EFFECT OF OKRA (*Abelmoschus esculentus*) MUCILAGE ON GLUCOSE LEVEL AND LIPID PROFILE OF ALLOXAN-INDUCED DIABETIC MICE

A.F.M Irfan Uddin Zim

Roll no : 0118/19

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

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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

---

(Prof. Dr. Jannatara Khatun)
Supervisor
Professor
Department of Animal Science & Nutrition

(Mohammad Mozibul Haque)
Co-supervisor
Lecturer
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

JUNE 2019

DEDICATED TO MY BELOVED

FAMILY

&

TEACHERS

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Abbreviations

%	:	Percentage
&	:	And
ANOVA	:	Analysis of variance
AOAC	:	Association of Official Analytical Chemists
AUC	:	Area under the curve
℃	:	Degree Celsius
dl	:	Deciliter
DC	:	Diabetic control
DPPH	:	2,2-diphenyl-1-picrylhydrazyl
et al	:	Et alii/ et aliae/ et alia
etc	:	Et cetera
g	:	Gram
GAE	:	Gallic acid equivalent
Kg	:	Kilogramme
mg	:	miligram
NS	:	Not significant
NC	:	Normal control
OGTT	:	Oral glucose tolerance test
PPM	:	Parts per million
PPS	:	Powdered peel seed
PM	:	Powdered mucilage
QE	:	Quercetin equivalent
SD	:	Standard drugs
SPSS	:	Statistical Package for Social Science
SEM	:	Standard Error of Mean
T1DM	:	Type 1 diabetes mellitus
T2DM	:	Type 2 diabetes mellitus
UV	:	Ultraviolet

Abstract

The raw mucilage of okra (*Abelmoschus esculentus)* has been used for the management of diabetes mellitus in many rural part of Bangladesh. However, there is dearth of knowledge about the efficiency of it. Hence, this study aimed to explore antidiabetic, anti-lipidemic activity of crude okra mucilage powder. In addition, the effectiveness of okra mucilage was compared with the isolated peel-seed of *Abelmoschus esculentus* after mucilage extraction. Crude protein and mineral contents and antioxidant activity of the sample were also assessed. The protein content of okra mucilage and peel-seed powder was found at 8.54±0.96 g/100g and 11.28±1.27 g/100g respectively. The mineral concentrations for okra mucilage and peel-seed mixture showed high amount of calcium, magnesium, phosphorus, potassium, sodium and iron. Three weeks administration of crude mucilage and peel-seed suspensions at a dose of 150 mg/kg and 200mg/kg significantly ($P<0.05$) reversed the abnormal changes of bodyweights, water consumption, feed consumption and fasting blood glucose levels of alloxan induced diabetic mice. The other biochemical parameters like cholesterol, triglycerides, low density lipoproteins, high density lipoproteins, and total protein were found to be significantly ($P<0.05$) improved after mucilage and peel-seed treatment. In vitro antioxidant activity of the mucilage and peel-seed powder were determined by the DPPH free radical scavenging assay, total phenol content and total flavonoid content. For the antioxidant activity, the IC_{50} value was 73.83 µg/ml for okra mucilage and 67.09 µg/ml for peel-seed mixture. Quantitative analysis displayed 65.98±0.3 mg gallic acid equivalent (GAE)/g and 68.84±0.3 mg GAE/g for phenolic contents and 9.50± 1.1 mg quercetin equivalent (QE)/g and 7.90±0.1 mg QE/g for flavonoid contents for peel-seed mixture and mucilage respectively. Finally, okra mucilage showed less efficacy in terms of improving diabetic status as well as in vitro antioxidant activity compared to peel-seed mixture.

Keywords: Okra, Mucilage, Antidiabetic, Anti-hyperlipidemic, Antioxidant.

Chapter 1: Introduction

Diabetes mellitus, a long term metabolic disease, is characterized by elevated glucose level in blood due to insulin secretion, insulin action, or both (American Diabetes Association, 2012). Insulin, which is synthesized by β-cells of the pancreas, assists in maintaining glucose concentration in blood by transporting it to the body cell and thus helps in producing energy (Ahmad, 2014). Hence, insulin resistance, dysfunction or destruction of beta cell leads to aberrant glucose metabolism in the body causing diabetes mellitus (Kahn et al., 2014). Underlying factors of diabetes mellitus includes environmental factors, genetic factors, lifestyle changes and high fat diet consumption (Ozougwu, 2013).

In 2017, there were globally 451 million people with diabetes which is projected to increase 693 million by 2045 (Cho et al., 2018). By the year 2045, India is expected to be the top one country with 134.3 million people with diabetes followed by china (119.8 million). Pakistan and Bangladesh will be in the 8th and 9th position respectively with 16.1 million and 13.7 million people with diabetes (International Diabetes Federation, 2017). People from low- and middle-income countries are the most sufferers (World Health Organisation, 2018). Rapid and ongoing socioeconomic transition are also influencing the prevalence of diabetes in developing countries (Guariguata et al., 2014).

Though absolute cure has not been found yet, proper management can keep a patient healthy. Without proper management diabetes can lead to severe damage to other organs including heart, kidneys, eyes, nerves and blood vessel (American Diabetes Association, 2012). Also, uncontrolled diabetes could be life threatening. General management of diabetes is defined by eating a healthy nutritious diet, avoiding sugar rich food, maintaining balanced weight, exercising in a regular manner, taking medications regularly and if needed taking insulin therapy. However, treatment with antidiabetic drugs is costly and, that is the reason people in developing or low income countries always have an intention to heal the consequences or symptoms of diabetes in a more natural way. Hence, research focusing on available medicinal plant for the treatment of chronic disease such as diabetes is going on continuously.

Okra is one of those plant product that can be used in the management of diabetes. Okra (*Abelmoschus esculentus*), a member of the Malvaceae family, is originated in Ethiopia

or India, and known as lady's finger in English, gombo in France, bamiah in Saudi Arabia, and bhendi in India (Lamont, 1999). It is generally known as dherosh in Bangladesh. Okra is a widely known vegetables that is cultivated all over the world and has been used for daily consumption in many countries. Okra plants are grown commercially in many countries including India, Japan, Turkey, Iran, Western Africa, Yugoslavia, Bangladesh, Afghanistan, Pakistan, Myanmar, Malaysia, Thailand, Brazil, Ethiopia, Cyprus and in the Southern United States**.** Given its robust nature, dietary fiber, and distinct seed protein balance of both lysine and tryptophan amino acids, Okra has been called "a perfect villager's vegetable" (Holser and Bost, 2004).

People generally eat the fruit and seed parts of Okra. Okra fruits are generally eaten as vegetables and also used in the preparation of salads, soups and stews (Gemede et al., 2015). Edible portion of fresh okra generally contains 90% water, 7.6g CHO, 2.0g protein, 0.1g fat, 3.2g dietary fiber, 0.3g total lipid and provide 33-38 kcal (Haytowitz and Matthews, 1984). However, recent study revealed that proximate composition varies in different okra accessions (Gemede et al., 2016).

Several health benefits of okra have been disclosed previously. Okra seed is a good source of protein and oil as well as rich in linoleic acid vital for human nutrition (Savello et al., 1980; Oyelade et al., 2003). Okra have been found to effective in the management of diabetes, digestive issues, colon health, body's cholesterol level and heart health (Gemede et al., 2015). Different parts of okra plant have also revealed the presence of the total phenolics, total flavonoids and antioxidant properties (Liao et al., 2012). Moreover, being a fiber rich vegetables, okra has a great potential to control blood glucose and plasma cholesterol levels in diabetic patients (Riccardi and Rivellese, 1991). Previous experiments have also demonstrated the in vivo and in vitro anti-hyperglycemic as well as anti-hyperlipidemic activities of different parts of okra (Dubey and Mishra, 2018)

Almost all parts of the okra including pods, seeds, stems, leaves, flowers, barks, roots and the cell walls of the fruit contains high amount of mucilage (Sengkhamparn et al., 2009; Ahiakpa et al., 2014). Okra mucilage are generally acidic polysaccharides composed of galacturonic acid, galactose, rhamnose, arabinose and glucose (Woolfe et al., 1977). Mucilage is also the main source of carbohydrates in okra (Kumar et al., 2009). Due to its high consistency, okra mucilage is sometimes added into the soups,

stews and sauces. Studies have also suggested its potential as a food, non-food products, and medicine (Kumar et al., 2010; Gemede et al., 2015). Previous research studies also portrays the okra mucilage as a potential pharmaceutical substance (Farooq et al., 2013). In vitro studies have suggested that okra polysaccharide has great potential in the management of hyperglycemia, oxidative stress, obesity and weight gain as it demonstrates inhibitory effect on α-glucosidase, α-amylase, lipase and intestinal glucose absorption as well as possess anti-oxidative and muscle glucose uptake activity (Ozougwu, 2013). However, there is only limited studies focuses on in vivo antidiabetic activities of okra mucilage.

Moreover, most of the previous researches on *Abelmoschus esculentus* focuses mainly on okra peel and seed. Though raw okra mucilage is consumed in many areas of Bangladesh as a natural substance to control diabetes, there is dearth of scientific knowledge about the efficiency of it. Therefore, the present study was conducted to determine the hypoglycemic and hypolipidemic potential of raw okra mucilage in alloxan induced diabetic mice. Moreover, antioxidant potential has also been studied.

Aims and Objectives:

The main objective of this thesis is to prepare mucilage powder from fresh okra vegetables and examine its effects on the blood glucose level and lipid profile of alloxan induced diabetic mice. Moreover, the antioxidant potential of okra mucilage will be evaluated. Finally, the effectiveness of okra mucilage and peel-seed of okra after mucilage extraction will be compared.

Chapter II: Review of Literature

2.1. Diabetes Mellitus

Diabetes mellitus, a chronic metabolic disorder, is characterized by elevation of glucose level in blood due to irregularity in insulin secretion, improper insulin action, or both. It happens if the body either not producing enough insulin or because the body cells do not properly respond to the insulin that is produced. Beta cells of pancreas maintain the adequate insulin secretion. However, a combination of genetic and environmental factors causes the beta-cell failure leads to hyperglycemia. This widely known metabolic disorder is affecting a large proportion of the population all over the world. Diabetes mellitus is a long term disease. This disease can directly or indirectly affect various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (Rother, 2007; American Diabetes Association, 2012; Cho et al., 2018;).

2.1.2. Classification

Diabetes mellitus is broadly divided into 2 groups, namely insulin dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM). This classification in mainly based on the requirement of insulin for treatment.

2.1.2.1. Type 1 diabetes mellitus (T1DM)

T1DM is widely known as insulin dependent diabetes mellitus (IDDM), previously known as juvenile onset diabetes mellitus. This condition results from a cellular-mediated autoimmune destruction of the beta cells of the pancreas. Beta cell is responsible for proper insulin secretion. Multiple genetic predispositions and other environmental factors are mainly the underlying causes of beta-cell destruction. Though the rate of beta-cell destruction is quite variable, the process is rapid in infants and children. However, the rate of destruction is slow in adults. That is the reason, type 1 diabetes mellitus only forms 5-10% of the total diabetes patients (American Diabetes Association, 2012).

2.1.2.2. Type 2 diabetic mellitus (T2DM)

The majority (95%) of diabetic patients have type II diabetes. T2DM is characterized by insulin insensitivity as a result of insulin resistance, declining insulin production, and eventual pancreatic beta-cell failure (Kahn, 1994). Impaired alpha-cell function is also associated with the pathophysiology of T2DM. These systematic problems in the

body leads to rising of glucagon and hepatic glucose levels during fasting. In addition with inadequate levels of insulin and increased insulin resistance, this condition ultimately results into hyperglycemia (Fujioka, 2007). Several studies show that insulin resistance precedes the defect in insulin secretion but individual will develop diabetes only when insulin secretion is inadequate to maintain glucose concentration close to normal (Fujioka, 2007; Kaku, 2010). This disease is the most prevalent and chronic metabolic disorder expanding firmly all over the world. This condition was formerly known as non-insulin dependent diabetes or maturity onset diabetes. Other than genetic factors, Physical inactivity, sedentary lifestyle, cigarette smoking and generous consumption of alcohol are the underlying causes of this condition (Olokoba et al., 2012).

2.1.2.3. Gestational Diabetes mellitus

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. Approximately 7% of all pregnancies are complicated by GDM, resulting in more than 200,000 cases annually. Gestational diabetes increases the risk of preeclampsia, stillbirth and delivery complication due to baby weight. Assessment of risks of developing gestational diabetes should take place during the first prenatal visit. However, these risks can be reduced by close monitoring of blood glucose through the whole pregnancy (American Diabetes Association, 2004).

2.1.2.4. Other specific types of diabetes

Some specific diseases, drugs or genetic conditions/syndrome are associated with development of chronic hyperglycemia. Pancreatic disease or removal of pancreatic tissue, endocrine diseases such as acromegaly, Cushing's syndrome, pheochromocytoma, glucagonoma, somatostatinoma, and primary aldosteronism, or the administration of certain hormones, drugs, and chemicals can cause hyperglycemia. Genetic syndrome, abnormalities in insulin receptors may also cause specific type of diabetes (Anonymous, 1979).

2.1.3. Etiology of diabetes mellitus

Generally, it is presumed that genetic and environmental factors play a contributing role for the onset of diabetes mellitus. Environmental factors trigger the diabetogenic process in a genetically susceptible individual. Family history among diabetic patients ranges 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 is also well documented. Resistance to insulin varied among different region of the world (Sharp et al., 1987). Study on various ethnic groups showed that environmental factors play a crucial role in the prevalence of diabetes (LaPorte et al., 1985). In case of gender, Bruno et al. Observed a significantly higher incidence of type I diabetes in males compared to females (Bruno et al., 1993). Another study reported that type II diabetes is more prevalent in men (16.7%) than in females (9.5%) in Mexican populations of all age groups (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, nutritional factors, obesity, severe and prolong stress, drugs can assist on the onset of diabetes (Adeghate et al., 2006). Regular physical activity increases insulin sensitivity and glucose tolerance (Kriska et al., 2001). Obesity has also been implicated as a risk factor for type II diabetes. Obesity is one of the causal factors for insulin resistance. Insulin resistance also occurs due to many factors including- hypertension, dyslipidemia, atherosclerosis, aging, medication, genetic and other rare conditions. Insulin resistance may improve with weight reduction and/or pharmacological treatment of hyperglycemia but is seldom restored to normal (American Diabetes Association, 2012).

2.1.4. Pathophysiology and pathogenesis of diabetes

Insulin is triggered by food especially foods having carbohydrate. Insulin is triggered by rise in blood glucose level after eating. Pancreas release insulin to control the elevation of glucose in blood. Insulin is used by body's cells to absorb glucose from the blood for use as fuel, for conversion to other needed molecules, or for storage. However, when insulin production from pancreas is hampered (T1DM) or insulin resistance occurs (T2DM), it ultimately raise the glucose level in blood rather than absorbed by other cells leading the onset of diabetes (Nathan et al., 2005; Santaguida et al., 2005).

On the other hand, when glucose level falls in the blood (Hypoglycemia), insulin production from beta cell gets reduced. In contrast, glucagon, which does opposite action of insulin, rises glucose level in the blood. In healthy subjects, consumption of meal high in glucose decrease the production of glucagon thereby reducing glucagon-induced stimulation of hepatic glucose production. Unfortunately, opposite situation

occurs 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 leading to chronic hyperglycemia in T2DM patient (Lund et al., 2014).

In T1DM patient, first indication of this disease is characterized by ketoacidosis. Others may indicate fasting hyperglycemia turning to severe hyperglycemia. At the final stage, there is little or no insulin secretion in the body leading to T1DM. The decrease in insulin secretory capacity is due to actual loss of beta cell mass. Though, children and adolescence are more susceptible to immune-mediated diabetes, it can also occur at the later stages of life (American Diabetes Association, 2012).

In simplest term, impaired beta cell function (insulin deficiency) and/or inefficient action (insulin resistance) are the central mechanisms of hyperglycemia.

2.1.5. Epidemiology of diabetes

The number of diabetic patient is increasing worldwide. The increased number is alarmingly high in some parts of the world. Dramatic changes in sedentary lifestyle and urbanization boosting the prevalence of diabetes worldwide. In 1980, the World Health Organization (WHO) estimated that there were 108 million people living with diabetes and this number increased fourfold in 2014 estimates (Zhou et al., 2016). It is estimated that 366 million people had DM in 2011; by 2030 this would have risen to 552 million (Anonymous, 2011). International Diabetes Federation (IDF) estimated the global 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 were 451 million (age 18–99 years) people with diabetes worldwide. These figures were expected to increase to 693 million by 2045. Moreover, It was 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 all-cause mortality among people within this age range (Cho et al., 2018). Similarly, the rise of type 2 diabetes in South Asia is estimated to be more than 150% between 2000 and 2035 (Nanditha et al., 2016). A recent meta-analysis showed that the prevalence of diabetes among adults had increased 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 was 15.2% compared with 8.3% among rural residents. In total, 56.0% of diabetics were not aware they had the condition and only 39.5% were

receiving treatment regularly. The likelihood of diabetes in individuals aged 55 to 59 years was almost double that in those aged 35 to 39 years (Akter et al., 2014). Almost one in ten adults in Bangladesh was found to have diabetes, which has recently become a major public health issue. Better detection, awareness, prevention and treatment is necessary to prevent the rise in diabetes.

2.1.6. Signs and symptoms

Sign and symptoms of marked hyperglycemia is characterized by polyuria (frequent urination), polydipsia (excessive thirst), weight loss, sometimes with polyphagia (excessive hunger), and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the non-ketotic hyperosmolar syndrome (American Diabetes Association, 2012). The symptoms may develop quite rapidly in Type 1 diabetes particularly in children, however, in Type 2 diabetes, symptoms usually develop much more slowly and may be subtle or completely absent. If diabetes is poorly controlled, then frequent weight loss can also occur. However, during onset of diabetes unexplained weight loss may also be experienced (Guariguata et al., 2014).

2.1.7. Diagnosis

Diabetes mellitus is characterized by elevated blood glucose level is diagnosed by demonstrating any one of the following (American Diabetes Association, 2012). More than 6.5% in A1C test can level a patient as a diabetic. A1C is a widely used marker of chronic glycemia, reflecting average blood glucose levels over a 2 to 3 month period of time. By fasting plasma glucose level a person can be labeled as a diabetic if it is at/or above 7.0 mmol/L (126 mg/dl). Patient having a 2-h plasma glucose more than 11.1mmol/L (200mg/dl) during an oral glucose tolerance test (OGTT) can be labeled as diabetic. The test should be performed as described by the World Health Organization (WHO, 2006) using a glucose load containing the equivalent of 75g anhydrous glucose dissolved in water. Random plasma glucose levels can also suspect diabetes. In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose is more than 11.1 mmol/l (200 mg/dl).

People are generally unaware of them having diabetes. 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 such as vision changes or unexplainable fatigue. Diabetes is often detected when a person suffers a problem that is frequently caused by diabetes, such as a heart attack, stroke, neuropathy, poor wound healing or a foot ulcer, certain eye problems, certain fungal infections, or delivering a baby with macrosomia or hypoglycemia. The most common trend is, however, two fasting glucose measurements of above 7.0 mmol/l for consideration diabetes mellitus (Santaguida et al., 2005; Hirsch, 2009).

2.1.8. Management of diabetes mellitus

Management of diabetes mellitus is focusing on supporting people to live with minimum or no risk of complications. In terms of T1DM, patients often required to use of insulin analogues and mechanical technologies (insulin pumps and continuous glucose monitors) for improved treatment of type 1 disease (Hirsch, 2009). Setting of realistic goals in management of T1DM is important for each child and family. Factors to be considered include a patient's age, developmental status, family involvement and social situation, economic factors, and hypoglycemic history in persons with established disease. Moreover, nutritional education and psychological support need to be provided accordingly. Though, in T1DM, more focus is given on

insulin management, optimal management requires proper recognition of the balance among insulin, exercise, and food (Haller et al., 2005). For T2DM, lifestyle modification along with nutritional and drug treatment can help in a great way. Maintaining body mass index at appropriate level together with continuing diet rich in fiber, low in saturated fat, abstinence from smoking and alcohol can significantly reduce the complications of T2DM (Willi et al., 2007; Chen et al., 2011a).

2.1.9. Drug Therapy

Glucose lowering medications and insulin administration can help to manage plasma glucose concentrations as close as possible to normal range.

2.1.9.1. Insulin administration

As T1DM is insulin dependent, insulin should be administered to type 1 diabetic patient. Insulin also suggested to T2DM patient as a supplement when oral drugs and nutritional therapy fails to control blood glucose concentrations. In market, 4 types of insulin

injections are available: rapid acting insulin, short acting insulin, intermediate acting insulin and long lasting insulin (Table 1). With multiple daily injections, a long acting insulin analogue provides basal insulin and a rapid-acting insulin is administered before meals, based on grams of carbohydrate consumed. 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 effects 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).

Table 1: Insulin types and action profile

Type and preparation		Constituents	Action profile (hour)		
			onset	peak	duration
Ultra-rapid acting	**Lispro**	Identical to human regular insulin with transposed lysine and proline in the β chain	0.2-0.5	0.5-2	3 - 4
Short-acting	**Regular**	Solution of unmodified zinc insulin crystals	0.5-1	2-3	6-8
Intermediate-acting	**NPH**	Protamine zinc, phosphate buffer	1.5	4-10	16-24
	Lente	Amorphous, acetate buffer	1.5-3	7-15	16-24
Long-acting	**Ultralente**	Amorphous and crystalline mix	3-4	9-15	22-28
Mixtures	**70/30**	NPH 70%, regular 30%	0.5-1	3-12	16-24
	50/50	NPH 50%, regular 50%	0.5-1	2-12	16-24

Source: (Chehade and Mooradian, 2000)

2.1.9.2. Oral anti-diabetic drugs

2.1.9.2.1. Biguanides

Metformin is the most widely used medicine under this category. It has proven to be an effective anti-hyperglycemic agent. In diabetic patient, biguanides suppresses hepatic glucose production, increases insulin sensitivity, enhances glucose uptake by phosphorylating GLUT-enhancer factor, increases fatty acid oxidation, and decreases the absorption of glucose from the gastrointestinal tract. It also enhances glucose uptake in the peripheral tissue, mainly the muscle (Chehade and Mooradian, 2000; Collier et al., 2006). It is noteworthy 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 in those individuals who appear to be secondary failures, a substantial blood glucose lowering occurs (DeFronzo and Goodman, 1995).

2.1.9.2.2. 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).

2.1.9.2.3. Meglitinides

Repaglinide and nateglinide are non-sulfonylurea secretagogues which act on the ATP-dependent K-channel in the pancreatic beta cells thereby 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 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)

2.1.9.2.4. 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

Many study has demonstrated the potential of diet modification to control hyperglycemia. Proper diet control can keep a patient way from taking extra doses of drugs. Nutritional management is necessary to slow the rate of development of diabetes complications. Generally, a healthy adults 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 less than 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. Decent nutritional management can also help to regulate weight management (American Diabetes Association, 2006).

Generally, moderate weight loss (5% of body weight) in subjects with type 2 diabetes is associated with decreased insulin resistance, improved measures of glycemia and lipemia, and reduced blood pressure (Klein et al., 2004). Continuing exposure to 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. There are data suggesting that consuming a high-fiber diet reduces glycemia in subjects with type 1 diabetes and glycemia, hyper-insulinemia, and lipemia in subjects with type 2 diabetes (Franz et al., 2002). 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). Overall, the evidence is strong that MNT is an effective and essential therapy in the management of diabetes.

2.2. Overview of okra (*Abelmoschus esculentus*)

Okra, scientifically known as "*Abelmoschus esculentus"*, also known as "Ladies finger" in some region, and in Bangladesh, its popular name is "Dherosh". It belongs to the Malvaceae family. The name Okra probably derived from one of Niger-Congo group of languages (the name for okra in the Twi language is nkuruma) (Yonas et al., 2014). The first recorded reference to okra was made by the Egyptians in 1216 A.D., although the plant explorer Vavilov indicated that there was strong evidence that the crop flourished even before that date in the tropical climate of Ethiopia, while others have identified its origin as India (Lamont, 1999). Okra is one of the world's oldest multipurpose crop due to its various uses of the fresh leaves, buds, flowers, pods, stems and seeds (Yonas et al., 2014). Immature fresh and green seed pods are consumed as vegetable. The edible immature seed pods generally harvested while they are still soft and the seeds are only partially developed. The color of immature pods varies from pale to dark green, red, or purple (Martin et al., 1981). *Abelmoschus esculentus* has been used throughout history for both medicinal and culinary purposes.

2.2.1. Okra constituents and uses

Okra has been used in a variety of ways in different geographical location throughout the history (Martin, 1982). Okra fruits is the most popular part to consider as a food. Okra pods have a unique flavor and mucilaginous texture. Fresh okra contains 90% water, 7.6% CHO, 2.0% protein, 0.1% fat, 3.2% dietary fiber, 0.3% total lipid and provide 33-38 kcal (Haytowitz and Matthews, 1984; Roy et al., 2014; Gemede et al., 2016). Okra also rich in various minerals including calcium, potassium and magnesium (Moyin-Jesu, 2007). Fresh pods are low in calories, practically no fat, high in fiber, and have several valuable nutrients, including about 30% of the recommended levels of vitamin C, 10 to 20% of folate and about 5% of vitamin A. Both pod skin and seeds are excellent source of zinc (Cook et al., 2000). Daily consumption of 100 grams of okra provides 20% of the calcium, 15% of the iron, and 50% the vitamin C of human dietary requirements. Okra pod contains important bioactive compounds such as

carotene, folic acid, thiamine, riboflavin, niacin, vitamin C, oxalic acid and amino acids (Roy et al., 2014).

Edible seeds can also be extracted from pods that are too mature to be eaten. Okra seeds are source of oil and protein. Okra seed is known to be rich in high quality protein especially with regards to its content of essential amino acids relative to other plant protein sources. Hence, it plays a vital role in the human diet (Farinde et al., 2006). Okra seeds contain high (70%) amount of unsaturated fatty acids such as linoleic and oleic acids (Lamont, 1999). The contents of total polyphenols and total polysaccharides were 29.5% and 14.8% in okra seeds and 1.25% and 43.1% in okra skin, respectively (Xia et al., 2015). Okra seeds contain polyphenolic compounds, oligomer catechins, flavone and zinc (Adelakun et al., 2009). Okra seeds can be used as non-caffeinated substitute for coffee. Okra seeds may also be roasted and ground to form a caffeine-free substitute for coffee (Martin, 1982).

Okra mucilage, a slimy and thick substance, is a viscous polysaccharide having potent physiological activities (Chukwuma et al., 2018). Mucilage from okra contains significant levels of protein, carbohydrate, neutral sugars, minerals, and other complex polysaccharides. The hydrolysis of okra mucilage yielded rhamnose, galactose, glucose, galacturonic and glucuronic acids (Woolfe et al., 1977). Okra mucilage has the potential for use as food, nonfood products, and medicine (Haruna et al., 2017). The food applications include a whipping agent for reconstituted egg whites, an additive in the formulation of flour-based adhesives, an additive for clarifying sugarcane juice. It is also used to modify the food quality in terms of food stability, texture, and appearance properties by acting as an emulsifier, thickener, gelling agent, or texture modifier (Noorlaila et al., 2015). Okra mucilage also contributes to improved functionality, especially water-binding, emulsifying, and foaming properties of food products (Jideani and Bello, 2009). A recent study revealed that pods of okra accessions contain a desirable amount of mucilage contents and are potential sources of natural antioxidants. The study also revealed that the mucilage of the pods of okra accessions was found to exhibit good functional properties and can offer a great potential in various food systems. Particularly, mucilage of okra had desirable water and oil absorption capacities as well as high emulsifying and foaming properties (Gemede et al., 2018).

2.2.2 Medicinal effects of Okra:

Okra, Abelmoschus esculentus, is a popular health food due to its high fiber, vitamin C, and folate content. This plant is popular and has been acclaimed to have various health benefits including anti-diabetic properties (Dubey and Mishra, 2017). Abelmoschus esculentus is well known for its nutritional value and healing properties such as anticancer, reduced heart attack, lower blood cholesterol, relieve intestinal disorder, relieve inflammation of the colon, relive diverticulitis, relieve stomach ulcer, neutralize acid, lubricate large intestine, treatment of lung inflammation, treatment of bowel, keep joints limber, as well as the treatment of sore throats, burns, reducing poisonings and psoriasis (Oyelade et al., 2003; Arapitsas, 2008a). A study reported that okra polysaccharide possesses anti complementary and hypoglycemic activity in normal mice (Tomoda et al., 1989). In vivo study of different okra parts also showed anti-diabetic activity (Sabitha et al., 2011). Okra is reported to have its hypolipidemic effect by decreasing absorption of cholesterol from diet (Nguyen et al., 2019). Okra is widely used in ethno medicine in diverse cultures. In Ayurveda, okra is used as an edible infusion and in different preparation for diuretic effect (Maramag, 2013). An infusion of the fruit mucilage is also used to treat dysentery and diarrhoea in acute inflammation and irritation of the stomach, bowels, and kidneys catarrhal infections, ardor urine, dysuria and gonorrhea. Seeds are used as antispasmodic, cordial and stimulant. Leaves and root extracts are served as demulcent and emollient poultice (Babu and Srinivasan, 1995).

Several study also provides important in vitro data on the effects of okra on various AlD-associated cellular processes in H63D variant HFE cells. These results suggest okra may be beneficial in people expressing the H63D variant to reduce the risk of Alzheimer's disease and other neurodegenerative diseases related to oxidative stress (Mairuae et al., 2015). The seed extracts of Abelmoschus esculentus L. possess antioxidant, anti-stress, and nootropic activities which promisingly support the medicinal values of ladies finger as a vegetable. Studies showed evidence that aqueous and methanolic seed extracts of Abelmoschus esculentus (200 mg/kg BW.) for seven days significantly ($P < 0.01$) attenuated scopolamine-induced cognitive impairment in the passive avoidance test in mice. These extracts significantly reduced the blood glucose, corticosterone, cholesterol, and triglyceride levels elevated by acute restraint stress (Doreddula et al., 2014; Sindhu and Puri, 2016). Another study on obese mice

documented reduced blood glucose and serum insulin levels and improved glucose tolerance when ethanolic extract of okra had been used (Fan et al., 2014a). The okra reduce serum cholesterol and therefore decreases the chance of heart disease. The use of okra is an efficient method to manage the body's cholesterol level (Nguyen et al., 2019). Okra also promote antioxidant ability by lowering malondialdehyde level and increasing superoxide dismutase and glutathione peroxidase levels. Another study reported that, the methanolic extract of Abelmoschus esculentus showed significant analgesic, anti-inflammatory, CNS depression and anti-diarrheal properties (Shammi et al., 2014).

In terms of mucilage, study have shown that 5 weeks administration of mucilage (2g/kg B.W.) extracted from okra decreased glucose, total cholesterol, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol levels but increased insulin level in diabetic (Hajian et al., 2016). Furthermore, another study demonstrated the in vitro glucose entrapment and α-glucosidase inhibitory ability of okra mucilage (Palanuvej et al., 2009). It is medically proven to be linked with anticancer, antimicrobial, hypoglycemic, anti-ulcer activities as well as its ability to bind cholesterol and bile acid carrying toxins by filtering the liver (Shui and Peng, 2004). Okra mucilage can also act as a hepatoprotective agent (Ameena et al., 2010). A recent study showed that, okra dry mucilage extracts may ameliorate oxidative stress. Dry okra mucilage extracts may also exert glycemic and weight gain control by enhancing muscle glucose uptake and impairing carbohydrate and fat digestion and small intestinal glucose absorption (Chukwuma et al., 2018).

2.3. Significance of plant based diet/medicine to improve diabetes mellitus

Nowadays, there is a growing interest in the use of plant-derived bioactive compounds in the treatment and management of diabetes, obesity and oxidative stress (Chukwuma et al., 2018). Over the last 2500 years, there have been very strong traditional systems of medicine such as Chinese, Ayurvedic, and the Unani, born and practiced, more in the eastern continent. These traditions are still flourishing, since; approximately 80% of the people in the developing countries rely on these systems of medicine for their primary health care needs (Tsay and Agrawal, 2005). In the last few years there has been a rapid growth in the field of herbal medicine. These medicine are gaining popularity both in developing and developed countries because of their natural and less side effects

(Dubey and Mishra, 2017). Moreover, the awareness on the diabetic issue has led to a vast discovery of new medications as well as natural products extracted from herbal plants. Natural flavonoids of these plants can promote hypoglycemia through increase glucose uptake and glycogen synthesis(Chan et al., 2012). About 800 plant species have been reported to possess antidiabetic properties. Several plant species have been used for prevention or management of diabetes by the Native Americans, Chinese, South Americans and Asian Indians (Mentreddy et al., 2005). A review study revealed that 108 plant species including common names such as Glycine max, *Tamarindus indica*, Citrus reticulate, *Azadirachta indica*, *Beta vulgaris*, *Momordica Charantia*, *Moringa oleifera* etc. were generally used for treatment of diabetes (Dubey and Mishra, 2018). The fruits were most commonly used plant parts and other parts (leaf, root, stem, bark, flower, and whole plant) were also useful for curing. In terms of diet, studies have also shown that individuals following a plant based eating pattern typically consume fewer calories and less fat, saturated fat, and cholesterol and have lower BMIs than non-vegetarians(Trapp and Levin, 2012). Both the American Academy of Nutrition and Dietetics and the American Diabetes Association (ADA) now include well-planned, plant-based eating patterns (vegetarian and vegan) as a meal-planning option in their nutrition recommendations for people with diabetes (Craig et al., 2009; Anonymous, 2012).

2.4. Mucilage and Diabetes

Generally, mucilage is viscous compound and can be found in common plant including psyllium (Plantago species), yellow mustard (*Sinapis alba*), flaxseed (*Linum usitatissimum*) and okra (*Abelmoschus Esculentus*) (Kaewmanee et al., 2014). Polysaccharides from various kinds of natural sources have demonstrated hypoglycemic activity in both in vivo and in vitro study by following several approach including- improvement of beta cell dysfunction, inhibition of alpha-amylase and alpha-glucosidase activities, enhancement of insulin action and by improving glucose metabolism (Wu et al., 2016). An acidic polysaccharide separated from Saccharina japonica exhibited hypoglycemic and hypolipidemic properties in alloxan induced diabetic mice (Wang et al., 2013) . Acidic polysaccharides from Tremella aurantia, Phellinus baumii also exhibited antidiabetic activities (Kiho et al., 1995; Hwang et al., 2005). Also, polysaccharide extracted from the root of Ophiopogon japonicus could significantly reduce blood glucose levels and increases the insulin level (Chen et al.,

2011b). Similarly, in terms of okra polysaccharide, studies have shown to attenuate blood glucose levels, body weight, and serum cholesterol levels as well as improve glucose tolerance in obese mice (Fan et al., 2013).

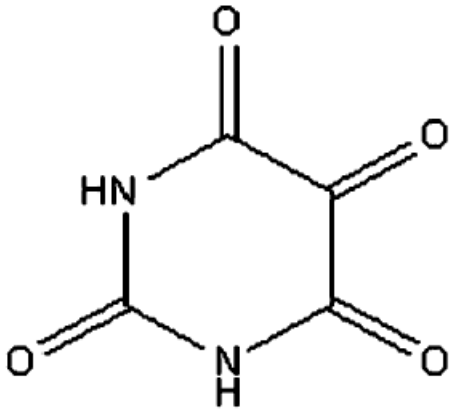
2.5. Summary of alloxan-induced diabetes

In chemically induced models of diabetes, endogenous beta cells are destroyed in high amount leading to little endogenous insulin production elevating the blood glucose level and reduced weight. 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 diabetes in animals. This is due to the action of specific necrosis of the pancreatic beta cells (Dunn et al., 1943).

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. Alloxan is reduced to dialuric acid and then re-oxidized back to alloxan, creating a redox cycle for the generation of superoxide radicals that undergo dismutation to form hydrogen peroxide and thereafter highly reactive hydroxyl radicals that cause fragmentation of beta cell DNA (Nerup et al., 1994; Szkudelski, 2001a; King, 2012).

Alloxan is a very unstable chemical compound with a molecular shape resembling glucose (Table 2). The alloxan molecule is structurally so similar to glucose that 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 therefore selectively enter beta cells in an unrestricted manner (Weaver et al., 1979; Gorus et al., 1982; Lenzen, 2008).

Table 2: Chemical properties of alloxan

	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 at acid pH
Mode of toxicity	Generation of ROS

Source: (Lenzen, 2008).

2.6. Conclusion

The use of alternate natural medicines from plants and herbs in the management of diabetes becoming popular especially in rural communities of developing countries. People want to consume easily available, accessible, cheap and relatively safe food which also give additional medicinal properties. The major issue often associated with this type of medicine is that of lack of scientific proof of their efficacy in therapy. Okra is an easily available vegetables. People in rural areas believe that consumption of mucilage extracted form okra can improve diabetes condition. Therefore, the present study intended to verify and validate the antidiabetic activity of okra mucilage.

Chapter III: Materials and Methods

3.1. Study area and Study period

The study period was seven months from March, 2019 to September, 2019. All tasks were conducted in Dept. of Applied Food Science and Nutrition, Dept. of Food Processing and Engineering, Dept. of Animal Science and Nutrition, Dept. of Physiology, Biochemistry and Pharmacology at Chattogram Veterinary and Animal Sciences University, Bangladesh.

3.2. Layout of experiment

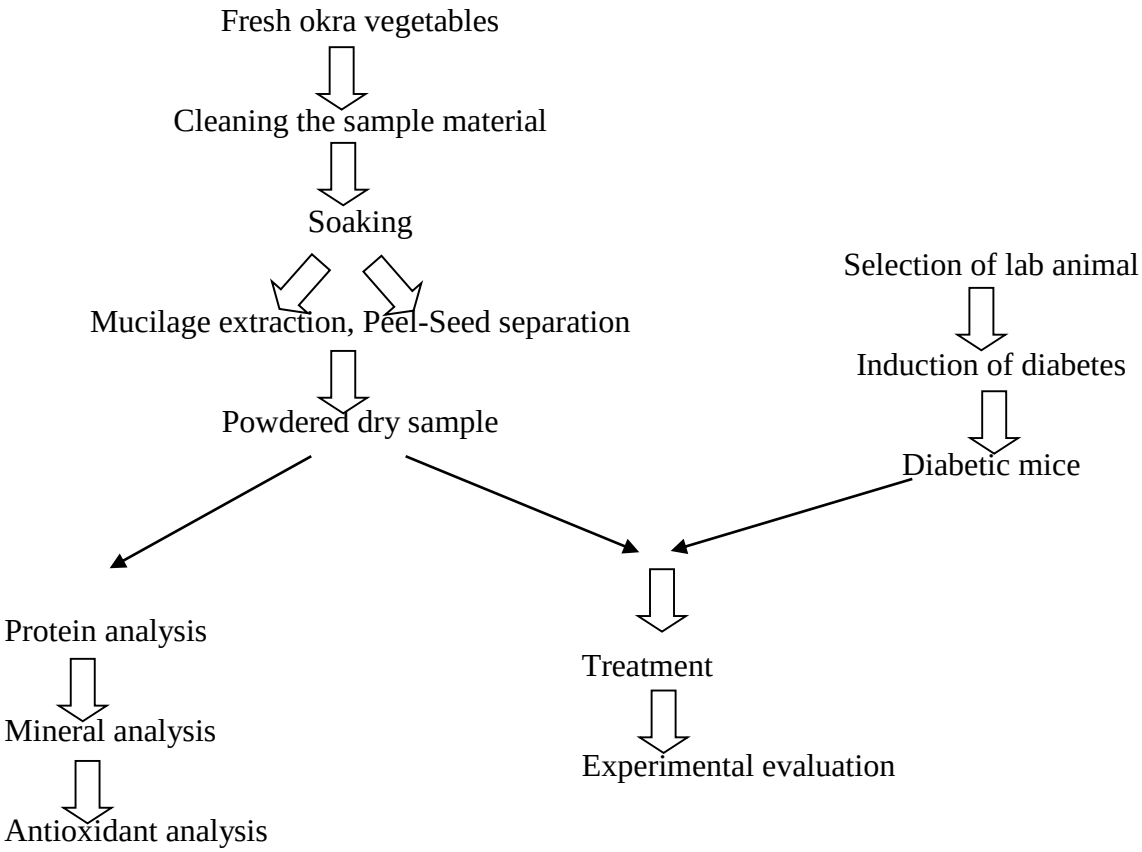


Figure 1: Flow chart of study design

3.3. Plant material/Sample collection

Fresh fruits of Okra (*A. esculentus*) were procured from local fruit and vegetable store in Chawkbazar market, Chittagong, Bangladesh. Okra fruits were then washed and stored in a cool and dry place until they were used.

3.4. Extraction of Mucilage

Mucilage extraction of okra was done by following the traditional method. Obtained okra was properly washed and soaked in distilled water for 8-9 hours. Thereafter, it was heated in a water bath with continuous agitation for 30 minutes at 60°C to favor the thorough release of the mucilage into the water. The concentrated viscous solution was then filtrated through a muslin cloth and remaining okra fruits were isolated for further use. The filtrated viscous solution was cooled to room temperature. The filtered mucilage was spreaded on a non-sticky paper over a tray. And then the mucilage solution was dried to constant weight at 45°C in a cabinet dryer. On average, approximately 24 hours were required for drying. Eventually, dried mucilage was ground into a fine powder by mortar and pestle. The powdered mucilage (PM) was then passed through #80 sieve size and packed in airtight containers. It was then stored in a desiccator for further use (Gemede et al., 2018).

3.5. Peel-Seed powder preparation

After the extraction of the mucilage, the separated fruits containing seeds were taken in a tray. They were washed with distilled water again. Later, they were dried to constant weight at 45°C in a cabinet dryer. Thereafter, crispy fruits and seeds were ground into a fine powder using mixer grinder. The powdered mixture (PPS) was then passed through #80mm sieve size and stored in an airtight container until the completion of the study.

Figure 2. Mucilage and Peel-Seed preparation

3.6. Experimental animals and Diet

A total of sixty healthy laboratory Swiss albino mice weighing between 23-27g were purchased from the animal house of the Department of Pharmacy, Jahangirnagar University, Bangladesh. The mice were kept in the animal house of the Department of Animal Science and Nutrition, Chattogram Veterinary and Animal Sciences University (CVASU). A standard laboratory conditions with appropriate temperature, humidity and 12-h light: 12-h dark cycle were maintained. The mice were placed in standard ventilated cages and free access to food and water was ensured. Mice were acclimatized for 7 days before the commencement of the study. During the entire period of the study the mice were fed with pellet diet as presented in (Table 3). The study had approval by the CVASU Institutional Animal Ethical Committee (Memo no.-CVASU/Dir(R&E)EC/2019/39(2)). Whole studies were carried out in scrupulous guidelines for the care of laboratory animal.

Figure 3. Experimental animals

Table 3. Composition of the diets for mice.

Composition of Diet	Quantity (%)
Wheat Flour	40%
Wheat Bran	19%
Fish Meal	10%
Mustard Oil Cake	10%
Maize powder	6%
Rice Police	4%
Milk Powder	4%
Full fat soy	3%
Soybean oil	1%
Salt	1%
Molasses	1%
Vitamin	1%

3.7. Acute toxicity study

Acute oral toxicity test of okra PM and PPS was carried out as per Organisation for Economic Cooperation and Development Guidelines (OECD) guidelines (OECD, 2001). The PM and PPS were diluted with distilled water and administered orally upto 500mg/kg body weight. The animals were observed for 24 h for behavioral or any adverse change.

3.8. Induction of Diabetes

Overnight fasted mice selected for diabetic group were intraperitoneally administered alloxan monohydrate (150mg/kg body weight) dissolved in ice-cold saline (0.9% NaCl). To prevent alloxan–induced hypoglycemia, the animals received 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).

Figure 4. Induction of diabetes

3.9. Experimental design

All mice were divided into 7 dietary groups with 8 animals per group. All treatments were given using oral gavage for three weeks. Groups were divided as follows:

Table 4. Experimental design

Group	Treatment
Group 1: (NC)	Normal control (NC) treated with distilled water.
Group 2: (DC)	Diabetic control (DC) (alloxan-induced diabetic mice) received only distilled water.
Group 3: (SD)	Diabetic mice treated with standard drug (SD) glibenclamide at a dose of 5 mg/kg body weight.
Group 4: (PPS1 treated)	Diabetic mice treated with powdered peel-seed (PPS) at a dose of 150 mg/kg body weight.
Group 5: (PPS2 treated)	Diabetic mice treated with powdered peel-seed (PPS) at a dose of 200 mg/kg body weight.
Group 6: (PM1 treated)	Diabetic mice treated with powdered mucilage (PM) at a dose of 150 mg/kg body weight.
Group 7: (PM2 treated)	Diabetic mice treated with powdered mucilage (PM) at a dose of 200 mg/kg body weight.

All mice continued to get the normal pellet diet and ad libitum water. Suspension of powdered mucilage (PM), powdered peel-seed mixture (PPS) and glibenclamide were prepared with distilled water just before the oral administration. 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.

Figure 5. Weighing of mice

3.10. Oral glucose tolerance test

Oral glucose tolerance test was conducted on overnight fasted control and treated mice after 3 weeks administration. After measuring the fasting blood glucose level, glucose solution (2g/kg body weight) was given to the animals by oral gavage. Blood was withdrawn again from the tail vein at 30, 60, 90 and 120 minute after glucose administration by using glucometer (Gluco Dr., Korea) (Tao Bu et al., 2012). Calculation of the area under the curve (AUC) was measured according to the following formula (Dong et al., 2014).

Area under the curve= (Basal glycemia + glycemia at 0.5 hour) * 0.25 + (glycemia at 0.5 hour + glycemia at 1 hour) *0.25+ (glycemia at 1 h + glycemia at 2 hour) *0. 5

3.11. 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.

Figure 6. Collection of blood sample

3.12. Biochemical tests

Total protein, total cholesterol, high-density lipoprotein (HDL), and triglyceride (TG) levels were measured by Humalyzer 3000 using commercial kit from Randox laboratories limited (United Kingdom). The low-density lipoprotein (LDL) levels were calculated according to the formula: (Friedewald et al., 1972)

LDL = Total Cholesterol − (HDL+ Triglyceride/5)

3.13. Methanolic extraction of PM and PPS

Dried plant materials (100 mg) were weighed into a conical flask. About 100 ml of 95% aqueous methanol was added. The suspension was stirred slightly in a water bath at below 40°C and then left at room temperature for 24 h. The extract was centrifuged for 10 min at 3000 rpm, and then filtered through Whatman No. 4 paper. Supernatants were collected for experimental procedure (Wojdyło et al., 2007).

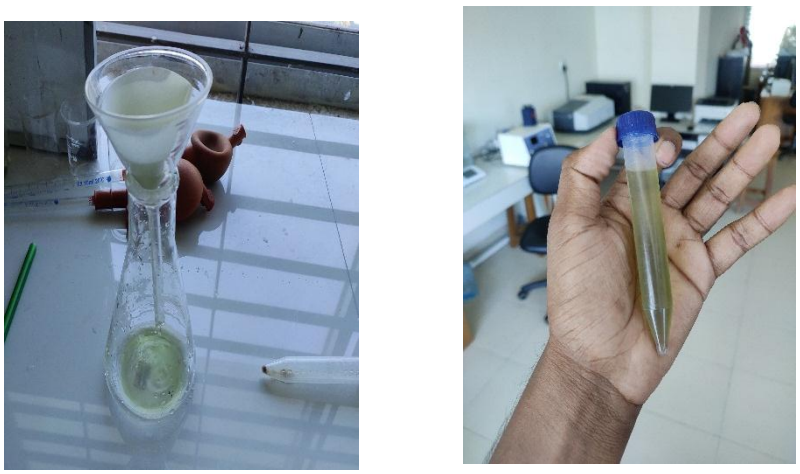


Figure 7. Sample preparation for antioxidant activity

3.14. Antioxidant activity

DPPH radical scavenging abilities of the test samples were determined by following the method as described by (Nariya et al., 2013; Gemede et al., 2018).

3.14.1. Preparation of DPPH solution (100 µm)

At first 4 mg of DPPH was dissolved in 100 ml of methanol (95%) in a dark condition.

3.14.2. Preparation of standard ascorbic acid solution

To prepare stock solution of 1 mg/ml, about 10 mg of ascorbic acid was dissolved in 10 ml of distilled water. Then serial dilution was performed in order to prepare different concentrated solution (2 µg/ml, 4 µg/ml, 8 µg/ml, 16 µg/ml, and 32 µg/ml).

3.14.3. Preparation of sample solution

Serial dilution was performed in order to prepare different concentrated solution (2 µg/ml, 4 µg/ml, 8 µg/ml, 16 µg/ml, 32 µg/ml).

3.14.4. Procedure

About 4 ml of DPPH solution was added to 1 ml of sample extracts or standards at different concentration. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance of the solution was measured at 517 nm using a UV-Vis spectrophotometer against blank. Control sample was prepared containing the same volume without any extract and reference ascorbic acid. Methanol was used as blank. IC_{50} was calculated from % inhibition. Scavenging of the DPPH free radical was measured using the following equation:

% inhibition = $\{(A_0 - A_1)/(A_0)\} \times 100$

A_0 = absorbance of DPPH alone

A_1 sample = absorbance of DPPH along with different concentrations of extracts.

3.15. Determination of total phenol content

3.15.1. Preparation of standard gallic acid solution

To prepare stock solution of 1 mg/ml, about 10 mg of gallic acid was dissolved into 10 ml of distilled water. Then serial dilution was performed in order to prepare different concentrated solution (2 µg/ml, 4 µg/ml, 8 µg/ml, 16 µg/ml, 32 µg/ml).

3.15.2. Procedure

The total phenol content of okra extracts was evaluated by the Folin-ciocalteu method as described by (Wojdyło et al., 2007)**.** About 1ml of sample extracts or standard at different concentrations were mixed with 2 ml of Folin–Ciocalteu reagent (10times diluted), and incubated at room temperature for 3 min. After that, 10 ml of 20% sodium carbonate was added to the mixture and left for incubation at room temperature for an hour. The absorbance of the mixture was measured at 765 nm with a Shimadzu UV–VIS-2600 spectrophotometer against a blank solution. The blank solution contained all the reagent mixture without extract or standard sample. Gallic acid standard curve was used to quantify total phenolic contents and the results were expressed as mg of gallic acid equivalent (GAE) per gram of dried weight. All determinations were performed in triplicate (n = 3).

3.16. Total flavonoid content determination

Flavonoid content in samples was measured by aluminum chloride colorimetric method as described by Shah and Hossain, (2014).

3.16.1. Preparation of 1M potassium acetate solution

0.9815 g of potassium acetate was dissolved in 10 ml water.

3.16.2. Preparation of 10% $AlCl_3$ solution

1g $AlCl_3$ was dissolved in 10ml water.

3.16.3. Preparation of standard quercetin solution

About 10 mg of quercetin was dissolved into 10 ml of distilled water. So the concentration of the solution was 1mg/ml. This is called stock solution. Then serial dilution was performed in order to prepare different concentrated solution (6 µg/ml, 12 µg/ml, 24 µg/ml, 48 µg/ml, 96 µg/ml).

3.16.4. Procedure

About 1ml of sample or standard at different concentration solution was taken in a test tube. After that, 0.2 ml of 10% aluminum chloride, 0.2 ml of 1 M potassium acetate, and 8.6 ml of distilled water were added. The reaction mixture was then incubate at room temperature for 30 min to complete the reaction. The absorbance of the mixture was measured at 420 nm. Quercetin was used to make the calibration curve. The calculation of total flavonoids content in the extracts was carried out in triplicate and

the results were averaged. The final result were expressed as mg of quercetin equivalent (QE) per gram of dried weight. All determinations were performed in triplicate (n = 3).

3.17. Mineral analysis

Mineral contents were determined by using biochemical analyzer (Humalyzer 3000). Commercially available biochemical kit (Randox®) was used for biochemical assay. For sample preparation, 5 g of powdered sample was taken into a conical flask. After that, 7.5 ml HNO_3 and 2.5 ml $HCLO_4$ was added into the conical flask. Then it as heated over an induction cooker at 200W until complete digestion. Then it was cooled. Finally, deionized water was added upto 100ml. The results were expressed as mg/100g after conversion from mg/dl.

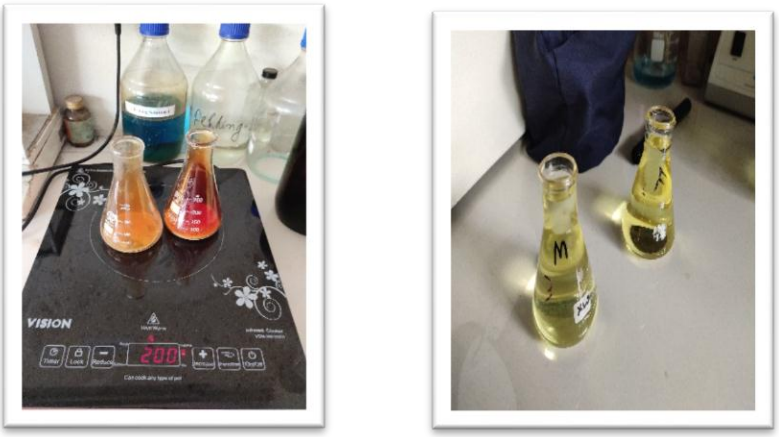


Figure 8. Sample preparation for mineral analysis

3.18. Crude protein determination

The crude protein was determined by kjeldahl method. About 0.3g sample was weighted into digestion tube. A mixture containing 72 g Potassium sulfate and 8 g Copper sulfate was prepared. About 4 g of this mixture was added to the digestion tube. Then, 5 ml of concentrated H_2SO_4 were added into the digestion tube. Digestion was carried out at 320°C for 30minutes. Sample was cooled down before addition of 25 ml of distilled water and 25 ml of 40% NaOH. About 10ml 4% boric acid with 3 drops of green bromocresol indicator was prepared as receiving solution in conical flask. Cooled tube

and receiving solution was placed into the distillation unit. After that, 25ml 40% NaOH was filled automatically into the tube and the distillation process was conducted for 4 minutes. The receiving solution turned to green color after the distillation process. The receiving solution was titrated with 0.2N HCL until it turned to grey color. The percentage of crude protein was calculated by formula.

Percentage of protein= Percentage of nitrogen * 6.25

Percentage of nitrogen= $\frac{(T-B)*N*14.007*100}{Weight\ of\ sample\ in\ mg}$

T= Volume of titration of sample.

B= Volume of titration for Blank.

N= Normality of HCL (0.2)

3.19. Statistical analysis

All statistical analysis was done using statistical package for social sciences (SPSS) version 25. One-way analysis of variance was used to evaluate the data. Data are presented as the mean± (Standard Error Mean) SEM. Differences in means were compared using the Tukey test. P values <0.05 were considered significant

Chapter IV: Results

4.1. Yield of dry mucilage

The mucilage yield from the okra was ranged from 4g to 6g/kg.

4.2. Toxicity study

The oral administration of mucilage powder and peel-seed mixture were found to be safe at a dose level of 500mg/kg body weight in mice. Neither any toxicological effect nor mortality was observed. Hence, the dose of 150 and 200 mg/kg body weight was selected.

4.3. Effect of mucilage and peel-seed on fasting blood glucose level

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, increased to 12.3±0.6 - 13.1±0.8 mmol/L in different groups after alloxan induction. Moreover, hyperglycemic effect in diabetic control group increased significantly ($P<0.001$) in every week compared to normal control. Highest blood glucose level was documented at 15.1±0.5 mmol/L in DC group at the end of the experiment. However, the groups treated with a standard drug (glibenclamide), powdered mucilage (PM) suspension and peel-seed mixture (PPS) suspension showed significant ($P<0.001$) decrease in glucose levels over the three-week period when compared to DC group (Table 5). Glibenclamide (5 mg/kg) treatment demonstrated the lowest blood glucose concentration in mice at 5.8±0.3 mmol/L. Similarly, PPS1, PPS2, PM1 and PM2, after 3-weeks treatment, resulted in 53.7%, 58.3%, 52.9% and 55.6% reductions respectively in fasting blood glucose levels compared with non-treated diabetic mice (7±0.1 mmol/L, 6.3±0.5 mmol/L, 7.1±0.4 mmol/L and 6.7±0.4 mmol/L vs. 15.1±0.5 mmol/L; $P<0.001$).

Table 5: Effect of PPS and PM on fasting blood glucose level in alloxan-induced diabetic mice.

Sample	Blood glucose (mmol/L)				
	Initial	**Week 0**	**Week 1**	**Week 2**	**Week 3**
NC	3.9±0.1	4.2±0.1^{a}	4.3±0.1^{a}	4.4±0.1^{a}	4.5±0.1^{a}
DC	4.1±0.1	12.3±0.6^{b}	14.1±0.4^{b}	14.6±0.6^{b}	15.1±0.5^{b}
SD	3.9±0.1	12.9±0.6^{b}	8.2±0.4^{c}	6.9±0.6^{c}	5.8±0.3^{ac}
PPS1	4.2±0.1	12.6±0.7^{b}	11.1±0.3^{d}	8.2±0.2^{c}	7±0.1^{c}
PPS2	3.9±0.1	12.6±0.3^{b}	10.7±0.3^{d}	7.5±0.7^{c}	6.3±0.5^{c}
PM1	4.1±0.2	12.3±0.8^{b}	10.9±0.4^{d}	8.3±0.3^{c}	7.1±0.4^{c}
PM2	4.2±0.1	13.1±0.8^{b}	10.9±0.4^{d}	7.6±0.5^{c}	6.7±0.4^{c}
P value	0.618	<0.001	<0.001	<0.001	<0.001

The mean difference is significant at the 0.05 level

Legends: NC=Normal control, DC=Diabetic control, SD=Standard drug, PPS1= Peel-Seed (150mg/kg), PPS2=Peel-Seed (200mg/kg), PM1=Mucilage (150mg/kg), PM2=Mucilage (200mg/kg). All data are expressed as mean± SEM. Means ± SEM within the column bearing different superscripts (a, b, c) are significantly different (P< 0.05).

4.4. Oral Glucose Tolerance Test (OGTT)

The OGTT study supported the trends in fasting glucose level test. Oral Glucose Tolerance Test (OGTT) in mice demonstrated that blood glucose concentration in all mice groups reached the highest levels after 30min of glucose administration (2 g/kg BW) and found to be decreased steadily with time. Figure 9 revealed that after 21days administration, glucose tolerance of PPS and PM treated group showed the similar significant ($P<0.001$) improvement as standard drug glibenclamide when compared to the DC group (7.7±0.2 mmol/L to 8.9±0.2 mmol/L vs 18.2±0.5 mmol/L). The calculation of the AUC (area under the curve) also indicated the significant ($P<0.001$) decrease of all treatment groups in contrast to the DC group (Figure 9). However, the AUC of all treatment group was still considerably higher than that of normal group.

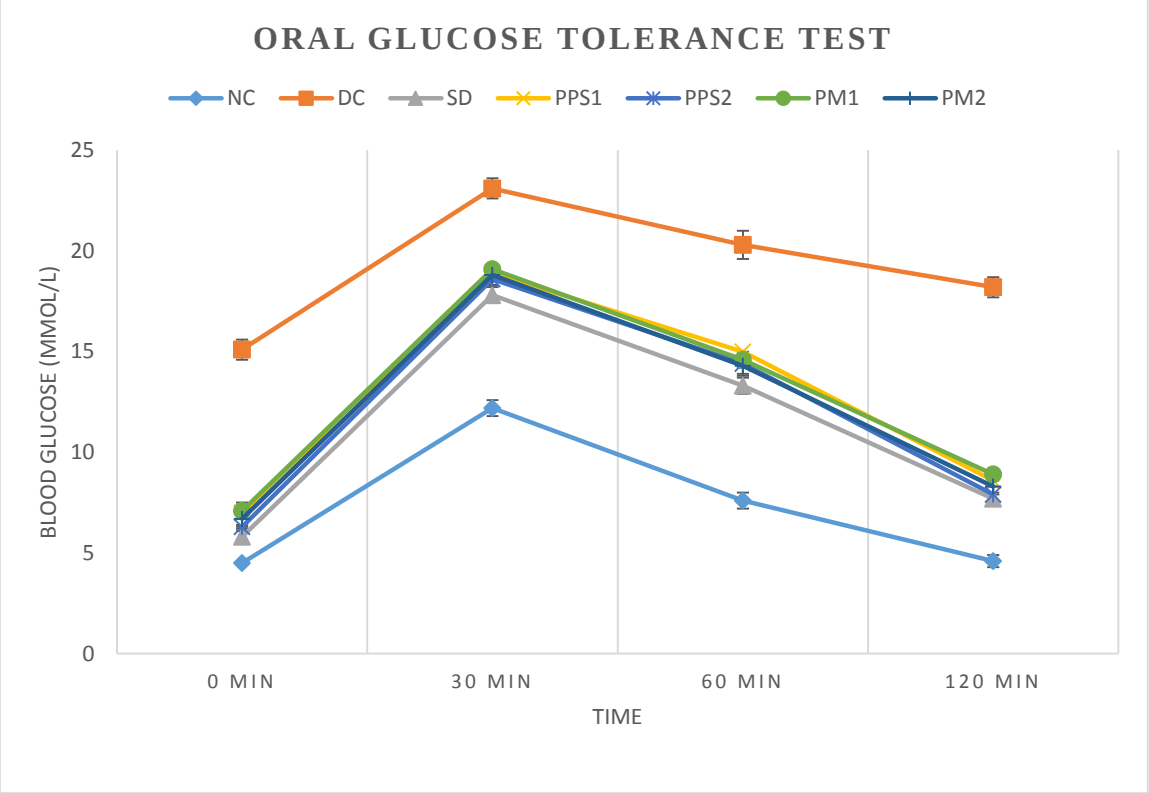


Figure 9: Oral glucose tolerance test

Area under the curve (AUC): NC=15.2±0.4^{a} h.mmol/L, DC=39.7±0.6^{b} h.mmol/L, SD=24.2±0.4^{c} h.mmol/L, PPS1=26.8±0.4^{d} h.mmol/L, PPS2= 25.6±0.6^{cd} h.mmol/L, PM1=26.7±0.4^{d} h.mmol/L, PM2= 25.9±0.6^{cd} h.mmol/L.

Legends: NC=Normal control, DC=Diabetic control, SD= Standard drug, PPS1= Peel-Seed (150 mg/kg), PPS2= Peel-Seed (200mg/kg), PM1=Mucilage (150mg/kg), PM2= Mucilage (200 mg/kg). Means ± SEM of AUC having different superscripts (a, b, c, d) denotes significant difference (P<0.05) between groups.

4.5. Effects of PPS and PM 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 Figure 10. On week 1, no significant variation was noted between the DC 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 (Figure 10).

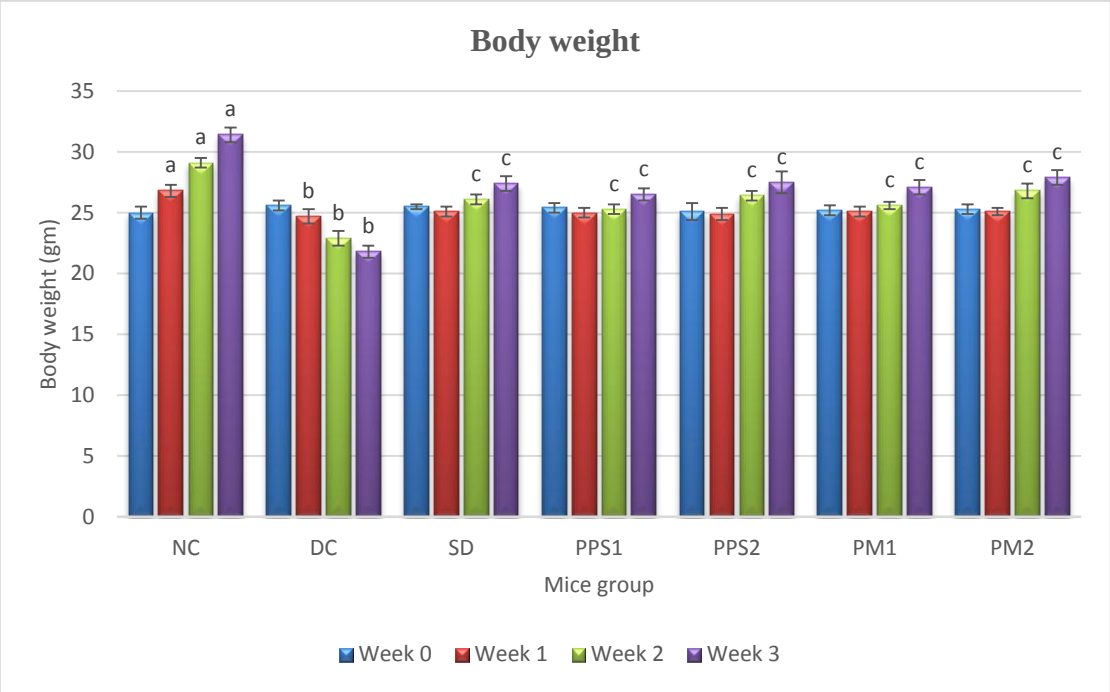


Figure 10: Effects of PPS and PM on body weight of mice

Legends: NC=Normal control, DC=Diabetic control, SD= Standard drug, PPS1= Peel-Seed (150 mg/kg), PPS2= Peel-Seed (200mg/kg), PM1=Mucilage (150mg/kg), PM2= Mucilage (200 mg/kg). Different Superscripts (a, b, c) within the same week denotes significant difference ($P<0.05$) between groups.

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

Food intake per day among different groups showed considerable variation (Figure 11). The NC mice consumed around 4.6 g/day food whereas the consumption rate in DC was statistically significantly higher ($P<0.001$) at 11.1 g/day. However, the food intake was significantly lower ($P<0.001$) in SD, PPS1, PPS2, PM1, and PM2 at 7.5, 8.1, 7.6, 8.1 and 7.7 g/day food respectively in contrast to diabetic group (Figure 11).

The normal control group drank only 5.8±0.5 ml/day of water, which was statistically significant ($P<0.001$) in contrast to diabetic control groups (14.4±0.7 ml/day) (Figure 12). All other alloxan induced groups- SD (8.5 ml/d), PPS1 (9.3ml/d), PPS2 (8.4 ml/d), PM1 (9 ml/d) and PM2 (8.8 ml/d) consumed considerably lower amount of water when compared to non-treated diabetic control groups.

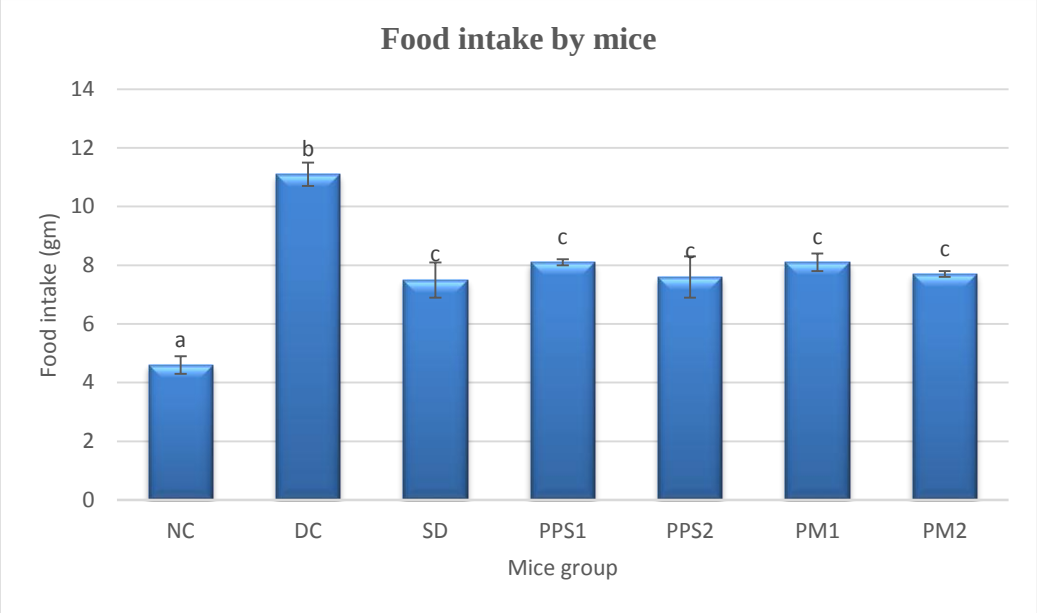


Figure 11. Average food intake of different mice group

Legends: NC=Normal control, DC=Diabetic control, SD= Standard drug, PPS1= Peel-Seed (150 mg/kg), PPS2= Peel-Seed (200mg/kg), PM1=Mucilage (150mg/kg), PM2= Mucilage (200 mg/kg). Different Superscripts (a, b, c) in the column denotes significant difference ($P<0.05$) between groups.

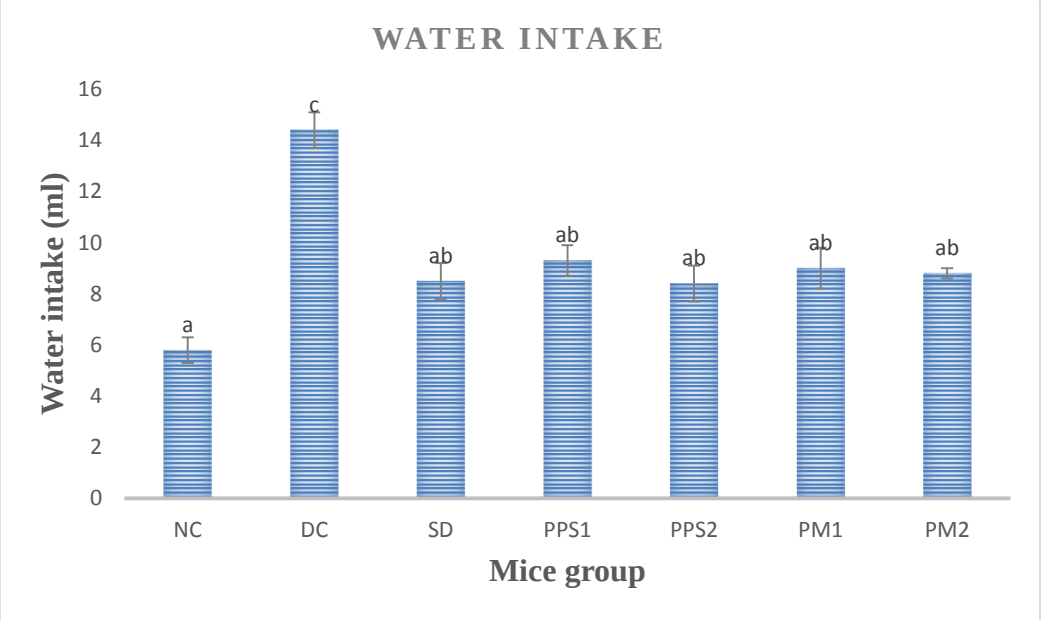


Figure 12. Average water intake of different mice group

Legends: NC=Normal control, DC=Diabetic control, SD= Standard drug, PPS1= Peel-Seed (150 mg/kg), PPS2= Peel-Seed (200mg/kg), PM1=Mucilage (150mg/kg), PM2= Mucilage (200 mg/kg). Different Superscripts (a, b, c) in the column denotes significant difference ($P<0.05$) between groups.

4.7. Lipid profile

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 peel-seed mixture significantly ($P<0.001$) increased the HDL level and reduced the cholesterol, triglycerides, and LDL levels in alloxan induced diabetic mice when compared with the diabetic control group (Table 6). It is also obvious that, at the same doses, PPS exerted superior hypolipidemic effect than that of PM.

4.8. Total protein content in blood

In the present study, a significantly ($P<0.001$) decreased total protein was observed in diabetic control mice than normal control mice. However, Total protein level was significantly ($P<0.001$) increased after the administration of both doses of PM and PPS compared with diabetic control mice (Table 6).

Table 6. Effect of PM and PPS on various biochemical parameters in mice.

Sample	Cholesterol (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	TP (g/dl)
NC	110.7 ± 3.1^{a}	106.9 ± 1.1^{a}	75.2 ± 1.5^{a}	14.2 ± 4.4^{a}	7.3 ± 0.1^{a}
DC	165.9 ± 6.3^{d}	174.6 ± 1.2^{d}	31.5 ± 1.6^{d}	99.5 ± 5^{d}	3.5 ± 0.2^{d}
SD	116.9 ± 3.6^{ab}	122.5 ± 3^{b}	49.7 ± 1.6^{b}	42.6 ± 1.5^{b}	6.4 ± 0.1^{bc}
PPS1	133.7 ± 2.1^{c}	138.4 ± 1.1^{c}	41.7 ± 1.2^{c}	64.3 ± 3.4^{ce}	6.1 ± 0.1^{bc}
PPS2	127.2 ± 0.9^{bc}	130.9 ± 1.7^{c}	44.4 ± 1.5^{bc}	56.6 ± 1.3^{bc}	6.7 ± 0.2^{b}
PM1	140.8 ± 2.1^{c}	154.8 ± 0.9^{e}	37.6 ± 1.3^{cd}	72.2 ± 2.8^{e}	6 ± 0.1^{c}
PM2	139.1 ± 0.8^{c}	160.1 ± 1.8^{e}	40.4 ± 1.3^{c}	66.7 ± 1.7^{ce}	6.4 ± 0.1^{bc}
P value	<0.001	<0.001	<0.001	<0.001	<0.001

Legends: NC=Normal control, DC=Diabetic control, SD= Standard drug, PPS1= Peel-Seed (150 mg/kg), PPS2= Peel-Seed (200mg/kg), PM1=Mucilage (150mg/kg), PM2= Mucilage (200 mg/kg). All data are expressed as mean± SEM. Means ± SEM within the column bearing different superscripts (a, b, c, d, e) are significantly different ($P< 0.05$).

4.9. In vitro antioxidant activity

4.9.1. DPPH free radical scavenging assay

Results for the DPPH free radical scavenging activity of methanolic extracts of PM and PPS shown in Figure 13. Both PM and PPS showed a dose dependent radical scavenging effect in DPPH assay. The half inhibition concentration (IC_{50}) value of ascorbic acid was 9.22 µg/ml. In contrast, the IC_{50} value for free radicals achieved by the PM and PPS are 73.83 µg/ml and 67.09 µg/ml respectively (Table 7, Figure 13). So, in comparison with ascorbic acid, it is clear that both powdered mucilage and peel-seed possess anti radical activity.

Table 7. DPPH radical scavenging activity of mucilage and peel-seed.

Serial No.	Concentration (µg/ml)	%inhibition of Ascorbic acid	%inhibition of mucilage	% inhibition of peel-seed
1	2	28.78	10.39	5.58
2	4	37.63	15.01	13.09
3	8	54.67	22.23	20.59
4	16	69.59	24.83	23.68
5	32	90.57	27.23	26.66
IC_{50} (µg/ml)		9.22	73.83	67.09

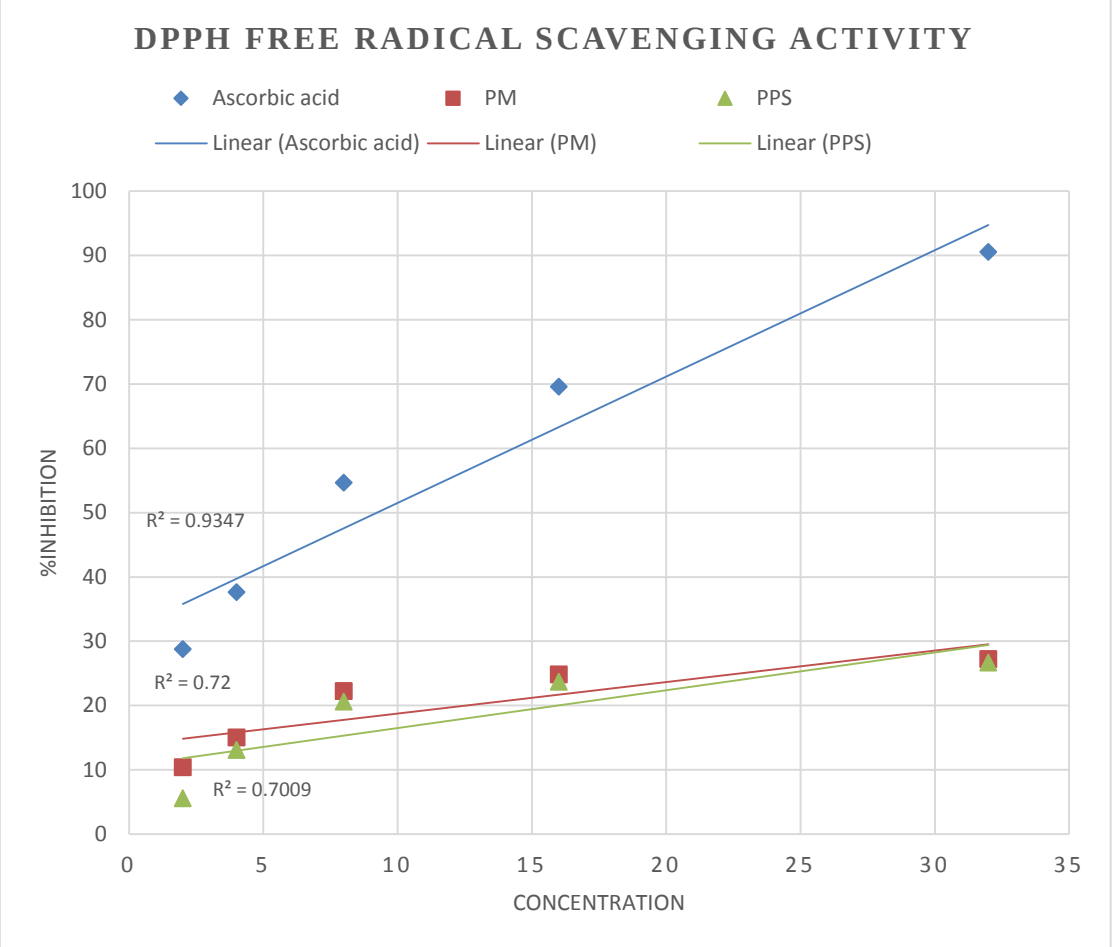


Figure 13. DPPH scavenging radical activity (% inhibition vs concentration graph for standard and sample)

4.9.2. Total phenol content

Phenol content was measured by using gallic acid calibration curve (Figure 14). Total phenol content of the methanolic extract of PM and PPS was found at 68.84±0.3 mg Gallic acid equivalent per gram and 65.98±0.3 mg Gallic acid equivalent per gram.

4.9.3. Total flavonoid content

Flavonoid content was determined by using quercetin calibration curve (Figure 15). Total flavonoid content of the methanolic extract of peel-seed (PPS) mixture was 9.50±1.1 mg Quercetin equivalent/g and for mucilage (PM) the value was 7.90±0.1 mg Quercetin equivalent/g.

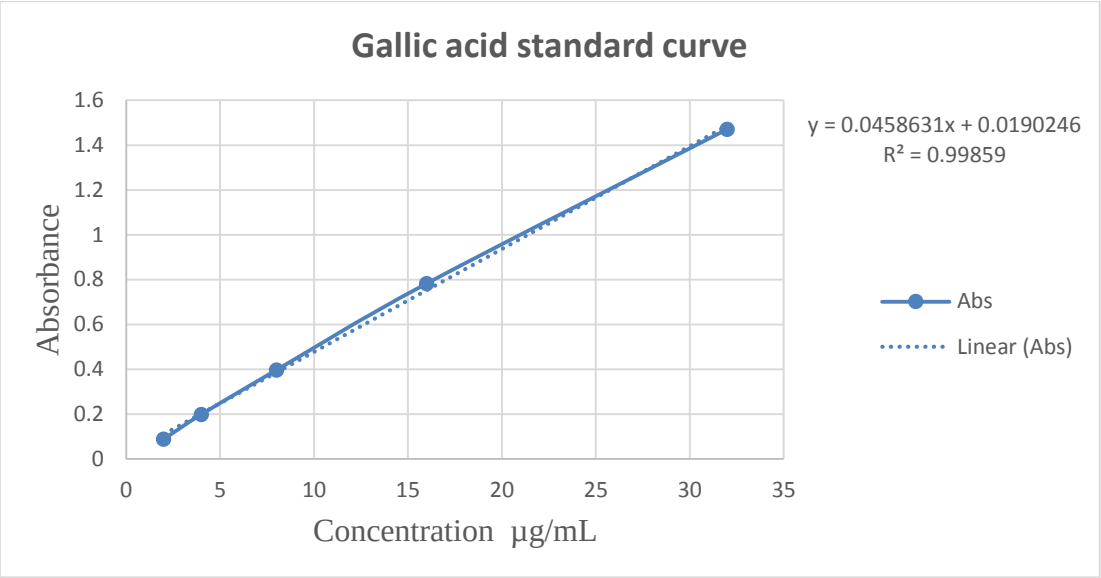


Figure 14. Standard curve of Gallic acid

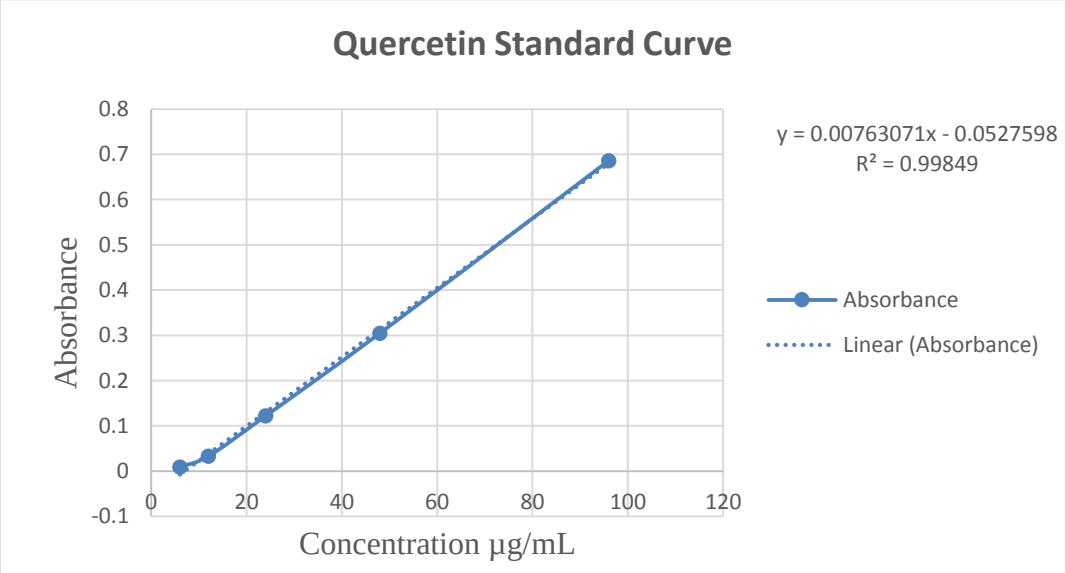


Figure 15. Standard curve of quercetin.

4.10. Mineral contents

Powdered peel-seed mixture had significantly (P<0.05) higher amount of potassium, calcium, magnesium and phosphorus in contrast to powdered mucilage (Table 8). Iron content was slightly higher in PPS compared to PM, though it was not significant. However, sodium content was found significantly higher in PM compared to PPS.

Table 8. Mineral contents of mucilage and peel-seed (mg/100g)

Sample	Sodium	Potassium	Calcium	Magnesium	Phosphorus	Iron
PM	5.72± 0.02	112± 1.4	120± 5.7	196± 1.4	50± 1.4	1.03±0.01
PPS	5.17 ±0.04	422 ±4.2	344± 8.5	324 ±4.24	306± 4.24	1.15±0.07
P value	0.004	<0.001	0.001	0.001	<0.001	0.143

Legends: PM and PPS represents powdered mucilage and powdered peel-seed mixture respectively. All values are expressed as Mean ± SD. The mean difference is significant at 0.05 level.

4.11. Protein content

The crude protein contents of the okra mucilage (PM) was 8.54±0.96 g/100g. And, a higher protein content was found in peel-seed (PPS) at 11.28±1.27 g/100g.

Chapter V: Discussions

The present study followed the traditional method for the mucilage extraction procedure. Mucilage yield was quite low compared to other researches following different extraction procedure. The yield of dry mucilage in the current study was 0.5% on average where other studies showed 1.25 to 4% dry mucilage yield from *Abelmoschus esculentus* (Chukwuma et al., 2018; Gemede et al., 2018). The variation in yield percentage may be due to the differences in extraction process, regional production processes or weather condition of the production area. Species type, maturity at harvesting time, effect of drying, genetic factor, the season of collection, and topographic variation such as rain distribution, temperature, and soil type may also have an effect on the yield percentage of mucilage (Gebresamuel & Gebre-Mariam, 2011). In terms of mineral content, both PM and PPS showed high amount of calcium, potassium, magnesium, sodium and iron. Gemede et al., (2016) also reported high amount of minerals in *Abelmoschus esculentus.*

The present research demonstrated an elevated fasting blood glucose levels in mice subjected to alloxan induction. By inhibiting the glucose sensor of beta cell known as glucokinase, alloxan impedes the secretion of glucose-induced insulin. Simultaneously, alloxan initiates a redox cycle as well as form superoxide radicals which than go through the process of dismutation to form hydrogen 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, 2001b; King, 2012;). Alloxan induction in mice, in the current study, also exhibited typical visible feature of diabetes mellitus including weight loss, polydipsia (excessive thirst) and polyphagia (excessive hunger). However, reverse situation was observed after the treatment.

This study confirms that oral administration of mucilage and peel-seed to the alloxan induced diabetic mice can ameliorate diabetes mellitus, as assessed by fasting blood glucose level, oral glucose tolerance test, body weight, food, and water consumption. The efficiency of hypoglycemic activity in mice may be due to the ability of the mucilage and peel-seed mixture 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. As expected, the untreated diabetic controls did not show any

improvement in terms of blood glucose level, body weight and even in food and water consumption pattern.

The results from the present study indicates a supportive action of the mucilage and peel-seed mixture in glucose utilization. Present experiment showed that raw mucilage and peel-seed mixture powder at a dose of 150mg/kg and 200 mg/kg successfully demonstrated the hypoglycemic effect of alloxan-induced diabetic mice as well as ameliorated oral glucose tolerance, and reduced the increased food and water intake. The magnitude of this reduction was found to be reliant on the dose of administration. Hypoglycemic effect of PM and PPS showed a proportionate relation with the increasing dose suggesting there usefulness in the treatment of diabetes mellitus. In terms of blood glucose level, after three weeks of the PM and PPS administration, dosage at 200 mg/kg differ marginally in degree when compared to standard glibenclamide administration at 5 mg/kg and contrasted strongly from non-treated diabetic control. Similar scenario was recorded in case of glucose tolerance, where blood glucose level in PPS2 and PM2 treated mice after 120 minutes was found to be similar with glibenclamide treated mice (7.9±0.3 mmol/L, 8.3±0.3 mmol/L vs. 7.7±0.2 mmol/L). Other treatments (PPS1, PM1) also exhibited significantly lower blood glucose concentration after two hours of glucose administration. Standard drug glibenclamide helps in the diabetes management by controlling insulin secretion and insulin action (Luzi and Pozza, 1997). The underlying mechanism of PM and PPS in controlling blood glucose level can be similar to the mechanism of glibenclamide.

Hyperlipidemia is associated with coronary artery disease in diabetes patients (O'Brien et al., 1998). High blood glucose is correlated with a high risk of dyslipidemia. Hyperglycemia tends to increase triglyceride and LDL as well decrease the HDL levels (Abbate and Brunzell, 1990). Similar scenario was observed in the present study where diabetic control group exhibited excess level of LDL, triglyceride and total cholesterol. Interestingly, good diabetes management is associated with overcoming the hyperlipidemia issues (Sosenko et al., 1980). The current study carried out on mice supported this theory by revealing that okra mucilage, peel-seed can help in the management of diabetes by controlling glycemic load in blood and thus helps in lowering the hyperlipidemic effect on diabetic mice. Administration of PM and PPS showed a positive impact on lipid profile of diabetic mice. Moreover, total protein level in the treatment group has also increased compared to diabetic group. These may be due

to high protein content present in powdered mucilage and peel-seed which has also been shown in the current study. Hence, this study concludes that both PM and PPS has potential in the treatment of dyslipidemia in diabetic subjects.

Research studies conducted before also showed the potential hypoglycemic and hypolipidemic effects of Abelmoschus esculentus. Previous experiment has reported that consumption of peel and seed powder can lower the glucose as well as cholesterol level in diabetic subjects (Sabitha et al., 2011). Even antidiabetic potential of okra fruits and seeds do not significantly alter due to the common processing treatment including boiling and roasting (Nguekouo et al., 2018). In the present study powdered peel-seed showed high efficacy than crude mucilage. This result is also supported by another previous study which showing higher effectiveness of okra seed than the mucilage (Hajian et al., 2016). There is, however, some differences between the design of the present and the previous study including animal model, diabetes induction procedure, daily dose administration and mucilage extraction procedure.

In terms of in vitro antioxidant activity, the phenol content, flavonoid content and DPPH scavenging radical activity of both PM and PPS revealed their efficiency as an efficient antioxidant agent. It has been known that dietary flavonoids and antioxidants play vital role in antidiabetic mechanism in the body (Bajaj and Khan, 2012; Babu et al., 2013). Previous study reported that okra seed and peel both contain high amount of polyphenolic compounds including quercetin derivatives, catechins in seeds and quercetin, hydroxycinnamic acid derivatives in skins (Arapitsas, 2008b). Gemede et al., (2018) also documented okra mucilage as a promising source of natural antioxidants. Interestingly, researchers have also disclosed that active antioxidants in polysaccharide such as mucilage can reduce the blood glucose level in normal as well as drug induced diabetic subjects (Li et al., 2006). Studies have also shown that hydroxycinnamic acid, a derivatives of cinnamic acid, can improve glucose hemostasis and insulin resistance, thus helps in the prevention of diabetes complications (Adisakwattana, 2017). Similarly, quercetin also possess hypoglycemic and hypolipidemic effect (Jeong et al., 2012). This is also confirmed by a study reported by Fan et al., (2014b) where isoquercitrin and quercetin 3-O-gentiobioside were found to be effective in reducing blood glucose levels and improving glucose tolerance in high fat diet-induced obese mice.

Overall, findings in the present study demonstrated that *Abelmoschus esculentus* mucilage and peel-seed possess in vitro antioxidant activity and has the potential to improve blood glucose and lipid profile of diabetic mice.

Chapter VI: Conclusion

Okra *(Abelmoschus esculentus)* is a popular vegetable in Bangladesh. Raw mucilage has been widely used in the management of diabetes in many parts of the country. The data procured from this present research also provide evidence that the mucilage of *Abelmoschus esculentus* possess hypoglycemic effects on blood glucose concentrations of diabetic mice. Mucilage at 200mg/kg dose showed more effectiveness. The investigation also found that okra mucilage can also be effective in cases of glucose tolerance impairment. The antioxidant activity of mucilage may have a pivotal contribution to the potential hypoglycemic action. Furthermore, the results obtained from this study proved that *Abelmoschus esculentus* mucilage has hypolipidemic activity. The results from this study also revealed that mucilage of *Abelmoschus esculentus* is rich in protein and minerals. Mucilage possess high amount calcium, potassium, magnesium, phosphorus, iron and sodium. The present study also investigated the efficiency of mucilage compared to the peel-seed mixture which was isolated after mucilage extraction. Though peel-seed mixture exhibited more potential activities than mucilage, no significant difference was observed between them. Finally, it can be concluded that the present study justify the traditional use of *Abelmoschus esculentus* mucilage 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 okra mucilage could be a solution. More pharmacological and biochemical studies are recommended to elucidate the mechanism of action of the antidiabetic and anti-hyperlipidemic activities of the mucilage. A thorough studies need to be done to understand the beneficial effect of mucilage on human subject.

Future works should involve freeze drying method to yield high quality mucilage. Moreover, incorporation of mucilage in different food products need to be studied. Higher level of protein and mineral content of mucilage suggested that it has potential food value and could be recommended as functional ingredient in our food industry. In addition, market has great demand on food having antioxidant activity. Natural antioxidants have gained increased demand for higher quality food intake. Thus, mucilage, as a source of natural antioxidant, can be used to many food products.

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Appendix A: Photo Gallery

Figure: Experimental works

Brief Biography

A.F.M Irfan Uddin Zim passed the Secondary School Certificate Examination in 2010 from Chittagong Govt. High School, Chittagong, and then Higher Secondary Certificate Examination in 2012 from Govt. Hazi Muhammad Mohsin College, Chittagong. He 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, Chittagong, Bangladesh. Now, he 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). He 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.