‘’Pharmacological Evaluation of Methanolic Extract of *Nigella sativa on* Hyperglycemia in Laboratory Animal Models’’

A research paper is submitted to the Department of Pharmacy, East West University in conformity with the requirements for the degree of Bachelor of Pharmacy

Submitted by

Nazir Hossain Munna

ID: 2014-1-70-021

Supervised by

Dr. JMA Hannan
Professor
Department of Pharmacy

East West University

Declaration by the candidate

I, **Nazir Hossain Munna**, hereby declare that the dissertation entitled **"Determination of Anti diabetic Efficacy of seeds of "*Nigella sativa*"** submitted by me to the Department of Pharmacy, East West University and in the partial fulfillment of the requirement for the award of the degree of Bachelor of Pharmacy(Honors), genuine and authentic research work carried out by us during the period 2017 of our research in the Department of Pharmacy, East West University, under the supervision and guidance of Dr. JMA Hannan, Professor, Department of Pharmacy, East West University. The thesis paper has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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Nazir Hossain Munna

ID: 2014-1-70-021

Department of Pharmacy

East West University

Certificate by the Supervisor

This is to certify that the thesis entitled **"Determination of Anti diabetic Efficacy of seeds of *"Nigella sativa*"** submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, was carried out by **Nazir Hossain Munna** (student ID: 2014-1-70-021) in 2017, under the supervision and guidance of me. The thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

_____

Dr. JMA Hannan
Professor and Supervisor
Department of Pharmacy
East West University, Dhaka.

Endorsement by the Chairperson

This is to certify that the thesis entitled **"Determination of Anti diabetic Efficacy of seeds of "*Nigella sativa*"** submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, was carried out by **Nazir Hossain Munna** (student ID 2014-1-70-021) in 2017, under the supervision and guidance of **Dr. JMA Hannan,** Professor, Department of Pharmacy, East West University.

_____

Dr. Chowdhury Faiz Hossain
Chairperson and Professor
Department of Pharmacy
East West University, Dhaka

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Thank You.

DEDICATION

This Research Paper Is Dedicated

To My Beloved **Parents**, Who Are My biggest inspiration…

Abstract

Diabetes is increasingly affecting a growing number of patients and seriously reducing their quality of life. Use of conventional drugs in diabetes management is expensive, thus, unaffordable to most patients. Furthermore most of these conventional drugs are associated with undesirable side effects. Incorporation of herbal medicine into conventional healthcare system may significantly improve the overall healthcare system. Evaluation of efficacy and safety by scientific method is necessary to validate herbal medicine utilization, in most cases even where efficacy of the plants has been established the standard dosage required to bring about healing is not clear. The intraperitoneal route of herbal extract administration was found to be more effective than the oral route. Our present studies were focused on the probable anti- hyperglycemic effect of methanolic extract of *Nigella sativa* in long-evans rats and the statistical significance of such effect. The seeds extract was subjected to anti-diabetic study through Inhibition of Carbohydrate Absorption (six segment method) and Intenstinal Disaccharidase activity method. In six segment, the amount of sucrose unabsorbed in different GIT segments were evaluated in control rats vs. rats fed with 2.5g/kg extract at 30 minutes,1 h, 2h & 4 h. In Disaccharide activity the amount of unabsorbed sucrose in pancreatic enzymes are evaluated in control rats vs rats fed with 100mg/kg extract The extract caused a significant decreased ($p<0.001$) in dose dependent inhibition of glucose absorption and showed Anti-hyperglycemic effects in long-evans rats weighing from 150-200 gm. The anti-diabetic effects were estimated by measuring the amount of glucose in the samples collected after the experiment. In conclusion, these observations provide evidence and possible mechanisms of action for the anti-diabetic properties of leaves of *Nigella sativa* claimed in Ayurveda medicine.

Keywords: Anti-Diabetic, *nigella sativa*, Anti-hyperglycemic, Glucose, Sucrose.

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1.1. Overview

Diabetes is a disease in which blood glucose, or blood sugar, levels are too high. With type 1 diabetes, our body does not make insulin. With type 2 diabetes, the more common type, our body does not make or use insulin well. Without enough insulin, the glucose stays in our blood. Diabetes mellitus is a disease that prevents our body from properly using the energy from the food we eat. Diabetes occurs in one of the following situations:

- The pancreas (an organ behind our stomach) produces little insulin or no insulin at all. (Insulin is a naturally occurring hormone, produced by the beta cells of the pancreas, which helps the body use sugar for energy.)

- The pancreas makes insulin, but the insulin made does not work as it should. This condition is called insulin resistance.

Our body is made up of millions of cells. To make energy, the cells need food in a very simple form. When we eat or drink, much of our food is broken down into a simple sugar called glucose. Glucose provides the energy your body needs for daily activities (Araki et al 1994).

1.1.1Epidemiology

The disease burden related to diabetes is high and rising in every country, fuelled by the global rise in the prevalence of obesity and unhealthy lifestyles. The latest estimates show a global prevalence of 382 million people with diabetes in 2013, expected to rise to 592 million by 2035. The aetiological classification of diabetes has now been widely accepted. Type 1 and type 2 diabetes are the two main types, with type 2 diabetes accounting for the majority (>85%) of total diabetes prevalence. Both forms of diabetes can lead to multisystem complications of microvascular endpoints, including retinopathy, nephropathy and neuropathy, and macrovascular endpoints including ischaemic heart disease, stroke and peripheral vascular disease. The premature morbidity, mortality, reduced life expectancy and financial and other costs of diabetes make it an important public health condition.

1.1.2 History

The first known mention of diabetes symptoms was in 1552 B.C., when Hesy-Ra, an Egyptian physician, documented frequent urination as a symptom of a mysterious disease that also caused emaciation. Also around this time, ancient healers noted that ants seemed to be attracted to the urine of people who had this disease.

In 150 AD, the Greek physician Arateus described what we now call diabetes as "the melting down of flesh and limbs into urine." From then on, physicians began to gain a better understanding about diabetes.

Centuries later, people known as "water tasters" diagnosed diabetes by tasting the urine of people suspected to have it. If urine tasted sweet, diabetes was diagnosed. To acknowledge this feature, in 1675 the word "mellitus," meaning honey, was added to the name "diabetes," meaning siphon. It wasn't until the 1800s that scientists developed chemical tests to detect the presence of sugar in the urine (Hruz and Mueckler, 2001).

1.1.3 Types of diabetes

There are two main types of diabetes:

1. Diabetes Insipidus
2. Diabetes Mellitus

1.1.3.1 Diabetes Insipidus

Diabetes insipidus is a rare disorder that occurs when a person's kidneys pass an abnormally large volume of urine that is insipid—dilute and odorless. In most people, the kidneys pass about 1 to 2 quarts of urine a day. In people with diabetes insipidus, the kidneys can pass 3 to 20 quarts of urine a day. As a result, a person with diabetes insipidus may feel the need to drink large amounts of liquids. Diabetes insipidus and diabetes mellitus which includes both type 1 and type 2 diabetes are unrelated, although both conditions cause frequent urination and constant thirst. Diabetes mellitus causes high blood glucose, or blood sugar,

resulting from the body's inability to use blood glucose for energy. People with diabetes insipidus have normal blood glucose levels; however, their kidneys cannot balance fluid in the body.

1.1.3.2 Diabetes Mellitus

Diabetes mellitus (or diabetes) is a chronic, lifelong condition that affects your body's ability to use the energy found in food. There are three major types of diabetes: type 1 diabetes, type 2 diabetes, and gestational diabetes.

All types of diabetes mellitus have something in common. Normally, your body breaks down the sugars and carbohydrates you eat into a special sugar called glucose. Glucose fuels the cells in your body. But the cells need insulin, a hormone, in your bloodstream in order to take in the glucose and use it for energy. With diabetes mellitus, either your body doesn't make enough insulin, it can't use the insulin it does produce, or a combination of both.

Since the cells can't take in the glucose, it builds up in your blood. High levels of blood glucose can damage the tiny blood vessels in your kidneys, heart, eyes, or nervous system. That's why diabetes -- especially if left untreated -- can eventually cause heart disease, stroke, kidney disease, blindness, and nerve damage to nerves in the feet (Xue and Kahn, 2006).

1.1.3.2.1 Type 1 diabetes

Type 1 diabetes is also called insulin-dependent diabetes. It used to be called juvenile-onset diabetes, because it often begins in childhood. Type 1 diabetes is an autoimmune condition. It used to be called insulin-dependent diabetes, and is caused by the body attacking its own pancreas with antibodies. In people with type 1 diabetes, the damaged pancreas doesn't make insulin. This type of diabetes may be caused by a genetic predisposition. It could also be the result of faulty beta cells in the pancreas that normally produce insulin. A number of medical risks are associated with type 1 diabetes. Many of them stem from damage to the tiny blood vessels in your eyes (called diabetic retinopathy), nerves (diabetic neuropathy), and kidneys (diabetic nephropathy). In addition, there is the increased risk of

heart disease and stroke. Treatment for type 1 diabetes involves taking insulin, which needs to be injected through the skin into the fatty tissue below. The methods of injecting insulin include

- Syringes
- Insulin pens that use prefilled cartridges and a fine needle
- Jet injectors that use high pressure air to send a spray of insulin through the skin
- Insulin pumps that dispense insulin through flexible tubing to a catheter under the skin of the abdomen\

A periodic test called the HbA1C blood test estimates glucose levels in your blood over the previous six to 12 weeks. It's used to help identify overall glucose level control and the risk of complications from diabetes, including organ damage.

1.1.3.2.2 Type 2 diabetes

By far, the most common form of diabetes is type 2 diabetes. It used to be called maturity-onset diabetes because it often starts in adulthood. Unfortunately, with the epidemic of obese and overweight children, more teenagers are now developing type 2 diabetes. Type 2 diabetes was also called non-insulin-dependent diabetes. Diabetes UK says it affects 85% to 95% of all people with diabetes.

Type 2 diabetes can also cause major health complications, particularly in the smallest blood vessels in the body that nourish the kidneys, nerves and eyes. Type 2 diabetes also increases your risk of heart disease and stroke.

With type 2 diabetes, the pancreas usually produces some insulin, but either the amount produced is not enough for the body's needs, or the body's cells are resistant to it. Insulin resistance, or lack of sensitivity to insulin, happens primarily in fat, liver and muscle cells.

People who are obese are at particularly high risk of developing type 2 diabetes and its related medical problems. Type 2 diabetes, however, can be controlled with weight management, nutrition and exercise. Type 2 diabetes tends to progress and diabetes medications are often needed.

An HbA1C test is a blood test that estimates glucose levels in your blood over the previous 6-12 weeks. Periodic HbA1C testing may be advised to see how well diet, exercise and medications are working to control blood sugar and prevent organ damage. The HbA1C test is typically done a few times a year.

The pancreas makes insulin, but it either doesn't produce enough, or the insulin does not work properly. Nine out of 10 people with diabetes have Type 2. This type occurs most often in people who are over 40 years old and overweight. Type 2 diabetes may sometimes be controlled with a combination of diet, weight management, and exercise. However, treatment also may include oral glucose-lowering medications (taken by mouth) or insulin injections (shots) (Herman and Kahn, 2006).

1.1.3.2.3 Difference between type 1 and type 2 diabetes

Table 1.1.3.2.3 Difference between type 1 and type 2 diabetes

Type 1 diabetes	**Type 2 diabetes**
Often diagnosed in childhood	Usually diagnosed in over 30 year olds
Often associated with higher than normal ketone levels at diagnosis	Often associated with high blood pressure and/or cholesterol levels at diagnosis
Not associated with excess body weight	Often associated with excess body weight
Treated with insulin injections or insulin pump	Is usually treated initially without medication or with tablets
Cannot be controlled without taking insulin	Sometimes possible to come off diabetes medication

(Graham et al., 2006)

1.1.3.3 Other types of diabetes

1.1.3.3.1 Gestational diabetes

Gestational diabetes occurs when there is a high blood glucose level during pregnancy. As pregnancy progresses, the developing baby has a greater need for glucose. Hormone changes during pregnancy also affect the action of insulin, which brings about high blood glucose levels.

Pregnant women who have a greater risk of developing gestational diabetes include those who:

- Are over 25 years old
- Are above their desired body weight
- Have a family history of diabetes
- Are Hispanic, African-American, Native American, or Asian-American.

Blood glucose levels usually return to normal after childbirth. However, women who have had gestational diabetes have an increased risk of developing Type 2 diabetes later in life (Yang et al., 2005).

1.1.3.3.2 Prediabetes

Prediabetes puts us at a higher risk of getting type 2 diabetes. Prediabetes is a wake-up call that we're on the path to diabetes. But it's not too late to turn things around. If we have it our blood sugar (glucose) level is higher than it should be, but not in the diabetes range. People used to call it "borderline" diabetes. Normally, our body makes a hormone called insulin to help control our blood sugar. When we have prediabetes, that system doesn't work as well as it should. We might not be able to make enough insulin after eating, or our body might not respond to insulin properly. Prediabetes makes us more likely to get heart disease or have a stroke. But we can take action to lower those risks (Nyenwe 2015).

1.1.4 Causes of diabetes

The causes of diabetes are not known. The following factors may increase the chance of getting diabetes:

- Family history of diabetes or inherited tendency
- African-American, Hispanic, Native American, or Asian-American race, Pacific Islander or ethnic background
- Being overweight (20 percent or more over your desired body weight)
- Physical stress (such as surgery or illness)
- Use of certain medications, including steroids and blood pressure medications
- Injury to the pancreas (such as infection, tumor, surgery, or accident)
- Autoimmune disease
- High blood pressure
- Abnormal blood cholesterol or triglyceride levels
- Age (risk increases with age)
- Alcohol (risk increases with years of heavy alcohol use)
- Smoking
- History of gestational diabetes or delivery of a baby weighing more than 9 pounds (4.1 Kg).
- Pregnancy

It is important to note that sugar itself does not cause diabetes. Eating a lot of sugar can lead to tooth decay, but it does not cause diabetes.

1.1.5 Symptoms of diabetes

The symptoms of diabetes include:

- Increased thirst
- Increased hunger (especially after eating)
- Dry mouth
- Frequent urination
- Unexplained weight loss (even though you are eating and feel hungry)
- Weak, tired feeling
- Blurred vision
- Numbness or tingling in the hands or feet
- Slow-healing sores or cuts
- Dry and itchy skin (usually in the vaginal or groin area)
- Frequent yeast infections

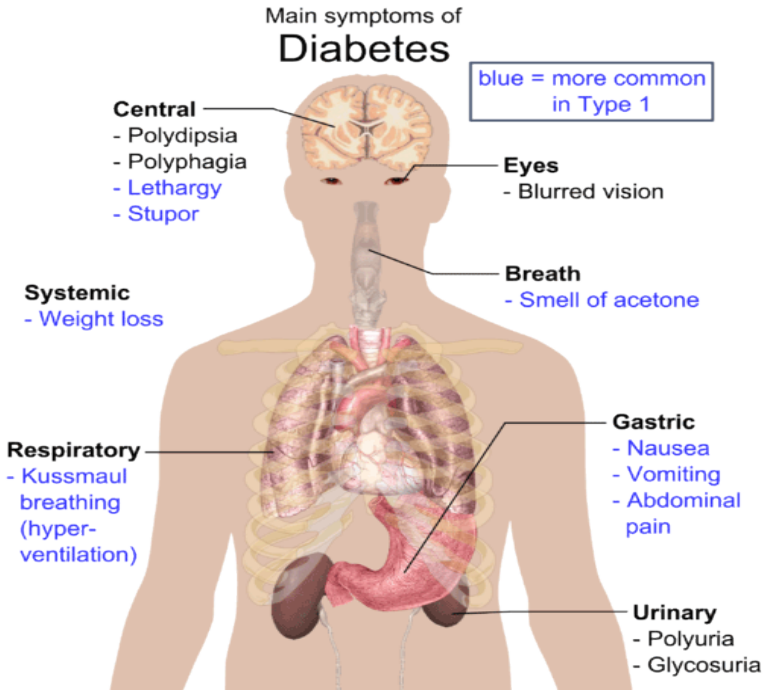

Figure 1.1.5: Symptoms of diabetes (Day & Bailey 1988)

1.1.5.1 Symptoms of low blood sugar

Most people have symptoms of low blood sugar (hypoglycemia) when their blood sugar is less than 60 mg/dl. When our blood sugar is low, our body gives out signs that we need food. Different people have different symptoms.

Common early symptoms of low blood sugar include the following:

- Feeling weak
- Feeling dizzy
- Feeling hungry
- Trembling and feeling shaky
- Sweating
- Pounding heart
- Pale skin
- Feeling frightened or anxious

Late symptoms of low blood sugar include:

- Feeling confused
- Headache
- Feeling cranky
- Poor coordination
- Bad dreams or nightmares
- Being unable keep your mind on one subject
- Numbness in your mouth and tongue
- Passing out

(Day & Bailey 1988)

1.1.6 Complications linked to badly controlled diabetes

Diabetes is a complex heterogeneous disease where multiple levels of abnormalities are present in various tissues. Defects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. The major long-term complications of diabetes mellitus are **macro vascular** diseases such as coronary and peripheral vascular diseases & **microvascular** diseases such as nephropathy, retinopathy and neuropathy.

1. **Eye complications** - glaucoma, cataracts, diabetic retinopathy, and some others.
2. **Foot complications** - neuropathy, ulcers, and sometimes gangrene which may require that the foot be amputated
3. **Skin complications** - people with diabetes are more susceptible to skin infections and skin disorders
4. **Heart problems** - such as ischemic heart disease, when the blood supply to the heart muscle is diminished. Stroke can occur.
5. **Hypertension** - common in people with diabetes, which can raise the risk of kidney disease, eye problems, heart attack and stroke
6. **Mental health** - uncontrolled diabetes raises the risk of suffering from depression, anxiety and some other mental disorders
7. **Hearing loss** - diabetes patients have a higher risk of developing hearing problems
8. **Gum disease** - there is a much higher prevalence of gum disease among diabetes patients
9. **Gastroparesis** - the muscles of the stomach stop working properly
10. **Ketoacidosis** - a combination of ketosis and acidosis; accumulation of ketone bodies and acidity in the blood.
11. **Neuropathy** - diabetic neuropathy is a type of nerve damage which can lead to several different problems.
12. **HHNS (Hyperosmolar Hyperglycemic Non-ketotic Syndrome)** - blood glucose levels rise up too high, and there are no ketones present in the blood or urine. It is an emergency condition.
13. **Nephropathy** - uncontrolled blood pressure can lead to kidney disease
14. **PAD (peripheral arterial disease)** - symptoms may include pain in the leg, tingling and sometimes problems walking properly

15. **Erectile dysfunction** - male impotence.
16. **Infections** - people with badly controlled diabetes are much more susceptible to infections
17. **Healing of wounds** - cuts and lesions take much longer to heal (Donnelly et al 2000)

1.1.7 Diagnosis of diabetes

Diabetes is diagnosed with fasting sugar blood tests or with A1c blood tests, also known as glycated hemoglobin tests. A fasting blood sugar test is performed after you have had nothing to eat or drink for at least eight hours.

Normal fasting blood sugar is less than 100 mg/dl (5.6 mmol/l). We do not have to be fasting for an A1c blood test.

Diabetes is diagnosed by one of the following:

- Blood sugar level is equal to or greater than 126 mg/dl (7 mmol/l).
- Two random blood sugar tests over 200 mg/dl (11.1 mmol/l) with symptoms.
- An oral glucose tolerance test with results over 200 mg/dl (11.1 mmol/l).
- A1c test is greater than 6.5% on two separate days.

An A1c test should be performed in a laboratory using a method that is certified by the National Glycohemoglobin Standardization Program (NGSP) and standardized to the Diabetes Control and Complications Trial (DCCT) assay.

- **The A1C test**

  - at least 6.5% means diabetes

  - between 5.7% and 5.99% means prediabetes

  - less than 5.7% means normal

- **The FPG (fasting plasma glucose) test**

  - at least 126 mg/dl means diabetes

  - between 100 mg/dl and 125.99 mg/dl means prediabetes

  - less than 100 mg/dl means normal

  An abnormal reading following the FPG means the patient has impaired fasting glucose (IFG)

- **The OGTT (oral glucose tolerance test)**

  - at least 200 mg/dl means diabetes

  - between 140 and 199.9 mg/dl means prediabetes

  - less than 140 mg/dl means normal

An abnormal reading following the OGTT means the patient has impaired glucose tolerance (Donnelly et al 2000).

1.1.8 Management of diabetes:

There is no cure for diabetes, but it can be treated and controlled. The goals of managing diabetes are to:

1. Keep our blood glucose levels as near to normal as possible by balancing food intake with medication and activity.
2. Maintain our blood cholesterol and triglyceride (lipid) levels as near the normal ranges as possible by decreasing the total amount of fat to 30% or less of our total daily calories, and by reducing saturated fat and cholesterol.
3. Control our blood pressure. (our blood pressure should not go over 130/80.)
4. Decrease or possibly prevent the development of diabetes-related health problems.

Keys to managing diabetes by:

- Planning what we eat and following a balanced meal plan.
- Exercising regularly.
- Taking medication, if prescribed, and closely following the guidelines on how and when to take it.
- Monitoring our blood glucose and blood pressure levels at home.
- Keeping our appointments with our health care providers and having laboratory tests completed as ordered by our doctor (Pari 2009).

1.2 Physiology of insulin secretion and action

Insulin is a hormone made by the pancreas that allows your body to use sugar (glucose) from carbohydrates in the food that you eat for energy or to store glucose for future use. Insulin helps keeps your blood sugar level from getting too high (hyperglycemia) or too low (hypoglycemia).

The cells in your body need sugar for energy. However, sugar cannot go into most of your cells directly. After you eat food and your blood sugar level rises, cells in your pancreas (known as beta cells) are signaled to release insulin into your bloodstream. Insulin then attaches to and signals cells to absorb sugar from the bloodstream. Insulin is often described as a "key," which unlocks the cell to allow sugar to enter the cell and be used for energy (Aljabre 2005).

1.2.1 Types of insulins used to treat diabetes and include:

1 Rapid-acting insulin: It starts working approximately 15 minutes after injection and peaks at approximately 1 hour but continues to work for two to four hours. This is usually taken before a meal and in addition to a long-acting insulin.

2 Short-acting insulin: It starts working approximately 30 minutes after injection and peaks at approximately 2 to 3 hours but will continue to work for three to six hours. It is usually given before a meal and in addition to a long-acting insulin.

3 Intermediate-acting insulin: It starts working approximately 2 to 4 hours after injection and peaks approximately 4 to 12 hours later and continues to work for 12-18 hours. It is usually taken twice a day and in addition to a rapid- or short-acting insulin.

4 Long-acting insulin: It starts working after several hours after injection and works for approximately 24 hours. If necessary, it is often used in combination with rapid- or short-acting insulin.

1.2.1.1 Types of Insulin and How They Work

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

(Kanter 2009)

1.2.2 Insulin biosynthesis

The secreted insulin consists of 51 amino acids with a molecular weight of 5.8 kDa. However, the insulin gene encodes a 110-amino acid precursor known as preproinsulin. As with other secreted proteins, preproinsulin contains a hydrophobic N-terminal signal peptide, which interacts with cytosolic ribonucleoprotein signal recognition particles (SRP). SRP facilitates preproinsulin translocation across the rough endoplasmic reticulum (rER) membrane into the lumen. This process occurs via the peptide-conducting channel, where the signal peptide from preproinsulin is cleaved by a signal peptidase to yield

proinsulin. Proinsulin then undergoes folding and formation of three disulfide bonds, a process requiring a diverse range of endoplasmic reticulum (ER) chaperone proteins such as the protein-thiol reductase. Subsequent to maturation of the three dimensional conformation, the folded proinsulin is transported from the ER to the Golgi apparatus where proinsulin enters immature secretary vesicles and is cleaved to yield insulin and C-peptide. Insulin and C-peptide are then stored in these secretory granules together with islet amyloid polypeptide (IAPP or amylin) and other less abundant β-cell secretary products.

Although insulin biosynthesis is controlled by multiple factors, glucose metabolism is the most important physiological event that stimulates insulin gene transcription and mRNA translation. In 3-day fasted rats, glucose injection increased relative proinsulin mRNA levels by three- to four-fold within 24 h and this effect was blocked by pharmacological inhibition of transcription with actinomycin D. These results suggest that glucose plays a central role in regulation of insulin biosynthesis which is controlled at least partially via alterations in proinsulin mRNA expression. In addition, glucose is an important factor for maintaining insulin mRNA stability. Results from in vitro studies demonstrated that insulin mRNA stability was reduced under lower glucose concentrations and increased under higher glucose concentrations. Interestingly, elevation of intracellular cAMP levels can prevent this reduction.

Most animals have only a single copy of the insulin gene, but rodents have two non-allelic insulin genes (insulin I and II). They differ in their number of introns and chromosomal locations. In all insulin genes the 5′-flanking region determines its tissue- and cell-type-specific expression. The transcriptional factor binding sites that determine insulin's exclusive expression in β-cells are located between −520 and +1 base pairs (bp) relative to the transcription start site (TSS) in both rat and human insulin genes. Among mammalian insulin genes, there is a conserved sequence located from −350 bp to the TSS, which controls cell-type-specific expression of insulin. Most transcriptional regulation occurs through interactions within these conserved sequences. Studies have shown that the sequence between −340 and +91 is the major insulin gene transcription enhancer region, which determines cell-specific and glucose-regulated insulin gene expression.

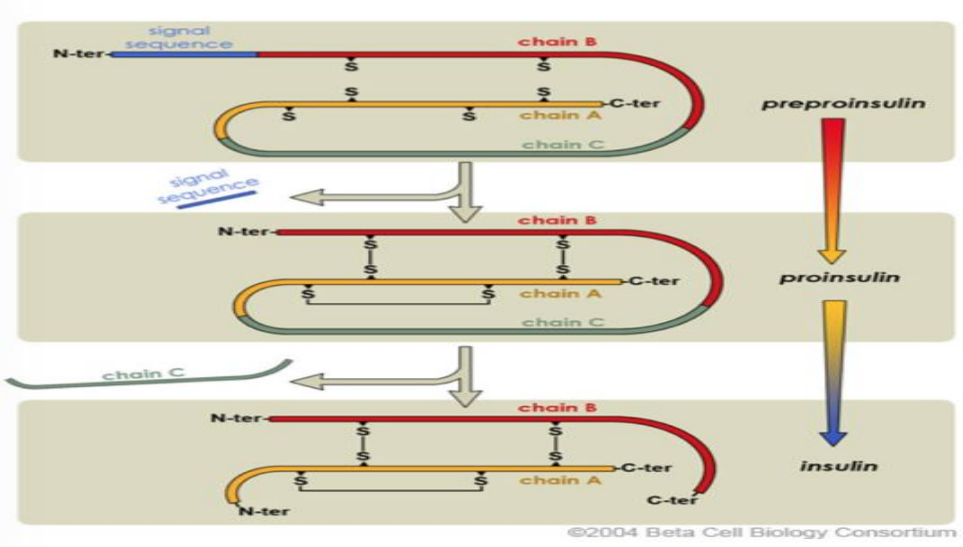

Figure 1.2.2: Insulin biosynthesis (Ahmad et al 2000)

1.2.2.1 Regulation of insulin secretion

Insulin is an important hormone required for normal metabolism. In healthy subjects, insulin release is exquisitely exact to meet the metabolic demand. Specifically, β-cells sense changes in plasma glucose concentration and response by releasing corresponding amounts of insulin. To sense the nutritional state, β-cells are clustered in islets that strategically connect to the vasculature. Islets form a dense network with small blood vessels and receive 10 times the amount of blood than cells in the surrounding exocrine regions. Capillaries surrounding islets show a remarkable number of small pores called fenestrae that allow for a greater nutrient exchange between the circulation and surrounding tissues. This structure enhances permeability, allowing unrestricted nutrient access so that β-cells can sense the nutritional state quickly. Fenestrations also permit rapid insulin diffusion into the blood. In addition to glucose, some amino acids and fatty acids also regulate insulin secretion. A schematic illustration of nutrient regulated insulin secretion is shown in figure 1.

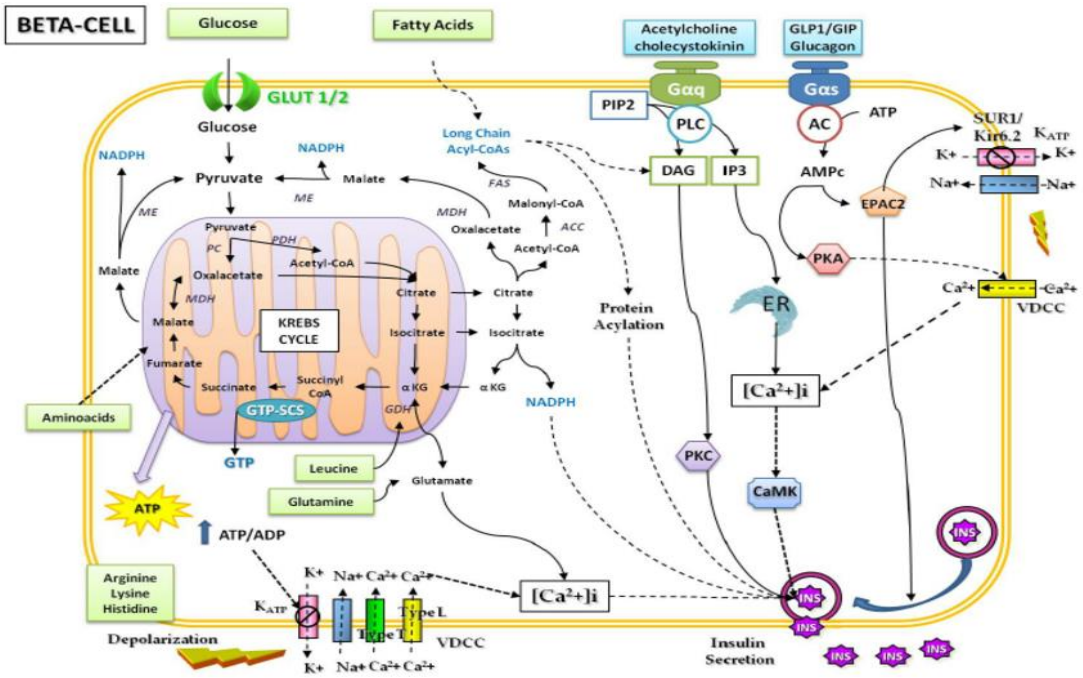

Figure 1.2.2.1 : Regulation of insulin secretion (Yada 1994)

1.2.2.2 Mechanism of insulin secretion

Insulin secretion occurs by the process of exocytosis in which the granule membrane fuses with the cell membrane, the membranes are disrupted at the point of fusion, and insulin crystals are discharged to the extracellular space. The process of exocytosis is the rate-limiting step for the physiologic insulin secretion. In this mechanism, cytoplasmic free calcium concentration and two second messenger systems, the cyclic-AMP and phosphoinositide systems are critically important for controlling the secretory steps and for setting the sensitivity of the release sites to the prevailing free calcium level. The levels of the second messengers are tightly regulated by various secretagogues, such as glucose, other nutrients, hormones, and neurotrasmitters. Such stimulators can be further divided into two categories including initiators and potentiators. The fuel hypothesis has been proposed and is the generally accepted model of glucose induced insulin secretion. It is based on the following observations. Firstly, glucose induced insulin secretion is tightly related to glucose utilization and oxidation and blocking glucose phosphorylation or glycolysis abolishes insulin secretion (Daniel & Gerald 1997).

In addition, non-metabolizable sugars, such as 3-O-methylglucose, galactose, and fructose characteristically do not induce insulin secretion whereas metabolizable nutrients such as the amino acid, leucine are potent stimulators of insulin secretion. As such, fuel metabolism plays a fundamental role in the initiation of insulin secretion. In contrast, the potent insulinotropic actions of other agents, including incretin hormones, require the presence of fuel secretagogues to mediate their actions and are referred to as potentiators of insulin secretion. The potentiation of insulin secretion by these agents is usually mediated by second messengers, such as cAMP, via binding and regulation of specific G protein-coupled receptor pathways. (McClenaghan et al 1996b, McClenaghan et al 1996c, Lindskog et al 1998)

1.2.2.2.1 ATP-sensitive K+ channels (K_{ATP} channels) – membrane depolarization – voltage dependent calcium channel (VDCC) pathway

Glucose is the main stimulator of insulin secretion and utilizes this pathway. Glucose (>5mM) is transported into pancreatic cells via GLUT2 and metabolized through glycolysis and Krebs cycle inside the mitochondria. This process leads to the elevation of the intracellular ATP. The increase of intracellular ATP, results in the increase of ATP/ADP ratio, causes closure of K_{ATP} channels and inhibits the efflux of potassium ions. Under basal glucose levels (0 – 3 mM), the membrane potential of –60 to –70 mV (Ashcroft et al 1992). However, with membrane depolarization via the closure of K_{ATP} channels, the resting cell membrane will be depolarized (raising to 0 mV from –70 mV) and results in the opening of the voltage- dependent calcium channels (VDCC). The intracellular Ca^{2+} concentration is increased by the influx of calcium via VDCC. Finally, the mobilization of secretory granules will be triggered and insulin will be discharged by exocytosis (Katagiri et al 1994).

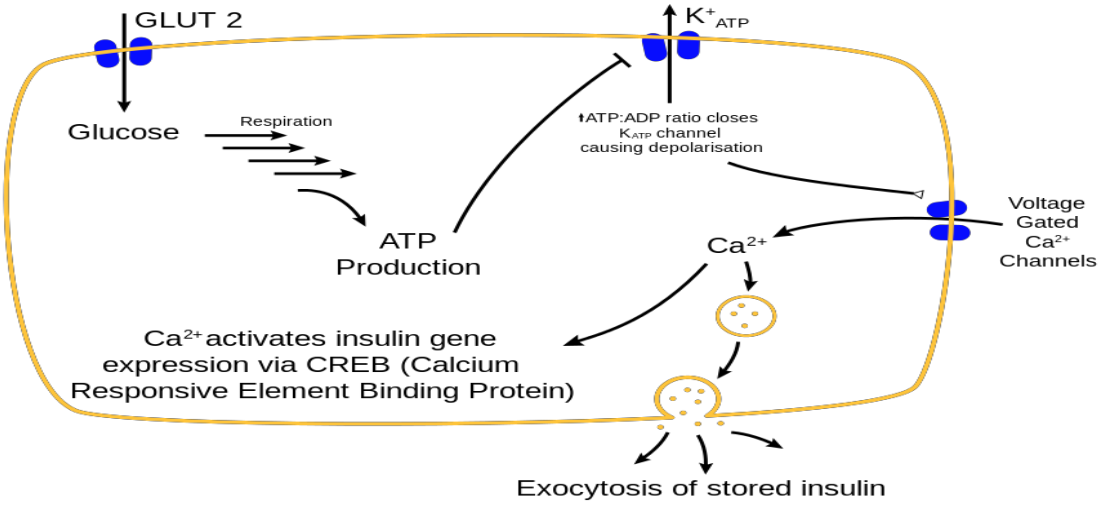

Figure 1.2.2.2.1: Mechanism of Insulin Secretion (Yada 1994)

Activation of certain key components of this pathway can trigger secretion. Firstly, amino acids, such as leucine, and keto acids, can generate intracellular ATP via metabolism resulting in a rise of the ATP/ADP ratio (Meglasson et al 1986). In this way these agents stimulate insulin secretion utilizing essentially the same pathway as glucose. In addition, the oral hypoglycemic agents, such as the sulphonylureas, tolbutamide and glibenclamide, can trigger insulin secretion by closure of K_{ATP} channels as a consequence of binding to the sulphonylurea binding subunit (SUR1). Moreover, membrane depolarization agents, such as KCl and arginine, have been shown to increase intracellular calcium via opening VDCCs (Ashcroft et al 1992). On the other hand, alanine depolarizes the cell membrane by co-transportation with Na^+ which depolarizes the cells and thereby increases intracellular calcium via activation of VDCCs (Yada 1994).

1.2.2.2.2 K_{ATP} channel independent pathway (amplification pathway)

Glucose can stimulate insulin secretion in pancreatic cells under conditions where K_{ATP} channels are fully opened by KCl and diazoxide. Interestingly, a significantly reduced first phase but maintained second phase of glucose induced insulin secretion was observed in SUR knockout mice .These observations suggest that glucose stimulated insulin secretion is not only via K_{ATP} channel–VDCC pathway but also by K_{ATP} channel-independent pathways (Henquin 2000).

1.2.2.2.3 Potentiation of insulin secretion via regulation of second messengers cAMP – Protein kinase A pathway:

Cyclic AMP augments glucose-induced insulin secretion through a number of mechanisms including increased opening of voltage-sensitive Ca^{2+} channels, calcium-induced Ca^{2+}-release, activation of ryanodine receptors in the ER and direct effects on exocytosis. Most actions of cyclic AMP in the cell seem to be mediated through protein kinase A (PKA)- catalysed phosphorylation events but direct effects of the cyclic nucleotide on exocytosis are partly PKA-independent. PKA-independent effects on exocytosis can be mediated by the cyclic AMP-binding protein cAMP-GEFII, interacting with Rim2, a target of the small G-protein Rab3.

Furthermore, incretin hormones, such as glucagons-like peptide 1 (GLP-1) and gastric–inhibitory-polypeptide (GIP), can enhance glucose-induced insulin secretion by binding to their own specific stimulatory G protein coupled receptors, thereby increasing intracellular cAMP by activation of adenylate cyclase. An increase in intracellular cAMP by activation of adenylate cyclase with forskolin has been shown to enhance glucose induced biphasic insulin secretion. Although it has been accepted that cAMP regulates insulin exocytosis due to protein phosphorylation; nonetheless, cAMP-dependent pathways still remained to be fully characterized (Kanno et al 1998).

Cyclic AMP is hydrolysed to its biologically inactive 5′ derivative by cyclic nucleotide phosphodiesterases (PDE1-PDE11) enzymes. Selective inhibition of phosphodiesterases (PDEs) augments insulin secretion by increasing cyclic AMP. Thus PDEs offer a target for developing drugs for the treatment of type 2 diabetes mellitus. IBMX, an inhibitor of cyclic AMP phosphodiesterase, has been shown to augment glucose-induced insulin secretion via increased levels of intracellular cAMP. Several selective PDE3 inhibitors augmented glucose- induced insulin secretion from rat and human islets. Org 9935 and siguazodan augmented insulin secretion in the insulin-secreting cell line BRIN-BD11. A novel piperazine hypoglycaemic agent was shown to inhibit PDE3 and PDE4 in islets and augmented insulin secretion (Ahmad et al 2000).

1.2.2.2.4 Phospholipase C- protein kinase C pathway:

Phospholipase C (PLC) is a key component of activation of the calcium-calmodulin and protein kinase C system. This activation is via hydrolysis of $PtdInsP_2$ into $InsP_3$ and diacylglycerol (DAG). As a result, IP_3 increases intracellular calcium via mobilazation of intracellular calcium stores in ER or microsomes. Elevation of intracellular calcium is directly associated with insulin exocytosis and along with DAG activates PKC which has been suggested to contribute in K_{ATP} channel independent pathways for insulin release. Neurotransmitters, such as acetylcholine, and the gastrointestinal hormone, cholecystokinin-8 (CCK-8), enhance glucose induced insulin secretion by activation of the PLC-PKC pathway following binding to specific muscarinic and CCK-8 receptors, respectively. Direct activation of PKC with the phorbol ester, phorbol 12-myristate 13 acetate (PMA) stimulates insulin secretion. However, down-regulation of PKC activity by chronic culture with phorbol esters has little effect on glucose-stimulated insulin secretion (McClenaghan & Flatt 1999b).

1.2.3 Hormone regulation of insulin secretion

1.2.3.1 Estrogen

β-cells are not considered classic estrogen targets; however, estrogen receptors are present in islets and the effects of 17β-estradiol on β-cells are well documented. The main physiological consequence of 17β-estradiol action on β-cells is the enhancement of insulin secretion. In humans, 17β-estradiol can increase insulin secretion in postmenopausal women. This insulinotropic effect is mediated by potentiating glucose-stimulated insulin secretion (GSIS). The effects of estradiol are initiated by its binding to estrogen receptors. Two types of estrogen receptor (ER) are present in β-cells: 1). the nuclear ERs (ERα and ERβ) and 2). the membrane ER (ERγ). It is reported that at physiological concentrations, 17β-estradiol can significantly decrease K_{ATP} channel activity in a reversible manner, which causes membrane depolarization and subsequent opening of voltage-gated Ca^{2+}channels, thereby potentiating glucose-induced intracellular [Ca^{2+}] oscillations. The modulation of K_{ATP}channel activity by estradiol may be mediated by activation of the cGMP-dependent protein kinase (PKG) pathways. The activated PKG can directly

phosphorylate transcriptional factor CREB. After phosphorylation, CREB can bind to CRE, which in turn modulates transcription of genes containing cAMP/Ca^{2+} response elements to potentiate glucose-induced intracellular [Ca^{2+}] oscillations that influence insulin secretion.

1.2.3.2 Melatonin

Melatonin is a hormone secreted by the pineal gland, which helps adjust the timing or reinforces oscillations of the biological clock. The direct effect of melatonin on β-cells was confirmed by the discovery of melatonin receptors on both clonal β-cells and human islets. Effects on insulin section are controversial. There are studies showing that melatonin exerts an inhibitory, neutral, or stimulatory effect on insulin secretion. However the inhibitory effect is consistent in replicated experiments with clonal β-cells. Melatonin attenuated glucose- and KCl-stimulated insulin secretion in rat islets. The inhibitory effect of melatonin on insulin release was later confirmed in rat islets. Consistently, chronic melatonin administration ameliorates hyperinsulinemia in vivo.

1.2.3.3 GLP-1

GLP-1, an incretin hormone, is secreted from small intestinal L-cells together with GIP in response to nutrient load. Incretin is responsible for augmentation of insulin secretion to meet the increased demand for insulin after a meal. Experiments have shown nutrient load from the oral route stimulates more insulin secretion than intravenous nutrient load. The analogs of both GLP-1 and GIP have been explored as a potential therapy for T2D for many years, and the long-lasting GLP-1 analog exenatide was introduced to clinics in 2005 as a prescription drug for T2D treatment. Upon activation of the GLP-1 receptor (GLP-1R), adenylyl cyclase is activated, leading to the generation of cAMP. The elevated cAMP then potentiates GSIS. This insulinotropic effect is dependent on glucose. When the extracellular glucose concentration is in the normal fasting range (lower than 4 mmol/L), GLP-1 is inactive in stimulating insulin secretion. Such glucose-dependent action of GLP-1 is very important in preventing hypoglycemia.

1.2.3.4 Leptin

Leptin is secreted by adipocytes and is known to influence insulin action in fat and liver cells. It is generally accepted that leptin exerts an inhibitory effect on insulin secretion. Leptin deficiencies are associated with hyperinsulinemia in both mice and humans. A large body of literature shows that leptin plays an inhibitory role in insulin secretion in clonal β-cells, cultured rodent islets, human islets, perfused rodent pancreas, as well as in mice. It is hypothesized that leptin's inhibitory effect is through antagonizing the action of elevated intracellular cAMP, since it was reported that leptin inhibits insulin secretion induced by 3-isobutyl-1-methylxanthine (IBMX), which elevates cAMP content by inhibiting phosphodiesterases (PDEs), the enzymes catalyzing the hydrolysis of cAMP. Leptin also potently inhibits glucoincretin- or GLP-1-induced insulin secretion, which augments GSIS through activation of the cAMP signaling pathways. Leptin was shown to inhibit insulin secretion by activating PDE 3B, a subtype of PDE.

1.2.3.5 Growth hormone

Growth hormone (GH) has targets in variety of cells but one of its best-known actions is to stimulate production of insulin-like growth factor-I (IGF-I) and its binding proteins. Recombinant human IGF-I was shown to decrease serum levels of insulin and C-peptide in normal human subjects. Ex-vivo studies using isolated rat islets confirmed that IGF-1 directly suppresses insulin secretion. This inhibitory effect is possibly mediated through activation of PDE3B, which is responsible for breaking down cAMP in β-cells, as stated above (Araki et al 1994).

1.2.4 Mechanism of insulin action

Insulin binds to specific, high-affinity receptors in the cell membrane of most tissues, including liver, muscle, and adipose. This is the first step in a cascade of reactions ultimately leading to a diverse array of biologic actions.

1.2.4.1 Insulin receptor

The insulin receptor is synthesized as a single polypeptide that is glycosylated and cleaved into subunits, which are then assembled into a tetramer linked by disulfide bonds. A hydrophobic domain in each subunit spans the plasma membrane. The extracellular subunit

contains the insulin-binding site. The cytosolic domain of the subunit is a tyrosine kinase, which is activated by insulin (Zimmermann 2009).

1.2.4.2 Insulin receptor substrates

The insulin receptor belongs to a subfamily of tyrosine kinases that includes the insulin-like growth factor (IGF)-I receptor and the insulin receptor-related receptor (IRR). These receptors are tetrameric proteins consisting of two glycoprotein subunits. Primary substrates of the insulin receptor include the four proteins, insulin receptor substrate (IRS)-1, -2, -3 and -4. The participation of IRS proteins in mediating intracellular signals from the insulin receptor is well documented.

1.2.4.3 Signal transduction

The binding of insulin to the subunits of the insulin receptor induces conformational changes that are transduced to the subunits, promoting a rapid autophosphorylation of specific tyrosine residue of each subunit.

The signaling mechanism involved in the various biologic responses to insulin remains somewhat elusive, but recent progress has shed light on a few pathways that are critical for its regulation of glucose and lipid metabolism. The action of insulin is characterized by a diverse variety of effects, including changes in vesicle trafficking, stimulation of protein kinases and phosphatases, promotion of cellular growth and differentiation, and activation, or in some cases, repression of transcription. The diverse mechanisms involve multiple signaling pathways that diverge at or near the receptor. It has also been documented that both phosphoinositide (PI) 3-kinase- independent and -dependent signaling pathways are a necessary component of insulin- stimulated GLUT4 translocation. Insulin-stimulated activation of PI 3- kinase is a crucial step linking signaling of GLUT4 translocation (Araki et al 1994).

1.2.5 Effects of insulin

1.2.5.1 Insulin and Carbohydrate Metabolism

Glucose is liberated from dietary carbohydrate such as starch or sucrose by hydrolysis within the small intestine, and is then absorbed into the blood. Elevated concentrations of glucose in blood stimulate release of insulin, and insulin acts on cells though out the body

to stimulate uptake, utilization and storage of glucose. The effects of insulin on glucose metabolism vary depending on the target tissue. Two important effects are:

Insulin facilitates entry of glucose into muscle, adipose and several other tissues.

The only mechanism by which cells can take up glucose is by facilitated diffusion through a family of hexose transporters. In many tissues - muscle being a prime example - the major transporter used for uptake of glucose (called GLUT4) is made available in the plasma membrane through the action of insulin.

It should be noted here that there are some tissues that do not require insulin for efficient uptake of glucose: important examples are brain and the liver. This is because these cells don't use GLUT4 for importing glucose, but rather, another transporter that is not insulin-dependent.

Insulin stimulates the liver to store glucose in the form of glycogen

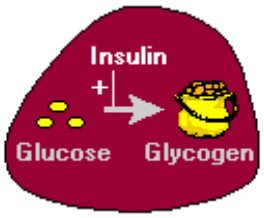

Figure 1.2.5.1 Glucose Storage (Carvalho et al., 2005)

A large fraction of glucose absorbed from the small intestine is immediately taken up by hepatocytes, which convert it into the storage polymer glycogen.

3. A well-known effect of insulin is to decrease the concentration of glucose in blood, which should make sense considering the mechanisms described above. Another important consideration is that, as blood glucose concentrations fall, insulin secretion ceases. In the absence of insulin, a bulk of the cells in the body become unable to take up glucose, and begin a switch to using alternative fuels like fatty acids for energy. Neurons, however,

require a constant supply of glucose, which in the short term, is provided from glycogen reserves.

When insulin levels in blood fall, glycogen synthesis in the liver diminishes and enzymes responsible for breakdown of glycogen become active. Glycogen breakdown is stimulated not only by the absence of insulin but by the presence of glucagon, which is secreted when blood glucose levels fall below the normal range

1.2.5.2 Insulin and Lipid Metabolism

The metabolic pathways for utilization of fats and carbohydrates are deeply and intricately intertwined. Considering insulin's profound effects on carbohydrate metabolism, it stands to reason that insulin also has important effects on lipid metabolism, including the following:

Insulin promotes synthesis of fatty acids in the liver

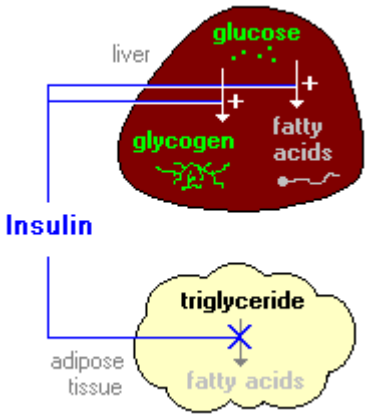

Figure 1.2.5.2 Synthesis of fatty acids (Carvalho et al., 2005).

As discussed above, insulin is stimulatory to synthesis of glycogen in the liver. However, as glycogen accumulates to high levels (roughly 5% of liver mass), further synthesis is strongly suppressed. When the liver is saturated with glycogen, any additional glucose taken up by hepatocytes is shunted into pathways leading to synthesis of fatty acids, which are exported from the liver as lipoproteins. The lipoproteins are ripped apart in the

circulation, providing free fatty acids for use in other tissues, including adipocytes, which use them to synthesize triglyceride.

2. Insulin inhibits breakdown of fat in adipose tissue by inhibiting the intracellular lipase that hydrolyzes triglycerides to release fatty acids.

Insulin facilitates entry of glucose into adipocytes, and within those cells, glucose can be used to synthesize glycerol. This glycerol, along with the fatty acids delivered from the liver, are used to synthesize triglyceride within the adipocyte. By these mechanisms, insulin is involved in further accumulation of triglyceride in fat cells.

From a whole body perspective, insulin has a fat-sparing effect. Not only does it drive most cells to preferentially oxidize carbohydrates instead of fatty acids for energy, insulin indirectly stimulates accumulation of fat in adipose tissue (Carvalho et al., 2005).

1.2.6 Glucose transport and GLUT4

GLUT4 is one of 13 sugar transporter proteins (GLUT1-GLUT12, and HMIT) encoded in the human genome (Joost and Thorens, 2001; Wood and Trayhurn, 2003) that catalyzes hexose transport across cell membranes through an ATP-independent, facilitative diffusion mechanism (Hruz and Mueckler, 2001). These sugar transporters display differences in their kinetics and respective substrate specificities, such that GLUT5 and perhaps GLUT11 are likely fructose transporters. GLUT4 is highly expressed in adipose tissue and skeletal muscle, but these tissues also express a selective cohort of these other transporters. In the case of skeletal muscle, GLUT1, GLUT5, and GLUT12 may significantly contribute to sugar uptake in addition to GLUT4 (Stuart et al., 2000, 2006), while in adipose tissue GLUT8, GLUT12, and HMIT are also expressed (Wood et al., 2003; Wood and Trayhurn, 2003). However, GLUT4 displays the unique characteristic of a mostly intracellular disposition in the unstimulated state that is acutely redistributed to the plasma membrane in response to insulin and other stimuli (Bryant et al., 2002; Czech and Corvera, 1999).
GLUT4 contains unique sequences in its N- and COOH-terminal cytoplasmic domains that direct its characteristic membrane trafficking capability. These include a distinctive N-terminal sequence with a potentially critical phenylalanine residue (Piper et al., 1993;

Araki et al., 1996; Melvin et al., 1999; Al-Hasani et al., 2002), as well as dileucine and acidic motifs in the COOH terminus (Corvera et al., 1994; Garippa et al., 1996; Shewan et al., 2000; Sandoval et al., 2000; Martinez-Arca et al., 2000). These motifs likely govern kinetic aspects of both endocytosis and exocytosis in a continuously recycling trafficking system. The COOH terminus LL and acidic motifs are also present in an aminopeptidase protein (IRAP) that in adipocytes is similarly sequestered within GLUT4-enriched intracellular membranes and highly responsive to insulin action (Johnson et al., 1998, 2001; Hou et al., 2006). These considerations place GLUT4 at the interface of two exciting fields of biology—insulin signaling and membrane trafficking.

GLUT4 Is a Key Determinant of Glucose Homeostasis

A central role for GLUT4 in whole-body metabolism is strongly supported by a variety of genetically engineered mouse models where expression of the transporter is either enhanced or ablated in muscle or adipose tissue or both. The whole-body GLUT4$^{-/-}$mouse itself may be less informative due to upregulation of compensatory mechanisms that may promote survival of these animals (Katz et al., 1995; Stenbit et al., 1996). However, heterozygous GLUT4$^{+/-}$ mice that display decreased GLUT4 protein in muscle and adipose tissue show the expected insulin resistance and propensity toward diabetes that is consistent with a major role of GLUT4 in glucose disposal (Rossetti et al., 1997; Stenbit et al., 1997; Li et al., 2000). Interestingly, overexpression of GLUT4 expression in skeletal muscle of such GLUT4$^{+/-}$ animals through crosses with transgenic mice normalizes insulin sensitivity and glucose tolerance (Tsao et al., 1999). Transgenic mice expressing high levels of GLUT4 in adipose tissue (Shepherd et al., 1993; Tozzo et al., 1995) or in skeletal muscle (Tsao et al., 1996, 2001) in turn are both highly insulin sensitive and glucose tolerant.

Conversely, conditional depletion of GLUT4 in either adipose tissue or skeletal muscle causes insulin resistance and a roughly equivalent incidence of diabetic animals (Zisman et al., 2000; Abel et al., 2001). This was particularly surprising in the former case since adipose tissue accounts for only a small fraction of total body glucose disposal (James et al., 1985). These tissue-specific depletions of GLUT4 have profound metabolic effects on other tissues. For example, mice with muscle-specific GLUT4 deficiency display

decreased insulin responsiveness in adipose tissue and liver (Zisman et al., 2000), while those with adipose-specific GLUT4 depletion exhibit muscle and liver insulin resistance (Abel et al., 2001). In the muscle-specific GLUT4 knockout mice, this is at least partially mediated through elevated blood glucose levels that occur in the conditional knockout animals, which secondarily impairs insulin signaling (Kim et al., 2001).

1.2.7 Current therapies for diabetes mellitus

Since diabetes conditions encompass a multiplicity of endocrine and metabolic disturbance, it is necessary to consider a wide range of pharmacological approaches to manage these. These may be required individually or in combinations to treat different features of the disease process. Ideal treatments will target the fundamental causes of insulin resistance, defective beta -normal glucose homeostasis cell function and loss of cell mass. Currently glycaemic control is achieved by dietary manipulation, oral hypoglycamics agents (for example sulphonylurase or biguanides) or insulin injections. Approximately 75% of diabetic patients in UK achieve glycaemic control without exogenous insulin treatment (Bailey & Flatt 1995).

1.2.7.1 Diet

The regulation of food intake is central to the treatment of diabetes mellitus and various dietary regimes have been considered to assist in the control of hyperglycemia. The control of diet should be the first treatment offered to type 2 patients before drugs are considered. The main goal of nutritional management is to correct obesity as weight loss will improve glucose control, lower blood pressure and lipid concentration, all of which may help in preventing or diminishing long term complications. Various dietary regimes have been considered to assist in the control of hyperglycemia. However, in most cases the dietary recommendations for type 2 diabetic patients are identical to those for the general population. Calorie restriction in the overweight and obese, with the emphasis on low- fat, high-carbohydrate and high-fibre is recommended (Stuart et al., 2000, 2006).

1.2.7.2 Insulin as drug

The discovery of insulin by Banting, Best and co-workers in 1922 dramatically improved the prospects of individuals with diabetes mellitus. As type 1 is characterized by insulin insufficiency caused by partial or total destruction of insulin releasing pancreatic beta cells,

patients with this condition required exogenous insulin replacement for treatment. The last decade has seen increasing refinement of exogenous insulin delivery in type 1 diabetes. In an attempt to reinstate normoglycemia, efforts have been made to match exogenous insulin delivery with the 24 h glucose profile. These have led to the introduction of continuous subcutaneous insulin infusion (CSII) and practice of multiple (4/d) subcutaneous insulin injections. Although intensive insulin regimes have unquestionably improved the control of diabetes they have not consistently achieved normoglycemia in clinical practice. In certain cases of type 2, exogenous insulin is required to achieve glycemic control.A number of insulin preparations have been developed since its discovery based on the duration of action. Although various procedures were attempted to prolong the duration of insulin action, the two forms endured; the production of neutral protamine hagedorn (NPH) insulin, where absorption is retarded by protamine and development of the lente series by the use of zinc-insulin complexes. Insulin can be broadly classified as having short, medium, or long duration of action, however their effects vary considerably from one patient to another and in the same patient from time to time (Eisenbarth 1986, Rossini et al 1993)

1.2.7.3 Ant-diabetic drugs

Those patients who fail to achieve glycemic control through dietary intervention measures require oral hypoglycemic agents. Approximately 50% of type 2 patients in the UK are treated with oral hypoglycemic agents. Although there are new oral hypoglycemic agents on the horizon, the choice at the present is primarily between sulphonylureas and biguanide (metformin).

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Sulphonylureas, developed after initial observations of sulphonamide in patients with typhoid fever (Bailey 2015), have been the foundation of antidiabetic therapy for many years. The various sulphonylureas differ in potency, pharmacokinetic properties and side effects. The sulphonyurea drugs have direct and immediate stimulating effects on the cell

mediated via the inhibition of K_{ATP} channels in the cell (James et al 2016) The potentiation of the stimulatory effect of the amino acids alanine and leucine by sulphonylureas through enhanced cell recognition has been documented (Nathan et al 2009)

Some authors claim an extrapancreatic action for sulphonylureas on the insulin receptor and at the post-receptor level which require the presence of endogenous insulin. Recently promotion of insulin exocytosis was demonstrated and was shown to be partly independent of K_{ATP} channels and dependent on protein kinase C.

Repaglinide has recently been introduced in the US. The reports of trials in patients with type 2 diabetes have demonstrated that it promptly increase insulin concentrations and reduce postprandial hyperglycemia without causing interprandial glucose concentration to fall bellow the normal range (Palmer et al 2016).

Metformin, the major biguanide in clinical use, was used before the characteristic insulin resistance was discovered. In contrast to sulphonylurea drug, metformin enhances the extrapancreatic actions of insulin in insulin resistance and hyperglycemic status but has no effect on glycemia of type 1 diabetic individuals. Metformin does not change insulin-receptor binding or alter phosphorylation and kinase activity of insulin receptors after insulin-mediated glucose uptake *in vitro* with metformin indicating a post-receptor site of action. In addition to insulin-mediated glucose disposal, metformin and related biguanides decrease hepatic glucose output and increase glucose utilization by the small intestine. Some of these effects are independent of insulin but in patients devoid of insulin these drugs are ineffective. The glucose-lowering efficacy of sulphonylureas and metformin in type 2 diabetes are reviewed elsewhere (Bailey 2015).

1.3 The need for new treatments for diabetes mellitus

The management of diabetes mellitus is on the threshold of a revolution. Approach as to the control of blood glucose and prevention of hyperglycemia are central to the treatment of diabetes mellitus .At present none of these therapies either alone or in combination can reinstate normal blood glucose homeostasis or eliminate long-term complications and many limitation exist in the use of antidiabetic drugs. In type 1 diabetes a more physiological means of insulin delivery is required. Insulin therapy affords effective glycemic control, yet its short comings such as ineffectiveness on oral administration, short

shelf life, requirement of constant refrigeration, and in the event of excess dosage – fatal hypoglycemia – limits its usage (Kalra 2013). Currently available sulfonylureas, the most commonly used pharmacologic agents in treatment of type 2 diabetes; have gradually increasing secondary failure rates reaching 50% at the end of 5 y of disease, though the initial response is good in 70-75% of patients. The biguanides are mainly used as adjuvants to sulfonylureas. The gastrointestinal intolerance limits their use in many patients. Thus, large number of patients with type 2 diabetes fails to achieve persistent good metabolic control. New therapies are needed which reinstate a normal metabolic environment and prevent long-term complications. The development of new antidiabetic drugs, which address the underlying metabolic lesions in type 2 diabetes, ideally requires new pharmacological treatments, which stimulate both the secretion and action of insulin (Bailey & Flatt 1986).

1.3.1 Traditional plants for diabetes treatment

Plants have formed the basis for the treatment of diseases in traditional medicine systems for thousands of years, and continue to play a major role in the primary health care of about 80% of the world's inhabitants. It is estimated that 66-80% of medicines used in developing countries are based on plants. Many of the currently available drugs have been derived directly or indirectly from plants. Within developed countries 25% of medicinal therapies contain active priciples derived from plants. Besides providing active raw materials, plants can offer molecules that serve as templates for the development of new drugs (Day & Bailey 1988).

World ethnobotanical information about medicinal plants reports that almost 800 plants are used in the control of diabetes mellitus. Over the last two decades, several comprehensive reviews have been written on the evidence that higher plants are of use in the treatment of diabetes, providing discussions of the botany, phytochemistry, pharmacology, and in some cases, toxicology, of the botanical agents. Literally hundreds of extracts of higher plants used in folk medicine for diabetes (or active principles derived from these plants) have been screened for their biologic activity in both *in vitro* and *in vivo* assays. The most extensive review evaluated available data on more than 1000 species of plants reported to have been used to treat diabetes and/or been investigated for antidiabetic activity, and indicated that approximate 80% of the traditional plants used for the treatment of diabetes

demonstrated some antidiabetic activity. In many instances the chemical constituent in the plant responsible for the biological activity has been isolated and identified, and information is also available concerning the mechanism of action. Galega officinalis (goat's rue), used in Europe as a treatment for diabetes since medieval times, yields a hypoglycemic principle rich in guanidine. Further derivatives of this principle have given rise to biguanides and the present anti-diabetic agent metformin (Famsworth 1971).

1.3.1.1 Medicinal Plants with reported Anti diabetic Effect

Table 1.3.1.1 Medicinal Plants with reported Anti diabetic Effect

Plant(Family)	**Part of Plant Used**	**Material**	**Result**
Annona Sqamosa (Annonaceae)	Fruit peel	Alcohol, ether, ethyl acetate	Significant increase body weight and diminished blood glucose level
Tamarandus indica Linn	Seeds	Aqueous extract	effective in type II diabetic rat model
Calamus erectus (Arecaceae)	fruit	Methanolic extract	Reduction of blood glucose level
Momordica Charantia (Cucurbitaceae)	Plant	Alcoholic extract	lower the blood sugar level
Dactyl lifera linn (Arecaceae)	dried dates	Aqueous extract	reduction in blood glucose level
Swertia Chirata (Gentianaceae)	Whole plant	Aqueous and 12% ethanolic extracts	Significant antidiabetic activity
Tamarandus indica Linn(Caesalpiniacee)	Fruit pulp	Ethanolic extracts	Antidiabetic effect
Tamarandus indica Linn(Caesalpiniacee)	Fruit pulp	Ethanolic extracts	Antidiabetic effect
Parmelia Perlata. Ach (Permeliaceae)	Leaves	Aqueous extract	reduced the fasting blood glucose and HbA1C level
Psidium guvajava (Myrtaceae)	Leaves	Ethanolic extract	reduction in blood glucose level

(Gilani et al., 1999; Asres et al., 2005; Shah et al., 1997; Singh et al., 2009)

1.4 *Nigella sativa*

Nigella sativa (*N. sativa*) (Family Ranunculaceae) is emerging as a miracle herb with a rich historical and religious background since many researches revealed its wide spectrum of pharmacological potential. *N. sativa* is commonly known as black seed. *N. sativa* is native to Southern Europe, North Africa and Southwest Asia and it is cultivated in many countries in the world like Middle Eastern Mediterranean region, South Europe, India, Pakistan, Syria, Turkey, Saudi Arabia (Khare 2004).

The seeds of *N. sativa* and their oil have been widely used for centuries in the treatment of various ailments throughout the world. And it is an important drug in the Indian traditional system of medicine like Unani and Ayurveda. Among Muslims, it is considered as one of the greatest forms of healing medicine available due to it was mentioned that black seed is the remedy for all diseases except death in one of the Prophetic hadith. It is also recommended for use on regular basis in Tibb-e-Nabwi.

Figure 1.4 ***Nigella sativa*** (Abel-Salam BK 2012)

1.4.1 Scientific Classification

Domain- Eukarya
Kingdom- Plantae (Plants)
Subkingdom- Tracheobionta (Vascular plants)
Superdivision- Spermatophyta (Seed plants)
Phylum- Magnoliophyta (Flowering plants)
Class- Magnoliopsida (Dicotyledons)
Subclass- Magnoliidae
Order- Ranunculales
Family- Ranunculaceae (Buttercup family)
Genus- *Nigella*
Species- *N. sativa*
(Abel-Salam BK 2012)

1.4.2 Pharmacognostical Characteristics

1.4.2.1 Morphology of the plant

N. sativa is an annual flowering plant which grows to 20-90 cm tall, with finely divided leaves, the leaf segments narrowly linear to threadlike. The flowers are delicate, and usually colored white, yellow, pink, pale blue or pale purple, with 5-10 petals. The fruit is a large and inflated capsule composed of 3-7 united follicles, each containing numerous seeds (Goreja 2003).

Figure 1.4.2.1 *N. sative* (whole plant, flower and seeds) (Cheikh-Rouhou et al 2008)

1.4.2.2 Characteristics of the seeds and powder

Macroscopically, seeds are small dicotyledonous, trigonus, angular, regulose-tubercular, 2-3.5mm×1-2 mm, black externally and white inside, odor slightly aromatic and taste bitter. Microscopically, transverse section of seed shows single layered epidermis consisting of elliptical, thick walled cells, covered externally by a papillose cuticle and filled with dark brown contents. Epidermis is followed by 2-4 layers of thick walled tangentially elongated parenchymatous cells, followed by a reddish brown pigmented layer composed of thick walled, rectangular elongated cells. Inner to the pigment layer, is present a layer composed of thick walled rectangular elongated or nearly columnar, elongated cells. Endosperm consists of thin walled, rectangular or polygonal cells mostly filled with oil globules. The powder microscopy of seed powder shows brownish black, parencymatous cells and oil globules (Khare 2004).

1.4.2.3 Chemical composition of black seeds

Many active compounds have been isolated, identified and reported so far in different varieties of black seeds. The most important active compounds are thymoquinone (30%-48%), thymohydroquinone, dithymoquinone, p-cymene (7%-15%), carvacrol (6%-12%), 4-terpineol (2%-7%), t-anethol (1%-4%), sesquiterpene longifolene (1%-8%) α-pinene and thymol *etc*. Black seeds also contain some other compounds in trace amounts. Seeds contain two different types of alkaloids; *i.e*. isoquinoline alkaloids *e.g*. nigellicimine and nigellicimine-N-oxide, and pyrazol alkaloids or indazole ring bearing alkaloids which include nigellidine and nigellicine. Moreover, *N. sativa* seeds also contain alpha-hederin, a water soluble pentacyclic triterpene and saponin, a potential anticancer agent. Some other compounds *e.g*. carvone, limonene, citronellol were also found in trace amounts. Most of the pharmacological properties of *N. sativa* are mainly attributed to quinine constituents, of which TQ is the most abundant. On storage, TQ yields dithymoquinone and higher oligocondensation products. The seeds of *N. sativa* contain protein (26.7%), fat (28.5%), carbohydrates (24.9%), crude fibre (8.4%) and total ash (4.8 %). The seeds are also containing good amount of various vitamins and minerals like Cu, P, Zn and Fe *etc*. The seeds contain carotene which is converted by the liver to vitamin A. Root and shoot are reported to contain vanillic acid (Al-Jassir 1992).

The seeds reported to contain a fatty oil rich in unsaturated fatty acids, mainly linoleic acid (50-60%), oleic acid (20%), eicodadienoic acid (3%) and dihomolinoleic acid (10%). Saturated fatty acids (palmitic, stearic acid) amount to about 30% or less. α-sitosterol is a major sterol, which accounts for 44% and 54% of the total sterols in Tunisian and Iranian varieties of black seed oils respectively, followed by stigmasterol (6.57-20.92% of total sterols) (Cheikh-Rouhou et al 2008).

1.4.3 Scientific researches and pharmacological potentials

The extensive researches using modern scientific techniques were carried out by various researchers on *N. sativa* since it is believed to be a miraculous herb that can cure multiple ailments and disorders. A number of pharmacological actions of *N. sativa* have been investigated in the past few decades.

1.4.3.1 Antibacterial activity

The antibacterial effect of ground black seeds was studied in a modified paper disc diffusion method. A clear inhibition of the growth of *Staphylococcus aureus* was observed by concentration of 300 mg/mL with distilled water as control, this inhibition was confirmed by using the positive control Azithromycin. The inhibition obtained was higher with *N. sativa* ground seeds from Hadramout than with *N. sativa* ground seeds from Ethiopia. The positive inhibition may be attributed to the two important active ingredients of *N. sativa*, TQ and melanin (Bakathir 2011). Different crude extracts of *N. sativa* were tested for antimicrobial effectiveness against different bacterial isolates which comprised of 16 gram negative and 6 gram positive representatives. These isolates showed multiple resistances against antibiotics, specially the gram negative ones. Crude extracts of *N. sativa* showed a promising effect against some of the test organisms. The most effective extracts were the crude alkaloid and water extracts. Gram negative isolates were affected more than the gram positive ones (Morsi 2000).

1.4.3.2 Antifungal activity

Methanolic extracts of *N. sativa* have the strongest antifungal effect followed by the chloroform extracts against different strains of *Candida albicans*. Aqueous extracts showed no antifungal activity. An intravenous inoculum of *Candida albicans* produced colonies of the organism in the liver, spleen and kidneys. Treatment of mice with the plant extract 24 h after the inoculation caused a considerable inhibitory effect on the growth of the organism in all organs studied. Khan *et al.* in 2003 reported that the aqueous extract of *N. sativa* seeds exhibits inhibitory effect against candidiasis in mice. A 5-fold decrease in Candida in kidneys, 8-fold in liver and 11-fold in spleen was observed in the groups of animals post-treated with the plant extract. These findings were also confirmed by Histopathological examination of the respective organs (Bita 2012). Antidermatophyte activity of ether extract of *N. sativa* and TQ was tested against eight species of dermatophytes: four species of *Trichophyton rubrum* and one each of *Trichophyton interdigitale*, *Trichophytonmentagrophytes*, *Epidermophytonfloccosum* and *Microsporum canis* using Agar diffusion method with serial dilutions of ether extract of *N. sativa*, TQ and griseofulvin. The MICs of the ether extract of *N. sativa* and TQ were between 10-40 and 0.125-0.250 mg/mL, respectively, while those of griseofulvin ranged from 0.00095 to 0.01550 mg/mL. These results denote the potentiality of *N. sativa* as a source for antidermatophyte drugs and support its use in folk medicine for the treatment of fungal skin infections.

1.4.3.3 Anti-schistosomiasis activity

The effect of NSO against the liver damage induced by *Schistosoma mansoni* (*S. mansoni*) infection in mice was studied by Mahmoud *et al*. When the NSO was given alone, it reduced the number of S. mansoni worms in the liver and decreased the total number of ova deposited in both the liver and the intestine. When NSO was administered in combination with PZQ, the most prominent effect was a further lowering in the dead ova number over that produced by PZQ alone. Infection of mice with *S. mansoni* produced a pronounced elevation in the serum activity of ALT, GGT, with a slight increase in AP level, while reduce serum albumin level. Administration of NSO succeeded partially to correct the previous changes in ALT, GGT, AP activity, as well as the Alb content in serum.

These results suggest that NSO may play a role against the alterations caused by *S. mansoni* infection (Mahmoud 2002).

1.4.3.4 Antidiabetic activity

The therapeutic potentials of α-lipoic acid (α-LA), L-carnitine, and *N. sativa* or combination of them in carbohydrate and lipid metabolism was evaluated in a Rat model of diabetes which was induced by single *i.p.* injection of streptozocin (STZ) 65 mg/kg. For evaluation of glucose metabolism, fasting blood glucose, insulin, insulin sensitivity, HOMA, C-peptide, and pyruvate dehydrogenase activity were determined. Either α-LA or *N. sativa* significantly reduced the elevated blood glucose level. The combination of 3 compounds significantly increased the level of insulin and C-peptide. Combination of α-LA, L-carnitine and *N. sativa* will contribute significantly in improvement of the carbohydrate metabolism in diabetic rats, thus increasing the rate of success in management of DM (Salama 2011). The effects of *N. sativa* aqueous extract and oil, as well as TQ, on serum insulin and glucose concentrations in streptozotocin diabetic rats were studied. Serum insulin and glucose concentrations, SOD levels, and pancreatic tissue malondialdehyde (MDA) were determined. Electron microscopy was used to identify any subcellular changes. Diabetes increased tissue MDA and serum glucose levels and decreased insulin and SOD levels. Treatment of rats with *N. sativa* extract and oil, as well as TQ, significantly decreased the diabetes-induced increases in tissue MDA and serum glucose and significantly increased serum insulin and tissue SOD. Ultrastructurally, TQ ameliorated most of the toxic effects of streptozotocine(STZ), including segregated nucleoli, heterochromatin aggregates (indicating DNA damage), and mitochondrial vacuolization and fragmentation. The aqueous extract of *N. sativa* also reversed these effects of STZ, but to a lesser extent. The *N. sativa* oil restored normal insulin levels, but failed to decrease serum glucose concentrations to normal. The biochemical and ultrastructural findings suggest that *N. sativa* extract and TQ have therapeutic and protect against STZ-diabetes by decreasing oxidative stress, thus preserving pancreatic β-cell integrity. The hypoglycemic effect observed could be due to amelioration of β-cell ultrastructure, thus leading to increased insulin levels. *N. sativa* and TQ may prove

clinically useful in the treatment of diabetics and in the protection of β-cells against oxidative stress (Abdelmeguid et al 2010).

1.4.3.5 Anticancer activity

In vitro study of TQ to determine whether or not TQ can increase survival and sustain the expression of the homing receptor CD62L in antigen-specific T cells. The results showed that stimulation of OT-1 (transgenic CD+) T cells with OVA antigen resulted in activation, as shown by a decrease in the surface expression of CD62L which coincided with significant apoptosis measured three and five days after antigen stimulation. Addition of low concentrations of TQ during CD85+ T-cell activation resulted in enhanced survival of the activated T cells and sustained expression of CD62L. These effects coincided with enhancement in the capability of CD8+ T cells to produce the effector cytokine interferon-gamma (IFNgamma). This is concluded that TQ has a beneficial effect in conditioning T cells *in vitro* for adoptive T-cell therapy against cancer and infectious disease (Salem 2011). The cytotoxic effects of different *N. sativa* seed extracts as an adjuvant therapy to doxorubicin on human MCF-7 breast cancer cells was reported. The study showed *N. sativa* lipid extract is cytotoxic to MCF-7 cells with LC_{50} of 2.720 ± 0.232 mg/mL, while its aqueous extract cytotoxicity exhibited when the applied concentration is high as about 50 mg/mL (Mahmoud 2012)

1.4.3.6 Anti-inflammatory and analgesic activity

The aqueous extract of *N. sativa* was found to possess anti-inflammatory and analgesic but not antipyretic activities in animal models while anti-inflammatory effect of the alcoholic extracts of *N. sativa* seeds and its callus on mix glial cells of rat with regard to their TQ content was investigated. The mix glial cells, inflamed by lipopolysaccharide, were subjected to anti-inflammatory studies in the presence of various amounts of TQ and the alcoholic extracts. Results confirmed that TQ content of the callus of leaf was 12 times higher than that measured in the seeds extract. Studies on the inflamed rat mix glial cells revealed significant reduction in the nitric oxide production in the presence of 0.2 to 1.6 mg/mL of callus extract and 1.25 to 20 µL/mL of the seed extracts (Alemi et al 2012).

1.4.3.7 Immunomodulatory activity

The potential immunomodulatory effects of *N. sativa* were investigated in light of splenocyte proliferation, macrophage function, and NK anti-tumor activity using BLAB/c and C57/BL6 primary cells. Results demonstrated that the aqueous extract of *N. sativa* significantly enhances splenocyte proliferation in a dose-responsive manner. In addition, the aqueous extract of *N. sativa* favors the secretion of Th2, versus Th1, cytokines by splenocytes. The secretion of IL-6, TNF-α, and NO; key pro-inflammatory mediators, by primary macrophages is significantly suppressed by the aqueous extract of *N. sativa*, indicating that *N. sativa* exerts anti-inflammatory effects *in vitro*. Finally, experimental evidence indicates that the aqueous extract of *N. sativa* significantly enhances NK cytotoxic activity against YAC-1 tumor cells, suggesting that the documented anti-tumor effects of *N. sativa* may be, at least in part, attributed to its ability to serve as a stimulant of NK anti-tumor activity. It was anticipated that *N. sativa* ingredients may be employed as effective therapeutic agents in the regulation of diverse immune reactions implicated in various conditions and diseases such as cancer (Majdalawieh 2010)

1.4.3.8 Cardiovascular activity

The acute (at 4 and 18 h) effects of diesel exhaust particles (DEP) on cardiopulmonary parameters in mice and the protective effect of TQ were investigated. Mice were given, intratracheally, either saline (control) or DEP (30 µg per mouse). At 18 h (but not 4 h) after giving DEP, there was lung inflammation and loss of lung function. At both 4 and 18 h, DEP caused systemic inflammation characterized by leucocytosis, increased IL-6 concentrations and reduced systolic blood pressure. SOD activity was decreased only at 18 h. DEP reduced platelet numbers and aggravated in vivo thrombosis in pial arterioles. In vitro, addition of DEP (0.1-1 µg/mL) to untreated blood-induced platelet aggregation. Pretreatment of mice with TQ prevented DEP-induced decrease of systolic blood pressure and leucocytosis, increased IL-6 concentration and decreased plasma SOD activity. TQ also prevented the decrease in platelet numbers and the prothrombotic events but not platelet aggregation in vitro (Nemmar 2011).

Chapter: 02

Objective of the Study

2.1 Research Objective:

The objective of this research work has therefore focused on the following point:

- To evaluate the anti-hyperglycemic effect of the methanolic extract of the seeds of plant *Nigella sativa* in long evans rats.

- To determine the anti-diabetic efficacy of the plant *Nigella sativa.*

Chapter: 03

Literature Review

3.1 Literature Review on *Nigella sativa*

We have studied on stem, bark and seed of this plant. Here is some literature review on different parts of this plant.

3.1.1 Effect of *Nigella sativa* on Glucose Concentration, Lipid Peroxidation, Anti-Oxidant Defence System and Liver Damage in Experimentally-Induced Diabetic Rabbits (Meral 2001)

This study was carried out to investigate whether Nigella sativa could decrease the lipid peroxidation, increase the anti-oxidant defence system and also prevent the lipid-peroxidation-induced liver damage in experimentally induced diabetic rabbits. Fifteen New Zealand male rabbits were divided into three experimental groups: control, diabetic and diabetic and N. sativa-treated. The diabetes mellitus (DM) was induced in the rabbits using 150 mg/kg of 10% alloxan. The diabetic + N. sativa-treated group was given extract of N. sativa seeds orally every day for 2 months after induction of DM. At the end of the 2-month experiment, blood samples were collected to measure malondialdehyde (MDA), glutathione (GSH), ceruloplasmin and glucose concentration, and livers were harvested for histopathological analysis. Treatment with N. sativa decreased the elevated glucose and MDA concentrations, increased the lowered GSH and ceruloplasmin concentrations, and prevented lipid-peroxidation-induced liver damage in diabetic rabbits.

3.1.2 Pharmacological and toxicological properties of *Nigella sativa* (Ali 2003)

The seeds of *Nigella sativa* Linn. (Ranunculaceae), commonly known as black seed or black cumin, are used in folk (herbal) medicine all over the world for the treatment and prevention of a number of diseases and conditions that include asthma, diarrhoea and dyslipidaemia. This article reviews the main reports of the pharmacological and toxicological properties of *N. sativa* and its constituents.

The seeds contain both fixed and essential oils, proteins, alkaloids and saponin. Much of the biological activity of the seeds has been shown to be due to thymoquinone, the major component of the essential oil, but which is also present in the fixed oil.

3.1.3 Neuropharmacological activity of *Nigella sativa* L. extracts (Al-Naggar 2003)

Pharmacological studies have been conducted on the aqueous and methanol extracts of defatted *Nigella sativa* L. seeds to evaluate their effects on the central nervous system (CNS) and on analgesic activity. The observations suggest that the two extracts of *Nigella sativa* possesses a potent CNS and analgesic activity (depressant action especially in the case of the methanolic extract)

3.1.4 Effects of *Nigella sativa* on oxidative stress and β-cell damage in streptozotocin-induced diabetic rats (Kanter 2004)

The aim of the present study was to evaluate the possible protective effects of Nigella sativa L. (NS) against β-cell damage from streptozotocin (STZ)-induced diabetes in rats. STZ was injected intraperitoneally at a single dose of 50 mg/kg to induce diabetes. NS (0.2 ml/kg/day, i.p.) was injected for 3 days prior to STZ administration, and these injections were continued throughout the 4-week study. Oxidative stress is believed to play a role in the pathogenesis of diabetes mellitus (DM). To assess changes in the cellular antioxidant defense system, we measured the activities of antioxidant enzymes (such as glutathione peroxidase (GSHPx), superoxide dismutase (SOD), and catalase (CAT)) in pancreatic homogenates. We also measured serum nitric oxide (NO) and erythrocyte and pancreatic tissue malondialdehyde (MDA) levels, a marker of lipid peroxidation, to determine whether there is an imbalance between oxidant and antioxidant status. Pancreatic β-cells were examined by immunohistochemical methods.

3.1.5 *Nigella sativa* seed extracts enhance glucose-induced insulin release from rat-isolated Langerhans islets (Rchid 2004)

Nigella sativa L. 'Black cumin' (Ranunculaceae) is one of the plants commonly used in Moroccan folk medicine for treatment of various ailments including diabetes mellitus. The present study was undertaken to investigate the effect of different N. sativa seed extracts on insulin secretion. Different fractions of the seed were prepared: the defatted fraction (HR II), which was divided into two subfractions: the first (HR III) containing acidic and neutral compounds and the second (HR IV) containing basic compounds. The insulin

secretory effects of these extracts were evaluated individually at different concentrations (0.01, 0.1, 1 and 5 mg/mL), in vitro in isolated rat pancreatic islets in the presence of 8.3 mmol/L glucose. The results show that addition of the defatted whole extract or of the basic subfraction of the seed in the incubation medium significantly increased glucose-induced insulin release from the islets. In the case of the acidic and neutral subfraction, the stimulatory effect was observed only for the higher concentration (5 mg/mL).

3.1.6 Effects of Methanolic Extract and Commercial Oil of Nigella sativa L. on Blood Glucose and Antioxidant Capacity in Alloxan-Induced Diabetic Rats (Houcher 2007)

Nigella sativa is a medicinal plant widely used in the Arabic and Islamic world against a number of human pathologies. In this present study the methanol extraction (85 % then 50 %) of plant seeds gave an important yield of 27 % of dry substance. The anti-hyperglycaemia effect of the crude methanolic extract and the commercial oil of these seeds were tested in alloxan-induced, intra peritoneal, diabetic rats (150 mg/kg). Effects of these two substances on other diabetes-linked factors such as the reducing power of the plasma and the osmotic fragility of erythrocytes. The daily orally administration of the crude methanolic extract (810 mg/kg/day) and the oil (2.5 ml/kg/day) for 25 days leads to a significant decrease of glycaemia, especially during the first 10 days of treatment (decreases of 58.09 and 73.27 % respectively). However, the dose of 270 mg/kg of crude methanolic extract had no effect, which is probably due to the low dose.

3.1.7 Antidiabetic Activity of *Nigella sativa*. Seed Extract in Cultured Pancreatic β-cells, Skeletal Muscle Cells, and Adipocytes (Benhaddou et al 2008)

The seeds of *Nigella sativa*. L. (NS), a plant of the Runanculaceae family, are used in traditional medicine in North Africa and the Middle East for the treatment of diabetes. Despite widespread use and a number of scientific studies, the target tissues and cellular mechanisms of action of this plant product are not well understood. This study evaluated the effects of NS seed crude ethanol extract on insulin secretion in INS832/13 and β TC-tet lines of pancreatic β-cells and on glucose disposal by C2C12 skeletal muscle cells and

3T3-L1 adipocytes. An 18-h treatment with NS amplified glucose-stimulated insulin secretion by more than 35% without affecting sensitivity to glucose. NS treatment also accelerated β-cell proliferation. An 18-h treatment with NS increased basal glucose uptake by 55% (equivalent to approximately two-fold the effect of 100 nM insulin) in muscle cells and approximately by 400% (equal to the effect of 100 nM insulin) in adipocytes; this effect was perfectly additive to that of insulin in adipocytes.

3.1.8 The in Vivo Antidiabetic Activity of Nigella sativa Is Mediated through Activation of the AMPK Pathway and Increased Muscle Glut4 Content (Benhaddou et al 2011).

The antidiabetic effect of N. sativa seed ethanol extract (NSE) was assessed in Meriones shawi after development of diabetes. Meriones shawi were divided randomly into four groups: normal control, diabetic control, diabetic treated with NSE (2 g eq plant/kg) or with metformin (300 mg/kg) positive control, both administered by daily intragastric gavage for 4 weeks. Glycaemia and body weight were evaluated weekly. At study's end, an Oral Glucose Tolerance Test (OGTT) was performed to estimate insulin sensitivity. Upon sacrifice, plasma lipid profile, insulin, leptin, and adiponectin levels were assessed. ACC phosphorylation and Glut4 protein content were determined in liver and skeletal muscle. NSE animals showed a progressive normalization of glycaemia, albeit slower than that of metformin controls. Moreover, NSE increased insulinemia and HDL-cholesterol, compared to diabetic controls. Leptin and adiponectin were unchanged. NSE treatment decreased OGTT and tended to decrease liver and muscle triglyceride content. NSE stimulated muscle and liver ACC phosphorylation and increased muscle Glut4.

3.1.9 Antidiabetic Properties of a Spice Plant Nigella sativa (Mathur 2011)

Seeds of *Nigella sativa* (black cumin/kalonji) used in pickles as spice, have also been traditionally used in treatment of many diseases including diabetes and hypertension. Among many activities exhibited by *N. sativa* and its constituents in animal experiments, antidiabetic property is most important. Thymoquinone (TQ), a volatile oil, is one of its active constituents but antidiabetic activity has also been shown by its aqueous extract and defatted extract. *N. sativa* may be beneficial in diabetic individuals and those with glucose

intolerance as it reduces appetite, glucose absorption in intestine, hepatic gluconeogenesis, blood glucose level, cholesterol, triglycerides, body weight and simulates glucose induced secretion of insulin from beta-cells in pancreas; improves glucose tolerance as efficiently as metformin; yet it has not shown significant adverse effects and has very low toxicity. In streptozotocin (STZ) induced diabetic rats it causes gradual partial regeneration of pancreatic beta-cells, increases the lowered serum insulin concentrations and decreases the elevated serum glucose.

3.1.10 A review on therapeutic potential of *Nigella sativa*: A miracle herb (Ahmad 2013)

Nigella sativa (N. sativa) (Family Ranunculaceae) is a widely used medicinal plant throughout the world. It is very popular in various traditional systems of medicine like Unani and Tibb, Ayurveda and Siddha. Seeds and oil have a long history of folklore usage in various systems of medicines and food. The seeds of *N. sativa* have been widely used in the treatment of different diseases and ailments. In Islamic literature, it is considered as one of the greatest forms of healing medicine. It has been recommended for using on regular basis in Tibb-e-Nabwi (Prophetic Medicine). It has been widely used as antihypertensive, liver tonics, diuretics, digestive, anti-diarrheal, appetite stimulant, anti-bacterial and in skin disorders. Extensive studies on *N. sativa* have been carried out by various researchers and a wide spectrum of its pharmacological actions have been explored which may include antidiabetic, anticancer, immunomodulator, analgesic, antimicrobial, anti-inflammatory,

Chapter: 04 Methods and Materials

4.1 Extraction techniques of medicinal plants

Extraction, as the term is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures. The products so obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use.

These include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, pilular (semisolid) extracts and powdered extracts. Such preparations popularly have been called galenicals, named after Galen, the second century Greek physician. The purposes of standardized extraction procedures for crude drugs are to attain the therapeutically desired portion and to eliminate the inert material by treatment with a selective solvent known as menstruum (Daniel & Gerald 1997).

4.1.1 Plant material collection

Plant seeds sample of *Nigella sativa* was used for the experiment which was processed in the laboratory. The Plant seeds were collected and washed with water several times.

4.1.2 Drying and grinding

The collected plant seeds were washed with water, separated from undesirable materials, partially dried by fan aeration and then fully dried in the oven at below 40°C for 2 days. The fully dried seeds were then grinded to a powdered form and stored in there refrigerator at +4°C for a few days.

4.1.3 Methanol extraction

500 gm of powered material was taken in a clean, flat bottomed glass container and soaked in 2500 ml methanol, sealed and kept for a period of 2 days with occasional shaking and stirring. It was then filtered first by cotton material and twice through whatman filter paper to obtain a finer filtrate. The filtrate (Methanol extract) obtained was evaporated by Rotary evaporator (Eyelan 1000, Tokyo Rikaki Kai Co. Ltd, Rotary vacuum, Japan) at 4 to 5 rpm and at 65◦C temperature.

4.1.4 Extraction procedure

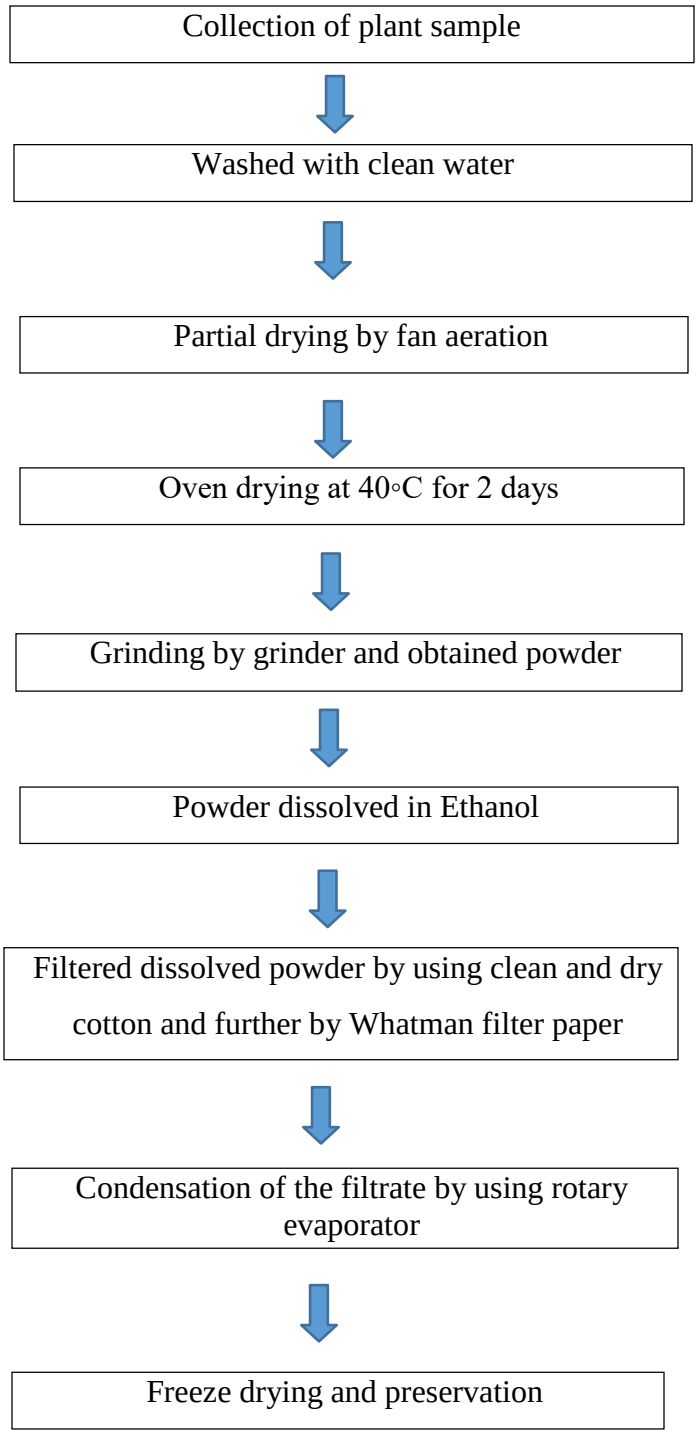


Figure 4.1.4: General Plant Extraction Procedure

4.2 Experimental animal models

Long Evans rats (male and female), weighing 150-200g of either sex are bred in ICDDRB and grown in the animal house of the Department of Pharmacy, East West University. All the animals acclimatized one week prior to the experiments. The animals were housed under standard laboratory conditions (relative humidity 55-65%, room temperature 25.0 ± 2°C, and 12 hours light dark cycle). The animals were fed with standard diet from ICDDRB and had free access to filtered water .The overall nutrient composition of the diet was 36.2% carbohydrate, 20.9% protein, 4.4% fat and 38.5% fiber with metabolizable energy content of 1.18 MJ/100 gm (282Kcal/100 gm). The animals were maintained in the laboratory and the treatment was scheduled.

Animal described as fasted were deprived of food for at least 12hr but allowed free access to drinking water.

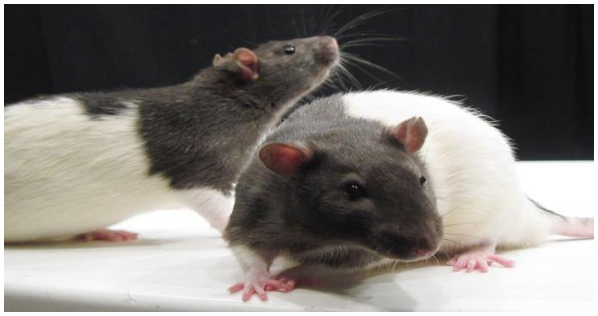


Figure 4.2: Long Evans Rats (Bailey & Flatt 1995)

4.2.1 Description of the model

This model was developed by Dr Long and Dr Evans in 1915.The long evans rat is the result of a cross between a female albino from the WISTAR Institute and a wild male (*Rattus norvegicus*) captured near Berkeley and offspring selection. The long evans rat is small and resistant to oncogenesis. This strain is widely used in behavioral, learning, ageing (visual acuity less affected than that of albino strains), addiction – especially to alcohol – studies.

4.2.2 Biomedical research

Rats have prevalence within biomedical research second only to humans and they share 90% of the genome with humans. Almost all disease-linked human genes we currently know of have equivalent genes within the rat genome, making them a suitable research tool.

Figure 4.2.2: Rat handling (Bailey & Flatt 1995)

Rats were the first mammalian species specifically domesticated to be used in the laboratory. Records dating back to the 1850s show these animals were derived from those bred by rat fanciers who collected them for their unique coat colors and behavioral characteristics. The success of the rat in research today has been linked to the Wistar Institute in America and their development of the Wistar albino strain. There are currently 117 albino strains of the laboratory rat, all of which can be traced genetically back to the one rat, likely to have arisen as a mutation from a hooded (piebald) rat strain. Since their development as a laboratory species, rats have been used to answer a wide range of basic science questions ranging from physiology, immunology, pharmacology, toxicology, nutrition, behavior and learning (Bailey & Flatt 1995).

4.3 Screening for the Possible Inhibition of Carbohydrate Absorption by Plant Material

4.3.1 Chemicals and reagents

Normal saline, 2N H_2SO_4, 1N NaOH, Sucrose (2.5g/Kg body weight of rat in 5ml deionized water)

Drug: 100mg/Kg body weight of rat

Kits: Glucose kit was used for the determination of Glucose.

4.3.2 Procedure of Six Segments

Rats were fasted for 20hours before experiment. Sucrose (2.5g/Kg/5ml, average 443 mg) with or without extract (effective dose of hypoglycemic effect). Each segment was washed out with ice- cold saline (10ml), acidified with H_2SO_4 (2ml) and centrifuged at 3000rpm for 10minutes. The supernatant thus obtained was boiled for 2hours to hydrolyze the Sucrose and then neutralized with NaOH (approximately 2.5ml). The blood glucose level and the amount of Glucose liberated from residual Sucrose in the gastrointestinal tract were measured by Glucose Oxidase (GOD- PAD) Method. Then the gastrointestinal sucrose content was calculated from the amount of liberated glucose.

4.3.3 Steps in six segments

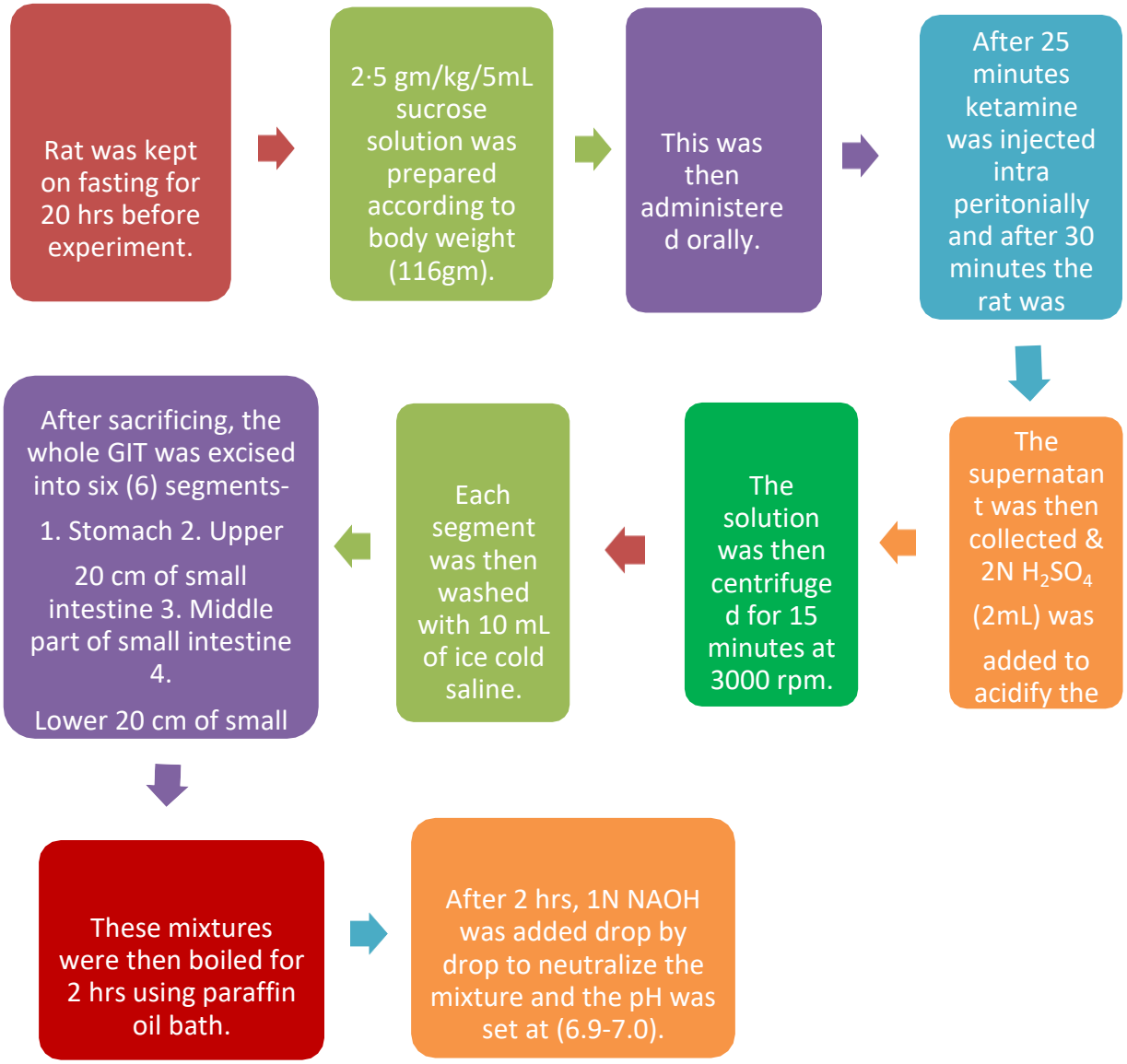


Figure 4.3.3: Steps in Six Segments

4.4 Assessment of the Effect of Plant Materials on Intestinal Disaccharidase Activity (Enzyme activity).

4.4.1 Assessment of conditions

All rats were fasted overnight (12hours) before being tested but still allowed free access to distilled water. Extract is administered orally to experiment group and water to control group.

4.4.2 Mucosa/Tissue Collection

After one hour of drug administration, rats are anesthetized with pentobarbital-Na/ether, the entire length of the small intestine (from pylorus to ileocaecal junction) is carefully removed from the pylorus to the ileocaecal junction. The lumen of the intestine is washed out with 50ml of ice cold saline. Intestine is then placed on ice-cold glass plates over ice and cut longitudinally. The mucosa is isolated but scrapping with glass microscope slides and homogenized with 10ml of saline for 20seconds at medium speed in a Heidolph Diax 600 homogenizer.

4.4.3 Enzyme activities

Disaccharidase activity is assessed using the Dahlqvist method with modifications. Twenty (20) µl of mucosal homogenate were added in duplicate to 40 mM sucrose and incubated at 37◦C for 60minutes. The glucose converted from sucrose and total protein (using Lowry's methods) in the homogenate are measured. Disaccharidase activity will be calculated by glucose concentration converted from sucrose as µmol-mg glucose/protein/h.

4.4.4 Steps in Enzyme Activity

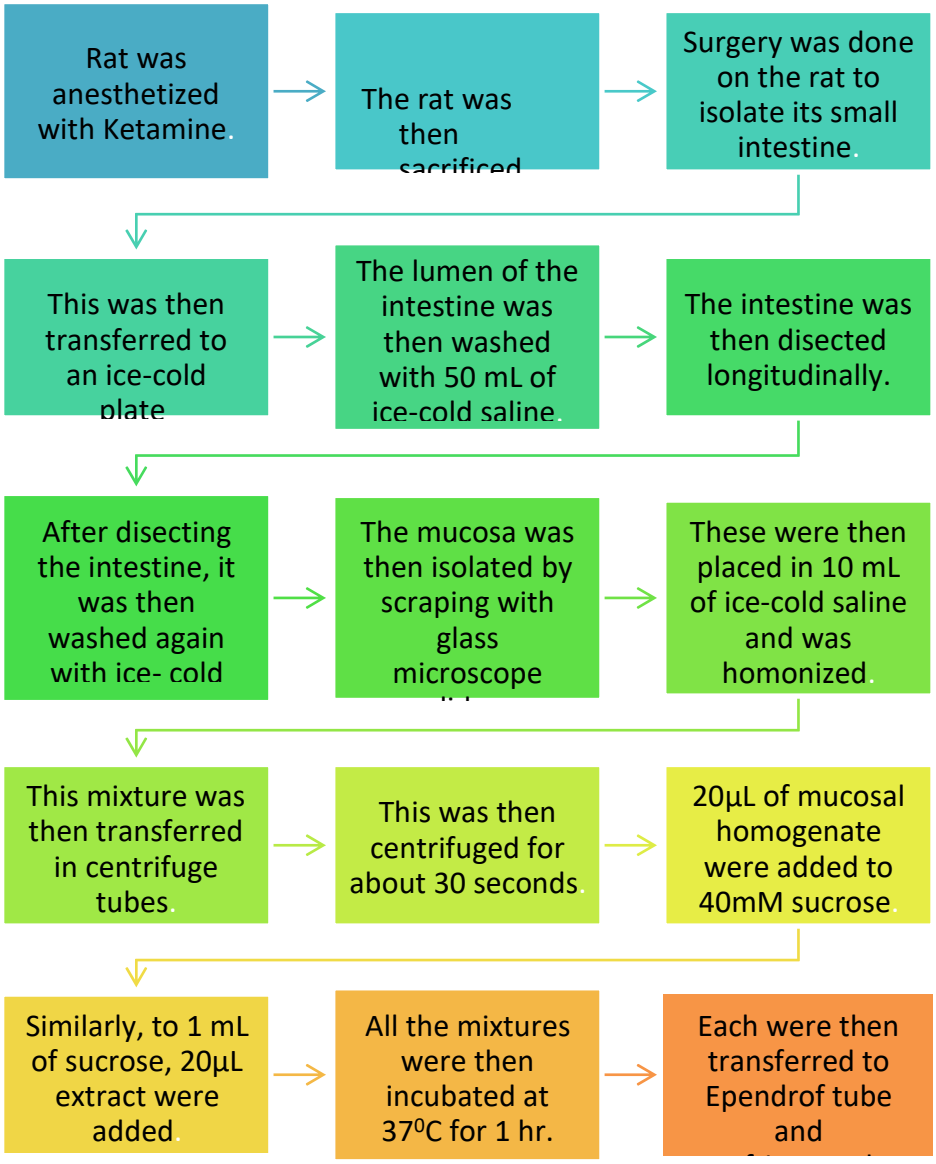


Figure 4.4.4: Steps in Enzyme Activity

4.5 Assessment of the Effect of Plant Materials on Gastrointestinal Motility:

One of the pivotal tasks of gastrointestinal tract is its ability to organize coordinated transport of luminal content which is perfectly adjusted to the digestive needs of the body. To achieve this gastrointestinal tract exhibits a wide repertoire of motor patterns that are based on spatiotemporal coordination of muscle activity. The gastrointestinal tract is able to monitor caloric density; osmolarity and pH of the luminal content and reacts with the initiation of the appropriate motility pattern.

The fascinating variety of motility patterns is best appreciated by imaging gut motility and transit of luminal content by Video fluoroscopy.

Motility disorders in the gut are major causes and concomitant phenomena of various functional, structural and inflammatory bowel diseases; one of the most prominent examples is irritable bowel disease (IBS).

4.5.1 Procedure:

1. For this experiment, 12 hours fasted mince is taken from the animal house.
2. Distilled water is administered to one mouse and marked it with marker as control mouse.
3. Plan extract is administered to another mouse and marked it as test mouse.
4. Barium sulphate milk is prepared by dissolving 10% (W/V) barium sulphate milk in 0.5% (W/V) sodium carboxymethyl cellulose (Na- CMC) suspension.
5. $BaSo_4$ milk is administered to all mice after 1 hour of administration of test drug.
6. Mice are sacrificed after 15 minutes of administration of the $BaSo_4$ milk.
7. The distance travelled by $BaSo_4$ milk is determined by scale.

4.5.2 Steps in Gastrointestinal Motility:

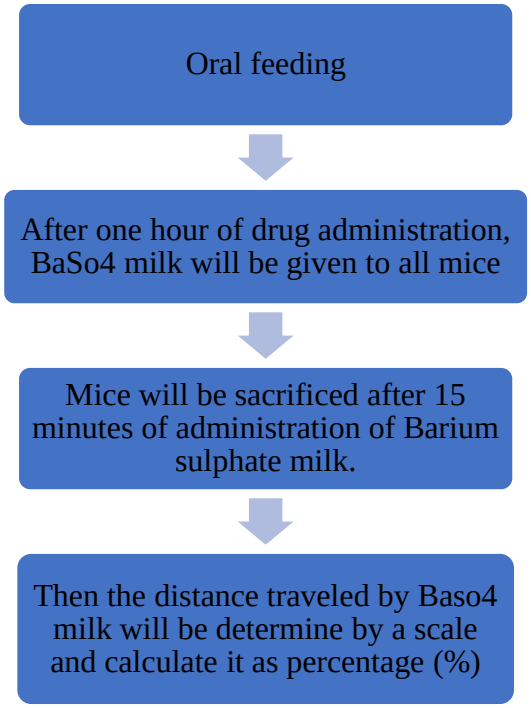


Figure 4.5.2: Steps in Gastrointestinal Motility

Chapter: 05

Result

5.1 Effect of *Nigella sativa* on Unabsorbed Sucrose Content in the Gastrointestinal Tract

Upon oral administration of sucrose along with *Nigella sativa* (2.5g/Kg), significant amount of unabsorbed sucrose was remained in the stomach, upper, middle, and lower intestine at 30 min and 1h. This amount of residual sucrose remained significant in caecum and large intestine till 4h (Table 5.1.1. - Table 5.1.6, Figure 5.1.1-5.1.6)

In Figures 5.1 – 5.6, blue color graph indicates control groups & orange color graph indicates *Nigella sativa* (drug) group.

Table 5.1.1 Unabsorbed Sucorse Content(mg) in Stomach

Parts	Group	Time			
		30 min	60 min	120 min	240 min
Stomach	Control	54.13±1.7	34.04±3.94	8.50±0.60	1.32±0.32
	Nigella sativa	57.22±0.72	37.87±1.87	21.97±1.97*	8.30±0.80*

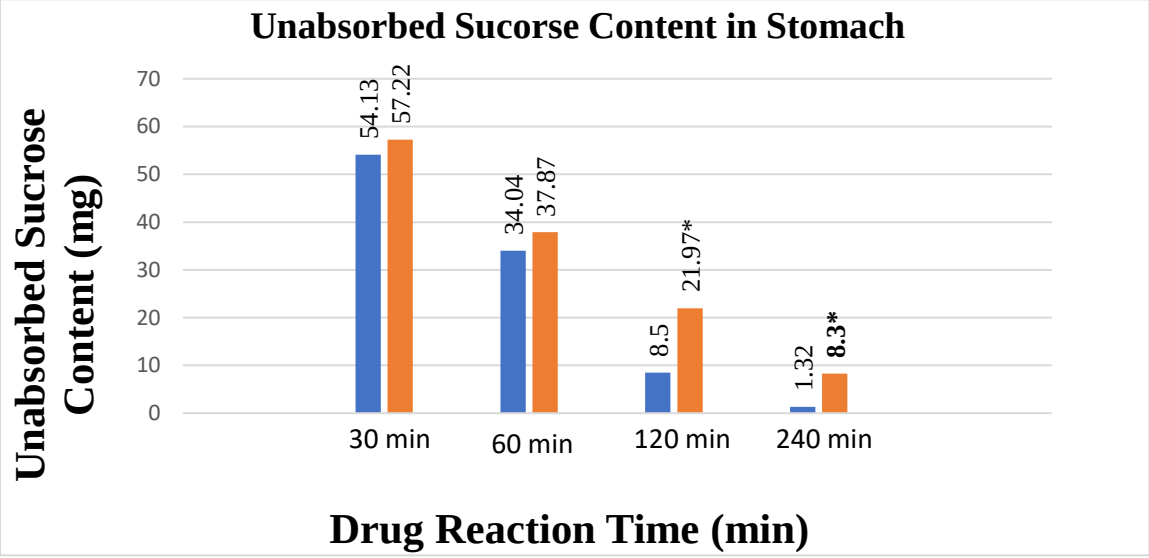


Figure 5.1.1: Anti Hyperglycemic Effect of *Nigella sativa* in Stomach

* p < 0.05, ** p < 0.01 *** p < 0.001

Table 5.1.2 Unabsorbed Sucrose content(mg) in Upper 20cm of Small Intestine					
Parts	**Group**	**Time**			
		30 min	60 min	120 min	240 min
Upper 20cm	Control	14.69±0.89	11.68±0.66	4.56±1.08	0.95±0.15
	Nigella sativa	19.15±0.96*	15.42±0.39*	6.14±0.54	1.59±0.19

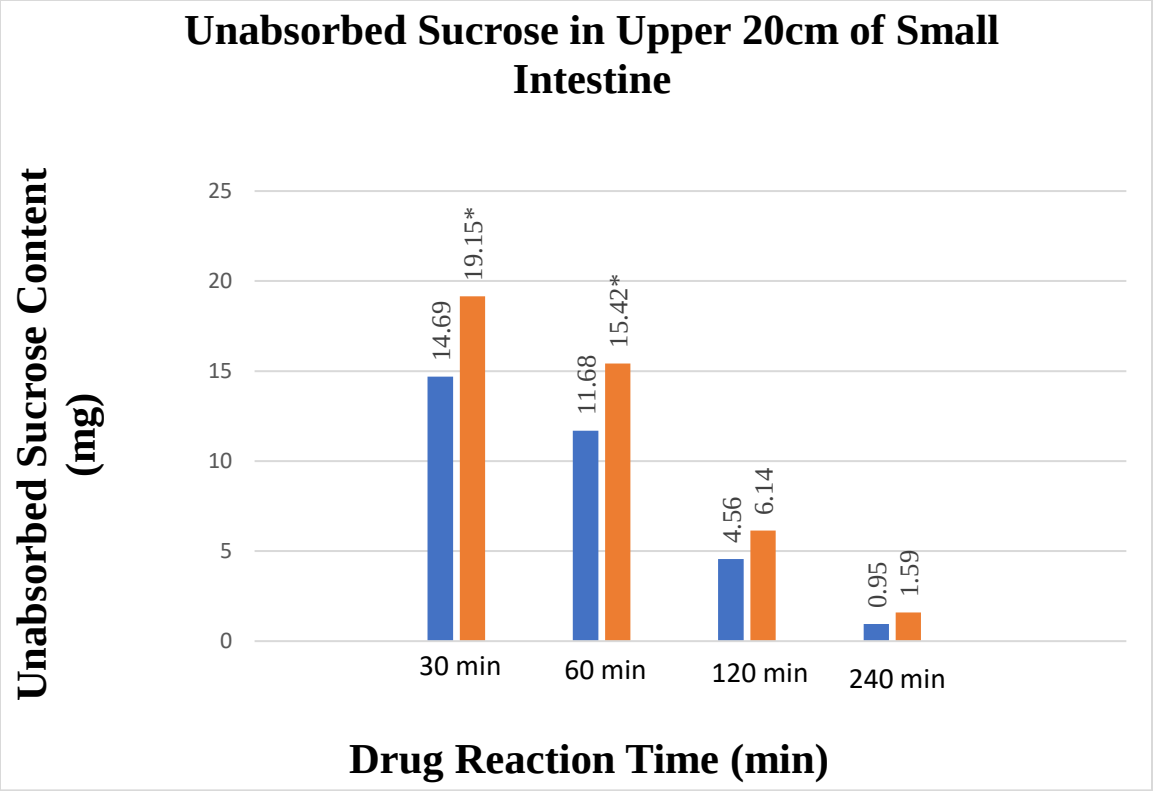


Figure 5.1.2: Anti Hyperglycemic Effect of *Nigella sativa* in upper 20 cm of small intestine.

* $p < 0.05$, ** $p < 0.01$ *** $p < 0.001$

Table 5.1.3 Unabsorbed Sucrose content(mg) in Middle part of Small intestine					
Parts	**Group**	**Time**			
		30 min	60 min	120 min	240 min
Middle	Control	20.17±1.95	17.48±0.72	7.99±0.05	1.26±0.08
	Nigella sativa	25.42±0.91	21.65±1.65	8.26±0.46	1.78±0.18

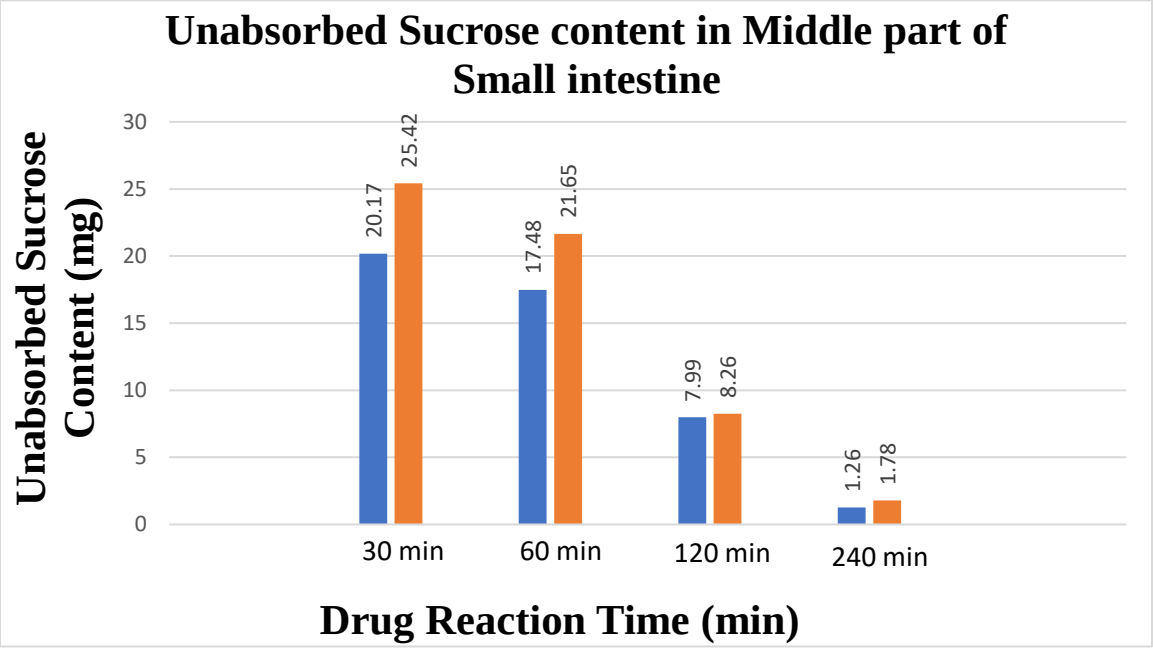


Figure 5.1.3: Anti Hyperglycemic Effect of *Nigella sativa* in middle part of small intestine

* $p < 0.05$, ** $p < 0.01$ *** $p < 0.001$

Table 5.1.4 Unabsorbed Sucrose Content(mg) in Lower 20cm of Small intestine					
Parts	**Group**	**Time**			
		30 min	60 min	120 min	240 min
Lower 20cm	Control	5.57±0.7	3.24±0.73	1.26±0.56	0.98±0.02
	Nigella sativa	9.23±1.03*	5.02±0.67	4.20±0.59	1.49±0.01**

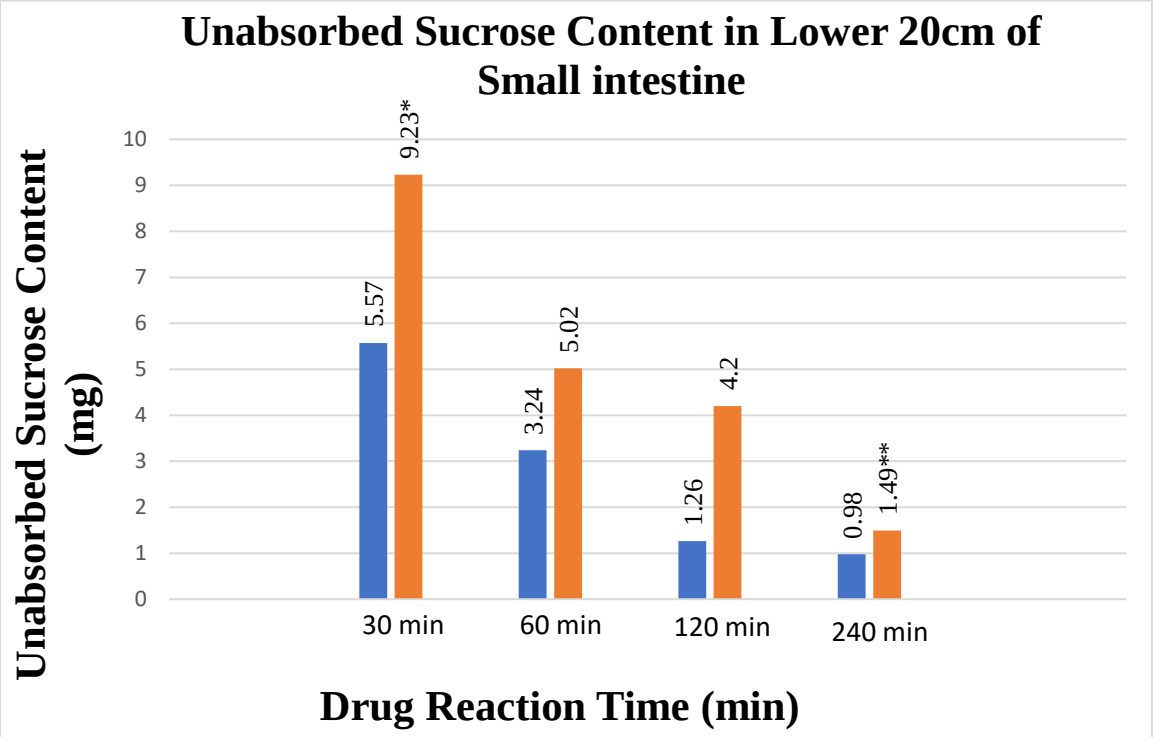


Figure 5.1.4: Anti Hyperglycemic Effect of *Nigella sativa* in lower 20 cm of small intestine

* $p < 0.05$, ** $p < 0.01$ *** $p < 0.001$

Table 5.1.5 Unabsorbed Sucrose Content(mg) in Cecum					
Parts	**Group**	**Time**			
		30 min	60 min	120 min	240 min
Cecum	Control	2.7±0.4	2.01±0.02	1.76±0.04	0.74±0.08
	Nigella sativa	5.74±0.96	3.94±0.06***	3.10±0.11**	1.32±0.06*

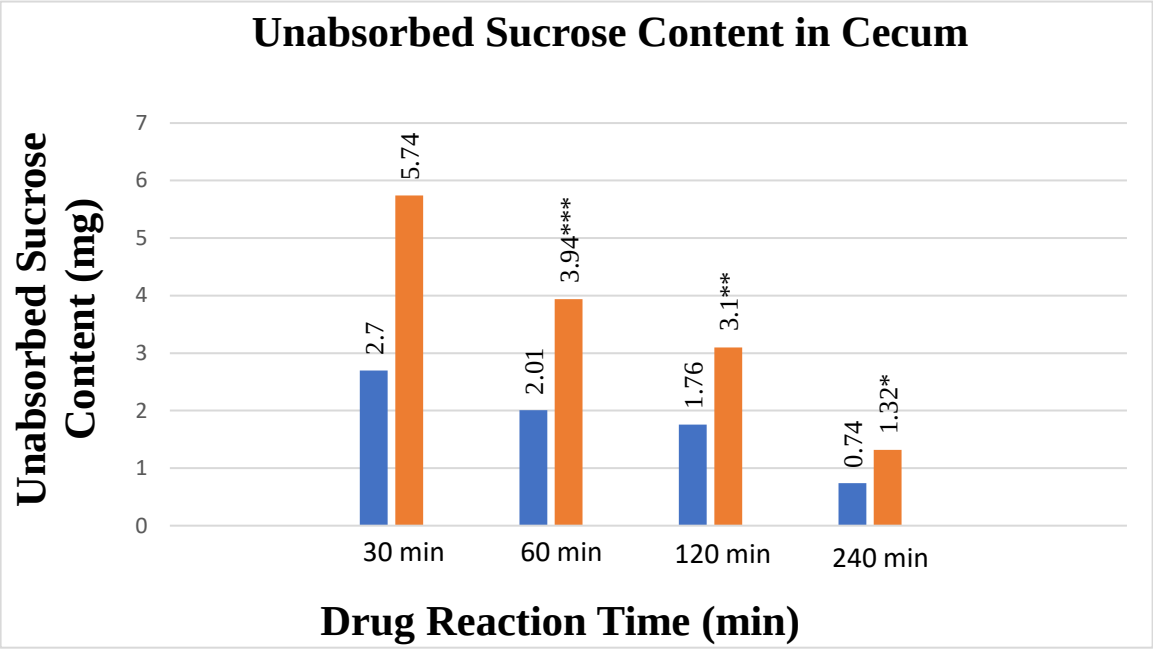


Figure 5.1.5: Anti Hyperglycemic Effect of *Nigella sativa* in cecum

* $p < 0.05$, ** $p < 0.01$ *** $p < 0.001$

Table 5.1.6 Unabsorbed Sucrose Content(mg) in Large intestine					
Parts	**Group**	**Time**			
		30 min	60 min	120 min	240 min
Large Intestine	Control	1.32±0.22	0.94±0.06	0.96±0.15	0.48±0.01
	Nigella sativa	5.19±0.79*	2.46±0.46*	2.33±0.17*	0.86±0.06*

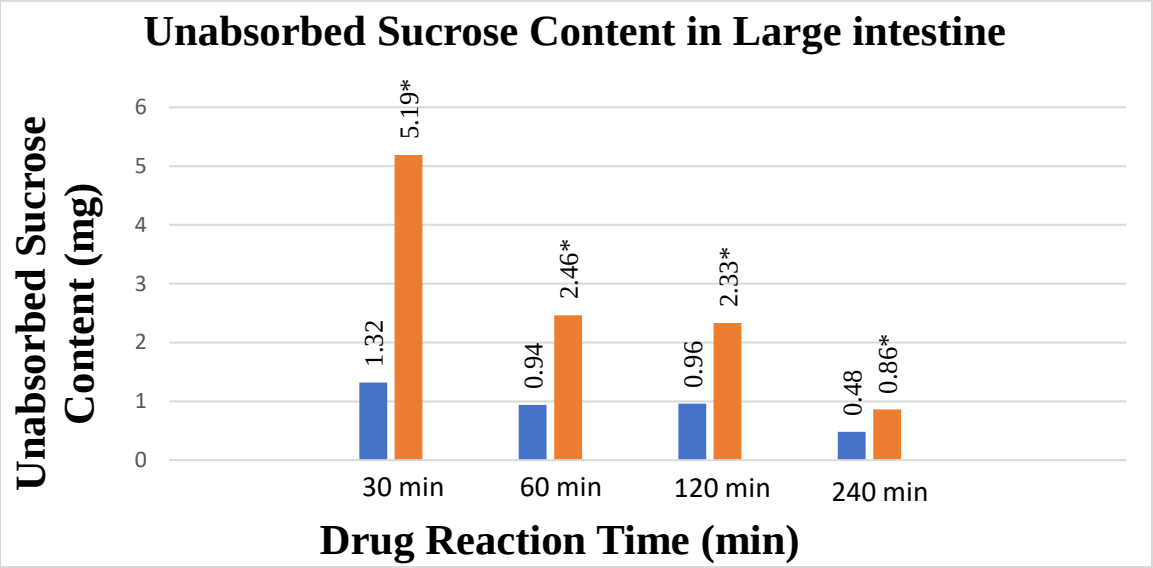


Figure 5.1.6: Anti Hyperglycemic Effect of *Nigella sativa* in large intestine

* $p < 0.05$, ** $p < 0.01$ *** $p < 0.001$

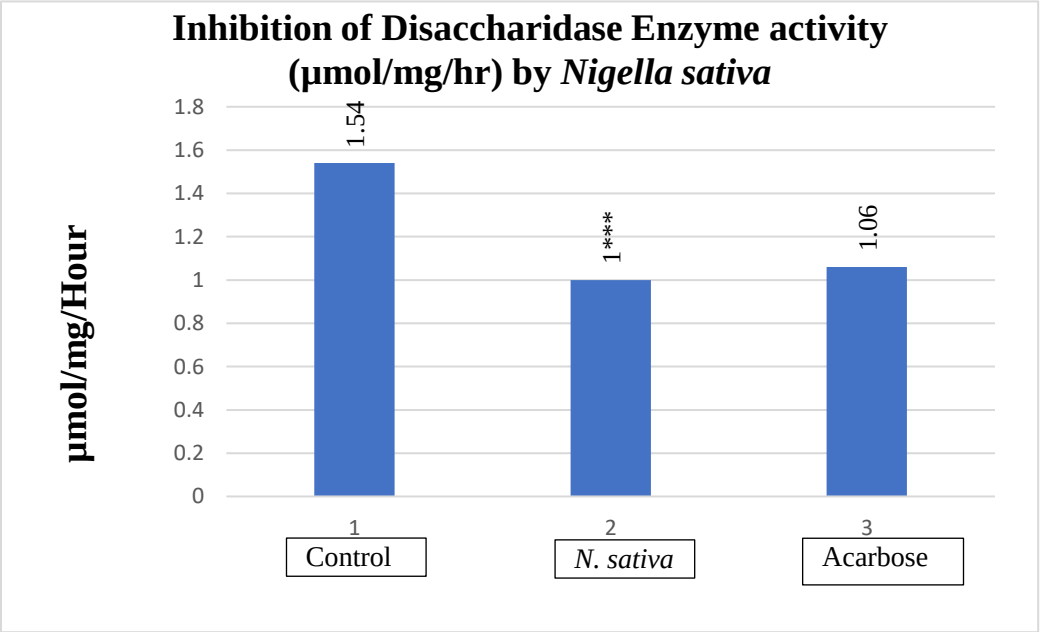
Figure 5.1.1-5.1.6 represents Effects of methanol extract of *Nigella sativa* on gastrointestinal sucrose content after oral sucrose loading in normal rats: Rats were fasted for 20 h before the oral administration of a sucrose solution (2·5 g/kg body weight) with (treated group) or without (control group) methanol extract of *Nigella sativa* (2.5g/kg body weight). Values are means and standard deviations represented by vertical bars. This is derived from repeated-measures ANOVA and adjusted using Bonferroni correction.

5.2 Effect of *Nigella sativa* on Intestinal Disaccharidase Enzyme Activity

Nigella sativa extract showed significant ($p<0.001$) inhibition of disaccharidase enzyme activity.

Table 5.2: Effect of *Nigella sativa* on Intestinal Disaccharidase Enzyme Activity

Group	Disaccharidase activity**(µmol/mg/h)** ± SEM
Control	1.54±o.02
Nigella sativa	1.00±0.02***
Standard (Acarbose)	1.06±0.02



* $p < 0.05$, ** $p < 0.01$ *** $p < 0.001$

Figure 5.2: Effects of methanol extract of *Nigella sativa* on intestinal disaccharidase activity in normal rats.

Rats were fasted for 20 h before the oral administration of methanol extract of *N. sativa* (2.5g/kg body weight) or water (control). Enzyme activity was determined at 60min. Acarbose (200 mg/Kg) was used as reference control for disaccharidase activity test. It significantly decreased ($p<0.001$) disaccharidase enzyme activity (derived from repeated-measures ANOVA and adjusted using Bonferroni correction).

5.3 Effect of *Nigella sativa* on Gastro-intestinal Motility

Methanolic extraxct of *Nigella sativa* shows significant result ($p<0.001$) on GI motility

Table 5.3 Effect of *Nigella sativa* on Gastro-intestinal Motility

Group	%GI Motility ± SEM
Control	49.33±1.95
Nigella sativa	64.82±2.82***
Standard (Bisacodyl)	84.68±0.31

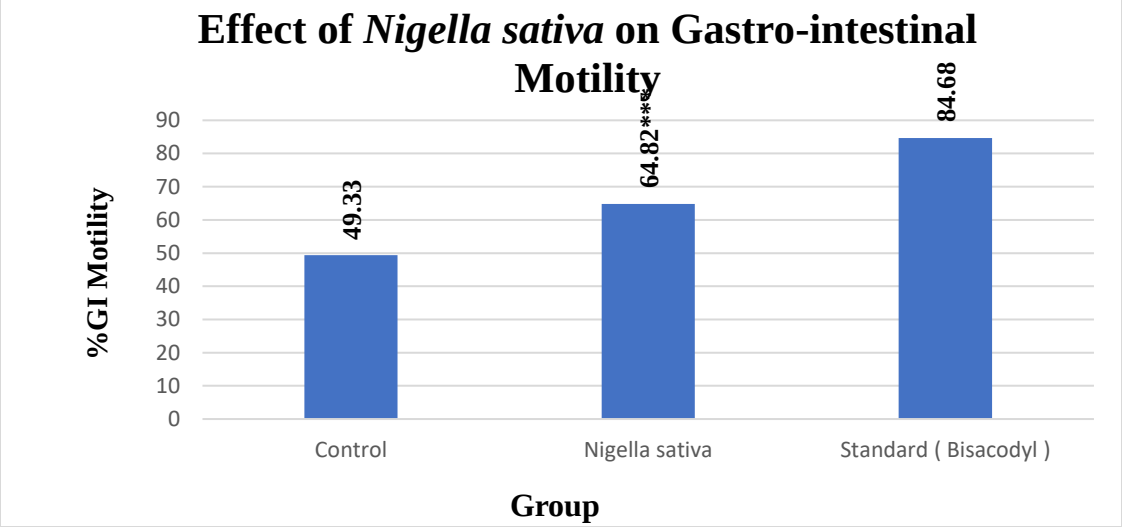


Figure 5.3: Effect of *Nigella sativa* on Gastro-intestinal Motility

Chapter: 06
Discussion & Conclusion

6.1 Discussion

Diabetes and its complications is becoming the third leading cause of death after cancer and cardiovascular diseases. Many serious side effects of insulin therapy and oral hypoglycaemic drugs necessitate the search for newer effective and safer class of compounds to overcome diabetic problems. In recent years, herbal products have started to gain importance as a source of antidiabetic medicines. It has been estimated that more than 1000 plant species are used as folk medicine for treating diabetes though most lack scientific evidence. Our study is directed to evaluate the anti-diabetic property of methanolic extract of stalks of *Nigella sativa* on normal rats. Additionally, unpublished, preliminary screening data, of this plant, showed highly promising hypoglycemic activity. Oral treatment with the defatted methanolic leaf extract showed hypoglycemic activity in normal rats. However, the tissue level mechanism of action of *Nigella sativa* antidiabetic property is yet to be investigated. According to established studies, the initiator of diabetic tissue damage is the hyperglycaemic states. The cells which are damaged by hyperglycemia cannot maintain a constant internal level of glucose which ultimately results in altered cellular mechanism and long-term changes in cellular macromolecular content. Postprandial glucose spike causes perturbation in endothelial cell function, and increased blood coagulation. An increase in the products of glycosylation is another result of hyperglycaemic states, which significantly influences the development of diabetic induced vascular disease. Thus, management of hyperglycaemic states in diabetes patients is the most important method of diabetes control. Commonly used diabetic drugs follow the basic mechanism of enhancing insulin secretion or enhancing sensitivity to insulin, improving peripheral glucose utilization, inhibiting glucose absorption and intestinal disacharidase enzymes. Through our studies on *Nigella sativa*, after using several techniques, we are trying to prove any of the above mentioned mechanism that this plant follows.

Six Segment test showed significantly higher amount of sucrose in stomach, upper, middle and lower intestine in *Nigella sativa* administered groups. The latter three part of GI are most important for absorption of nutrients including sugar. Disaccharides in its own form does not get absorbed due to lack to sucrose carriers, as carriers monosaccharaides only are present in the GI tract. Therefore, it is imperative that disaccharides get converted to monosaccharaides first for absorption. Higher sucrose content in the GI tract clearly reflects a reduced sucrose digestion throughout the GI tract. This in turn, s shown by a significantly higher concentration of sucrose

reaching the large intestine and caecum, which eventually remains unabsorbed and egested with faeces.

In the intestinal disaccharidase activity assay, *Nigella sativa* was shown to have reduced the catabolism of sucrose and starch respectively. Since complex carbohydrates and disaccharides have first to be broken down into simpler monosaccharaides, it follows that any inhibition of this catabolic process would retard sugar absorption, which would in turn, be shown as a lower glycemic peak.

Dietary fibers of plant ingredients or powders can often provide a barrier to diffusion caused due to its high viscosity and ability to bind to glucose. Because, dietary fibers are capable of significantly reducing the transit time in GI Tract of ingested food. Reduced transit time is responsible for lesser time available for di-and polysaccharides in the meal to be digested and absorbed.

Methanolic extract of *Nigella sativa* show significant effect on GI motility

So, our results can be fully attributed to the significant increase amount of unabsorbed sucrose was remained in 6 different parts of intestine and decrease in disaccharide enzyme activity which validates anti-hyperglycemic activity of *Nigella sativa.*

Further research is underway, in our labs, for identifying the active molecules responsible for inhibiting α-amylase and disaccharidase enzyme activity. We also intend to study if there is any significant lipid lowering or obesity controlling ability of *Nigella sativa* in diabetic models.

6.2 Conclusions

Nigella sativa plant is rich in phytochemicals and has been in use since ancient times to treat a wide range of diseases in traditional system medicine. The present study would be helpful to create awareness among people for taking control measures based on, herbal plants against infectious diseases. Further more detail clinical researches are needed to explore its medicinal value in order to establish it as a standard drug. Our studies confirm the previous findings showing anti-hyperglycemic action of *Nigella sativa.* Additionally, we have elucidated that *Nigella sativa* has significant capabilities of inhibiting absorption of glucose by inhibition of intestinal disaccharidase enzyme. Therefore, its traditional use, as mentioned above is justified and calls for further research, to optimize its anti-diabetic activity.

Chapter: 07

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