Effects of *Gynura procumbens* Leaf Based Meal on Glucose Level, Lipid Profile and Mineral Content of Alloxan-Induced Diabetic Mice

MITHILA NATH

Roll No: 0118/07

Registration No: 549

Session: July-December 2019

A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Applied Human Nutrition and Dietetics

Department of Applied Food Science and Nutrition

Faculty of Food Science and Technology

Chattogram Veterinary and Animal Sciences University

Chattogram- 4225, Bangladesh

December 2019

Authorization

I, the author, confirm of authenticity of all the works in this report. All the information collected from books, national and international journals, websites and other references have been acknowledged accordingly.

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Mithila Nath
December, 2019

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made

----------	**----------**
(Dr. Md. Manirul Islam)	**(Taslima Ahmed)**
Supervisor	Co-supervisor
Professor	Assistant Professor
Department of Animal Science and Nutrition	Department of Applied Food Science and Nutrition

----

(Md. Altaf Hossain)
Chairman of the Examination Committee

Department of Applied Food Science and Nutrition
Faculty of Food Science and Technology
Chattogram Veterinary and Animal Sciences University
Chattogram-4225, Bangladesh

December 2019

Acknowledgements

I, sincerely appreciate the almighty God for his graces, strength, faithfulness and love all along the completion of my thesis. His magnanimity has made me excel and successful in making my thesis possible.

My unalloyed appreciation goes to my amiable, ever supportive and humble supervisor, Professor **Dr. Md. Manirul Islam** for his voluminous and invaluable contributions and instructions throughout my research. His encouragement and high degree of freedom helps me to do my thesis work is highly appreciated.

It's a great pleasure for me to express my heartfelt thanks and profound gratitude to my respectable teacher **Md. Altaf Hossain**, Assistant Professor, Department of Applied Food Science & Nutrition for his suggestions and co-operation.

I, also offer special thanks to my co-supervisor **Ms.Taslima Ahmed,** Assistant Professor, Department of Applied Food Science & Nutrition for her suggestions & inspiration.

I would like to thank **DR. Kona Adhikary** and **DR. Priunka Bhowmik** for their guiding support during the whole experimental period.

Furthermore, I would also like to acknowledge with much appreciation the crucial role of all the staffs of the **Department of Animal Science and Nutrition** in every step throughout the research period. I sincerely thank to all the members of the **Department of Physiology, Biochemistry and Pharmacology**, for their help in using their laboratory. I would like to express my gratitude to Bangladesh and Advanced Studies & Research, CVASU, for providing fund for this research work.

Finally, an honorable mention goes to my families and friends for their understandings and supports in completing this thesis. Without their help, it would have been impossible to overcome the difficulties faced during the research period.

DEDICATION

DEDICATED TO MY RESPECTED AND BELOVED PARENTS AND TEACHERS

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Abbreviations

%	:	Percentage
&	:	And
ANOVA	:	Analysis of variance
AOAC	:	Association of official analytical chemists
Dl	:	Deciliter
DC	:	Diabetic control
DPPH	:	2,2-diphenyl-1-picrylhydrazyl
et al	:	Et alii/ et aliae/ et alia
Etc	:	Et cetera
G	:	Gram
GP	:	*Gynura Procumbens*
Kg	:	Kilogramme
mg	:	Milligram
NS	:	Not significant
NC	:	Normal control
OGTT	:	Oral glucose tolerance test
T1DM	:	Type 1 diabetes mellitus
T2DM	:	Type 2 diabetes mellitus
SD	:	Standard deviation
SPSS	:	Statistical Package for Social Science
DM	:	Diabetes mellitus

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Abstract

Gynura procumbens (GP) leaf is used as an anti-diabetic medicinal plant at Southeast Asia. A 28 day feeding trial was conducted to evaluate the effects of *Gynura procumbens* leaf meal on growth performance, glucose level, lipid profile and mineral content on alloxan- induced diabetic mice. A total of 36 mice at 14 days old were assigned to four treatment groups: NC (Normal Control), T_0 Control (Basal diet), T_1 (Basal diet + 0.5% Dry leaf on DM basis), T_2 (Basal diet + 1.0% Dry leaf on DM basis) having 3 replications consisting of 9 mice each in a completely randomized design. The results revealed that glucose level were significantly ($P<0.001$) controlled at the last 3 weeks when compared to control and normal groups. There were no significant variations for blood Phosphorus, Ca and total protein. Three weeks dietary supplementation of GP leaf at feed 0.5% and 1.0% significantly ($P<0.05$) changes of bodyweights, water consumption and feed consumption of alloxan-induced diabetic mice. Other biochemical parameters like cholesterol, triglycerides, low density lipoproteins (LDL), high density lipoproteins (HDL) were found to be significantly ($P<0.001$) improved after treatment. Moreover, in the proximate CHO, crude protein, crude fat increased while ash, moisture, crude fiber decreased at treatment groups compared to basal diet. Overall, *Gynura procumbens* leaf showed trustworthy result on anti-diabetic effect included lipid profile.

Keywords: Alloxan-induced mice, *Gynura procumbens*, Lipid profile, Growth performance, Blood mineral (Ca, P).

Chapter I: Introduction

Diabetes is one of the non-communicable diseases (NCDs) that make the greatest contribution to morbidity and mortality worldwide. It is a lifelong metabolic disease, characterized by means of improved glucose level in blood due to insulin secretion, insulin action, or both (American Diabetes Association, 2012). Insulin, which is synthesized through β-cells of the pancreas, helps in keeping glucose concentration in blood by means of transporting it to the physique telephone and hence helps in producing power (Ahmad, 2014). Hence, insulin resistance, dysfunction or destruction of beta cell (β) leads to flip pathway of glucose metabolism in the body causing diabetes mellitus (Kahn et al., 2014). Environmental factors, genetic factors, way of life changes and excessive fat, weight loss program consumption (Ozougwu, 2013) are also accountable for diabetes mellitus.

According to WHO (2016) about 422 million people globally had diabetes, most living in the growing countries. Unfortunately, extra than 80% of diabetes deaths manifest in low- and middle-income countries. But more than 70% of people with diabetes live in low- and middle-income countries. The occurrence of diabetes is growing in Bangladesh in each city and rural areas (World Health Organization, 2020). A latest scoping overview (1994-2013) published that the incidence of type 2 diabetes assorted from 4.5% to 35.0% in Bangladesh. It increases healthcare use and expenditure and imposes a big monetary burden on the healthcare systems. The International Diabetes Federation estimated 7.1 million human beings with diabetes in Bangladesh. This wide variety is estimated to double by means of 2025. Moreover an equal range with undetected diabetes. During 90s, the country had a few low diabetes affected population. According to the International Diabetes Federation, the occurrence will be 13% via 2030. Bangladesh used to be ranked as the 8th best possible diabetic populous (13.7 million) country in the time length of 2010-2011. About 129,000 deaths have been attributed to diabetes in Bangladesh in 2015, as pronounced with the aid of main lookup company ICDDR ,B (Mohiuddin, 2019).

Diabetes prompted 1.5 million deaths in 2012. Higher-than-optimal blood glucose caused an additional 2.2 million deaths, by using increasing the dangers of cardiovascular and different diseases. Forty-three percent (43%) of these 3.7 million deaths appear earlier than the age of 70 years. The share of deaths to excessive blood glucose or diabetes that takes place prior to age 70 is higher in low- and middle-income countries than in high-income countries (World Health Organization, 2016).

Diabetes and its issues deliver about financial loss to people with diabetes and their families. Health structures and country wide economies through direct clinical charges impacts plenty and loss of work and wages. While the important fee drivers are clinic and outpatient care, a contributing thing is the upward jostle in price for analogue insulin which are more and more prescribed in spite of little proof that they provide sizable blessings over less expensive human insulin.

At the era of modern-day century, many anti-diabetic drugs have been developed for diabetic patients; most of them are produced by way of chemical / biochemical agents. In health care offerings and scientific care, medicines have to be used however or combined. However, cure with anti- diabetic tablets are costly. And, that is the cause human beings in developing or low income international locations always have an intention to heal the penalties or signs and symptoms of diabetes in a greater natural way. Hence, lookup focusing on reachable medicinal plant for the therapy of chronic ailment such as diabetes is going on continuously.

Gynura procumbens (Lour.) Merr (family Compositae), also acknowledged regionally as “Sambung Nyawa”, is cultivated in Southeast Asia, in particular Indonesia, Malaysia and Thailand, for medicinal purposes. This plant is viewed to be beneficial for hypertension, anti-inflammation, anti-herpes simplex virus, prevention of rheumatism, and therapy of eruptive fevers, kidney troubles, colon cancer, hemorrhoids and diabetes (Perry et al., 1980).

Gynura procumbens (GP) is frequently recognized as toughness two spinach which two is two one of the treasured two medicinal flora of Asterceaes. It is typically

famous two in South-East Asian countries for its usual medicinal properties. It is commonly used as a traditional medicine for the cure of inflammation, herpes simplex virus, rashes, fever, rheumatism, kidney diseases, migraines, diabetes mellitus, cancer and hypertension.

Gynura procumbens leaf has been used for a lengthy time as a medium of regular remedy and handed down from generation to era from Indonesia when you consider that nevertheless royal period, because spices and herbs also this us of a colonized by way of country of Portuguese and Dutch until Japan. Currently Gynura herbal plant life are disbursed and there are a range of nations such as Africa and China, for the life of the plant in Bali. In Thailand, it has been used traditionally as a topical anti-inflammatory and anti-allergy agent. (Jiratchariyakul et al., 2000). In Indonesia, the stem and leaves are used as an antipyretic in eruptive fevers. The dried leaves rubbed with oil and mashed are used as a salve for rashes. *Gynura procumbens* is additionally used as a treatment for kidney trouble and hypertension (Perry, 1980). In Malaysia, the flowers are broadly used in people medication to self- medicate a variety of prerequisites and illnesses ranging from migraines, constipation, hypertension, diabetes mellitus, and cancer.

Different research have proven that *Gynura procumbens* is capable to deal with eruptive fevers, rash, kidney diseases, migraine, constipation, hypertension, diabetes mellitus and most cancers (Perry, 1980). Akowuah et al. (2002) found that the leaves of GP have bioactive compounds such as flavonoids, saponins, tannins and terpenoids. Previous research have exposed the effectiveness of GP as a natural anti-hyperglycaemic agent. (Zurina et al., 2010) mentioned that unique strategies of plant extraction resulted in one of a kind outcome. It used to be found that 25% ethanolic extract of *Gynura procumbens* gave the best result in lowering blood glucose level. It was once also determined that the GP extract stimulant the metformin mechanism (Algariri et al., 2013) through elevating the glucose consumption in the muscle cells (Zurina et al., 2010). However, it's practicable in fertility on diabetic topics remains rarely known. Noor and Rahayu (2012) suggested that GP methanolic extract is able to enhance sperm quality. Based on these findings, the objectives of this study is to

observe the effects of *Gynura procumbens* leaf meal on glucose level, growth performance and lipid profile in diabetic induced mice.

1.1. Significance of the Study

Diabetes is a hypocritical chronic disease; non-compliant patients are in a threat of moderate to extreme issues in Bangladesh. Annually diabetes is responsible for 5% of all deaths globally, and its occurrence is increasing steadily. As said via International Diabetes Federation (IDF), approximately 75–80% of humans with diabetes die due to cardiovascular complications. Diabetes of all types can lead to complications in many parts of the body and can enlarge the ordinary risk of death prematurely. Possible issues consist of coronary heart attack, stroke, kidney failure, leg amputation, vision loss and nerve damage. In pregnancy, poorly controlled diabetes will increase the hazard of fetal death. However, plants are natural assets to manage specific diseases. *Gynura procumbens* is said as historically used flora which helps to reduce cholesterol, to control diabetes, to forestall most cancers activities (Hew & Gam, 2011).

The present study was focused on and tried to give a clear idea about the determination of hypoglycemic, hypo-lipidemic and mineral content of the plant of alloxan- induced diabetic mice. The research was done for taking the leaf as easiest way to control blood sugar and lipid profile.

1.2 Aims and Objectives:

1. To examine the effects GP powder on the blood glucose levels of alloxan-induced mice.
2. To observe the effect of plant powder on lipid profile of diabetic mice.
3. To evaluate the blood mineral (Ca, P) and total protein of the blood sample.

Chapter-II: Review of Literature

2.1. Diabetes mellitus

Diabetes mellitus is diagnosed as being a syndrome, a series of issues that have hyperglycemia and glucose intolerance as their hallmark, due both to insulin deficiency or to the impaired effectiveness of insulin's action, or to a mixture of these. In order to recognize the normal physiological procedure taking place throughout and after a meal. Food passes thru the digestive system, where nutrients, together with proteins, fats and carbohydrates are absorbed into the bloodstream. The presence of sugar, a carbohydrate, signals to the endocrine pancreas to secrete the hormone insulin. Insulin causes the uptake and storage of sugar through nearly all tissue sorts in the body, particularly the liver, musculature and fat tissues (Roussel, 1998).

Unfortunately, there is no therapy for diabetes but however by way of controlling blood sugar ranges through a wholesome diet, exercise and medicine the danger of long-term diabetes issues can be decreased. Long-term complications that can be skilled are:

- Eyes
- Cataracts and retinopathy (gradual detrimental of the eye) that may additionally lead to blindness
- Kidneys – kidney ailment and kidney failure
- Nerves – neuropathy (gradual damaging of nerves)
- Feet – ulcers, infections, gangrene, etc.
- Cardiovascular machine – hardening of arteries, coronary heart sickness and stroke (Heart foundation, 2003).

The revolutionary nature of the ailment necessitates consistent reassessment of glycaemic manage in people with diabetes and suitable adjustment of therapeutic regimens. When glycaemic manage is no longer maintained with a single agent, the addition of a 2d or 0.33 drug is generally greater fine than switching to some other single agent.

2.1.2. Classification of Diabetes Mellitus

A primary requirement for orderly epidemiologic and clinical research on and for the management of diabetes mellitus is a suitable classification. Furthermore the manner of grasp the etiology of a disease and reading its natural history includes the potential to discover and differentiate between its more than a few varieties and location them into a rational etiopathologic framework (Harris and Zimmet, 1997). In 1979 the National Diabetes Data showed the contemporary classification of diabetes and different classes of glucose intolerance, primarily based on research on this heterogeneous syndrome. Two most important forms of diabetes are identified in Western countries; insulin dependent diabetes mellitus (IDDM, type I diabetes) and non-insulin dependent diabetes (NIDDM, type II diabetes).

2.1.2.1. Insulin Dependent Diabetes Mellitus (IDDM)

Type I diabetes, is commonly characterized through the snappy onset of extreme symptoms, dependence on exogenous insulin to maintain lifestyles and proneness to ketosis even in the basal state, all of which is induced with the aid of absolute insulin deficiency. IDDM is the most frequent type of diabetes among young people and younger adults in growing countries, and was formally termed juvenile diabetes (Harris and Zimmet, 1997). It is a disorder with circulating insulin is genuinely absent however plasma glucagon is elevated, and the pancreatic β cells fail to respond to all insulinogenic stimuli (Nolte and Karam, 2001). It is concept to end result from an infectious or toxic environmental contingency in people whose immune systems are genetically predisposed to boost a lively autoimmune response towards pancreatic β mobile antigens.

An underlying genetic defect relating to pancreatic β cell replication or feature might also predispose an individual to the development of β cell failure after viral infections. On the different hand, particular HLA genes may additionally enlarge susceptibility to a diabetogenic virus or might also be linked to positive immune response genes that predispose patients to a unfavorable autoimmune response against their personal islet cells (auto aggression). Observations that pancreatic β cell damage seems to be lessened

when immunosuppressive drugs such as cyclosporine or azathioprine are given at the initial manifestation of type I diabetes support the importance of auto-aggression by the immune system as a foremost aspect in the pathogenesis of this type of diabetes (Nolte and Karam, 2001).

2.1.2.2. Non-Insulin Dependent Diabetes Mellitus (NIDDM)

Patients with NIDDM are not dependent on exogenous insulin for prevention of ketouria and are now not susceptible to ketosis. However, they may require insulin for the correction of fasting hyperglycemia. If this can't be done with the use of food regimen or oral agents, they may additionally enhance ketosis beneath different situations such as extreme stress precipitated via infections or trauma (Harris and Zimmet, 1997). The pathogenesis in type II diabetes is that the pancreas produces insulin however the body does not utilize the insulin correctly. This is specially due to peripheral tissue insulin resistance where insulin-receptors or different intermediates in the insulin signaling pathways within physique cells are insensitive to insulin and as a result glucose does no longer without difficulty enter the tissue leading to hyperglycemia or increased blood glucose concentrations (Albright, 1997). Obesity is a common chance factor for this type of diabetes, which usually effects in impaired insulin motion and most patients with type II diabetes are obese (Nolte and Karan, 2001) and will subsequently require multiple anti-diabetic agents to keep ample glycaemic control (Gerich, 2001).

2.1.3. Aetiology of diabetes mellitus

Commonly, it is an idea that genetic and environmental factors play a contributing role for the onset of diabetes mellitus. Environmental elements set off the diabetogenic procedure in a genetically environment friendly individual. Family records among diabetic sufferers levels from 25 to 50% (Raffel and Goodarzi, 2013). Evidence of genetic involvement in the etiology of type I diabetes is that 95% of type I diabetes patients carry HLA-DR3, HLA-DR4, or both. Genetic factors with type II diabetes mellitus are also properly documented. Resistance to insulin various among different region of the world (Sharp et al., 1987). Study on a variety of ethnic corporations

showed that environmental factors play a crucial role in the incidence of diabetes (La Porte et al., 1985). In case of gender, Bruno et al. determined a notably greater incidence of type I diabetes in males in contrast to girls (Bruno et al., 1993). Another study reported that type II diabetes is more regularly occurring in men (16.7%) than in women (9.5%) in Mexican populations of all age companies (Lerman et al., 1998). Moreover, ethnicity, location, seasonal variation, toxic agents, viruses and infection can initiate the onset of type 1 diabetes mellitus. Considering type 2 diabetes mellitus, urban and rural residency, physical inactivity, body weight, fat distribution, dietary factors, obesity, severe and prolong stress, drugs can aid on the beginning of diabetes (Adeghate et al., 2006). Regular physical pastime increases insulin sensitivity and glucose tolerance (Kriska et al., 2001). Insulin resistance additionally takes place due to many elements including- hypertension, dyslipidemia, atherosclerosis, aging, medication, genetic and other rare conditions. Insulin resistance may also improve with weight reduction and/or pharmacological cure of hyperglycemia however is hardly ever restored to normal (American Diabetes Association, 2012).

2.1.4. Pathophysiology and pathogenesis of diabetes

β cells synthesized insulin. Insulin is triggered through meals specially having carbohydrate. Insulin is prompted by using rising in blood glucose degree after eating. Pancreases launch insulin to control the elevation of glucose in blood. Insulin is used to soak up glucose from the blood to cells two as the usage of fuel, for conversion to different needed molecules, or for storage. However, when insulin manufacturing from pancreas is impeded (T1DM) or insulin resistance happens (T2DM), it in the end extend the glucose degree in blood alternatively than absorbed by way of different cells main the onset of diabetes (Nathan et al., 2005; Santaguida et al., 2005).

Generally, when glucose level falls in the blood (Hypoglycemia), insulin production from beta cell receives reduced. In contrast to, glucagon, which does opposite action of insulin, raises glucose level in the blood. In healthy subjects, consumption of meal high in glucose decreases the manufacturing of glucagon thereby lowering glucagon-induced stimulation of hepatic glucose production. Unfortunately, in reverse situation takes

place in patients with type 2 diabetes rising high plasma glucagon levels in the fasting state. High glucose ingestion can't help them to reduce the plasma glucagon level in the blood thereby main to chronic hyperglycemia in T2DM affected person (Lund et al., 2014).

In T1DM patient, first indication of this disease is identified through ketoacidosis. Others may also point out fasting hyperglycemia turning to severe hyperglycemia. At the final stage, there is little or no insulin secretion in the physique main to T1DM. The restriction in insulin secretion capacity is due to actual loss of beta cell mass. Though, younger people and childhood are more prone to immune-mediated diabetes, it can also manifest at the later stages of lifestyles (American Diabetes Association, 2012).

In a word, impaired beta cellphone feature (insulin deficiency) and/or inefficient action (insulin resistance) are the central mechanisms of hyperglycemia.

2.1.5. Epidemiology of diabetes

The quantity of diabetic affected person is increasing day by using day worldwide. The expanded wide variety is terribly excessive in some parts of the world. Dramatic modifications in sedentary life-style and urbanization boosting the prevalence of diabetes worldwide. In 1980, the World Health Organization (WHO) estimated that there were 108 million human beings living with diabetes and this number multiplied quadruple in 2014 estimates (Zhou et al., 2016).International Diabetes Federation (IDF) estimated the international prevalence to be 151 million in 2000, 194 million in 2003, 246 million in 2006, 285 million in 2009, 366 million in 2011, 382 million in 2013 and 415 million in 2015. In 2017 there had been 451 million (age 18–99 years) people with diabetes worldwide. These figures were anticipated to make bigger to 693 million by 2045. . It is estimated that 366 million people had DM in 2011; through 2030 this would have risen to 552 million (Anonymous, 2011). Moreover, It was once estimated that approximately 5.0 million deaths were attributable to diabetes among people aged 20–99 years in 2017. Hence, diabetes accounted for 9.9% of the global mortality amongst people inside this age range (Cho et al., 2018). Similarly, the upward jab of type 2 diabetes in South Asia is estimated to be more than 150% between 2000 and 2035

(Nanditha et al., 2016). A current meta-analysis showed that the occurrence of diabetes among adults had extended substantially, from 4% in 1995 to 2000 and 5% in 2001 to 2005 to 9% in 2006 to 2010 (Saquib et al., 2012). Among urban residents, the prevalence of diabetes used to be 15.2% compared with 8.3% among rural residents. In total, 56.0% of diabetics have been no longer aware they had the condition and only 39.5% were receiving remedy regularly. The likelihood of diabetes in men and women aged 55 to 59 years was almost double that in these aged 35 to 39 years (Akter et al., 2014).

Almost one in ten adults in Bangladesh was determined to have diabetes, which has recently become a major public fitness issue. Better detection, awareness, prevention and cure are vital to stop the rise in diabetes.

2.1.6. Diagnosis

Diabetes mellitus is characterized by elevated blood glucose level is diagnosed by demonstrating any one of the following:

Glycated hemoglobin (A1C) test: This blood test, which doesn't require fasting, indicates average blood sugar level for the past two to three months. It measures the proportion of blood glucose attached to hemoglobin, the oxygen-carrying protein in red blood cells.

- An A1C level of 6.5 percent or higher on two separate tests indicates that presence of diabetes.
- An A1C between 5.7 and 6.4 percent indicates pre-diabetes.
- Below 5.7 is considered normal.

If the A1C test results aren't consistent, the test isn't available, or, the A1C test inaccurate

— Such as pregnant or have an uncommon form of hemoglobin (known as a hemoglobin variant)

— may use the following tests to diagnose diabetes:

Random blood glucose test: A blood sample is going to be taken at a random time. Regardless of when last ate, a random blood glucose level of 200 milligrams per deciliter (mg/dL) — 11.1 milimoles per liter (mmol/L) — or higher suggests diabetes.

Fasting blood glucose test: A blood sample is going to be taken after an overnight fast. A fasting blood glucose level but 100 mg/dL (5.6 mmol/L) is normal. A fasting blood glucose level from 100 to 125 mg/dL (5.6 to 6.9 mmol/L) is taken into account pre-diabetes. If it's 126 mg/dL (7 mmol/L) or higher on two separate tests ensures diabetes.

Oral glucose tolerance test: For this test, fasting overnight and fasting blood sugar level is measured. Then have to drink a sugary liquid and blood sugar levels are tested periodically for the next two hours.

- A blood glucose level but 140 mg/dL (7.8 mmol/L) is normal.
- A reading of more than 200 mg/dL (11.1 mmol/L) after two hour indicates diabetes.
- A reading between 140 and 199 mg/dL (7.8 mmol/L and 11.0 mmol/L) indicates pre-diabetes
- Tests for gestational diabetes

At high risk of gestational diabetes — for example, obesity at the start of pregnancy; or had gestational diabetes during a previous pregnancy; or have a mother, father, sibling or child with diabetes.

At average risk of gestational diabetes — have a screening test for gestational diabetes sometime at trimester — typically between 24 and 28 weeks of pregnancy.

In most cases, the diagnosis of diabetes is usually made in various ways. These include ordinary health screening, detection of hyperglycemia during other medical investigations, and secondary symptoms like vision changes or unexplainable fatigue. Diabetes is often detected when a person suffers a problem that is frequently caused by diabetes, such as thirsty, frequent urination, extreme hunger, unexplained weight loss, presence of ketone in urine, fatigue. Irritability, blurred vision, slow healing sores, infections Type 1 diabetes can develop at any age, though it often appears during

childhood or adolescence. But Type 2 diabetes can develop at any age which is common in people older than 40 (Santaguida et al., 2005; Hirsch, 2009).

2.1.7. Management of diabetes mellitus

Diabetes is a commonly chronic condition the control of which demands the combining efforts of the patients and a group of specialized care providers. The patient participation, motivation and enthusiasm are critical for achieving optimal control of the disease. The successful management of diabetes requires more than just controlling the plasma glucose levels. It requires a multidisciplinary approach (Fauci et al.,2008; Rothe et al., 2008). Giving the fact that most patients will have developed one or more complications of diabetes at the time they show up at the health care provider and the diagnosis is set, then the case management will focus on two directions: a) history and physical examination, in order to check for any signs and symptoms of acute hyperglycemia, and b) screening for long-term or chronic complications related to DM. According to the International Diabetes Federation (IDF) the idea behind diabetes management is that, although monitoring and controlling the level of plasma glucose is essential, the optimal management of diabetes requires also the investigation of potential DM complications and their management, accompanied by efforts to modify the risk factors for different diabetes-related conditions. Diabetes care and management might also be dependent on a certain number of other factors such as social and economic factors. Cultural factors and employment factors are also very important as they relate to life-style, including smoking, drinking, physical activity, the patterns of feeding, stress and a whole range of other activities which could serve as risk factors for triggering diabetes.

The International Diabetes Federation refers the self-monitoring of blood glucose as often as necessary, testing for HbA1c several times per year, education of patients with refreshment once per year, examination of eye and foot once or twice a year, blood pressure measurement, lipid profile once a year and vaccinations against influenza as good approaches toward the ongoing management of diabetes.

In this regards, diabetic patient education is an ongoing process, which should reinforced once or twice annually and which could not be completed by a single visit to the doctor or nurse. Different education approaches could be used: individual or group education. There is evidence that individual education is more effective in controlling HbAc1 concentration level compared to group education or usual care approach (Sperl-Hillen et al., 2001-10; Khunti et al., 2010). Other studies have highlighted the effectiveness of diabetes case management and education in terms of costs saving and clinical outputs, especially for low-income populations (Gilmer, 2007).

2.1.8. Drug Therapy

Drug therapy with oral hypoglycemic agents and insulin are components of diabetes management that also include diet, exercise/activity, monitoring, and education. Major concerns for practitioners using drug therapy are selection of the appropriate therapy for a particular patient, and drug interactions and side effects. Drug therapy is based on knowledge of the medications, glucose goals, as determined by practitioners and patients and families. When using insulin, practitioners need to consider the differences between insulin therapy for insulin-dependent and non-insulin-dependent diabetes; how to initiate, adjust, and supplement insulin; situations that require variations in insulin therapy; and injection mechanics (Anonymus, 2012)

2.1.9. Insulin Administration

2.1.9.1. Insulin

Insulin therapy is vital for replacing the insulin your body doesn't produce for T1DM. Sometimes, people with T2DM or gestational diabetes need insulin therapy if other treatments haven't been able to keep blood glucose levels within the desired range. Insulin therapy helps prevent diabetes complications by keeping blood glucose within practice range.

Patients with type 1 diabetes, of course, need insulin immediately after diagnosis. An exception is patients with Latent Type I Diabetes of the Adult (LADA), whose glucose are often controlled on noninsulin medications for a short time, although for a much shorter period than for patients with type 2 diabetes. Because of the difficulties of using

insulin (from both the patient and provider perspectives fail. Although many guidelines recommend insulin in type 2 diabetes patients with high A1C levels, this is not always necessary. Almost all patients with newly diagnosed of type 2 diabetes will respond quickly to maximal doses of a sulfonylurea with reduction of the initial dose often necessary to avoid hypoglycemia (Peters et al., 1996; Babu et al., 2015). Because of the progressive loss of insulin secretion in type 2 diabetes, however, patients with an extended duration of disease and really high A1C levels do require insulin.

Most children who have symptomatic T1DM at diagnosis require a total insulin dose of approximately 1 μ/kg/d at diagnosis. Pubertal children typically are more insulin resistant and often require 1 to 1.5 μ/kg/d. The selected insulin regimen, given alone or in combination with oral agents, should be tailored to the individual needs of the patient. Sometime, adverse effect such as hypoglycemia was also observed in some patient. It could be happened due to erratic meal timing, excessive insulin dosage or unplanned exercise (Chehade and Mooradian, 2000; Haller et al., 2005).

There are many types of insulin on the market, all of which must be injected into the fat under the skin in order for it to reach the bloodstream. Injections can be done using a:
Syringe: A needle connected to a hollow tube that holds the insulin and a plunger that pushes the insulin down into and thru the needle
Insulin pen: A device that looks like a pen that holds insulin but has a needle for its tip
Insulin pump: small machine (worn on a belt or kept in a pocket) that holds insulin, pumps it through a small plastic tube and through a tiny needle inserted under the skin where it stays for several days.

Table 1. Types of Insulin and How They Work.

Insulin Type	How fast it starts to work (onset)	When it peaks	How long it lasts (duration)
Rapid-acting	About 15 minutes after injection	1 hour	2 to 4 hours
Short-acting, also called regular	Within 30 minutes after injection	2 to 3 hours	3 to 6 hours
Intermediate-acting	2 to 4 hours after injection	4 to 12 hours	12 to 18 hours
Long-acting	Several hours after injection	Does not peak	24 hours; some last longer

Source: Insulin basics. American Diabetes Association

2.1.9.2. Oral anti-diabetic drugs

2.1.9.2.1. Biguanides

Metformin is the most extensively used medicine under this category. It has proven to be an effective anti-hyperglycemic agent. In diabetic patient, biguanides represses hepatic glucose production that increases insulin sensitivity, enhances glucose uptake by phosphorylating GLUT-enhancer factor, increases carboxylic acid oxidation and decreases the absorption of glucose from the gastrointestinal tract. It also improves glucose uptake in the peripheral tissue, mainly the muscle (Chehade and Mooradian, 2000; Collier et al., 2006). It is remarkable that individuals who are primary or secondary failures to sulphonylurea agents are unlikely to respond to metformin alone. However, when metformin is combined with sulphonylurea agents who appear to be secondary failures, a substantial blood glucose lowering occurs (DeFronzo and Goodman, 1995).

2.1.9.2. Sulphonylurea

Sulphonylurea works by binding to sulfonylurea receptor on beta cell surface to stimulate secretion of insulin from the pancreatic beta cells. Sulphonylureas are divided into two groups: 1st generation drugs (tolazamide, tolbutamide, acetohexamide, and chlorpropamide) and 2nd generation drugs (glibenclamide glimepiride, glipizide and gliclazide). Second generation drugs are superior to the first generation drugs.

Occurrence of hypoglycemia was often associated under the usage of these drugs (Chehade and Mooradian, 2000; Olokoba et al., 2012).

2.1.9.3. Meglitinides

Repaglinide and nateglinide are non-sulfonylurea secretagogues which act on the ATP-dependent K-channel in the pancreatic beta cells which stimulating the release of insulin from the beta cells, similar to sulfonylurea, though the binding site is different. Meglitinides are given before meals for postprandial blood glucose control. Pre-prandial administration allows flexibility just in case a meal is missed without increased risk of hypoglycemia. The relatively low risk of hypoglycemia will be an interesting feature of this new class of antidiabetic agents, especially in the elderly population and in patients with hypoglycemia unawareness (DeFronzo and Goodman, 1995)

2.1.9.4. Alpha-Glucosidase Inhibitors

These agents are most effective for postprandial hyperglycemia. However, administration should be avoided in patients with significant renal impairment. Miglitol and voglibose are other commercially available α-glucosidase inhibitors (Olokoba et al., 2012).

2.1.10. Diet Therapy

The ideal diet for diabetic patients remains to be fixed. Recommendations generally require low-fat high-carbohydrate diets. The primary purpose of this recommendation is to reduce the risk for coronary heart disease, a major killer of diabetic patients. Some investigators also suggest that high-carbohydrate diets also improve glucose tolerance, even in patients with non-insulin-dependent diabetes mellitus (NIDDM). Another potential advantage of a : low-fat diet (high percentage of carbohydrate) is that it's going to promote weight reduction. High-fat diets are thought by many investigators to stimulate weight gain. In summary, the best dietary approach in obese patients with NIDDM, who are relatively early in the course of their disease, is to attempt weight reduction with a low fat diet. However, if this diet should fail, it probably would be better to reduce carbohydrate intake and replace some of it with monounsaturated. Likewise, in more advanced NIDDM, with deficiency of insulin secretion, high-

carbohydrate diets probably should be avoided, because they accentuate hyperglycemia. Therefore, the ideal diet for diabetic patients may depend on the presence or absence of obesity, the response to a weight-reduction diet, and the stage of progression of J-cell dysfunction (Diabetes Care, 1991).

Usually, a healthy adult should consume 45–65% of total energy from carbohydrate, 20–35% from fat, and 10–35% from protein. However, optimal mix of carbohydrate, protein, and fat needs to be followed on individual circumstances. Saturated fat should be limited to but 7% of total calories. Intake of trans fat should be minimized. For individuals with diabetes and normal renal function, protein intake should be 15–20% of total calories (American Diabetes Association, 2006).

Many short-term studies have shown that moderate weight loss (5% of body weight) in subjects with type 2 diabetes is related to decreased insulin resistance, improved measures of glycaemia and lipidaemia, and reduced blood pressure (Klein et al., 2004). Modified diet help to control lipid concentration as well as maintain blood pressure. However, low-carbohydrate diets, restricting total carbohydrate to less than 130 g/day, are not recommended in the management of diabetes. Foods with low glycemic indexes include oats, barley, bulgur, beans, lentils, legumes, pasta, pumpernickel (coarse rye) bread, apples, oranges, milk and yogurt. Fiber, fructose, lactose, and fat are dietary constituents that also tend to lower glycemic response (Mayer-Davis et al., 2006). People with diabetes are encouraged to consume a variety of fiber-containing foods such as legumes, fiber-rich cereals, fruits, vegetables, and whole grain products because they provide vitamins, minerals, and other substances important for good health.

Clinical trials/outcome studies of medical nutrition therapy (MNT) have reported decreases in HbA1c of 1% in type 1 diabetes and 1–2% in type 2 diabetes, depending on the duration of diabetes (Pastors et al., 2002, 2003). There are data suggesting that consuming a high-fiber diet reduces glycaemia in subjects with type 1diabetes and glycaemia, hyper-insulinaemia, and lipidemia in subjects with type-2 diabetes (Franz et al., 2002).

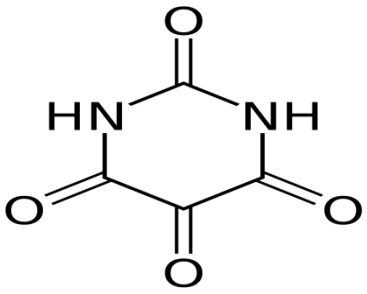
2.2. Alloxan- Induced Diabetes:

Alloxan and Streptozotocin, both are cytotoxic glucose analogues, are the most common accepted chemical in this category (Lenzen, 2008). About two hundred years ago, two scientist, Wöhler and Liebig, (1838) synthesized a pyrimidine derivative, which is latter called alloxan (Lenzen and Panten, 1988). In 1943, another reported that alloxan could induce in animals and the action of specific necrosis of the pancreatic beta cells (Dunn et al., 1943).

In addition, the cytotoxic action of alloxan is mediated mainly by the generation of reactive oxygen species (ROS). Dialuric acid is that the reduction product of alloxan has been noted to determine a redox cycle with the formation of superoxide radicals, which undergo dismutation to hydrogen peroxide (H2O2) and highly reactive hydroxyl radicals are formed by the Fenton reaction. Further, the huge increase in cytosolic calcium concentration ultimately causes rapid destruction of beta cells of pancreatic islets (Rohilla and Ali, 2012).

Alloxan is a very unstable chemical compound with a molecular shape similar to glucose (Table 2). Thus the GLUT2 glucose transporter in the beta cell plasma membrane accepts this glucomimetic and transports it into the cytosol. Alloxan does not inhibit the function of the transporter and can selectively enter beta cells in an unrestricted manner (Weaver et al., 1979; Gorus et al., 1982; Lenzen, 2008). The diabetic effect of alloxan is mainly attributed to rapid uptake by the beta cells and the formation of free radicals, which beta cells have poor defense mechanisms.

Table 2. Chemical properties of Alloxan

Properties	**Alloxan**
Chemical Name:	2,4,5,6-Tetraoxypyrimidine;
	2,4,5,6-pyrimidinetetrone
Chemical Structure	
Chemical Properties	Very hydrophilic, beta cell-toxic glucose analogue (partition coefficient -1.8) weak acid, Chemically unstable (half-life of 1.5 min at pH 7.4 and 37^0C, decomposing to alloxanic acid); Stable acid pH
Mode of toxicity	Generation of ROS

2.3. *Gynura procumbens*:

Gynura procumbens (GP) is commonly known as longevity spinach belonging to the family (Asteraceae) that grows extensively in Southeast Asia, particularly in Indonesia, Malaysia and Thailand.

GP grows up to a height of 1-3 m, with a bloated stem and purple tint. The leaves are ovate-elliptic or lanceolate, 3.5 to 8 cm long and 0.8 to 3.5 cm wide. Flowering heads are narrow, yellow and 1 to 1.5 cm long (Gao et al., 2007). It is traditionally used for the treatment of eruptive fever, rash, kidney disease, constipation, hypertension, diabetes mellitus, migraines, urinary tract infection, rheumatism, viral diseases of skin (Chin et al., 2006).

Some of these traditional claims are validated in scientific and pharmacological studies, including anti-herpes virus (Phillipson et al., 2001) anti-inflammatory (Mann, 1992) and Cragg et al., 2002) and anti-hyperlipidemic and anti-hyperglycemic(Griggs, 1997;

Solecki, 1975; Heinrich, 2004) anti-hypertensive (Duke, 2008) activities. Recently received great attention of GP is in the pharmacology of anti-diabetic medicinal plants probably because of its experimental evidence and efficiency in the management of diabetes mellitus.

The leaves of this plant are often consumed in the diet, and research shows that leaves contents are non-toxic (Gershenzon, 2007; Vanijajiva, 2009). Besides, recently conducted researches are evident that GP oils play roles in dentistry, dermatology, gynecology, and pediatrics as a means of hygienic care. The GP leaves yields diversified phytoconstituents including macro elements and microelements, alkaloids, saponins, chlorophyll, carotenoids, essential oils, etc. The following is an extensive and up-to-date review about the history, distribution, phytochemistry, toxicity and pharmacological properties of GP with an urge of further advancements in the medicinal uses of the herb worldwide.

Figure 1. *Gynura procumbens* leaf

2.3.1. History

The plant was discovered many centuries ago at the African continent. The Gynura (Asteraceae-Senecioneae) comprises 44 species and is distributed from tropical Africa to South and East Asia and Austraialasia with one species in tropical Australia. The highest species diversity is found in Southeast Asia, but the genus is well understood particular in Thailand. It has been used both for food and as a remedy for illnesses. During the last 15 years, the plant has attracted the attention of the international scientific community because the source of the many powerful biologically active substances (Zerikly et al., 2009).

2.3.2. Distribution

Gynura procumbens (GP), a fast-growing herbaceous plant, is widely found in Borneo, Java, the Philippines, and Peninsular Malaysia. It is a topical plant from China and known as ‘baibingca’ (Backer and Vandenbrink, 1965). It is also found in Myanmar and some Asian countries such as Indonesia and Thailand. This plant grows easily from stem cuttings. Seeds are not available. It is best grown in well-draining, fertile soil that's kept moist in the least times. This plant prefers Semi-shade although it can be slowly adapted to grow in full sun, provided the plant does not dry out at the roots. Growth should resume once the plant has accustomed to its new growing condition (Perry, 1980).

2.3.3. Phytoconstituents

Considerable work has already been done to identify and isolate the chemical constituents from different extracts of GP. Frequent studies have exposed that various extract of GP leaves contains several active chemical constituents like flavonoids, saponins, tannins, terpenoids and steroil glycosides (Phillipson, 2001; Jeffreys, 2004). Previous studies had also shown that GP leaves extracts contained rutin, kaempferol and two potential antioxidant components which are kaempferol-3-O-rutinoside and astragalin (Phillipson, 2001). Flavonoids are polyphenolic compounds with potential beneficial effects on human health; they reportedly have anti-allergic, antiplatelet, antiviral, anti-inflammatory, antitumor and activities.

The genus Gynura includes about forty species basically disbursed in tropical Africa to South and East Asia and Australasia with one species in tropical Australia, of which 10 species had been recorded in the south of China.

Gynura procumbens belongs to Asteracea household and is an annual evergreen shrub with fleshy stems (Jiratchariyakul et al., 2004). It was first of all described and the identify validly published by Joao de Loureiro in 1790. It was once Elmer Drew Merrill, however, who reclassified it into today's valid botanical systematic in 1923 (Backer et al., 1965).

Common name: In our country it is regarded as Gynura, Diabetics plant.

2.3.4. Habitat and distribution of *Gynura procumbens*

Gynura procumbens is a common medicinal plant dispensed in western and central Africa and from southern China during continental South-East Asia and Papua New Guinea (Perry et al., 1980).

It was first described in 1838 and it was found abundantly in Borneo, Java. The Philippines and Peninsula Malaysia (Burkill et al., 1966) and broadly dispensed in Indonesia, Malaysia and Thailand (Zahra et al., 2011). It contains about forty four species native to the humid tropics of Africa to Southeast Asia. However, this plant is not native to Bangladesh. But at present it is cultivating all over the country.

2.3.5. Culinary uses

The leaves of the plant are eaten as a vegetable. In fact, in Malaysia *Gynura procumbens* young shoots are eaten raw as "ulam". Having slight taste two of the leaves are used uncooked in salads, added to soups. Stir-fries, casseroles, condiments and sauces, rice dishes and different savory meals. It is also used for sauces, as flavoring and in sandwich for making taste. Dried or fresh cutted leaves and stems, are used for making herbal tea.

2.3.6. Traditional uses

Gynura procumbens has traditionally been used for the remedy of eruptive fevers, rash, kidney disease, migraine, constipation, hypertension, diabetes mellitus and cancer. In Indonesia some people used its leaves as medication of uterine cancer, breast cancer and blood cancer. It is also used for kidney issues in Indonesia (Java) and as febrifuge in Indo-China. The dried and mashed leaves are mixed with oil and applied as a poultice for rashes (Perry et al., 1980). In Thai folks medicine, semi-succulent leaves of *Gynura procumbens* are utilized externally to deal with topical inflammation, rheumatism, viral infection of the skin and generic body-pain. In Indonesia, it has been used to deal with fevers, skin rashes and as tonic to deal with ringworm infection. In the Philippines, in

particular in Mindanao, the plant is grown in the rice fields to manipulate insect pests. In Malaysia, *Gynura procumbens* is used as folk remedy to treat diabetes. In Malacca, boiled leaves are used for dysentery. In south-east Asia it is used for male reproductive health and performance, which include prostate function. Females have taken the herb for breast firming, menstrual cycle problems and vagina contraction (Shipard, 2003).

2.3.7. Medicinal use

Anti-hypertensive remedy: *Gynura procumbens* has been used as a therapy for lowering high blood pressure for decades. Scientific research has confirmed its efficacy as an effective remedy (Hoe et al., 2011).

Anti-herpetic actions: Research has verified anti-herpetic actions of GP. It does so by using reducing replication capability of virus. It has powerful anti-microbial properties, which make it superb in opposition to the little trouble makers invading our body (Jarikasem et al., 2013)

Lowering blood sugar levels: Research indicates that *Gynura* lowers blood glucose levels and can be used as an high-quality remedy against diabetes. Its leaf extract has bioactive components that make certain anti-diabetic action (Algariri et al., 2013).

Anti-oxidant effects: Research indicates that G P has strong anti-oxidant potential. This amazing plant has phenol compounds trapped in its leaves. These phenolic compounds scavenge free radicals in our body (Rosidah et al., 2008).

Anti-cancer nature: It is a rising natural anti-cancer remedy. Proteins extracted from its leaves include miracle in, peroxidaes, thaumatin -like proteins. These proteins manipulate unusual cell growth. Thus have therapeutic results on tumors. Especially tumors of the colon. Gynura is additionally tremendous in assuaging breast cancer (Nurulita et al., 2012).

Anti-hyperlipidemic effect: Along with glucose decreasing effects, ethanolic extract of Gynura procumbens leaves have anti-hyperlipidemic effects. It can lower LDL, cholesterol and triglycerides levels in our body (Zhang et al., 2000).

Anti-ulcerogenic activity: *Gynura procumbens* has been used in gastrointestinal ailments. Research indicates that Gynura has anti-ulcerogenic activity. It protects mucosa (the internal layer of gut) from damage, for that reason ensures a healthful intestine (Mahmood et al., 2010).

Recovering wound healing: Wound restoration is a physiological procedure that starts offevolved proper alter the harm occurs. G P suggests its efficacy in speeding up wound harm at a notably fast (Iskander et al., 2002).

Anti-inflammatory effect: *Gynura procumbens* has been well-known for anti-inflammatory effects. It contains many bioactive constituents, alkaloids and steroids. These alkaloids play vital position in warding off inflammation. Steroids existing in this plant are additionally accountable of anti-inflammatory actions (Iskander et al., 2002).

Immune modulating effects: *Gynura procumbens* is one of those medicinal plants that have immune modulating effects. They stimulate increase human lymphocytes- cells of immune systems. Consuming these flowers additionally influences growth of herbal killer (N K) cells- any other variety of immune cells. Both these cell- lymphocytes and NK- make vital section of immune system and make sure that bodily defenses are high enough (Sriwanthana et al., 2007).

***Gynura procumbens* protects heart**: GP produces nitric oxide, which is a effective vasodilator and lowers blood pressure. It also lowers cholesterol and triglyceride ranges in the body which makes positive atherosclerosis would not appear (Lee et al., 2007).

Impacts on kidney: *Gynura procumbens* use as remedy for the management of kidney diseases. Researchers have found that Gynura has wholesome influences on innovative kidney disease. Bioactive compounds current in its leaf extract inhibit proliferation of mesangial cells (cells existing in kidney). They additionally reduce expression of some hazardous proteins, as a consequence act as bioactive agents for kidneys (Lee et al., 2007).

Fertility activity: GP decrease toxins- oxidative stress- from body and expand each the motility and volume of sperm (Hakim et al., 2008).

Anti-Photoaging activity: GP shows anti-photoaging activity. It prevents manufacturing of free radicals through UV rays and makes certain security from UV caused harm and premature getting older (Kim et al., 2011).

Taste making agent: Gynura procumbens has a protein miraculin which makes up extra than 0.1 of its protein extract. Miraculin protein is used to make taste of bitter remedy better and useable (Hew et al., 2011).

Anti-fungal effects: *Gynura procumbens* have excessive anti-fungal activity. So, it can be claimed that except having anti-viral effects, it also wards off funngal infections (Nazmul et al., 2011).

2.3.8. Commercial uses

Among the current patents related to *Gynura procumbens,* the majority of them are for preparations of standard Chinese medicine intended for the therapy of a number ailments which includes uterine cancer, cervical spondylosis, and continual skin ulcer Besides, it has also been used as an ingredient in special diets for patients with medical conditions such as coronary heart and liver disease In the food industry, it has been integrated into products such as tea, kirnchi, coffee powder, chocolate, sweet and chewing gum The applications of *Gynura procumbens* in non-public care and cosmetic products have also been reported which such as hand-washing solution, hand sanitizer, oral spray, facial masks, and pores and skin care lotions. These patents have tested the high industrial price of *Gynura procumbens* and its variety of uses in a quantity of industries (Tan et al., 2016).

2.3.9. Pharmacological potentials of *Gynura procumbens*

A number of research have been carried out to investigate its pharmacological activities, due to the extensive application of this plant .From literature survey it was once observed that normally the leaves of *Gynura procumbens* are used in the treatment of a number of diseases.

Anti-herpes activity

The plant extracts have anti-replicative action against herpes simplex virus stated via Jiratchariyakul et al. (2000). Again, Nawawi et al. (1999) said that methanol and water extracts showed anti HSV-1 activity in vitro therapeutic analysis the use of an experimental animal model, *in vivo*.

Jarikasem et al. (2013) reported that ethanol extract of *Gynura procumbens* showed virucidal and antireplicative actions against herpes simplex virus I-ISV-I and HSV-2 (ICso 625.0 and 675.0 µg/mL, respectively.) and avoided the viral replication with IC5o 584.0 and 568.0 g/mL, respectively .Water extract of *Gynura procumbens* had virucidal motion against both viral types.

Anti-diabetic activity

Rasadah et al. (2002) showed that the methanol extract of *Gynura procumbens* used to be able to enlarge insulin secretion in the insulin-secreting cell line, BRlN-BDI. Akowuah et al. (2001) showed that hypoglycaemic studies of the methanol extract confirmed substantial hypoglycaemic effect in streptozotocin-induced diabetic rats however exerted no huge reduction in blood glucose degree of normal rats.

Akowuah et al. (2002) showed that pharmacological screening of the n-butanol fraction from the leaf has a hypoglycemic effect. Blood glucose levels in streptozotocin precipitated type 2 diabetic rats have been decreased by way of the administration of 1 g/kg of the n-butanol fraction. The blood glucose levels in the diabetic rats dealt with with n-butanol fraction had been lowered via 32.42% and 40.77 % at hour 5 an hour 7, respectively.

Hassan et al. (2010) suggested that aqueous extract of *Gynura procumbens* leaves (1000 mg/kg) used to be administered orally as a single dose to streptozotocin-induced diabetic male and lady Sprague Dawley rats. An extensive minimize in fasting blood glucose level used to be discovered for the duration of seven hour after extract administration (22-30 %) however no great insulin secretion were determined compared to saline-treated diabetic manipulate group.

Algariri et al. (2014) later examined ethyl acetate, n-butanol, ethanol and aqueous extract of *Gynura procumbens* for anti- hyperglycemic activity in acute and sub-chronic conditions. The impact of a single dose (500 mg/kg or one thousand mg/kg or 2000 mg/kg) of these fractions on blood glucose in streptozotocin (STZ)-induced diabetic rats (SDRs) used to be evaluated.

Anti hyperlipidaemic activity

Pharmacological investigations Zhang et al. (2000) confirmed that ethanolic (95 p.c v/v) extract of the leaves of *Gynura procumbens* reduced serum cholesterol and triglyceride levels in Streptozotocin precipitated diabetic rats. The extract, at single doses of 50, 150 and 300 mg/kg orally, suppressed the improved serum glucose levels in diabetic rats; 150 mg/kg was once found to be the optimal hypoglycemic dose. The extract alternatively did not significantly the increased serum glucose degrees in regular rats, unlike glibenclamide. Metformin, however not glibenclamide, increased glucose tolerance in the diabetic rats. When the most suitable dose was given to diabetic rats for 7 days, the extract considerably decreased serum LDL, cholesterol and triglyceride levels in these rats.

Anti-inflammatory activity

Iskander et al. (2002) stated that ethanolic extract of *Gynura procumbens* exhibited anti-inflammatory activities. Steroids isolated from the plant have been demonstrated to possess anti- inflammatory activity.

Anti-ulcer activity

Gynura procumbens ethanolic (95 % v/v) leaf extract promotes ulcer protection as ascertained by considerable reduction of ulcer area and histologically decreases in ulcer areas, reduction or absence of edema and leucocytes infiltration of sub-mucosal layer.

Anti-hypertensive activity

The hypertensive activity of *Gynura procumbens* was first investigated by Lam et al. (1998). They described that the leaves of *Gynura procumbens* show anti-hypertensive

things to do in rat through inhibiting angiotensin-converting enzyme (ACE) that cause vasodilatation through inhibition of calcium channels.

Hoe et al. (2007) confirmed that an aqueous fraction of *Gynura procumbens* decreases the mean arterial pressure (MAP) of rats. Aqueous extract of *Gynura procumbens* leaves (500 mg/kg/day) administered orally to spontaneously hypertensive male rats (aged 10 weeks old) for 4 weeks considerably decreased systolic blood pressure, lactate dehydrogenase and creatine phosphate kinase and appreciably extended nitric oxide in comparison to distilled water treated group.

Cardiovascular activity

Hoe et al. (2011) said that butanol fraction (BU) from the ethanol (96 % v/v) extract of *Gynura procumbens* includes putative hypertensive compounds that appear to inhibit calcium inflow through receptor-operated and/or voltage-dependent calcium channels to cause vasodilatation and a consequent fall in blood pressure. They administered BU fraction of *Gynura procumbens* leaves (2.5-20 mg/kg) intravenously to person male albino Sprague Dawley rats for 10 minutes. They located that the dose of 10 and 20 mg/kg considerably decreased the coronary heart rate. The imply arterial pressure was once considerably reduced (EDso = 4.77 mg/kg of BU).

Kaur et al. (2012) pronounced that aqueous extract of leaves (1 and 2 mg/mL) substantially showed vasorelaxant impact on phenylephrine-induced contraction in isolated rat's aortic ring in contrast to control. The extract (0.25-1 mg/mL) drastically lowered isoprenaline-induced beats per minute in isolated rat's proper atrium compared to control. The extract (1 and 2 mg/mL) also appreciably lowered the energy of isoprenaline caused cardiac muscle contraction in isolated rat's left atrium compared to control.

Abrika et al. (2013) pronounced that n-butanol and methanol extract from the *Gynura procumbens* leaf have positive isotropic activities. The mechanism of effective ionotropic activities can be attributed to a direct motion on the SA node, which leads to

the limit in conduction or to the depression of myocardium of the heart, similar to the impact of quinidine, 3-adrenergic blocker tablets or calcium channel blockers.

Diabetes patient in Bangladesh and round the world is growing day by day. In Bangladesh, people are living under poverty line where greater than 70% people living in rural areas much less than $2.5 a day in Bangladesh (Rahim, 2017). The use of alternate natural drug treatments from plants in the administration of diabetes becoming popular particularly in rural communities of developing countries. People prefer to consume easily available, accessible, cheap and especially safe food which additionally provide extra medicinal properties Insufficient knowledge of health are leading people to go through extra complications of diabetes. Traditional plants are high quality which will be useful to minimize diabetes .Medicines and Insulin injections are added a burden to a lower income family. Raw flowers can be used commercially. This will create an innovation in food industry. Therefore, the existing study intended to verify and validate the anti -diabetic activity of GP Plants.

Chapter III: Materials and Methods

3.1. Study area

The study was carried out at Chattogram Veterinary and Animal Sciences University, Khulshi, Chattogram, Bangladesh. The experimental shed was used for animal trial including analysis under the Department of Animal Science and Nutrition and different analysis were carried out in Department of Physiology Biochemistry and Pharmacology.

3.2. Study period and climatic condition

The standard research used to be carried out from March 2019 to July 2019. The weather of Chattogram was not too warm or too cold (30° to 40°). Humid summer season was started from March and at the end of the experiment, the climate was rainy. The animal trial had to conduct avoiding excessive climate condition.

3.3. Preparation of Leaf meal

3.3.1. Collection of leaves

Fresh, mature healthy *Gynura procumbens* leaves were collected from Lalkhan Bazar nursery of Chattogram. The fresh leaves were sorted to reject unwanted and insect affected parts of the leaves. Then they were entirely washed with clean water to take away all dirt. The washed leaves had been left at room temperature for 2-3 days.

3.3.2. Drying & Storage

The washed leaves were dried in cabinet drier at 60°C for three days.The dried leaves had been grounded in a clean blender and formed course powder. After that the course leaves powder was sieved using a sieve of 80μm mesh size to acquire quality leaves powder and kept at 25°C in an airtight container.

3.4. Layout of the experiment

A whole of thirty six (36) female healthy laboratory Swiss albino mice weighing between 23-27g were bought from the animal residence of the Department of

Pharmacy, Jahangirnagar University, Bangladesh. The study had approval of the CVASU Institutional Animal Ethical Committee and research had been carried out in scrupulous recommendations for the care of laboratory animal.

For the experiment, thirty six mice were randomly distributed in completely randomized design with following treatments: NC as Normal Control, T_0 as Diabetic control, T_1 (basal diet with 0.5% dry leaf supplement), T_2 (basal diet with 1.0% dry leaf supplement).

All mice had been divided into 4 groups with 9 animals each. All dietary treatments had been given orally for three weeks, as follows:

Table 3: Layout of Experiment:

Dietary Treatment Groups	**Replications**	**No. of mice per replications**	**No. of mice per treatment**
NC=Normal Control	R1	3	9
	R2	3	
	R3	3	
T_0=Diabetic Control (Basal diet)	R1	3	9
	R2	3	
	R3	3	
T_1=(Basal diet+0.5% dry leaf powder)	R1	3	9
	R2	3	
	R3	3	
T_2=(Basal diet+1.0% dry leaf powder)	R1	3	9
	R2	3	
	R3	3	
Total			36

3.5. Management and Feeding

3.5.1. Preparation of the shed

For the experimental animal, extra cares were given. Firstly, it was cleaned and washed thoroughly with tap water and caustic soda using brushes and scrapers. All the cages, brooding boxes, corners, ceiling, feed storing racks and fans were cleaned also. Then the shed was disinfected spraying 0.5% (v/v) phenyl solution. The shed was left for two days to completely dry out. The house was then fumigated using formalin (40%

formaldehyde) and potassium permanganate and left for 24 hours. During fumigation, it was make sure that the room was completely sealed. Lime was spread around the shed to maintain strict biosecurity of the shed. Feeder and drinkers were cleaned using water, detergent followed by 0.3% potassium permanganate solution. Personal hygiene was maintained properly by using face mask, hand gloves, footwear and clothing. Strictly followed everything to avoid contamination.

3.5.2. Housing

The mice house was well-ventilated, wire-floored, closed cages. Room humidity was 30%-70% and temperature was 18-26°C. Each cage was solid-bottom caging with wood-shaving bedding. Each cage was provided with drinker and drinker to ensure *ad-libitum*. Male and female mice were kept at separate cage. A standard laboratory conditions with 12-h light: 12-h dark cycle were maintained.

3.5.3. Feeding and watering

The mice were supplied with self -made feed which is defined as basal diet. While purchasing, the freshness of the product and its expiry date were checked. The supplements were mixed uniformly with the feed before feeding to the mice. In Control, only basal diet was offered. In treatment groups, 0.5% dry leaf supplement (T1), 1.0% dry leaf supplement (T2) dry matter basis were mixed with basal diet. Feed and water were supplied *ad-libitum* to all groups of mice in two different times in a day (10.00 and 6.00 o'clock) throughout the experimental period .Cucumber slice was added to fill up their water balance at dry season. Mice were acclimatized for 7 days before the commencement of the study. For first week basal diet was offered to all. Fresh drinking water was supplied to the mice at free choice. The bodyweights and fasting blood glucose levels of the mice were recorded every week during the experiment period. Feed and water consumption were also recorded during the study period. The nutritive value of the diets is presented in Table 4.

Table 4: Nutritive value of basal diet.

Composition of Diet	Quantity (%)
Wheat Flour	26.30
Wheat Bran	19.00
Fish Meal	10.00
Mustard Oil Cake	4.00
Maize powder	22.00
Rice Police	3.00
Milk Powder	13.06
Full fat soy	12.00
Soybean oil	6.00
Salt	0.25
Choline chloride	0.10
Enzyme	0.01
Vitamin mineral premix	0.25
DCP	1.00
Soybean meal	12.00

3.6. Induction of Diabetes

Selected mice were kept overnight fasted for making diabetic were intraperitoneally administered alloxan monohydrate (150mg/kg body weight) dissolved in ice-cold saline (0.9% NaCl). To prevent hypoglycemia, the animals were given 5% glucose solution for the next 24 h. After 4 days observation blood glucose level was figured out by using Glucometer. Mice having more than 3-4 fold increase in their blood glucose level were considered diabetic (Tao Bu et al., 2012).

3.7. Determination of growth parameters.

While measuring growth parameters, the body weight was recorded per replication on weekly basis. The final body weight was recorded at the last day of the experiment. In addition, feed consumption for each replication was determined by deducting the feed residue from supplied feed. Feed conversion was calculated as the weight of feed consumed divided by body weight gain.

3.8. Feed Intake

Feed intake is determined by subtracting the refusal feed collected every morning before supplying of feed from the weighed feed provided to the birds for *ad-libitum* feeding. The average daily feed intake was calculated using the formula:

$$\text{Average daily feed intake} = \frac{\text{Weight of supplied feed – weight of refused feed}}{\text{Number of mice}}$$

3.9. Collection of Blood Samples

At the end of the experiment, blood samples were collected by cardiac puncture from overnight fasted anesthetized (by diethyl ether) animals. Serum was separated from blood after 40 to 60 minutes by centrifugation at 3500 rpm for 10 minutes. Obtained serum samples were stored at -30°C until analysis.

3.10. Biochemical tests

The blood of three mice from each replication was collected in 5 ml syringe using 23 Gauge needle. The blood was immediately transferred to vacutainers containing clot activator. The vacutainers were centrifuged at 3000 rpm for about 20 minutes to separate the serum from blood. The separated serums were then separated using micropipette and collected in eppendorf tube. The serums were then stored in freezer at –20°C. From these serums different biochemical tests such as total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) were determined using biochemical analyzer (Humalyzer 3000, Human ® Diagnostics, Germany**)** in the Post Graduate laboratory of Department of Animal Science and Nutrition by following the directions supplied with the kits (Randox® Laboratories limited, UK). The low-density lipoprotein (LDL) levels were calculated according to the formula (Friedewald et al., 1972):

$$LDL = \text{Total Cholesterol} - (HDL + \text{Triglyceride}/5)$$

3.11. Proximate Analysis of basal diet

From these samples, chemical analysis was once carried out in accordance to the standardized formulation by using AOAC International (2006). The analysis of proximate components was once carried out in the Nutrition Laboratory below the Department of Animal Science and Nutrition, CVASU, to decide the dry Matter (DM), crude protein (CP), crude fiber (CF) and ash.

Total Carbohydrate contents were determined by subtracting method i.e.

% Carbohydrate = 100 – %(Protein + Moisture + Fiber + Fat + Ash).

3.12. Determination of Moisture:

Moisture was determined by oven drying method. 1.5 g of well-mixed sample was accurately weighed in clean, dried crucible (W1). The crucible was allowed in an oven at 100-105° C for 6-12 h o until a constant weight was obtained. Then the crucible was placed in the desiccator for 30 min to cool. After cooling it was weighed again (W2). The percent moisture was calculated by following formula:

$$\% \text{ Moisture} = \frac{(W1\text{-}W2)\times 100}{\text{Wt of sample}}$$

Where, W1 = Initial weight of crucible + Sample
W2 = Final weight of crucible + Sample

3.13. Determination of Ash:

For the determination of ash, clean empty crucible was placed in a muffle furnace at 600° C for an hour, cooled in desiccator and then weight o of empty crucible was noted (W1). One gram of each of 1 sample was taken in crucible (W2). The sample was ignited over a burner with the help of blowpipe, until it is charred. Then the crucible was placed in muffle furnace at 550° C for 2-4 h. The appearances of gray white ash o indicate complete oxidation of all organic matter in the sample. After ashing furnace

was switch off. The crucible was cooled and weighed (W3). Percent ash was calculated by following formula:

$$\% \text{ Ash} = \frac{\text{Difference wt. of ash} \times 10}{\text{Wt. of sample}}$$

Difference in wt. of Ash= W3 -W1

3.14. Determination of Protein:

Protein in the sample was determined by Kjeldahl method. The samples were digested by heating with concentrated sulphuric acid (H_2SO_4) in the presence of digestion mixture. The mixture was then made alkaline. Ammonium sulphate thus formed, released ammonia which was collected in 2% boric acid solution and titrated against standard HCl. Total protein was calculated by multiplying the amount of nitrogen with appropriate factor (6.25) and the amount of protein was calculated.

Protein in the sample was determined by Kjeldahl method. 0.5-1.0 g of dried samples was taken in digestion flask. Add 10-15 ml of concentrated $H_2SO_4$and 8 g of digestion mixture i.e. K_2SO_4: CuSO4 (8: 1). The flask was swirled in order to mix the contents thoroughly then placed on heater to start digestion till the mixture become clear (blue green in color). It needs 2 hrs to complete. The digest was cooled and transferred to 100 ml volumetric flask and volume was made up to mark by the addition of distilled water. Distillation of the digest was performed in Markam Still Distillation Apparatus. Ten milliliters of digest was introduced in the distillation tube then 10 ml of 0.5 N NaOH was gradually added through the same way. Distillation was continued for at least 10 min and NH_3 produced was collected as NH_4OH in a conical flask containing 20 ml of 4% boric acid solution with few drops of modified methyl red indicator. During distillation yellowish color appears due to NH_2OH. The distillate was then titrated against standard 0.1 N HCl solution till the appearance of pink color. A blank was also run through all steps as above. Percent crude protein content of the sample was calculated by using the following formula:

% Crude Protein = 6.25* x %N (* Correction factor)

$$\% \text{ Protein} = \frac{(S-B)\times N\times 0.014\times D\times 100}{\text{Wt. of the sample}}$$

Where, S = Sample titration reading
B = Blank titration reading
N = Normality of HCl
D = Dilution of sample after digestion
V = Volume taken for distillation
0.014 = Milli equivalent weight of Nitrogen

3.15. Determination of Crude Fat:

Dry extraction method for fat determination was implied. It consisted of extracting dry sample with some organic solvent, since all the fat materials e.g. fats, phospholipids, sterols, fatty acids, carotenoids, pigments, chlorophyll etc. are extracted together therefore, the results are frequently referred to as crude fat. Fats were determined by intermittent soxhlet extraction apparatus. Crude fat was determined by ether extract method using Soxhlet apparatus. Approximately 1 g of moisture free sample was wrapped in filter paper, placed in fat free thimble and then introduced in the extraction tube. Weighed, cleaned and dried the receiving beaker was filled with petroleum ether and fitted into the apparatus. Turned on water and heater to start extraction. After 4-6 siphoning allow ether to evaporate and disconnect beaker before last siphoning. Transferred extract into clean glass dish with ether washing and evaporated ether on water bath. Then placed the dish in an oven at 105 C for 2 hrs and cooled it in a desiccator. The percent crude fat was determined by using the following formula:

$$\% \text{ Crude Fat} = \frac{\text{Wt. of ether extract x 100}}{\text{Wt. of sample}}$$

3.16. Determination of Crude Fiber:

Weighed 0.153 g sample (W0) weighed and transferred to porous crucible. Then placed the crucible into fiber unit and kept the valve in “OFF” position. After that added 150 ml of preheated H_2SO_4 solution and some drops of foam-suppresser to each column. Then opened the cooling circuit and turned on the heating elements (power at 90%).

When it started boiling, reduced the power at 30% and left it for 30 min. Valves were opened for drainage of acid and rinsed with distilled water thrice to completely ensure the removal of acid from sample. The same procedure was used for alkali digestion by using KOH instead of H_2SO_4. Dried the sample in an oven at 150° C for 1 h. Then allowed the sample to cool in a desiccator and weighed (W1). Kept the sample crucibles in muffle furnace at 55° C for 3-4 hrs. Cooled the samples in desiccator and weighed again (W2).

$$\% \text{ Crude Fiber} = \frac{(W1\text{-}W2)\times 100}{W0}$$

3.17. Statistical analysis

All statistical analysis was completed by the use of statistical package deal for social sciences (SPSS) model 16. One-way analysis of variance was used to evaluate the data. Data are presented as the mean ± (Standard Deviation) SD. Differences in mean have been compared using the Tukey test. *P values* ≤ 0.05 had been considered significant.

Chapter IV: Results

4.1. Yield of dry leaf powder

The yield from the *Gynura procumbens* leaves was 100g/kg.

4.2. Effects of *Gynura procumbens* leaves on blood glucose level in mice

Fasting blood glucose was measured in mice in every weeks (Table 5). Distinct boost in blood glucose level was observed in all samples induced by alloxan- monohydrate. The fasting blood glucose level was around 4 mmol/l in all groups at initial stage. The glucose concentration in blood, however, decreased to 7.6±0.25 to 5.13±1.05 mmol/L in different groups after alloxan induction. Moreover, hypoglycemic effect in diabetic control group changed significantly ($P<0.001$) in every week compared with normal control. T_0 which was the diabetic control group demonstrated the higher blood glucose level for using only basal diet. But T_1 & T_2 were the treatment group where dry leaf powder was used to see the effectiveness of diabetes.

Table 5. Effects of *Gynura procumbens* leaves on blood glucose level in mice.

Parameters	Blood Glucose (mmol/L)				*P value*
	NC	T_0	T_1	T_2	
Initial	4.06±0.47	6.1±0.55	5.13±1.05	5.4±1.05	0.083
Week 0	$4.1±0.5^{a}$	$6.±0.75^{b}$	$6.23±0.55^{b}$	$5.5±0.83^{b}$	0.006
Week 1	$4.06±0.25^{a}$	$7.4±0.26^{c}$	$5.3±0.50^{b}$	$5.9±0.51^{b}$	<0.001
Week 2	$4.4±0.15^{a}$	$7.6±0.25^{c}$	$5.5±0.58^{b}$	$5.9±0.60^{b}$	<0.001
Week 3	$4.3± 0.45^{a}$	$7.2±0.20^{c}$	$6.06±0.51^{b}$	$6.5±0.28^{cb}$	<0.001

^{abc} means with different superscripts in the same row differ significantly.
NC=Normal control, T_0=Diabetic Control without treatment, T_1= 0.5% GP leaf (Basal diet + 0.5% GP leaf on DM basis), T_2= 1.0%GP leaf (Basal diet + 1.0 % GP leaf on DM basis).
All data are expressed as mean± SD.

4.3. Effects on body weight of mice

The average body weight of all mice in various groups was about 25g at the beginning of the experiment. The weight of normal control mice continued to increase evenly and the diabetic control group lost weight consistently to the end of the experiment as shown in (Table 6). At first week, no significant variation was noted between the NC and other treatment groups. However, at the end of the experiment, all mice under treatment exhibited significant ($P < 0.001$) increase in body weight in contrast to diabetic control.

Table 6. Effects of *Gynura procumbens* dry leaves on body weight of mice.

Parameters (g)	**Treatments**				***P* value**
	NC	**T_0**	**T_1**	**T_2**	
Week0	24.97±1.2	25.53±1.1	25.57±0.6	25.46±1.2	0.63
Week1	$26.64±1.2^b$	$24.62±1.5^a$	$25.14±0.9^a$	$24.95±1.2^a$	<0.001
Week2	$29.12±1.13^c$	$23.00±1.5^a$	$26.22±1.2^b$	$25.35±1.1^b$	<0.001
Week3	$31.46±1.5^c$	$21.94±1.4^a$	$27.66±1.7^b$	$26.60±1.3^b$	<0.001

^{abc} means with different superscripts in the same row differ significantly.
NC=Normal control, T_0=Diabetic Control without treatment ,T_1= 0.5% GP leaf (Basal diet + 0.5% GP leaf on DM basis), T_2= 1.0%GP leaf (Basal diet + 1.0 % GP leaf on DM basis).
All data are expressed as mean± SD.

4.4. Food and water consumption of alloxan-induced diabetic mice

Food intake per day among different groups showed considerable variation (Figure 2). The NC mice consumed around 4.6 g/day food whereas the consumption rate in T_0 was statistically significantly higher ($P<0.001$) at 11.1 g/day. However, the food intake was significantly lower ($P<0.001$) and at T_1 and T_2 at 7.8g/day, 8.06g/day food respectively in contrast to diabetic group (Figure 2).

The normal control group drank only 5.7±1.3 ml/day of water, which was statistically significant ($P<0.05$) in contrast to diabetic control groups (13.5±0.8 ml/day) (Figure 3).

All other alloxan induced groups- T_1 (8.3ml/d) &T_2 (9.3 ml/d) consumed considerably lower amount of water when compared to non-treated diabetic control groups.

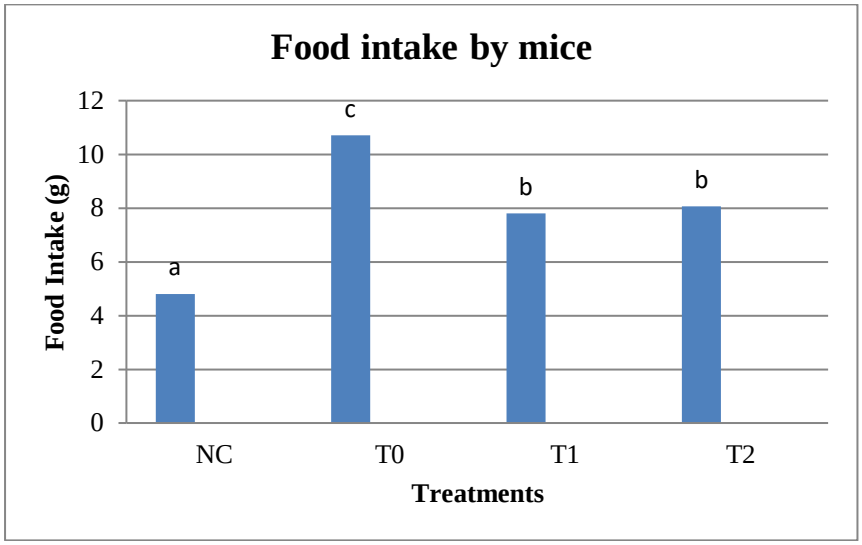


Figure 2. Food Intake by Mice.

[abc] means with different superscripts in the same row differ significantly.
NC=Normal control, T0=Diabetic Control without treatment, T1= 0.5% GP leaf (Basal diet +0.5% GP leaf on DM basis), T2= 1.0%GP leaf (Basal diet + 1.0 % GP leaf on DM basis). All data are expressed as mean± SD.

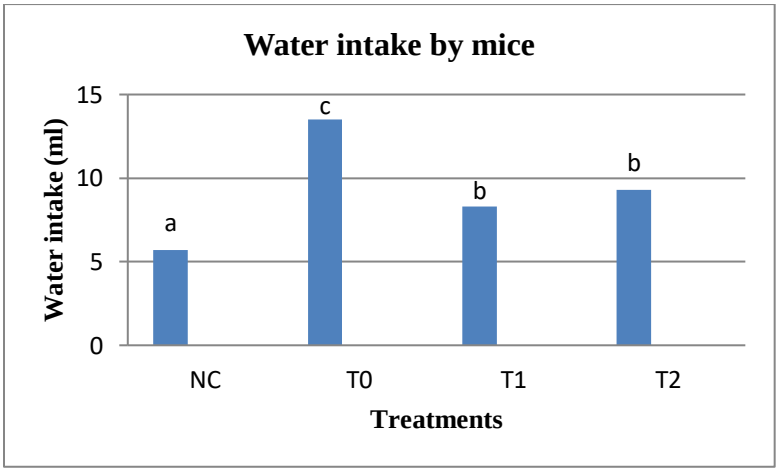


Figure 3. Water Intake by Mice.

[abc] means with different superscripts in the same row differ significantly.
NC=Normal control, T_0=Diabetic Control without treatment , T_1=0.5% GP leaf (Basal diet + 0.5% GP leaf on DM basis), T_2= 1.0%GP leaf (Basal diet + 1.0 % GP leaf on DM basis). All data are expressed as mean± SD.

4.5. Blood Lipid profile of mice

High density lipoprotein (HDL) decreased as well as total cholesterol, Triglycerides, and Low density lipoprotein (LDL) levels increased significantly in diabetes induced groups compared to normal control group. It is also apparent from the present study that mucilage as well as treatment groups significantly ($P<0.001$) decreased the HDL level and reduced the cholesterol, triglycerides, and LDL levels in alloxan induced diabetic mice when compared with the diabetic control group (Table 7).

Table 7. Effects of *Gynura Procumbens* on lipid profile in mice.

Parameters	**Treatments**				***P value***
	NC	**T_0**	**T_1**	**T_2**	
Cholesterol	114.07 ± 3.09^{a}	169.7 ± 3.55^{c}	137.07 ± 3.09^{b}	132.5 ± 2.96^{b}	<0.001
TG	112.4 ± 2.08^{a}	175.5 ± 3.61^{d}	146.4 ± 2.90^{c}	135.5 ± 4.37^{b}	<0.001
HDL	80.10 ± 1.45^{d}	36.43 ± 0.60^{a}	45.96 ± 4.14^{b}	54.43 ± 4.56^{c}	<0.001
LDL	11.48 ± 1.66^{a}	98.14 ± 3.83^{d}	61.81 ± 2.07^{c}	51.02 ± 4.62^{b}	<0.001

^{abc} means with different superscripts in the same row differ significantly.
NC=Normal control, T_0=Diabetic control,T_1=Dry leaf powder mixed with feed(0.5%),T_2=Dry leaf powder mixed with feed (1%) All data are expressed as mean± SD. All data shows highly significant.

4.6. Mineral contents & Total Protein of Blood

Mineral content analysis was done by blood sample of mice .Phosphorus, Ca & Tp values were non-significant .In the present study, decreased total protein was observed in diabetic control mice than normal control mice. However, Ca level was increased at T_1. Phosphorus level was similar with diabetic control mice (Table 8).

Table 8. Effects of *Gynura procumbens* on Blood Total Protein & Mineral content in mice.

Parameters	Treatments				*P value*
	NC	T_0	T_1	T_2	
Phosphorus	3.5±0.9	3.1±0.4	3.8±0.9	3.1±0.3	0.60
Calcium	8.73±0.7	9.2±1.2	10.15±0.9	9.2±0.4	0.16
Total Protein	5.8±0.3	4.7±0.5	4.8±0.7	4.5±1.01	0.34

[abc] means with different superscripts in the same row differ significantly.
NC=Normal control, T_0=Diabetic control,T_1=0.5% GP leaf (Basal diet + 0.5% GP leaf on DM basis), T_2= 1.0%GP leaf (Basal diet + 1.0 % GP leaf on DM basis)
All values are expressed as Mean ± SD.

4.7. Proximate Analysis

The result shows that the moisture percentage differs significantly among all dietary groups. In Basal Diet, there was a significant value in crude protein which was observed in all dietary group compared to others ($P<0.01$). The highest value was (20.96±1.3) at T_0. The crude fat decreased in T_1. The total ash content possessed significant change among all dietary groups.

Table 9. Effects of *Gynura procumbens* leaves on feed chemical composition.

Parameters	Treatments			*P value*
	T_0	T_1	T_2	
CHO	$31.83±0.15^{d}$	$25.83±0.80^{c}$	$23.50±0.70^{b}$	<0.001
Crude Protein	$20.96±1.3^{c}$	$17.13±0.60^{b}$	$18.13±0.60^{b}$	<0.001
Crude Fat	$1.60±1.6^{c}$	$0.07±0.005^{a}$	$0.83±0.50^{b}$	<0.001
Ash	$10.14±0.16^{a}$	$11.20±0.17^{b}$	$12.36±0.11^{c}$	<0.001
Moisture	$18.29±0.16^{d}$	$11.16±0.15^{b}$	$12.23±0.20^{c}$	<0.001
Crude Fiber	$5.75±0.17^{a}$	$7.79±0.05^{b}$	$8.60±0.06^{c}$	<0.001

[abcd] means with different superscripts in the same row differ significantly.
T0= Basal Feed, T1=0.5% GP leaf (Basal diet + 0.5% GP leaf on DM basis), T2= 1.0% GP leaf (Basal diet + 1.0 % GP leaf on DM basis)
All values are expressed as Mean ± SD.

4.8. Chemical Composition of *Gynura procumbens*

The graph shows that the chemical composition of GP leaf (Figure 4).

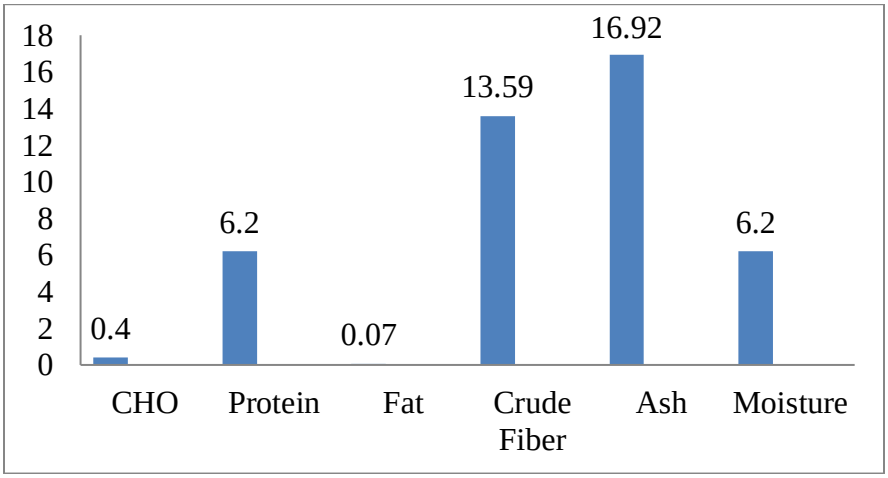


Figure 4. Chemical composition of *Gynura procumbens*.

(All values are expressed as Mean ± SD.)

Chapter V: Discussions

Medicinal plants consider as an effective source not only for traditional and modern medicines but also herbal medicine has been shown to have genuine utility and about 80% of rural population depends on it as primary health care. The beneficial health effects of many plants, used for centuries as seasoning agents in food and beverages, have been claimed for preventing food deterioration (Kaur and Arora, 2009).

This study confirms that *Gynura procumbens* (GP) leaf powder has effect on the alloxan induced diabetic mice can ameliorate diabetes mellitus (DM), as assessed by fasting blood glucose level, body weight, food, and water consumption. At the end week, the result of blood glucose was similar at the treatment groups and at the control group showed the higher level of blood glucose because of not controlling diabetes. The efficiency of hypoglycemic activity in mice may be due to the ability of the GP powder meal to prevent free radicals which is the major cause of alloxan induced hyperglycemia. This theory also supported by the health condition of diabetic control mice. By inhibiting the glucose sensor of beta cell referred to as glucokinase, alloxan impedes the secretion of glucose-induced insulin. Simultaneously, alloxan initiates a redox cycle furthermore as form superoxide radicals which than undergo the method of dismutation to make peroxide causing formation of highly reactive hydroxyl radicals by fenton reaction ultimately ensuing the death of beta cells and establish the condition of insulin-dependent diabetes (Szkudelski, 2001; King, 2012;). Alloxan induction in mice, within the current study, also exhibited typical visible feature of DM including weight loss, polydipsia (excessive thirst) and polyphagia (excessive hunger).

One of the parameters to consider the amelioration of diabetic state is to ascertain the effect of treatment on the body weight (Al-Attar and Zari, 2010). In diabetes mellitus, deranged glucagon-mediated regulation of cyclic adenosine monophosphate (AMP) formation in insulin deficiency leads to accelerated proteolysis (Rajasekaran et al., 2005). Since structural and tissue proteins contribute to 30 to 40% of total body weight, the excessive breakdown of tissue proteins due to diminished insulin response as well as

the unavailability of carbohydrate for energy metabolism in diabetes mellitus results in decreased body weight. Normalization of carbohydrate, protein and fat metabolism would alleviate the diabetic symptom of body weight loss; therefore body weight holds one of the key in evaluating the effectiveness of an anti-diabetic treatment (Al-Attar and Zari, 2010). In the present study, treatment on diabetic mice with *G. procumbens* meal showed decrease in body weight loss, which indicates the prevention of muscle tissue damage and protein wasting that is due to hyperglycemic condition in ameliorating diabetic state in alloxan-induced diabetic mice. *G. procumbens* meal treated groups which showed significant reduction in body weight gain. Normal mice and control groups were gained weight at the last week compared to treatment groups.

It was found that there was a numerical decrease in feed intake among treatment groups compared to control which was in agreement with the work of Oloruntola et al. (2018) where only numerical reduction in feed intake was observed. Different scenario was observed regarding the effects of GP leaf meal on feed intake similar to the normal mice where the control group felt hungry for the diabetes.

In this study biochemical parameters showed no significant differences while measuring the lipid profile. A significant decrease in serum cholesterol, triglyceride and LDL in treatment groups compared to control. However, serum HDL level increased in all treatment groups relative to control. A similar finding reported by Karthikesan et al. stated that CGA altered lipids, lipoproteins, and enzymes during lipid metabolism in STZ-nicotinamide-induced type 2 diabetes mellitus rats by decreasing the plasma and tissue (liver and kidney) lipids, cholesterol, triglycerides, free fatty acids, and low-density lipoproteins, respectively This finding might cause the blood pressure to reduce and return to normal range in atherosclerosis. (Runao et al., 2005). No significant variation was found in blood phosphorus, calcium and total protein content of this experiment.

Proximate analysis of basal and GP leaf meal diets showed lower content of carbohydrate, crude protein, crude fat while increased ash and crude fiber content.

Previous studies had a few research on dry powder proximate analysis. So the comparison was not significant. Other meal and feed had significantly differences.

Overall, findings in the present study are consistent with previous researches reporting that, lowers blood glucose level, anti-hyperglycemic effect as well as anti-hyperlipidemic effect.

Chapter VI: Conclusion

Medicinal plants have always been considered as healthy source of life for all people due to its rich in therapeutic properties and being 100 % natural. *Gynura procumbens* is a wonderful plant with high medicinal value. This powder showed 1% dose were more effectiveness. The data procured from this present research also provide evidence that the leaf powder possess hypoglycemic effects on blood glucose concentrations of diabetic mice. Furthermore, the results obtained from this study proved that has hypolipidemic activity. It posses HDL level higher according to LDL level. The results from this study also revealed that powder of *Gynura procumbens* is rich in fiber. Standard value of calcium, phosphorus and total protein on blood found at treatment groups than control group. The final result of body weight showed the higher differences between treatment group and control group. Hence, all the data obtained from the present study justify the traditional use of GP leaf in the treatment or management of diabetes in some part of Bangladesh.

Chapter VII: Recommendations & future perspectives

People, nowadays, want to use a wide range of natural remedies for the prevention and treatment of chronic disorders, including diabetes mellitus. And, in this case *Gynura procumbens* could be a solution. More pharmacological and biochemical studies are recommended to elucidate the mechanism of action of the anti-diabetic and anti-hyper-lipidemic activities A thorough studies need to be done to understand the beneficial effect of leaf on human subject.

Moreover, incorporation of dry powder in different food products needs to be studied. Higher level of protein and mineral content of GP suggested that it has potential food value and could be recommended as functional ingredient in our food industry.

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Appendix A: Photo Gallery

Figure: Sample Preparation

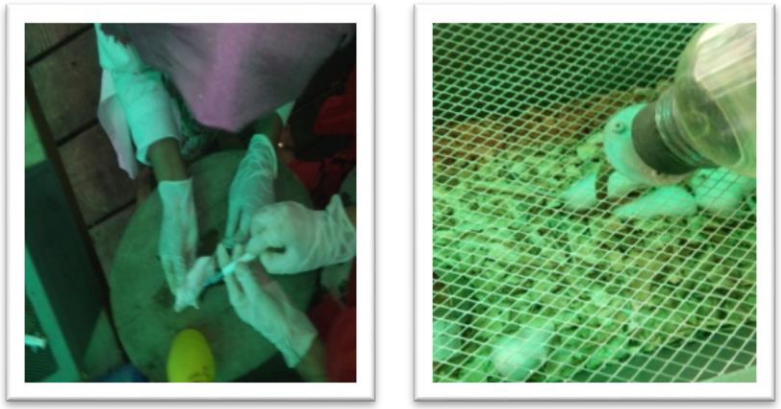

Figure: Experimental Works

Figure: Laboratory Works

Brief Biography

The author passed the Secondary School Certificate Examination in 2010 from Dr. Khastagir Govt. Girls' High School, Chattogram, and then Higher Secondary Certificate Examination in 2012 from Bangladesh Mohila Samiti Girls' High School & College, Chattogram. She obtained her B.Sc. (Hon's) in Food Science and Technology from the Faculty of Food Science and Technology at Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. Now, she is a candidate for the degree of Master of Science in Applied Human Nutrition and Dietetics under the Department of Applied Food Science and Nutrition, Chattogram Veterinary and Animal Sciences University (CVASU). She has an immense interest to work in improving health status of people through proper guidance and suggestions and to create awareness among people about food safety and nutrition.