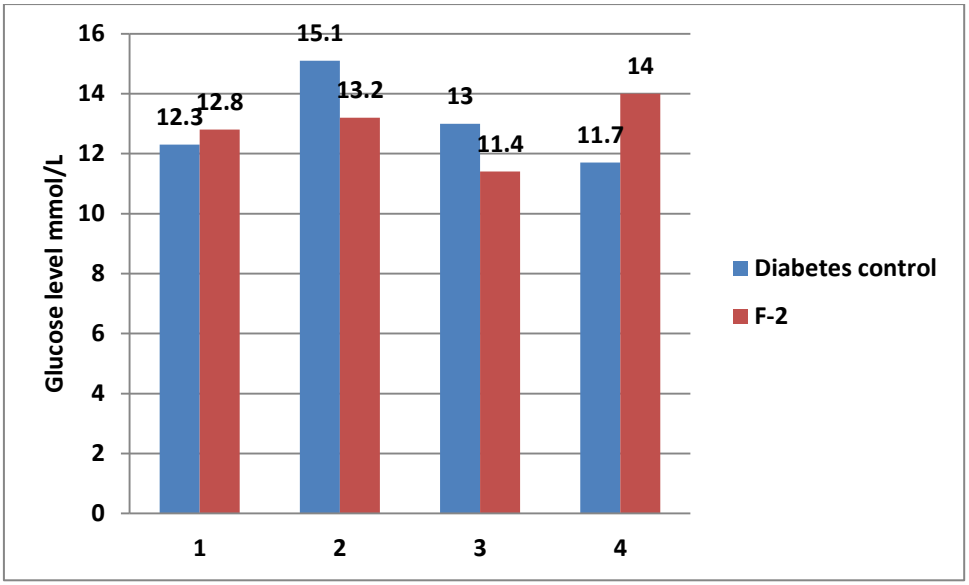

Figure 4.5: Blood glucose level in G-II (Diabetes control) and G-V (Formulation-2)

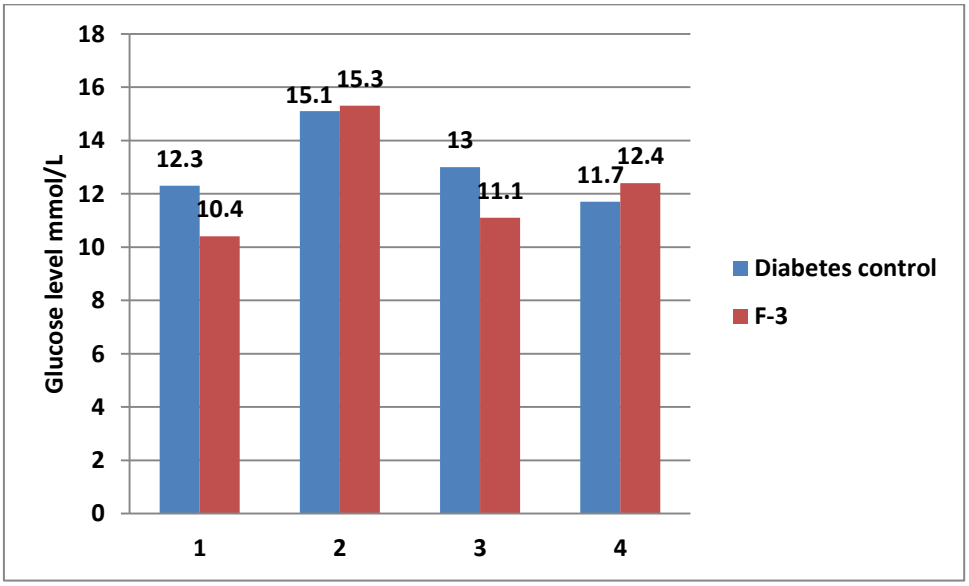

Figure 4.6: Blood glucose level in G-II (Diabetes control) and G-VI (Formulation-3)

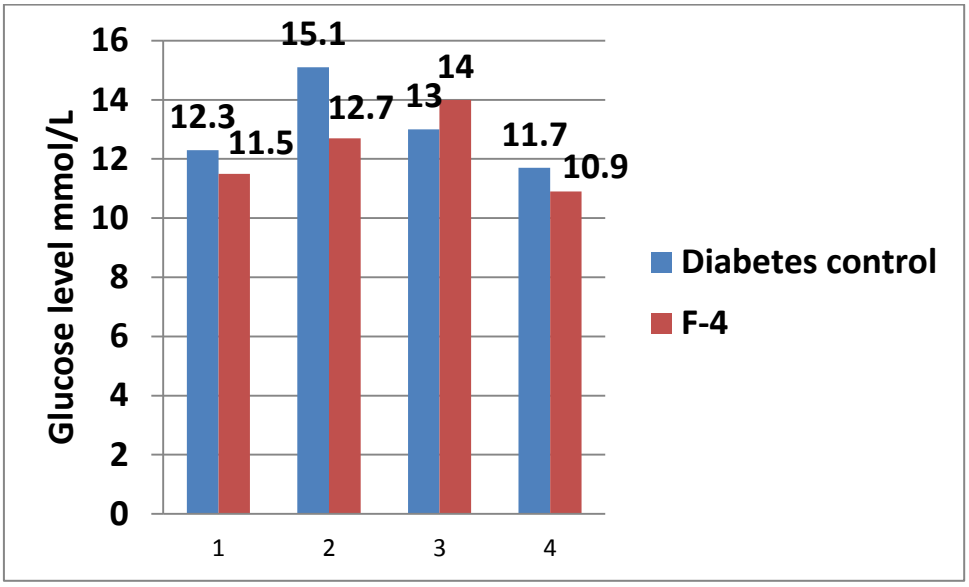

Figure 4.7: Blood glucose level in G-II (Diabetes control) and G-VII (Formulation-4)

4.4 Blood glucose level after administration of Gliclazide standard and formulations

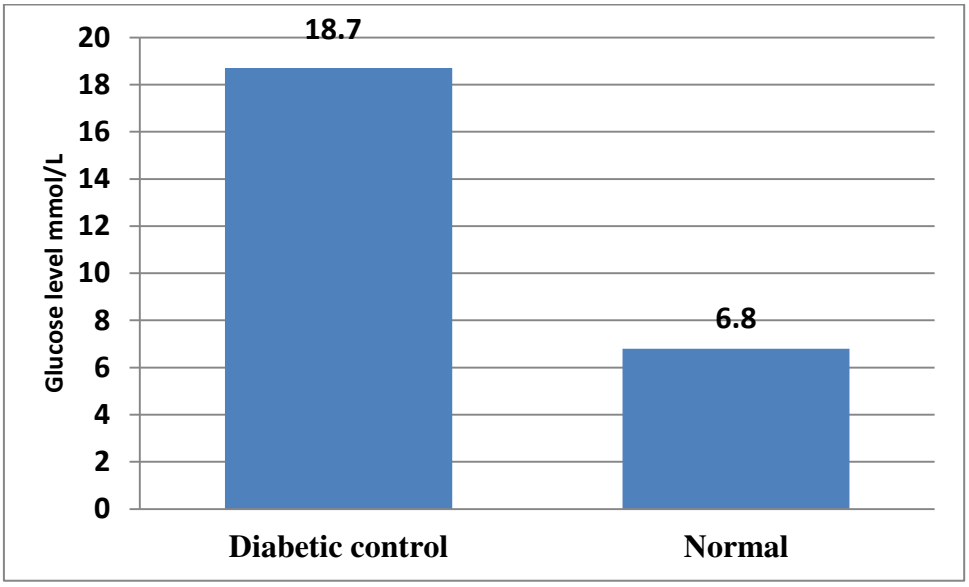

Figure 4.8: Blood glucose level in G-I (Normal control) and G-II (Diabetes control)

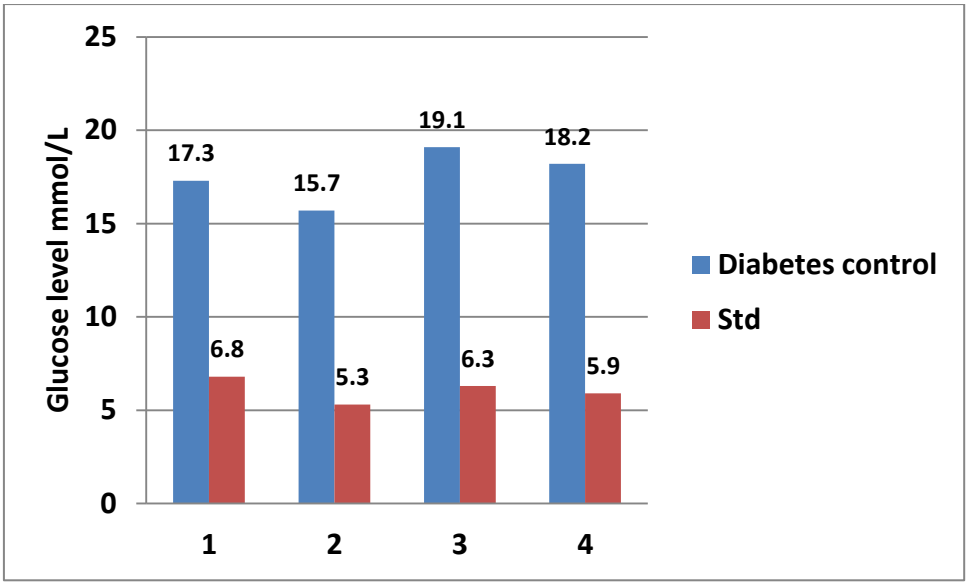

Figure 4.9: Blood glucose level in G-II (Diabetes control) and G-III (Std)

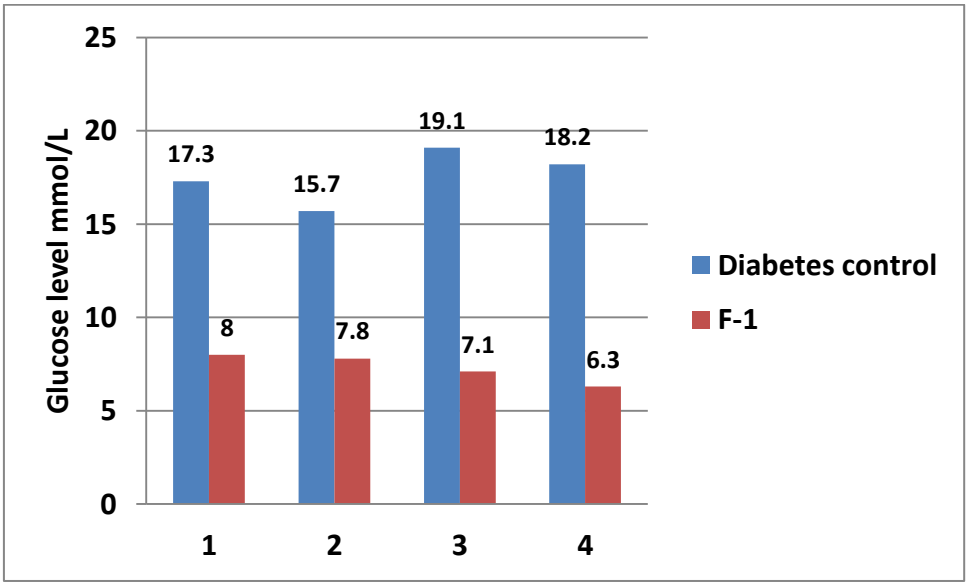

Figure 4.10: Blood glucose level in G-II (Diabetes control) and G-IV (Formulation-1)

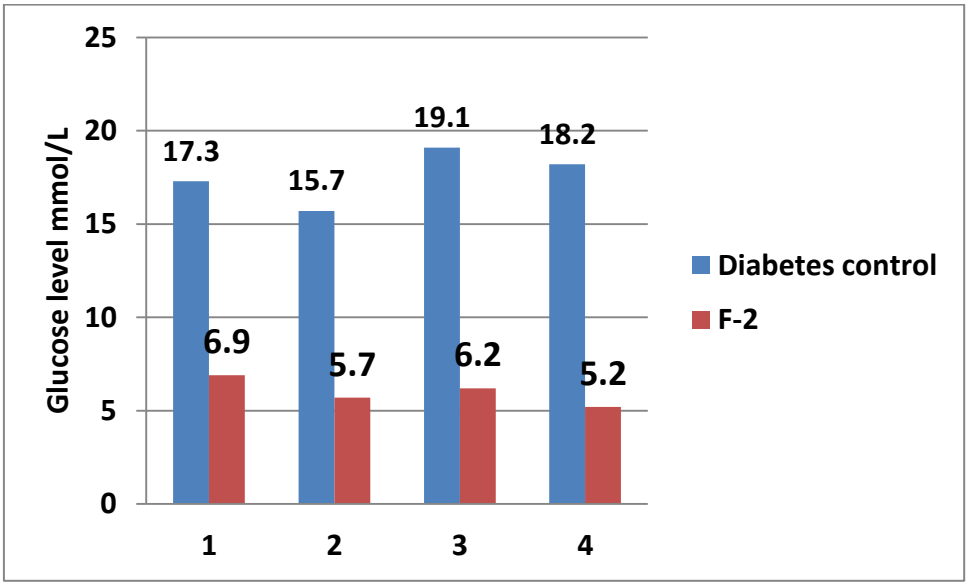

Figure 4.11: Blood glucose level in G-II (Diabetes control) and G-V (Formulation-2)

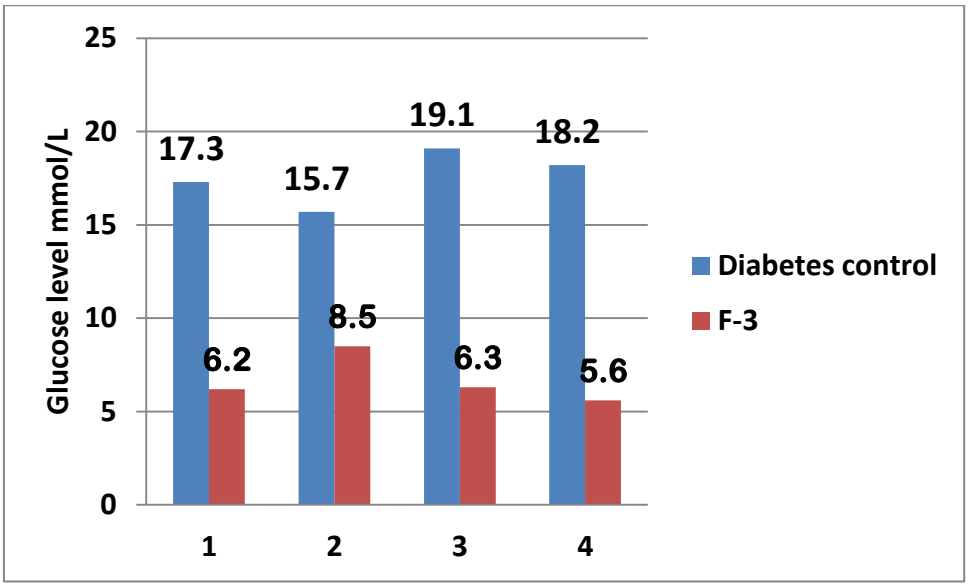

Figure 4.12: Blood glucose level in G-II (Diabetes control) and G-VI (Formulation-3)

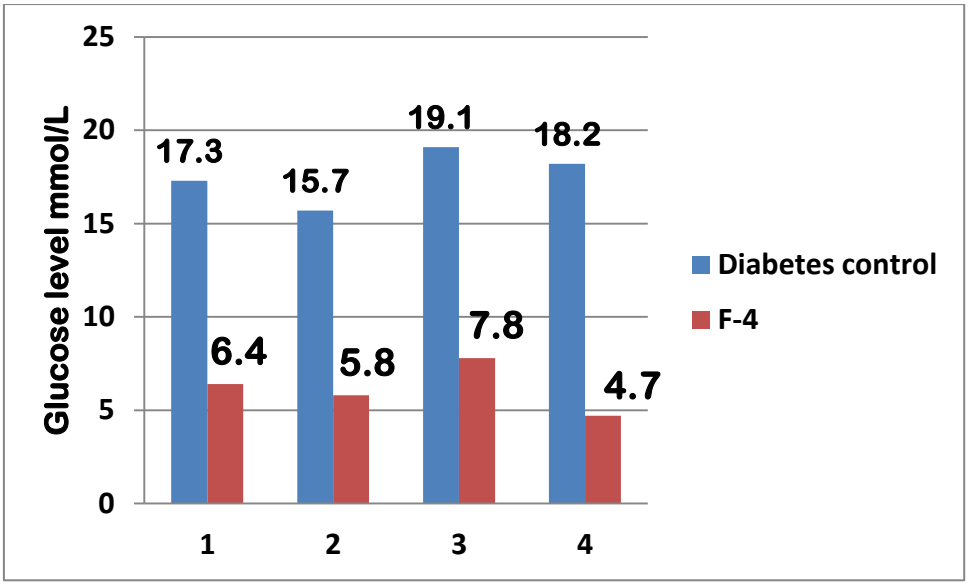

Figure 4.13: Blood glucose level in G-II (Diabetes control) and G-VII (Formulation-4)

4.5 The tested products demonstrated significant anti-hyperglycemic potentialities

The therapeutic efficacy of the products F1, F2, F3 and F4 on blood glucose levels were investigated in the control and alloxan induced diabetic mice using gliclazide as standard anti-diabetic agents. The mean blood glucose level of normal and diabetic control mice were shown in Fig. 4.15 and that of the drug treated animals (after oral administration of a single dose) after 5 hours were shown in Fig.4.16. It was shown that all the products significantly reduced the blood glucose of alloxan induced diabetic mice's ($P<0.05$). A significant reduction ($P<0.01$) in blood glucose of 54.75%, 61.93%, 56.5%, 61% and 70% were observed after 5hr with single dose of products F1, F2, F3, F4, F5, F6 and standard gliclazide respectively. All of the products reduced blood glucose level in diabetic mice's, which are consistent with that of standard drug gliclazide.

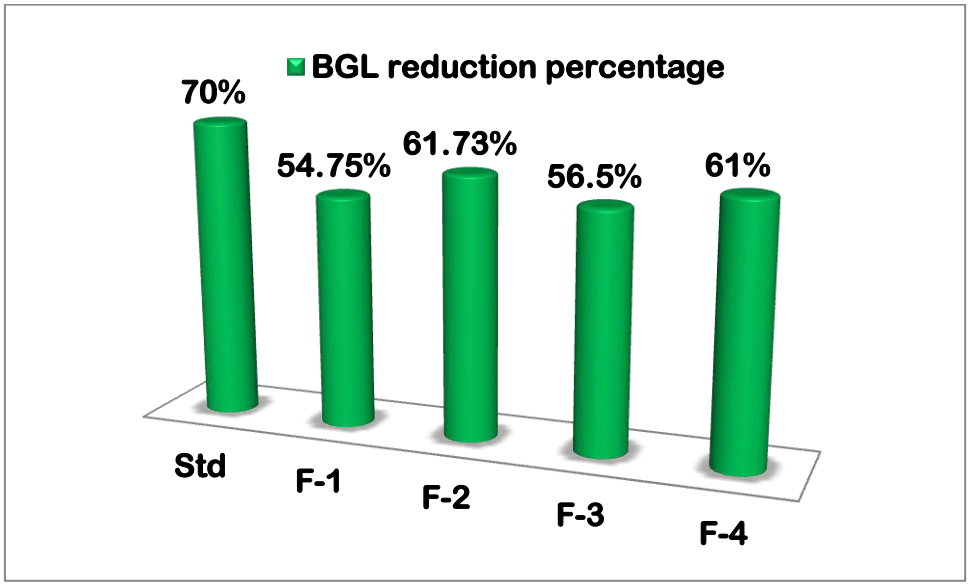

Figure 4.14: Percentage of reduction of blood glucose level

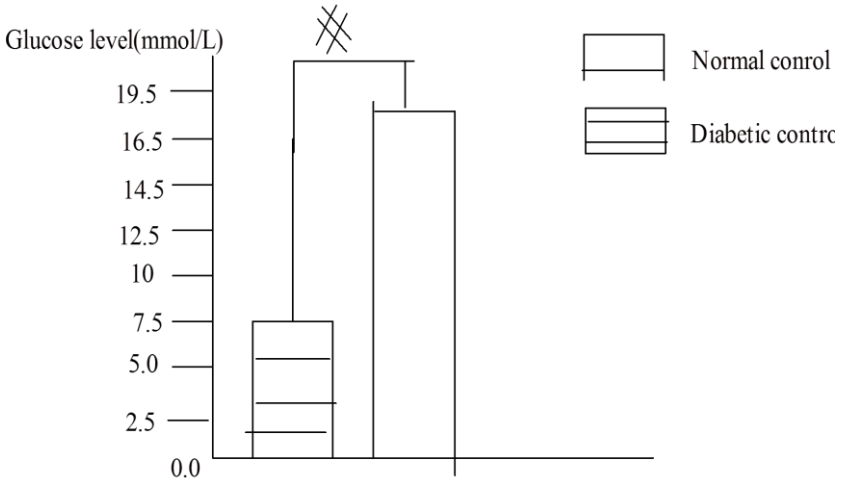

Figure-4.15: Blood glucose levels of normal and diabetic mice's. Each bar indicates the mean (±SEM) blood glucose levels. "#" indicates $P< 0.01$.

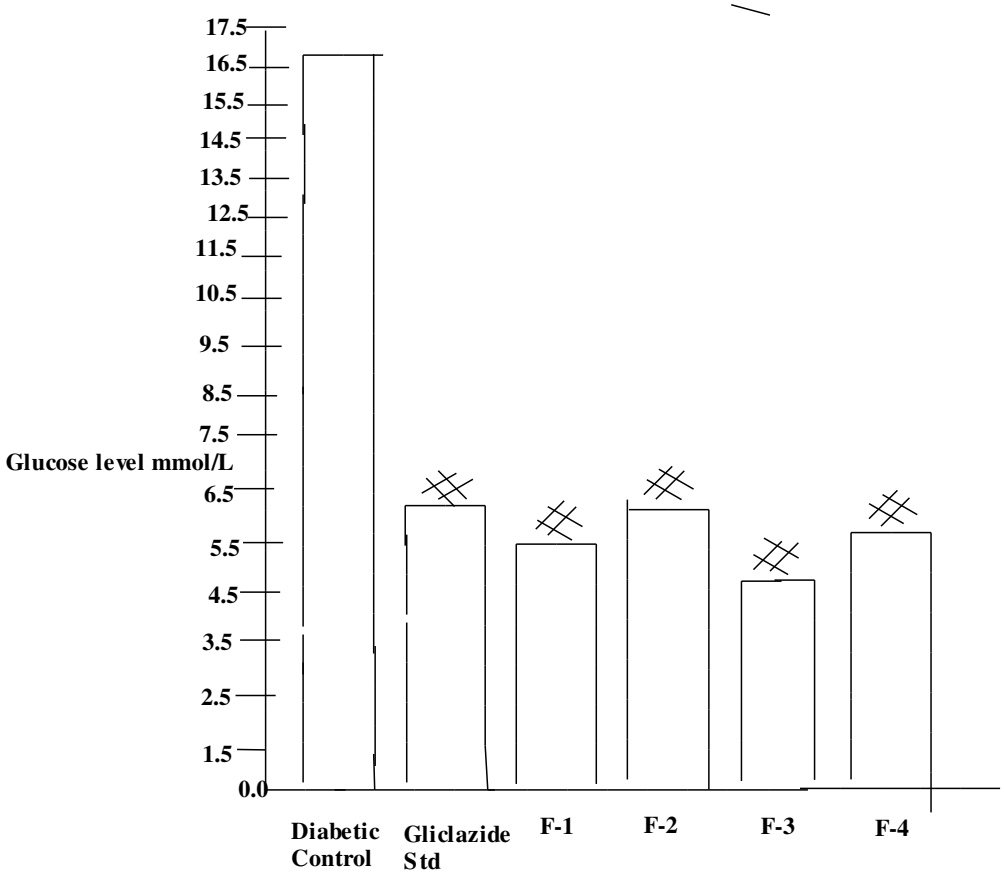

Figure-4.16: Effects of products F1, F2, F3, F4 and standard gliclazide after oral administration in alloxan-induced diabetic mice's. Each bar indicates the mean (± SEM) blood glucose levels. There are significant differences from control in the mean glucose levels. "#" indicates $p<0.01$.

4.6 Discussion

This study is a unique experimental scheme because it combined both pharmaceutical and pharmacological approach for bioequivalency assessment of gliclazide finished product. We have used animal model for successful biological evaluation of the gliclazide, which was consistent with their active gliclazide content and dissolution properties and bioequivalence requirements [69]. Since diabetes is a disease that is not cured completely, it needs regular physical exercise, dieting, continuous monitoring and medication. Among oral anti-diabetic formulations, gliclazide is an ideal drug being used frequently. As reported recently around 10% of total population of Bangladesh is now suffering from diabetes [70]. According to WHO statistics 347 million people worldwide is suffering from diabetes, which is estimated to be the 7th leading cause of death worldwide by 2030.

Although oral hypoglycemic agents and insulin is the mainstay of diabetes and are effective in controlling hyperglycemia, they have prominent side effects and fail to significantly alter the course of diabetic complications [72]. Alloxan, a ß-cytotoxin, induces "chemical diabetes" (alloxan diabetes) in a wide variety of animal species by damaging the insulin secreting pancreatic (ß-cell, resulting in a decrease in endogenous insulin release, which paves the ways for the decreased utilization of glucose by the tissues [71].

As an ideal anti-diabetic medicine, gliclazide tablet is supposed to be used by huge number of diabetic patients. From this point of view, it is quite easy to understand the importance of this study, which concerned with safety and efficacy for large number of population worldwide.

CHAPTER FIVE

CONCULASION

Conclusion

Our study revealed that gliclazide tablets marketed by Bangladeshi companies fulfill their requirements to be an ideal product. They have shown potential blood glucose lowering efficacy in alloxan-induced hyperglycemic in mice's. Assay and dissolution data of all the products follow USP specification. So it is not excessive to comment that Bangladeshi pharma companies are producing quality gliclazide tablets, following the physical and chemical specifications such as mixing, temperature control, granulation time, and addition of proper disintegrants, binders and other excipients. Our study has provided evidence that the tablet formulations of gliclazide marketed by Bangladeshi manufacturers confirm all the standard specifications and can be used safely by the patients of diabetes in Bangladesh and abroad without any confusion in terms of efficacy unless in tolerated. Although the present study gives some preliminary idea further study is necessary for details investigation of other formulation in Bangladesh.

CHAPTER SIX

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