Evaluation of Hypoglycemic Activities of Herbal Drug Methi in Steptozotocin Induced Diabetic Rats

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

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APPROVAL

This Project, **Evaluation of Hypoglycemic Activities of Herbal Drug Methi in Steptozotocin Induced Diabetic Rats** submitted by Md. Mohibul Alam to the Department of Pharmacy, Daffodil International University, has been accepted as satisfactory for the partial fulfillment of the requirement for the degree of Bachelor of Pharmacy and approved to its style and contents.

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DECLARATION

I hereby declare that, this project report is done by me under the supervision of Dr. M Obayed Ullah, Assistant Professor Department of Pharmacy, Daffodil International University, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy. I am declaring that this Project is my original work. I also declare that neither this project nor any part thereof has been submitted elsewhere for the award of Bachelor or any degree.

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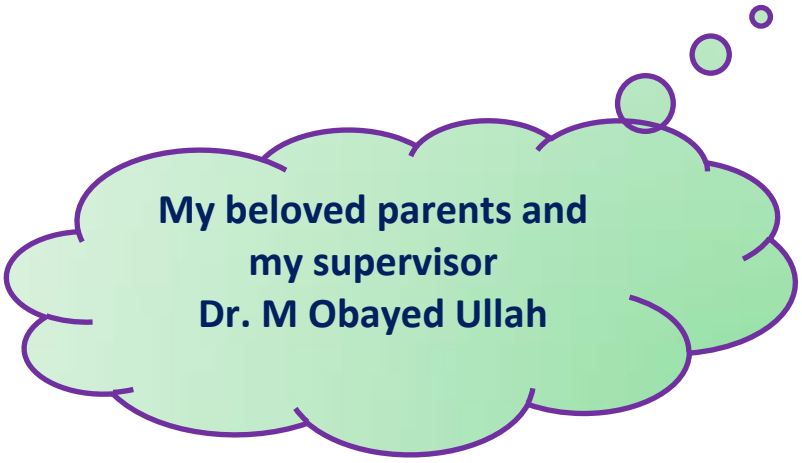

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Abstract

Trigonella foenum-graecum, is commonly known as Fenugreek or Methi, is available in Bangladesh. Though there are various therapeutic approaches to reduce the metabolic disturbance of diabetes and its secondary complications, herbal formulation are now getting preference due to fewer side effects. The present study was carried out to evaluate the anti-diabetic properties of *Trigonella foenum-graecum* on type 2 diabetic model rats, effects of water suspension of Fenugreek (F), who were studied in Streptozotocin (STZ)-induced diabetic rats.

Experimental diabetes was induced by injection a single dose of STZ (90mg/kg, i.p.). Adult male "Long Evans" rats were divided into three groups, for 28 days. Body weight, blood glucose level, were measured.

The blood glucose level and body weight were the salient features recorded in diabetic control rats. The Fenugreek supplements significantly decreased the levels of blood glucose, near to the normal control value.

Our data suggest that Fenugreek (methi) seeds powder supplementation may be beneficial for preventing diabetic complications.

Key Words: Fenugreek, Streptozotocin, Diabetic rats, Hypoglycemia.

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ABBREVIATIONS

g	: gram
L	: liter
mg	: milligram
mmoll/l	: milimol per liter
STZ	: streptozotocin
STD	: standard

Chapter One

Introduction

1.1 General Introduction

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia, with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [1]. There is increasing evidence that complications related to diabetes are associated with oxidative stress induced by the generation of free radicals [2]. Oxidative stress is a condition of imbalance due to excess formation of free radicals and decreased activity of antioxidant defense systems. The levels of reactive oxygen species (ROS) are regulated by a variety of cellular defense mechanisms consisting of enzymatic and non-enzymatic systems [2]. Dietary antioxidants become very important when oxidative stress in living organisms causes endogenous antioxidant depletion (especially glutathione) and they appreciably contribute to the improvement of antioxidant status in various biological fluids3,4. Natural remedies from medicinal plants are considered to be effective and safe alternative treatment for hyperglycemic and liver toxicity [5]. There is a growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience, and relatively cost effective. Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown [6]. Fenugreek (*Trigonella foenum graecum*) is an old herbal remedy used to treat metabolic and nutritive dysfunctions [7, 8]. Accordingly, in the present study streptozotocin induced diabetic rats were used to evaluate the possible hypoglycemic effects of a water suspension of fenugreek (Methi).

1.2 Prevalence of Diabetic Mellitus

The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. Globally, diabetes prevalence is similar in men and women but it is slightly higher in men above 60 years of age and in women at older ages. Overall, diabetes Prevalence is higher in men than women. [9]

The 10 countries estimated to have the highest numbers of people with diabetes in 2000 and 2030 are listed in the following table.

Table 1.1 List of countries with the highest number of estimated cases of diabetes for 2000 and 2030. [10]

Table 1: Countries with Highest Number of Estimated Cases of Diabetes (in millions) for 2000 and 2030.[2]

Ranking	2000		2030	
	Country	People with diabetes	Country	People with diabetes
1	India	31.7	India	79.4
2	China	20.8	China	42.3
3	U.S.	17.7	U.S.	30.3
4	Indonesia	8.4	Indonesia	21.3
5	Japan	6.8	Japan	13.9
6	Pakistan	5.2	Pakistan	11.3
7	Russian Federation	4.6	Russian Federation	11.1
8	Brazil	4.6	Brazil	8.9
9	Italy	4.3	Italy	7.8
10	Bangladesh	3.2	Bangladesh	6.7
World Total (millions)		**177**		**366**

Adapted from Wild S et al. *Diabetes Care*, 2004.

1.3 Prevalence of Diabetes Mellitus in Bangladesh

The prevalence of T2DM and impaired fasting glucose (IFG) and their risk factors in the urban population of Bangladesh exceeding 11.2 and 5.9%, respectively. The prevalence of diabetes in the urban population has increased alarmingly in recent years. Older age, obesity, higher income, family history of diabetes and reduced physical activity were proved to be the significant risk factors for diabetes and IFG. Bangladesh Institute of Research and Rehabilitation in Diabetes

Endocrine and Metabolic Disorders (BIRDEM), every year diagnose a total of about 20,000 new cases of diabetes.[11]

Table 1.2 Health expenditure for diabetes in 2010-SEA (IDF Bulletin, 2011)

Country/Territory	2010 Population (20-79 years)(000's)	Mean health expenditure (USS) per person with diabetes
SEA Total		154
Bangladesh	93862.1	21
Bhutan	413.0	97
India	713498.4	55
Maldives	186.0	554
Mauritius	876.7	254
Nepal	15556.5	30

1.4 Classification of Diabetic Mellitus

The most widely accepted classification of diabetes is the etiological classification of disorders of glycaemia [12]

A. Type 1 Diabetes Mellitus.

B. Type 2 Diabetes Mellitus.

C. Gestational diabetes.

D. Secondary Diabetes Mellitus.

E. Other Specific Types of Diabetes

1.4.1 Type 1 Diabetes

This is also known as juvenile, early-onset, or insulin-dependent diabetes. It usually first develops in children or young adults. With type 1 diabetes the illness usually develops quite quickly, over days or weeks, as the pancreas stops making insulin. It is treated with insulin injections and a healthy diet. [13]

1.4.2 Symptoms of Type 1 Diabetes

Symptoms that usually occur when first develop type 1 diabetes are:

1. Feeling thirsty a lot of the time
2. Passing a lot of urine
3. Tiredness, weight loss, and feeling generally unwell

The above symptoms tend to develop quite quickly, over a few days or weeks. After treatment is started, the symptoms soon settle and go.[14]

1.4.3 Diagnosis of Type 1 Diabetes

A simple dipstick test can detect sugar (glucose) in a sample of urine. This may suggest the diagnosis of diabetes. However, the only way to confirm the diagnosis is to have a blood test to look at the level of glucose in blood. If this is high then it will confirm that the person have

diabetes. Some people have to have two samples of blood taken and they may be asked to fast (this means having nothing to eat or drink, other than water, from midnight before the blood test is performed). [15]

1.4.4 Treatment of Type 1 Diabetes

Too much insulin can make the blood glucose level go too low (hypoglycemia, sometimes called a 'hypo'). This can cause feeling of sweaty, confused, and unwell, and may lapse into a coma. Emergency treatment of hypoglycemia is with sugar, sweet drinks, or a glucagon injection (a hormone which has the opposite effect to insulin). Then y should eat a starchy snack such as a sandwich. [16]

1.4.5 Type 2 Diabetes

This is also known as maturity-onset, late-onset, or non-insulin-dependent diabetes. Type 2 diabetes usually develops after the age of 40 (but sometimes occurs in younger people). It is more common in people who are overweight or obese.

With type 2 diabetes, the illness and symptoms tend to develop gradually (over weeks or months). This is because in type 2 diabetes, people still make insulin (unlike type 1 diabetes). However, either does not make enough for their body's needs, and/or the cells in their body are not able to use insulin properly. This is called insulin resistance.[17]

1.4.6 Symptoms of Type 2 Diabetes

The symptoms of Type 2 Diabetes often come on gradually and can be quite vague at first. Many people have diabetes for a long period of time before their diagnosis is made. [18]

The four common symptoms are:

1. Being thirsty a lot of the time
2. Passing large amounts of urine
3. Tiredness
4. Weight loss

1.4.7 Diagnosis of Type 2 Diabetes

A simple dipstick test may detect sugar (glucose) in a sample of urine. However, this is not sufficient to diagnose diabetes definitely. Therefore, a blood test is needed to make the diagnosis. The blood test detects the level of glucose in your blood. If the blood glucose level is high then it will confirm that the person have diabetes. Some people have to have two samples of blood taken and may be asked to fast (this means having nothing to eat or drink, other than water, from midnight before the blood test is performed). [19]

1.4.8 Fasting Plasma Glucose (FPG) Test

FPG test measures blood glucose in a person who has not eaten anything for at least 8 hours.

Table 1.3 this test is used to detect diabetes and pre-diabetes. [12]

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

1.4.9 Treatment of Type 2 Diabetes

Hypoglycemia (which is often called a 'hypo') occurs when the level of glucose becomes too low, usually under 4 mmol/L. People with diabetes who take insulin and/or certain diabetes tablets are at risk of having a hypo. A hypo may occur if they have too much diabetes medication, have delayed or missed a meal or snack, or have taken part in unplanned exercise or physical activity.

Symptoms of hypoglycemia include: trembling, sweating, anxiety, blurred vision, tingling lips, paleness, mood change, vagueness or confusion. [19]

1.5 Gestational Diabetes

Gestational diabetes mellitus (GDM) resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2–10% of all pregnancies and may improve or disappear after delivery.[20] However, after pregnancy approximately 5–10% of women with gestational diabetes are found to have diabetes mellitus, most commonly type 2. Gestational diabetes is fully treatable, but requires careful medical supervision throughout the pregnancy. Management may include dietary changes, blood glucose monitoring, and in some cases insulin may be required. [20]

1.6 Other types

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

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

Some cases of diabetes are caused by the body's tissue receptors not responding to insulin (even when insulin levels are normal, which is what separates it from type 2 diabetes); this form is very uncommon. Genetic mutations (autosomal or mitochondrial) can lead to defects in beta cell function. Abnormal insulin action may also have been genetically determined in some cases. Any disease that causes extensive damage to the pancreas may lead to diabetes (for example, chronic pancreatitis and cystic fibrosis). Diseases associated with excessive secretion of insulin-antagonistic hormones can cause diabetes (which is typically resolved once the hormone excess is removed). Many drugs impair insulin secretion and some toxins damage pancreatic beta cells. [21]

1.7 Medication

There are various medicines that can reduce the blood glucose level. Different ones suit different people. It is fairly common to need a combination of medicines to control blood glucose level. Some medicines work by helping insulin to work better on the body's cells. Others work by boosting the amount of insulin made by the pancreas. Another type works by slowing down the absorption of glucose from the gut. There is also a type which suppresses a hormone called glucagon, which is released into the bloodstream by the pancreas and stops insulin from working. [19]

1.8 Some common medications are:

1. Metformin
2. Sulfonylurea
3. Nateglinide and repaglinide
4. Thiazolidinediones
5. Acarbose
6. Insulin

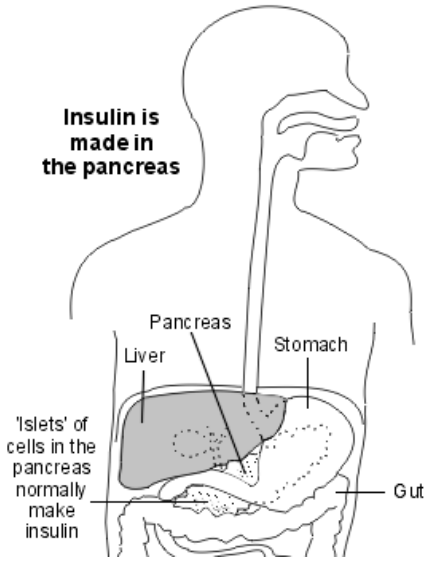

Fig1.1: Insulin in Pancreas

Note: Insulin injections lower blood glucose. Only some people with type 2 diabetes need insulin. It may be advised if blood glucose level is not well controlled by tablets.

Chapter Two

Literature Review

Methi or Fenugreek

Trigonella foenum-graecum, known as fenugreek, has long been used as a spice and an herbal remedy across the Middle East. People harvest and roast dried seeds of the plant for food flavoring and medicinal purposes. Various components of fenugreek are responsible for its beneficial effects, including blood sugar regulation and cholesterol reduction. [22]

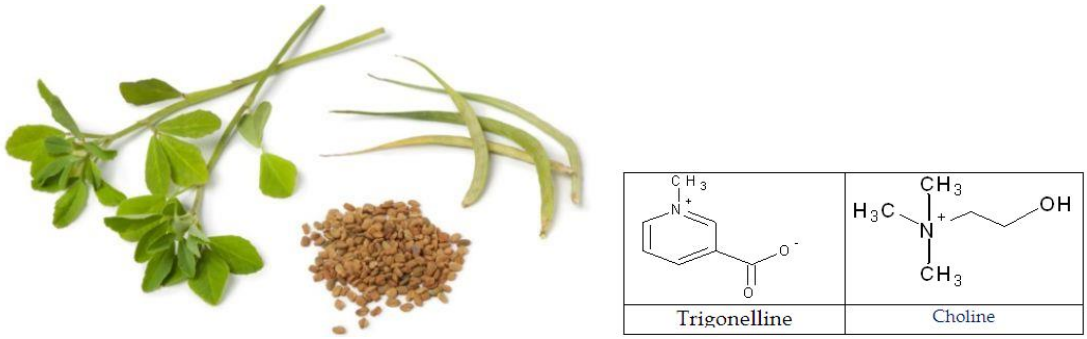

Fig.2.1 Leave, seeds of methi and its chemical structure

Metformin

Metformin is an oral diabetes medicine that helps control blood sugar levels.

Metformin is for people with type 2 diabetes. Metformin is sometimes used in combination with insulin or other medications, but it is not for treating type 1 diabetes.

Metformin may also be used for purposes not listed in this medication guide. [23]

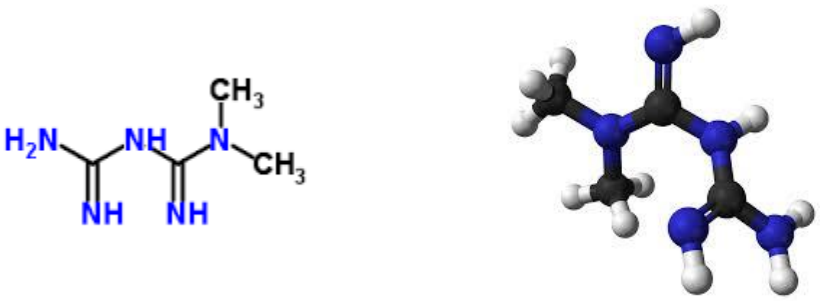

Fig2.2 Chemical and 3d structure of Metformin

Chapter Three

Materials and Methods

3.1 Experimental Animals:

Adult male "Long Evans'' rats weighing 200-300 g, purchased from the Bangladesh Institute of Research & Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), were used in the study. The rats were kept in a controlled environment and were allowed free access to standard pellet diet (15mg per day each) and water during study.

3.2 Plant Material and Preparation:

The dry powder of Fenugreek (F), *Trigonella foenum graecum*, was obtained from a local herbal market. Known weight of their powders was used as suspension in constant distilled water volume.

3.3 Experimental Design:

Rats were divided into normal control (NC), given distilled water orally, standard(STD) and diabetic groups, made diabetic by intraperitoneal (i.p.) injection of Streptozotocin (STZ) (90 mg/kg) freshly prepared in Citrate buffer (90mg/10ml). That confirms intraperitoneal dose 10µL/gm streptozotocin solution, 1pup get 70µl streptozotocin solution. Three month later after STZ administration, diabetic rats with OGTT test (Dose 2.5g/kg body weight) were randomly divided into three groups, diabetic control (DC), diabetic treated with Fenugreek (10mg/kg), treated with Metformin (10mg/kg) and treated with Water (NC). Each group contains three rats. All groups of rats were experimented for 28 days.

3.4 Statistical Analysis:

Data are presented as mean +standard deviation (S.D.).

Chapter Four

Result and Discussion

4.1 Body Weight

Table4.1: Show the body weight of rats Group-I (Herbal Methi), Group-II standard (Metformin), Group-III control after 28 days of treatment.

Group	0 Day Weight gm.	7 Day Weight gm.	14 Day Weight gm.	21 Day Weight gm.	28 Day Weight gm.
Group-I Fenugreek (Methi)	232±10.39	208.66±23.00	227.33±29.48	226±32.18	189.33±11.02
Group-II (Metformin)	198.66±15.14	159±9.89	148±19.79	215±49.49	144±25.45
Group-III (Water)	264.66±21.93	243.33±9.45	263.33±8.32	220.66±72.28	224±12.72

Data represent mean±S.D. (n=3) for each group

Following chart show the body weight of sample, standard and controlled,

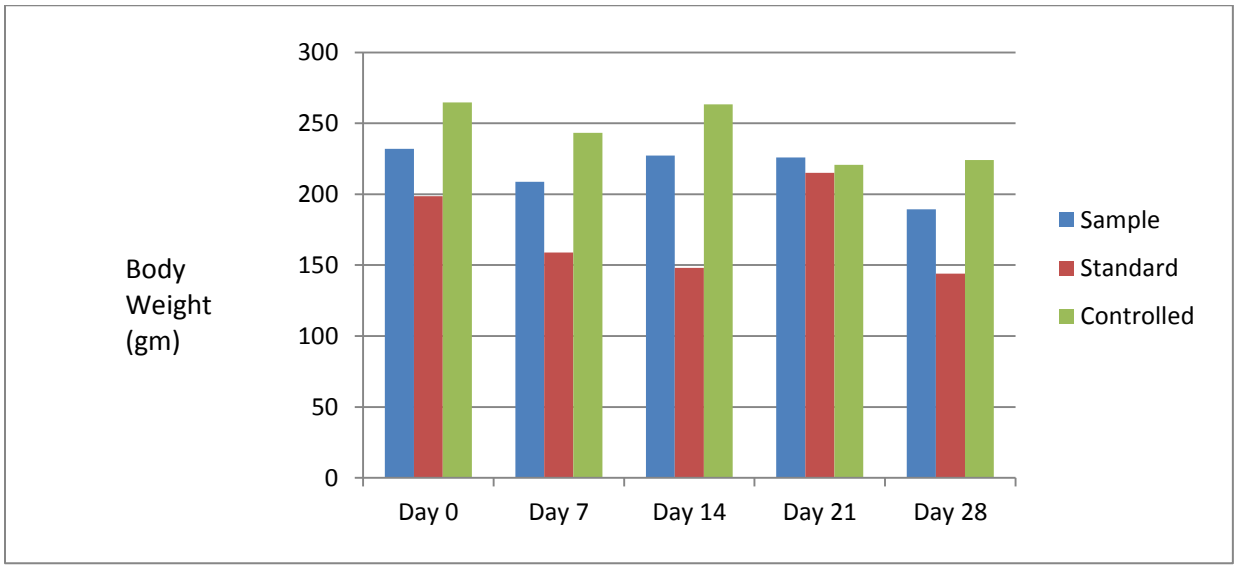

Fig4.1: Diagrammatical representation of body weight

A significant changed in body weight was detected in diabetic groups as compared to the standard and controlled groups, when the herbal drug Methi was administered at a given dose. But we got some abnormalities in this result, our standard drug Metformin gave fewer efficacies than the sample and controlled (water) that is contradictory. We have to go for further research.

3.2 Blood glucose level

Chart show the effect of 28 days treatment with powder aqueous suspension of Methi(sample), Metformin (standard), and water (controlled) on streptozoticin induced diabetic rats.

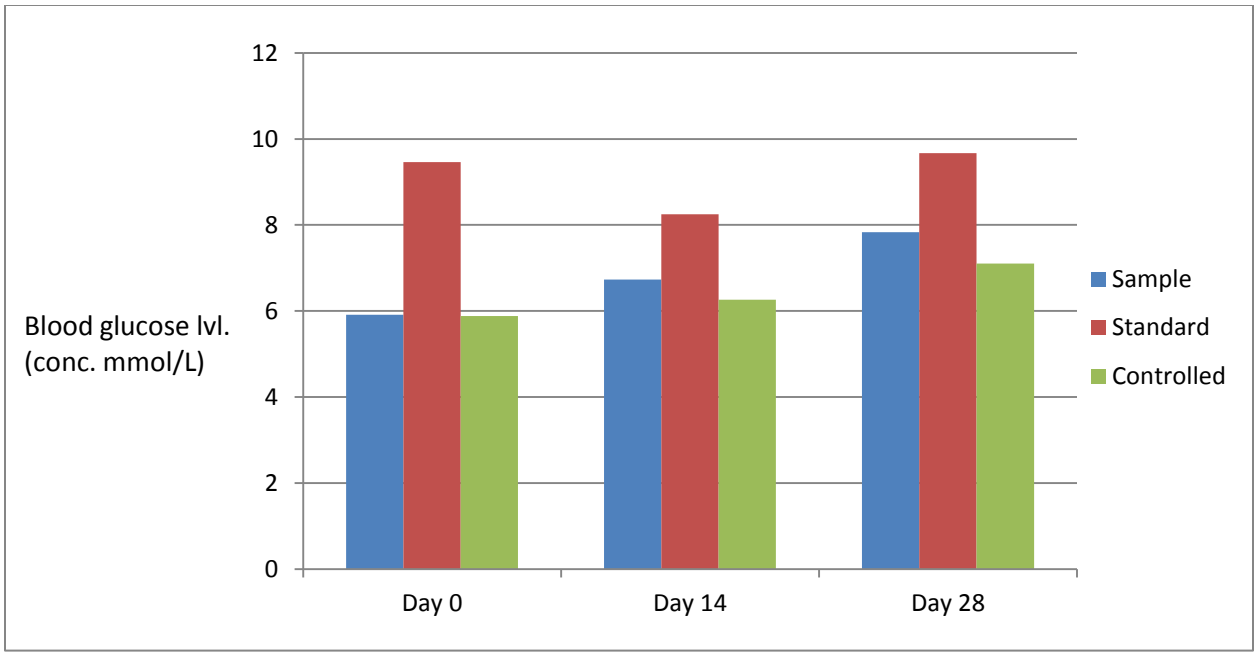

Fig4.2: Diagrammatical representation of blood glucose level

In this result (blood glucose level) we also got some abnormalities, our sample drug Methi deceased the blood glucose level more than the standard drug Metformin, this is also contradictory to the evidence.

4.3 Discussion

In the present study, we observed change in blood glucose level and body weight when steptozotocin diabetic rats were administered Methi (sample). The capacity of such powder (methi) to counteract hyperglycemia is an essential trigger during experimental diabetes. But we didn`t get the precise result during experimental study. But the results obtained showed Fenugreek seeds powder administration, decreased blood glucose level. We also found abnormalities in standard (metformin) and controlled group (water), that's why we have to go for further study or research.

Chapter Five

Conclusion

Conclusions:

Fenugreek (Methi) seeds powder suspension exhibited a hypoglycemic function and would be helpful in the prevention of diabetic complications. However, the precise hypoglycemic mechanisms of these seeds were not investigated in this work and further investigations in purifying the active compounds from the seeds will be necessary to elucidate the precise mechanisms of their hypoglycemic actions.

References:

[1] NAYAK BS, MARSHALL JR, ISITOR G, ADOGWA A. Hypoglycemic and hepatoprotective activity of fermented fruit juice of Morinda citrifolia (Noni) in diabetic rats. Evid Based Complement Alternat Med 2011; 2011: 875293.

[2] KIM HK, KIM MJ, CHO HY, KIM EK, SHIN DH. Antioxidative and anti-diabetic effects of amaranth (Amaranth usesculantus) in streptozotocin-induced diabetic rats. Cell Biochem Funct 2006; 24: 195-199.

[3] RECORD IR, DREOSTI IE, MC INERENEY JK. Changes in plasma antioxidant status following consumption of diets high or low in fruit and vegetables or following dietary supplementation with an antioxidant mixture. Br J Nutr 2001; 85: 459-464.

[4] PAPETTI A, DAGLIA M, GRISOLI P, DACARRO C, GREGOTTI C, GAZZANI G. Anti- and pro- oxidant activity of Cichorium genus vegetables and effect of thermal treatment in biological system. Food Chem 2006; 97: 157-166.

[5] CHO SY, PARK JY, PARK EM, CHO MS, LEE MK, JEON SM, KIM MJ, PARK YB. Alternation of hepatic antioxidant enzyme activities and lipid profile in streptozotocin-induced diabetic rats by supplementation of dandelion water extract. Clin Chim Acta 2002; 317: 109-117.

[6] GUPTA RK, KESARI AN, MURTHY PS, CHANDRA R, TANDON V, WATAL G. Hypoglycemic and antidiabetic effect of ethanolic extract of leaves of Annono squamosal L in experimental animals. J Ethnopharmacol 2005; 99: 75-81.

[7] ESKANDER EF WON JH. Hypoglycemic and hyperinsulinemic effects of some Egyptian herbs used for the treatment of diabetes mellitus (type II) in rats. Egypt J Pharm Sci 1995; 36: 331-342.

[8] CHEVASSUS H, MOLIMIER N, COSTA F, GALTIER F, RENARD E, PETIT P. A fenugreek seed extract selectively reduces spontaneous fat consumption in healthy volunteers.

[9] King H, Aubert RE, Herman WH (1998): Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. Diabetes Care 21:1414-1431.

[10] Wild SH, Roglic G, Sicree R, Green A, King H (2004): Global Burden of Diabetes Mellitus in the year 2000 and projection for 2003. Diabetes Carevol 27 no. 5 1047-1053.

[11] Sayeed M.A., Rumi M.A.K, Ali, L, Banu A., Hussain Z.A. and Khan A.K.Azad (2007). Effect of socioeconomic risk factors on the difference in prevalence of diabetes between rural and urban population in Bangladesh, Biological medical research council; 33(1), 1-12.

[12] WHO Consultation. (1999). Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Geneva: World Health Organization; Report no. 99.2.

[13] Diabetes - type 1; NICE CKS, Dec 2010

[14] Driving and diabetes; Diabetes UK.

[15] Diagnosis and management of type 1 diabetes in children, young people and adults; NICE Clinical Guideline (July 2004).

[16] Management of diabetes; Scottish Intercollegiate Guidelines Network - SIGN (March 2010)

[17] Diabetes - type 2; NICE CKS, July 2010.

[18] Diabetes Risk Score; Diabetes UK.

[19] Type 2 diabetes: the management of type 2 diabetes (update); NICE Clinical Guideline (May 2008).

[20] "National Diabetes Clearinghouse (NDIC): National Diabetes Statistics 2011". U.S. Department of Health and Human Services. Retrieved 22 April 2014.

[21] "Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications" (PDF). World Health Organisation. 1999.

[22] http://www.thehealthsite.com/

[23] http://www.drugs.com/metformin