Effect Of Nigella Sativa Seed Extract On Glucose, Lipid Profile, Liver Function Tests, Oxidative Stress and Histological Changes In Pancreas, Kidney, Liver and Tibial Nerve In Normal and Streptozotocin Induced Diabetic Rats

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By

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CERTIFICATE

This is to certify that this thesis entitled *"Effect Of Nigella Sativa Seed Extract on Glucose, Lipid Profile, Liver Function Tests, Oxidative Stress and Histological Changes in Pancreas, Kidney, Liver and Tibial Nerve in Normal and Streptozotocin Induced Diabetic Rats"* is a bonafide work of Mr. Shaik Hussain Saheb and was carried out under our supervision and guidance in the Department of Anatomy, Shri B. M. Patil Medical College, Hospital & Research Centre, Vijayapura, Karnataka, India.

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To

Lord Almighty

My Teachers
&
My Family Members

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SYMBOLS	ABBREVIATIONS & NOMENCLATURE
A	Absorbance
ADP	Adenosine Diphosphate
ALT	Alanine Transaminase
ANOVA	one-way analysis of variance
AST	Aspartate Transaminase
ATP	Adenosine tri phosphate
B	Blank
BW	Body weight
CE	Cholesterol ester
CHE	Cholesterol Esterase
CCL4	Carbon Tetrachloride
CHOD	Cholesterol dehydrogenase
CLIA	Chemiluminescence immunoassay
CPCSEA	Committee For the Purpose of Control and Supervision of Experiments on Animals
CVD	Cardiovascular diseases
DPA	Dihydroxyacetone phosphate
DC	Diabetic Control rats
D-GAL N	D-Glalactosamine
DHB	Dichloro hydroxyl benzene sulfonic acid
Dl	Deci liter
DM	Diabetes Mellitus
DNA	Deoxy ribo nucleic acid
DNS	Diabetic rats treated with Nigella sativa seed powder
DPX	Distyrene plasticizer and xylene
DTQ	Diabetic rats treated with thymoquinone
DW	Distil Water
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme Linked Immuno Absorbent Assay
FeCl3	Ferric Chloride
GAA	Glacial Acetic Acid
GK	Glycerol Kinase
GLUT2	Glucose transporter 2
GSH	Glutathione
H_2O_2	Hydrogen peroxide
H_2So_4	Sulphuric acid
HbA1c	Glycated Hemoglobin
HCL	Hydrochloric acid
HDL	High Density Lipoproteins
IAEC	Institutional Animal Ethics Committee
IU	International Unit
LDH	Lactate dehydrogenase
LDL	Low Density Lipoproteins
LPO	Lipid Peroxidation
LPS	Lipo polysaccaride
LFT	Liver function tests

SYMBOLS	ABBREVIATIONS & NOMENCLATURE
MDA	Malondialdehyde
MDH	Malate dehydrogenase
mg	Milli Gram
mg2	Magnesium
Min	Minute
mL	Milli Liter
NAD	Nicotinamide Adenine Dinucleotide
NADH	Nicotinamide Adenine Dinucleotide Hydrogenase
NaNO3	Sodium Nitrate
NC	Normal control rats
nm	Nano meter
NO	Nitric oxide
NNS	Normal rats treated with Nigella sativa seed powder
NS	Nigella Sativa
NTQ	Normal rats treated with thymoquinone
OD	Optical density
OGTT	Oral glucose tolerance test
OSI	Organosomatic Index
PH	Potential of hydrogen
POD	Peroxidage
RNS	Reactive Nitrogen Species
RLU	relative light units
ROS	Reactive Oxygen Species
S	Standard
Sec	Second
SGOT	Serum Glutamate Oxaloacetate Transaminase
SGPT	Serum Glutamate Pyruate Transaminase
SOD	Super oxide dismutase
SOP	Standard Operating Procedure
Std.	Standard
STZ	Steptozotocin
T	Test
TBA	Thio Barbituric Acid
TCA	Tri Chloro Acitic Acid
TG	Triglycerides
TQ	Thymoquinone
THQ	Thymohydroquinone
U	Unit
USD	United State Dollars
Vit-C	Vitamin-C
Vit-E	Vitamin-E
VLDL	Very Low Density Lipoproteins
B-cells	Beta cells of Islet of Langerhans
µL	Micro Liter
4AAP	4-Aminoantipyrine

ABSTRACT

ABSTRACT

Introduction: Diabetes is a group of metabolic diseases in which there are high sugar levels over a prolonged period. Symptoms of high glucose levels lead to increased thirst, hunger and frequent urination. If left untreated, diabetes can cause many complications. Acute complications include diabetic ketoacidosis and nonketotic hyperosmolar coma. Serious long-term complications include stroke, kidney failure, cardiovascular disease, diabetic foot and diabetic retinopathy. Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced. Diabetes can be treated by medically, diet balance and physical activities. However plant-derived and herbal remedies continue to be popular alternative for diabetes treatment. Nigella sativa is an extensively used herb in Arab medicine and Indian system of medicine Ayurveda. The present study was conducted to assess the effect of Nigella sativa seed and thymoquinone (major bio-active component of Nigella sativa seed) in the treatment of streptozotocin induced diabetic albino Wister rats.

Material and Methods: Laboratory bred adult albino Wister rats weighing between 175 - 250gm were used in the study. The acclimatized animals were divided into six groups of six rats each. Group I rats were normal control rats; group II were normal rats treated with Nigella sativa seed powder (300mg/kg body weight); group III were normal rats treated with thymoquionone(4mg/kg body weight); group IV were streptozotocin induced diabetic control rats; group V were streptozotocin induced diabetic rats treated with Nigella sativa seed powder(300mg/kg body weight) and group VI were

streptozotocine induced diabetic rats treated with thymoquinone(4mg/kg body weight). The duration of study was 45 days. At the end of 45 days blood was collected for biochemical tests such as glucose, insulin, MDA, SOD, Vitamin C & E, lipid profile and liver function tests. Oral glucose tolerance test (OGTT) was performed at the end of 45 days of study in all the groups. For the histopathological observations pancreas, liver, kidney and nerve tissues were collected and processed. IAEC was taken and CPESEA guide lines were followed.

Results: The diabetic untreated rats showed significant increase in serum glucose, total cholesterol, LDL – cholesterol, VLDL – cholesterol, triglycerides, AST, ALT, ASP and MDA compared with normal control rats. After treatment with Nigella sativa seed powder and thymoquinone in induced diabetic rat groups the above mentioned parameters were lowered significantly. Insulin, SOD, Vitamin C, Vitamin E and HDL – cholesterol were decreased significantly in diabetic untreated group. After treatment with Nigella sativa seed powder and thymoquinone the same parameters were increased significantly in induced diabetic rat groups. The diabetic rat groups treated with Nigella sativa seed powder and thymoquinone showed tolerance to oral glucose tolerance test (OGTT) compared with diabetic control rats. There was no significant change of any parameter between normal control rat groups and normal rat groups treated with Nigella sativa seed powder and thymoquinone. Histopathological observations of pancreas, liver and kidney of induced diabetic treated rat groups revealed that treatment with Nigella sativa seed powder and thymoquinone reversed the histopathological changes seen in induced diabetic control rats. There was no significant histopathological changes

observed between normal control rats and normal rat group treated with Nigella sativa seed powder and thymoquinone. This indicates nontoxicity of Nigella sativa seed and thymoquinone.

Conclusion: The biochemical parameters in induced diabetic rats were normalised with treatment of Nigella sativa seed powder and its major bioactive component thymoquinone. There was no toxic effect observed in normal groups treated with Nigella sativa seed powder and thymoquinone. This observation was supported by non-significant changes in biochemical parameters between normal control rats and normal rat groups treated with Nigella sativa seed and thymoquinone and furthermore supported by histological observations. The biochemical results and histopathological observations clearly showed beneficial effect of Nigella sativa seed powder and thymoquinone in diabetic treated groups. Hence these phytochemical substances may be considered as antidiabetic agents as well as beneficial to the overall diabetic health.

Key words: Nigella sativa seed, Thymoquinone, Diabetes mellitus, Streptozotocin, Hyperglycaemia, Antioxidants, Dyslipidaemia, Liver enzymes.

CHAPTER 1

INTRODUCTION

1.1.Diabetes mellitus

Diabetes mellitus (DM) is usually known to as diabetes and it is a group of metabolic disorders in which mainly there are high glucose levels over a long period. Symptoms of high glucose levels lead to increased thirst and hunger and frequent urination. If left untreated, diabetes can cause many complications. Acute complications include diabetic ketoacidosis and nonketotic hyperosmolar coma. Serious long-term complications includes stroke, kidney failure, cardiovascular disease, diabetic foot and diabetic retinopathy. The reason for diabetes is either the pancreas is not releasing sufficient insulin or the cells of the body are not responding properly to the insulin[1, 2].

In diabetes mellitus there are three important types:

- Type 1 DM due to pancreas failing to produce sufficient insulin. Earlier it was called as ``insulin-dependent diabetes mellitus``.
- Type 2 DM in which the cells of the body fail to respond to insulin properly due to insulin resistance. As the disease progresses, a lack of insulin may also develop. Earlier it was called as "non-insulin dependent diabetes mellitus". The primary cause is excessive body weight and not enough exercise[1].
- Gestational diabetes which occurs in pregnant women. In this type there need not any previous history of diabetes but a high glucose level is developed in pregnancy period[3].

Prevention and treatment of diabetes involve a healthy diet, physical exercise, weight management and avoiding smoking. The diabetic persons should control

the blood pressure and proper care of their foot is very important. Type 1 DM may be managed with insulin injections[1]. Type 2 DM may be treated with proper medications, with or without insulin and proper physical workouts[3]. Gestational diabetes usually resolves after the birth of the baby[4]. In 2014, an estimated 387 million people had diabetes throughout world[5], out of which Type2 DM were about 90%. This represent 8.3% of the adult population, with equal rates in both women and men[6]. In between 2012 and 2014, diabetes resulted in an estimated 1.5 to 4.9 million deaths each year[5]. Diabetes at least doubles a person's risk of death[1]. The number of people with diabetes is expected to increase to 592 million by 2035[5]. The economic cost of diabetes worldwide in 2014 was estimated around 612 billion USD[7].

1.2. Risk Factors for Type 2 Diabetes[8, 9]

People who develop type 2 diabetes are more likely to have the following characteristics:

- Age 45 years or more
- Overweight or obese
- Physically inactive
- Parent or sibling with diabetes
- History of giving birth to a baby weighing more than 9 pounds
- History of gestational diabetes
- High blood pressure—140/90 or above—or being treated for high blood pressure

- High-density lipoprotein (HDL)cholesterol below 35 milligrams per deciliter (mg/dL) or a triglyceride level above 250 mg/dl
- Polycystic ovary syndrome also called PCOS
- Prediabetes— HbA1C level of 5.7 to 6.4 %; fasting plasma glucose level of 100–125 mg/dl, called impaired fasting glucose; or 2-hour oral glucose tolerance level of 140–199, called impaired glucose tolerance
- History of cardiovascular disease.

1.3. Effect of diabetes on various biochemical parameters

Homeostasis of blood glucose levels

Glucose is the main source of energy for the cells in our body, but it is too big to simply diffuse into the cells by itself. Instead, it needs to be transported into the cells. Pancreas is producing the hormone Insulin which facilitates glucose transport into the cells. Insulin helps in glucose transport into the cells from the bloodstream, thus lowering blood sugar level. In fact, insulin actually stimulates glycogen formation from glucose. All these functions of insulin help to lower sugar level in the blood. In general the glucose level in the blood is estimated in terms of milligrams per decilitre (mg/dl), with the normal range of 70 to 110 mg/dl. In general, if glucose level is out of this range, the amount of insulin and glucagon released by the pancreas will be used to bring sugar levels back within the normal range. In normal individuals that insulin and glucagon signalling are having not all-or-nothing responses. When the system is functioning

properly, there is always some insulin and some glucagon being produced by the pancreas that is acting to bring a balance between glucose release into the blood and glucose uptake into cells[10, 11].

Many studies have proved that oxidative stress is to participate in the progression of diabetes which plays an important role during diabetes, including impairment of insulin action and elevation of complication incidence. Antioxidants have very good roll in the treatment of diabetes both Type 1 and Type 2. Increase in the levels of oxygen and nitrogen free radicals (ROS/RNS) has been linked with non-enzymatic glycation of proteins, oxidation of glucose and lipid peroxidation which lead to diabetes mellitus and its complications. Most of the studies have shown the oxidative stress effect in diabetes and with the complications related to eye, heart, kidney and liver. Thus, oxidative stress is a more worrying factor in metabolic disorders specially diabetes Type 2 [12, 13, 14].

In diabetic conditions oxidative stress is produced and is likely involved in progression of pancreatic beta-cell dysfunction. This may be due to low levels of antioxidant enzymes, pancreatic beta-cells being very vulnerable to oxidative stress. Pancreatic beta-cell dysfunction leads to lower levels of insulin, resulting in hyperglycaemia and the cells show intolerance to glucose. Higher levels of glucose lead to increased percentage of HbA1c[15].

Lipids

Diabetes mellitus produces disturbed changes in the lipid profile, which leads to the cells more susceptible to lipid peroxidation[16]. Experimental studies show that the

presence of polyunsaturated fatty acids in cell membrane leads to attack by free radicals due to the presence of multiple bonds[17]. Lipid hyperperoxides (LHP) through intermediate radical reactions produce such fatty acids that generate highly reactive and toxic lipid radicals that form new lipid hyperperoxides[18]. A critical biomarker of oxidative stress is lipid peroxidation which is the most explored area of research when it comes to ROS[19]. Malondialdehyde (MDA) is formed as a result of lipid peroxidation that can be used to measure lipid peroxides after reacting it with thiobarbituric acid[20]. These conditions lead to increased levels of MDA, total cholesterol, triglycerides, LDL-C, VLDL-C, ratio between total cholesterol and HDL-C, ratio between total HDL-C and LDL –C and decreased levels of HDL-C.

Vitamins

Vitamins are very important because they play important role in different biochemical processes. Vitamin A, C and E act as antioxidants by detoxifying the free radicals. The changes in vitamin levels are significant biomarkers of oxidative stress. The more utilization of these vitamin levels leads to decreased levels of same vitamins[18].

Superoxide dismutase (SOD)

Superoxide dismutase provides first line defence against ROS mediated cell injury by catalysing the proportion of superoxide, the primary ROS in oxygen metabolism, to molecular oxygen and peroxide. This condition leads to lower levels of SOD in diabetes[21].

Liver function tests

Theories behind liver function test parameters elevation in diabetes state that the liver helps to maintain normal blood glucose concentration in the fasting and postprandial states. Loss of insulin effect on the liver leads to glycogenolysis and increase in production of hepatic glucose. Abnormalities of triglyceride storage and lipolysis in insulin-sensitive tissues such as the liver are an early manifestation of conditions characterized by insulin resistance and it also can be detectable earlier than fasting hyperglycaemia, the precise genetic, environmental and metabolic factors and sequence of events that lead to the underlying insulin resistance[22]. The excess in free fatty acids found in the insulin-resistant state is known to be toxic to hepatocytes. Putative mechanisms include high concentration of cell membrane disruption, toxin formation, mitochondrial dysfunction and inhibition and activation of key steps in the regulation of metabolism[23]. Other potential explanations for elevated transaminases in insulin-resistant states include peroxisomal beta-oxidation, recruited inflammatory cells and oxidant stress from reactive lipid peroxidation.

1.4. Diabetes – Herbal care

There are two important principles in the management of Type 2 diabetes: one is to increase the production and effectiveness of insulin as a part of therapeutic management and the other is to decrease the glucose load to the body as part of dietary management. The drugs are of two types: some drugs are managing the diabetes by increasing the insulin production from pancreatic β- cells (Sulphnylurea) and some drugs are working

like insulin (Biguanides). These drugs play very important role in treatment of diabetes, but due to failures in achieving ideal result and increasing side effects, there has always been a need and desire for a natural, more effective and economically feasible alternative with fewer side effects. Herbs are the main say in the alternative treatments, as there are very satisfying for the patients to get their aliments treated with traditional recipes. These are safe and easy to be administrated, provided these are properly recognized and their pharmacological properties established.

1.5. NIGELLA SATIVA

Scientific classification [24, 25 ,26 ,27]

Kingdom	: Plantae
Division	: Angiosperms
Class	: Eudicots
Order	: Ranuculales
Family	: Ranuculaceae
Genus	: Nigella
Species	: Nigella Sativa

Fig 1: Nigella Sativa Plant, Flower and Seeds

Common Names

English : Black-caraway, Black-cumin, Fennel-flower, Roman-coriander.

Hindi : Kalonji, Kalajira,

Sanskrit : Mugrela, Upakuncika, Kalajaji

Kannada : Kari jirige

Bengali : Kalo jira

Among the traditional remedies, prescriptions of Nigella sativa for the treatment of various diseases are well-known since prehistoric era and especially its seeds for over 4000 years all over the world[28]. It is commonly known as black seed or black cumin. Pre-Islamic Arabian world knew the seed well, as the Egyptians used to keep the black seeds with mummies in their tombs, thinking that it would help them in the life thereafter. This indicates that Nigella sativa was used by Egyptians in different walks of their lives. The Holy Prophet Muhammad (PBUH) appreciated the use of black seed as a remedy for every illness except the death[29]. The plant of Nigella sativa is half a meter tall, having blue flowers and triangular black seeds with pungent smell[28]. Seeds contain a considerable amount of fixed and volatile oils[30], proteins, alkaloids and saponins[31].

Modern trials have proved that its seeds alone or in combination with other drugs are highly effective in diabetes mellitus[32], vitiligo, skin diseases[33], inflammation[34] and improve lipid profile through increasing HDL cholesterol and decreasing LDL cholesterol and triglycerides[35]. Therapeutic effects of Nigella sativa are thought to be due to nigellone and thymoquinone contents. The reported pharmacological properties include protection against disease and chemical induced renal and hepatic toxicity, anti-inflammatory, analgesic, antipyretic, anti-microbial and antineoplastic activities. The oil decreases blood pressure, cholesterol, triglycerides and glucose and increases respiration, haemoglobin and packed cell volume. In diabetics, it has insulinotropic action[36] and decreases hepatic gluconeogenesis[37], thus lowering blood glucose[38]. Increased intensity of staining for insulin and preservation of beta-cell numbers are the evidences to this effect[39,40]. Nigella sativa has been tried as anti-diabetic mostly in rats and other Type 2 like animal models with considerable efficacy[37].

Many previous studies proved that Nigella sativa seed is indeed a true panacea, having the capacity to treat everything from small allergies to hypertension and diabetes. A study is relating Nigella sativa to multi-drug resistant bacteria. This is very important because these multi-drug resistant bacteria are becoming more dangerous to public health[41]. According to a study, the important phytochemicals present in Nigella sativa seed are thymoquinone (TQ), thymohydroquinone (THQ) and thymol. This study proved the antifungal nature of Nigella sativa components, thymol, TQ and THQ against 30 human pathogens[42].

Thymoquinone is one of most important components which have been studied since five decades. Many previous studies proved thymoquinone having anti-oxidant, anti-inflammatory and anticancer properties and used in encephalomyelitis, diabetes, asthma and carcinogenesis[43]. Thymoquinone is acting as an effective superoxide radical scavenger. In addition it also helps to preserve antioxidant enzymes glutathione peroxidase and glutathione-S-transferase. These are major detoxifiers and play important role in cellular antioxidant defence systems because of their hepatoprotective nature from toxins[43,44].

Thymohydroquinone is important natural acetylcholinesterase(AChE) inhibitors. These inhibitors are chemicals that stop enzyme activity and these play very important role in treatment of Alzheimer's disease, autism, glaucoma, neurodegenerative conditions, schizophrenia, and Parkinson's disease[45,46].

Thymol is one of the active ingredients of Nigella sativa seed which acts as natural monoterpene that has a number of useful qualities[47,48]. It is used as a tuberculocide and medical and general purpose disinfectant. A study of antitumor activity of thymoquinone and thymohydroquinone proved that the two phytochemicals in Nigella sativa seed can reduce the tumour cells up to 52%[49].

A study demonstrated the hepatoprotective nature of Nigella sativa seed and thymoquinone[50]. Another research study stated that Nigella sativa and thymoquinone can cause partial regeneration of pancreatic beta-cells and these can increase the lowered

serum insulin levels and decrease the elevated sugar levels[51]. The same study stated that Nigella sativa seed can act like as metformin, which can increase glucose tolerance.

Hence the present study was an attempt to investigate the effects of Nigella sativa seed powder and its major bioactive component, thymoquinone on various biochemical parameters and histopathological changes in metabolic organs in normal and streptozotocine induced diabetic rats.

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CHAPTER 2

REVIEW OF LITERATURE

2.1 Diabetes Mellitus

2.1.1. Historical Background

The history on the discovery of Diabetes Mellitus is fascinating. Papyrus ebers of ancient Egypt (1500 BC) mentioned a number of remedies for combating the passing of too much urine (polyuria). In Ayurveda it recorded that insects and flies were attracted to the urine of some people that the urine tasted sweet and this was associated with certain diseases. Susruta (1000 BC) diagnosed Diabetes Mellitus. Greeks like Aristaeus, Celsius and Galen described the pathological condition of diabetes. Aristaeus was the first person to differentiate between diabetes insipidus and diabetes mellitus. Paul of Aegina refined further the diagnosis of "dypsacus" (diabetes), considered the dehydration in diabetes due to weakness of the kidneys and excess moisture from the body. Apollonius of Memphis probably coined the name "diabetes" sometime around 250 BC. The literature of those days reflects the early understanding of a disease that drained patients of more fluid than they consume. In the first century AD, the Greeks described diabetes as a melting down of the flesh and limbs into urine. Mellitus means in Latin honey and gradually the word "mellitus" word was appended to diabetes because of its link with sweet urine. It was not until 1798 that John Rollo actually documented excess sugar in the blood as well as the urine [1, 2, 3].

2.1.2. Types of Diabetes Mellitus

Diabetes Mellitus (DM) is a metabolic disorder. The characteristic features are the presence of chronic hyperglycemia accompanied by greater or lesser impairment in the metabolism of carbohydrates, lipids and proteins. There are three

types of diabetes, Type 1 diabetes, Type 2 diabetes and gestational diabetes. All these three types of diabetes mellitus have some common features. Normally, our body breaks down the sugars and carbohydrates we eat into a special sugar called glucose. Glucose fuels the cells in our body. The cells need insulin, a hormone, in our bloodstream in order to take in the glucose and use it for energy. In diabetes mellitus, either the pancreas does not secret enough insulin or the body cannot use the insulin it produces or a combination of both. Since the cells cannot take in the glucose, it builds up in blood. High levels of blood glucose can damage the tiny blood vessels in kidneys, heart, eyes or nervous system. That is why diabetes if left untreated can eventually cause heart disease, stroke, kidney disease, blindness and damage to nerves in the feet.

Type 1 Diabetes: It is a more severe form and is insulin-dependent. Previously it was also called as juvenile diabetes, because Type 1 diabetes usually develops in children and teenagers but it can develop at any age. In Type 1 diabetes, pancreas does not produce insulin. This type is an autoimmune condition and it is due to body attacking its own pancreas with antibodies. In Type 1 diabetic patients, the pancreas is damaged and it cannot produce insulin. This type of diabetes may be caused by a genetic disorder. Type1 diabetes can affect major organs in body including heart, blood vessels, nerves, eyes and kidneys. Keeping blood glucose level in control can dramatically reduce the risk of many complications. When this type of diabetes is untreated it can develop long-term complications gradually. Management of healthy blood glucose can help lower the risk of complications. The major long term complications of type 1 diabetes are atherosclerosis, neuropathy, nephropathy,

diabetic retinopathy and diabetic foot damage. Treatment for type 1 diabetes involves subcutaneous injection of insulin[4, 5].

Type 2 Diabetes: Type 2 diabetes is a progressive condition and it is most common, accounting for 95% of diabetic cases in adults. Type 2 diabetes was called adult-onset diabetes but with the epidemic of obese and overweight kids, more teenagers are now developing Type 2 diabetes. Type 2 diabetes was also called non-insulin dependent diabetes. Often it is considered that Type 1 diabetes is more dangerous than Type 2 diabetes. Nevertheless, Type 2 diabetes can still 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 the risk of heart disease and stroke. In Type 2 diabetes, the pancreas usually produces small amount of 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 are at high risk of developing type 2 diabetes, particularly the overweight or obese people who are having more than 20% over than their ideal body weight. The overweight people have insulin resistance. When With insulin resistance, the pancreas has to work more hard to produce more insulin, but still there is not enough insulin to keep sugars normal. There is no cure for diabetes. Type 2 diabetes can be controlled by increasing physical activity, nutrition and weight management. Type 2 diabetes tends to progress and medications are often needed. The effect of diabetes 2 on body, glucose builds up in the blood instead of going into cells, the cells are not able to function properly. Associated problems along with hyperglycaemia includes, dehydration -

the increased glucose in the blood leads to excess glucose in the urine because the kidneys can't deal with the high sugar levels. The increase glucose in urine draws frequent urination and it is causing dehydration. Diabetic coma – the dehydration in type 2 diabetes causes heavy fluid losses, they may develop this life-threatening complication. Damage to the body tissues –the treated or long time expose of higher blood glucose levels may damage the nerves and capillaries cause stroke, cardiac and renal diseases. The Symptoms of diabetes type 2 includes, increased hunger, increased thirst, dry oral cavity, frequent urination, unusual weight loss, weakness, blurred vision, recurrent infections[4, 5].

Gestational Diabetes

Diabetes that is triggered by pregnancies is called gestational diabetes. Gestational diabetes is usually diagnosed during late pregnancy. Because high blood sugar in the mother is circulated through the placenta to the baby, gestational diabetes must be controlled to protect the baby`s growth and development. If the mother is diagnosed with diabetes earlier in her pregnancy, she may have had diabetes before she became pregnant. Treating gestational diabetes can help both mother and baby stay healthy. The hyperglycaemic condition of mother`s blood circulated through the placenta to the baby can affect the health of baby and growing tissues, risks to the unborn baby are even greater than risks to the mother. The risks in gestational diabetes to baby include abnormal weight before birth, respiratory problems at birth and higher obesity and diabetes risk later in life. In gestational diabetes mother can face risks like damage to heart, kidney, nerves, eye and overweight baby may lead to cesarean section[4, 5].

Other Forms of Diabetes

A few rare kinds of diabetes can result from specific conditions. For example, diseases of the pancreas, certain surgeries and medications or infections can cause diabetes. These types of diabetes account for only 1% to 5% of all cases of diabetes[6].

2.1.3. Mechanism of diabetes mellitus and its complications

Carbohydrates, the major component of our food are broken down into simpler sugars like glucose and then absorbed into the body. Glucose is a source of energy, is transported into the blood and it has to enter into the cell for metabolism. Insulin facilitates the entry of glucose into the cell, where it burns through Krebs cycle in the mitochondria under the influence of multiple enzymes and energy is released in the form of adenosine triphosphate (ATP). Body tissues like muscles get energy from ATP to execute mechanical functions. In the absence of or ineffective insulin or a disturbance at the insulin receptor level or its destruction by the antibodies, the body is deprived of energy as it cannot use the ideal source of energy, the glucose. When insulin is absent from the body it is Type 1 diabetes mellitus and when it is scanty or ineffective it is Type 2 diabetes mellitus. The result is underutilization of glucose, which leads to its accumulation in the blood leading to hyperglycemia, a hallmark of diabetes mellitus. The body constantly requires energy to perform different functions like walking, working as well as sleeping and sitting. Even in sleep the organs are constantly at work like digesting food and breathing and all these functions need energy. In diabetes mellitus being unable to use glucose, the body resorts to other sources of energy like lipids, fats and proteins. When lipids and

fats are used in more quantity, their breakdown products are produced in abundance like cholesterol and triglycerides. The excess of cholesterol accumulates in blood. A close relationship exists between levels of blood cholesterol or other lipids and the development of atherosclerosis. In this disorder, plaques containing cholesterol are deposited on the walls of arteries, it happens usually in small and medium sized vessels and it leads to reduce in the diameter of lumen and the flow of blood. Clotting of blood, as in the coronary arteries which causes heart attack, is most likely to develop at places where arterial walls are roughened by such plaques. This blockage of the blood vessels is the main cause of the complications of diabetes mellitus. The chronic condition requires careful management to avoid multi-organ damage and derangements of lipid metabolism, thus preventing its complications including cardiovascular diseases, nephropathy, blindness and nerve damage[7, 8].

2.1.4 Management of Diabetes Mellitus

Type 1 diabetes mellitus can be treated by insulin therapy only.

In Type 2 diabetes efforts are made to convert the supply of insulin or reduce the glucose load to the body, an attempt to decrease the insulin. Thus the following principles of management of diabetes can be proposed and practiced.

- Providing alien insulin to the body through injections like Humulin or NPH insulin etc.
- Increasing the production of effective insulin within the body, which can be achieved through.
    - Drugs that stimulate the production of insulin from the β-cells of islets of Langerhans in pancreas like Sulphonylureas. These act mainly by

augmenting insulin secretion and consequently are effective only when some residual pancreatic beta-cell activity is present. During long term administration they also have an extra pancreatic action causing hypoglycaemia. But this is uncommon and usually indicates excessive dosage. The hypoglycaemia may persist for many hours and must always be treated in hospital.

  - Use of herbs which increases the production of insulin by maintaining the health and integrity of insulin producing cells and modulating the immune function.

- Use of drugs that increase the glucose utilization like Biguanides- metformin which works effectively by decreasing gluconeogenesis and by increasing peripheral utilization of glucose. Since it acts only in the presence of endogenous insulin it is effective only when there are few active functioning beta cells present in pancreas.
- Decreasing the production of glucose from non-carbohydrate sources like amino acids and fat, gluconeogenesis especially in the liver.
- Decreasing the glucose load to the body by
  - Restricting the carbohydrates in the diet.
  - Decreasing the absorption of glucose from the gut by Acarbose an inhibitor of intestinal alpha glucosidases which delays the digestion and absorption of starch and sucrose[9].

2.2. Nigella sativa

2.2.1. Historical Background

Nigella sativa was first discovered in Tutankhamen's tomb. It played an important role in ancient Egyptian practices. Although its exact role in Egyptian culture is not known, items entombed with a king were carefully selected to help him in the after life[10, 11]. The earliest reference to Nigella sativa seed is found in the book of Isaiah in the bible. The black cumin is not threshed with a threshing sledge, nor a cart wheel rolled over the cumin. It is beaten out with a rod[12]. The Hebrew word for Nigella sativa seed, "ketsah," refers to Nigella sativa, belongs to the order Ranunculaceae which cultivates in Egypt and Syria for its seed, this was clarified in Easton's Bible Dictionary[13]. Dioscoredes, the Greek physicist at unity century, reported that black seed is used to treat headaches, nasal congestion, toothache and intestinal worms. In addition it is also used to promote menstruation and increase milk production in nursing mothers[14]. A-Biruni who composed a treatise on the early origin of Indian and Chinese drugs, mentions that the black seed is a kind of grain called alwanak in the Sigzi dialect[15]. Later, this statement was justified by Suhar Bakht who explained that the black seed as habb-i-Sajzi (viz. Grains Sigzi) allows people to use it as a nutritional ingredient in the 10^{th} and11^{th} century. In a medical system in Greco-Arab/Unani-Tibb, which originated from Hippocrates, Galen and Ibn Sina, black seed is a valuable remedy in treating gastrointestinal dysfunction and hepatitis and is described as a stimulant to different conditions and high fever reliever[16]. Ibn Sina, in his book "The Canon of Medicine" described the black seed as "stimulating the body's energy and helping recovery from fatigue or lack of spirit"[17]. Black seed is also included in the list of natural drugs of Al-Tibb al-Nabawi

and the traditional saying is "Hold on to the use of the black seed for it has a remedy for every illness except death." This prophetic reference in describing black seed as "having a remedy for all illnesses" may not be so exaggerated as it at first appears. Many research studies conducted recently has provided evidence which indicates that Nigella sativa seed having an ability to improve and boost human immune system significantly if used over a time of period. The prophetic phrase, "hold on to the use of the seed" also emphasizes consistent usage of the seed[18].

In the Middle and Far East countries for centuries Nigella sativa seeds has been used traditionally and successfully to treat ailments including rheumatism and related inflammatory diseases, bronchial asthma and bronchitis, to treat digestive disturbances, to increase milk production in nursing mothers, to support the body's immune system, to promote digestion, elimination and to fight parasitic infestation. Its oil has been used to treat skin conditions such as eczema and boils and is used tropically to treat cold symptoms. The many uses of black seed have earned for this medicinal herb the Arabic approbation ``habbatul barakah`` meaning "the seed of blessing"[19, 20].

Nigella sativa has been used as a natural remedy for many ailments parts of Asia and Africa for centuries and is now well known in the USA and Europe. Seeds of Nigella sativa had been in use for over 4000 years all over the world[21, 22]. According to Birdwood, Nigella sativa is the black cumin of Bible, Melanthine of Hippocrates and Dioscorides and Gith of Pinty. Analic mentioned its use as carminative external application for skin eruptions, seasoning for food and protection for linen against insect[23]. The ancient Greek and Roman clinicians were aware of the beneficial effects of Nigella sativa and used it in combination with honey[24, 25].

Although native place of Nigella sativa is not exactly known, this herbaceous plant belongs to countries around the Mediterranean Sea like Egypt, Turkey and Italy[26]. Roxburgh believes this plant to be a native of India so also called as Nigella indica[27].

2.2.2. Morphological features of Nigella sativa

Nigella sativa is a pretty erect herb, 30-60 cm high. Its leaves are alternate and bipinnately dissected. The stipules are small. The flowers are terminal, pedunculated and whitish blue or purplish in color. Sepals are five, regular, deciduous, imbricate and petaloid. Petals are five with long claws and small bifid limbs. Stamens are numerous. Carpals are 3-10, sessile, connate below and, each with several horizontal ovules. Two seriates on the suture and style is usually long. Fruit is a capsule, dehiscing along ventral suture of free portion of individual carpal. Dried fruit and seeds are the main plant components and are mostly used medically. Seeds of Nigella sativa are black in color and triangular in shape. The seed is about one eighth inch long having a rough interior and a white oily kernel. On rubbing, the seed diffuses a pleasant odor of lemon with a slight soupcon of carrot[28].

2.2.3. Chemical Composition of Nigella sativa

According to Ahmed Aftab et al[29] many active compounds have been isolated, identified and reported in different varieties of black seeds. The most important active compounds are thymohydroquinone, dithymoquinone, thymoquinone (30%-48%), 4-terpineol (2%-7%), sesquiterpene longifolene (1%-8%) α-pinene, t-anethol (1%-4%), p-cymene (7%-15%), carvacrol (6%-12%) and thymol etc. Black seeds also contain some other compounds in traces. These contain two

different types of alkaloids; isoquinoline alkaloids like nigellicimine- N-oxide and nigellicimine and indazole ring bearing alkaloids or pyrazol alkaloids like nigellicine and nigellidine. The seeds also contain alpha-hederin, a water soluble pentacyclic triterpene and saponin, a potential anticancer agent[30]. Other compounds like carvone, limonene, and citronellol are also found in traces. Most of the pharmacological properties of Nigella sativa are mainly due to quinine constituents, out of which thymoquinone is the most important quinine constituent and main reason behind medicinal properties of seed. On storage, thymoquinone yields dithymoquinone and higher oligocondensation products. The seeds of Nigella sativa contain protein (26.7%), fat (28.5%), carbohydrates (24.9%), crude fibre (8.4%) and total ash (4.8%). Various vitamins and minerals like Cu, P, Zn and Fe also contents of Nigella sativa seed in good amount. The seeds contain carotene which is converted by the liver to vitamin A. Root and shoot are reported to contain vanillic acid[31]. Many previous studies reported that the Nigella sativa seeds to contain a fatty oil rich in unsaturated fatty acids, mainly linoleic acid, eicodadienoic acid, oleic acid and dihomolinoleic acid. In Nigella sativa seed saturated fatty acids also present about 30% or less. α-sitosterol is a major sterol which accounts for around 55% of the total sterols of black seed and stigmasterol one of the major sterol after α-sitosterol [32, 33]. In some studies it is reported that the other components includes nigellone, avenasterol-5-ene, avenasterol- 7 - ene , camp esterol, cholesterol , citrostadieno l , cycloeucalenol, gramisterol, lophenol, obtusifoliol, stigmastanol, stigmasterol-7-ene, β-amyrin, butyro- spermol, cycloartenol, 24-methylenecycloartanol, taraxerol, tirucallol, volatile oil (0.5-1.6%), fatty oil (35.6-41.6%), oleic acid, esters of unsaturated fatty acids with C15 and higher terpenoids, esters of dehydrosteari,

hederagenin glycoside, aliphatic alcohol, linoleic acid, melanthin, melanthigenin, β-unsaturated hydroxy ketone, tannin, resin, reducing sugar, glycosidal saponin and protein [34,35,36].

2.2.4. Nutritional Value of Nigella sativa

The earlier studies have shown Nigella sativa to have a high nutritional potential i.e. protein (22%), fat (38 - 40%) and carbohydrates (32%)[37]. Haq et al mentioned in their study that the whole black seed proteins have also been fractionated and characterized by sodium dodecyl sulphate polyacrylamide gel electrophoresis[38]. The mineral and vitamin contents per kg seeds are iron (105 rag), copper (18 rag), zinc (60 mg), phosphorus (527 rag), calcium (1860 rag)[39], thiamin (15.4 rag), niacin (57 mg), pyridoxine (5.0 rag) and folic acid (160 pg)[40]. A qualitative study of Nigella sativa and a number of other plant extracts of Saudi origin used in folk medicine has revealed the presence of sterols, triterpenes, tannins, flavonoids, cardiac glycosides, alkaloids, saponins, volatile oils, coumarins, volatile bases, glucosinolates and anthraquinones[41].

2.3. Effects of Nigella sativa on diabetes

Of the traditional remedies, prescriptions of Nigella sativa for the treatment of various diseases are well known since prehistoric era. Nigella sativa is one of the plants commonly used in Moroccan folk medicine for treatment of various ailments including diabetes mellitus. Previous studies found that its anti-diabetic effect is multifaceted like regulating blood glucose, insulinotropic, prevention of

gluconeogenesis, preserving the integrity of beta cells of islets of Langerhans in pancreas etc[42].

2.3.1. Effect of Nigella Sativa on Blood Glucose

El-Dakhakhny et al[43] studied the effect of Nigella sativa on blood glucose concentrations in streptozotocin diabetic rats. They also studied the effect of nigellone and thymoquinone on insulin secretion of isolated pancreatic islets in the presence of 3, 5.6 or 11.1 mM glucose. Their results showed Nigella sativa significantly lowering blood glucose concentrations in diabetic rats after 2, 4 and 6 weeks. In an experimental study, Alsaif[44] reported that blood glucose lowering effect of black seed was due to improved insulin insensitivity in diabetic rats. Abdelmeguid et al[45] reported that the anti-hyperglycemic effect of black seed and its active component thymoquinone could be due to reduction of oxidative stress, thus preserving pancreatic β -cell integrity lead to insulin levels increase. The black seed oil also contains many bioactive constituents such as thymoquinone, p-cymene, pinene, dithymoquinone and thymohydroquinone[46]. In a study, Khanam M[47] reported that Nigella sativa showed the lowering of blood glucose in diabetic group but the change was not statistically significant. In studies of Salama RH[48], Qidwai W et al[49], Sabzghabaee AM et al[50] and Datau EA et al[51] were also found similar findings in diabetic treated groups with Nigella sativa.

2.3.2. Insulinotropic Effect of Nigella sativa

Fararh et al[52] reported that Nigella sativa seed extract given orally has insulinotropic properties; it increases the production of insulin from the β-cells of the Islets of Langehans. This effect has been extensively studied in Streptozotocin and

Nicotinamide induced diabetic animal models. This was evidenced by the increase in serum insulin as measured by enzyme immunoassay and increased staining areas with positive immuno-reactivity for the presence of insulin in Islets of Langerhans by immunohistochemical staining method in which anti-insulin monoclonal antibody was used. Studies of Omar et al[53] and Rchid et al[54] have shown significant decrease in blood glucose level and increased Islet cell regeneration as evidenced by insulin immunohistochemical staining. Oxidative stress is believed to play a role in its pathogenesis. Nigella sativa extract decreases lipid peroxidation and serum NO and increases antioxidant enzyme activity, thus ameliorating pancreatic β-cell integrity and their numbers. Studies of Kanter et al[55,56], Badary et al[57] and Hajhashemi et al[58] showed the evidence of insulinotropic effect on increased intensity of staining for insulin and preservation of β-cell numbers in the Nigella sativa treated diabetics. Its anti-inflammatory effect also plays a role in preserving the integrity of β-cells. Moneim et al[59] reported that the Nigella sativa and sulphonilureas like gliclazide have synergistic action as for insulinotropic effect is concerned. An extra pancreatic hypoglycemic effect of Nigella sativa has been observed in the study of Le et al[60] with an insulin-sensitizing action of Nigella sativa extracts by enhancing the activity of the two major intracellular signal transduction pathways of the hormone's receptor and decrease in hepatic gluconeogenesis. In a study, Ahmad Bilal et al[61] reported that Nigella sativa showed the change in insulin level but the change was not statistically significant.

2.3.3. Nigella sativa and Histopathology of Pancreatic Beta-cells

Kanter et al[55] investigated the effect of Nigella sativa on histopathology of pancreatic β-cells, in streptozotocin-induced diabetic rats. Blood insulin and glucose concentrations were also measured. The results of this study reported that Nigella sativa treatment caused decrease in the elevated serum glucose, increase in the lowered serum insulin concentrations and partial regeneration or proliferation of pancreatic beta cells in STZ-induced diabetic rats. This study concluded that the hypoglycemic action of Nigella sativa could be partly due to amelioration in the β-cells of pancreatic islets causing increase in insulin secretion. Another study[56] of same researchers evaluated the possible protective effects of Nigella sativa against β-cell damage in streptozotocin induced diabetic rats. Oxidative stress is believed to play a role in the pathogenesis of diabetes mellitus. To assess changes in the cellular antioxidant defense system, they measured the activities of antioxidant enzymes such as glutathione peroxidase (GSHPx), superoxide dismutase (SOD) and catalase (CAT)) in pancreatic homogenates. They 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 beta-cells were examined by immunohistochemical methods. Streptozotocine induced a significant increase in lipid peroxidation and serum NO concentrations and decreased antioxidant enzyme activity. Nigella sativa treatment provided a protective effect by decreasing lipid peroxidation and serum NO and increasing antioxidant enzyme activity. In immunohistochemical study of Kanter M et al[56], cells islet of Langerhans shown weak insulin immunohistochemical staining and degeneration was observed in untreated STZ-induced diabetic rats.

Increased intensity of staining for insulin and preservation of beta-cell numbers were apparent in the Nigella sativa treated diabetic rats. This study stated that Nigella sativa treatment decreasing oxidative stress and preserving pancreatic beta-cell integrity due to this Nigella sativa showing the therapeutic protective effect in diabetes. It was also suggested that Nigella sativa may be clinically useful for protecting β-cells against oxidative stress.

2.3.4. Effect of Nigella sativa on Lipid Profile, Lipid Peroxidation and Anti-oxidant Defense System

Dahri et al[62] observed significant decrease in serum low density lipoprotein cholesterol level and increase in serum high density lipoprotein cholesterol level when rats were treated with Nigella sativa. The study of Bahram et al[63] showed significant decrease in the development of hyperlipidemia in Nigella sativa seed treated group and serum lipid profile and malondialdehyde were significantly lower, compared to control group. Some studies have mentioned different beneficial properties of Nigella sativa as extract, oil or active compounds such as thymoquinone. But only a few have studied the effects of whole or crushed seed, including its hypolipidemic and antioxidant properties. One study assessed the effect of crushed black seed on some blood parameters including serum levels of cholesterol and triglyceride[64]. Study of Ibraheim[65] showed significant decrease in the concentration of total cholesterol and triglycerides. Study of Zaoui et al[66] indicated that oral treatment with Nigella sativa decreased serum cholesterol and triglycerides. In another study Nigella sativa administration to rats significantly decreased serum total cholesterol, LDL-C and triglycerides and increased HDL-C[43].

Le et al[60] reported a significant decrease in plasma triglycerides and an increase in HDL-C levels in black seed extract-oral treated rats, compared to the control group. In the study of Dehkordi and Kamkhah[67] a significant reduction was observed in serum TC and LDL-C of patients with mild hypertension after 8 weeks of oral administration of Nigella sativa seed extract. The results of Bamosa et al[68] demonstrated a decrease in serum total cholesterol, LDL-C, HDL-C and triglycerides during intraperitoneal injection of thymoquinone in rats. Morikawa et al[69] in an invitro study investigated hypotriglyceridemic effect of nigellamines, that is black seed diterpene alkaloids, equivalent to the hypolipidemic agent, clofibrate. Ali and Blunden[70] stated in their study that the hypolipidemic effect of black seed does not seem to be due only to one component, but rather to the synergistic action of its different constituents including thymoquinone and nigellamine, soluble fiber - mucilage, sterols, flavonoids and high content of polyunsaturated fatty acids. The hypolipidemic action of thymoquinone and its mechanism is not fully established, however it is proposed that the main reason of hypolipidemic action of thymoquinone may due to its antioxidant property. In a studies of Elrehany M[71], Sabzghabaee AM et al[50] reported that Nigella sativa treatment in diabetic groups not showed significant change in parameters of lipid profile.

Regarding the effect of black seed on lipid peroxidation and antioxidant defense system, Kanter et al[55] found that treatment with Nigella sativa decreased blood MDA levels and increased the antioxidant defense system activity in carbon tetrachloride treated rats. In an another study conducted by same authors in the experimental spinal cord injury in rats proved that Nigella sativa treatment can reduce the spinal cord MDA and treatment also prevented from inhibition of CAT,

SOD and GPX enzyme activities[56]. The study of Meral et al[72] indicated that Nigella sativa extract decreased the elevated blood MDA concentration and increased the lowered glutathione and ceruloplasmin concentrations in diabetic group. The antioxidant effect of black seed seems to be due to thymoquinone, flavonoids and antioxidant vitamins like ascorbic acid. It has been shown that Nigella sativa and TQ inhibit non-enzymatic lipid peroxidation in liposomes and both work as a scavenger of various reactive oxygen species including superoxide anion and hydroxyl radicals[73]. In addition flavonoids are a class of polyphenolic compounds that seem to have antioxidant properties by suppressing reactive oxygen and nitrogen species formation, scavenging reactive oxygen and nitrogen species and protecting the antioxidant defense system[74].The sequels of diabetes mellitus are deranged lipid and carbohydrate metabolism leading to their increased levels. Nigella sativa decreases triglycerides, total cholesterol and increases the healthy cholesterol i.e. HDL cholesterol[66,26,35]. These effects potentate when Nigella sativa is given in combination with gliclazide. The combination significantly decreases cholesterol, triglyceride and total lipids, while a significant increase in serum insulin, total protein and liver glycogen levels was observed[60,75].

Meral et al[72] observed Nigella sativa decreased the elevated glucose and MDA concentrations, increased the lowered glutathione and ceruloplasmin concentrations and prevented lipid peroxidation induced liver damage in diabetic rabbits. Thus Nigella sativa might be used in diabetic patients to prevent lipid peroxidation, increase anti-oxidant defense system activity and also to prevent the liver damage. Hosseinzadeh et al[75] reported that thymoquinone and Nigella sativa

may have protective effects on lipid peroxidation process during ischemia-reperfusion injury in rat hippocampus.

A comprehensive study was conducted by Moneim et al[59] to evaluate the effects of Nigella sativa and gliclazide on serum glucose, insulin, total lipids, triglyceride, cholesterol, total protein and liver glycogen levels in diabetic rats. The results indicated that treatment with gliclazide significantly decreased the raised fasting blood glucose level as well as blood glucose while treatment with Nigella sativa significantly decreased blood glucose level during OGTT when compared to diabetic control. Combined treatment with gliclazide and Nigella sativa significantly decreased cholesterol, triglyceride and total lipids, while a significant increase in serum insulin, total protein and liver glycogen levels was observed.

2.3.5. Effect of Nigella sativa on Liver Function Tests

El-Dakhakhny et al[43] investigated the effects of weeks oral intake of nigalla sativa on some liver function tests in D-galactosamine or carbon tetrachloride-induced hepatotoxicity male albino rats. In another experiment, the effect of the Nigella sativa on serum lipid profile was examined in male spontaneously hypertensive rats of stroke prone strain and Wistar Kyoto rats. The study showed that daily administration of Nigella sativa did not adversely affect the serum transaminases (ALT and AST), alkaline phosphatase and serum bilirubin or prothrombin activity in normal albino rats. When Nigella sativa was given for four weeks prior to induction of hepatotoxicity by D-galactosamine or carbon tetrachloride, it was able to give complete protection against d-galactosamine and partial protection against carbon tetrachloride hepatotoxicity. Nigella sativa showed

a favorable effect on the serum lipid pattern, where the administration of Nigella sativa caused a significant decrease in serum total cholesterol, low density lipoprotein, triglycerides and a significant elevation of serum high density lipoprotein level. In another investigation by Al-Jishi and Hozaifa[76] the effects of Nigella sativa on liver function tests on male albino rats were studied and favorable results were shown by Nigella sativa. Many previous studies concluded that the active constituents of Nigella sativa are thymoquinone, dithymoquinone, thymohydroquinone and thymol[77]. Several studies have shown the various therapeutic actions of Nigella sativa. It has activity against diabetes[54,62] radical scavenging activit[78,79], prevents lipid peroxidation and increases the antioxidant defense system[72].

2.3.6. Effect of Nigella sativa on Kidney, Liver and Nerve

Renal dysfunction is a common complication in diabetes that is involved in oxidative stress changes[80,81]. The amelioration of renal hemodynamic and function changes in diabetics has been elucidated by supplementation with antioxidants. Nigella sativa seed was believed to be responsible for restoration in renal dysfunction in nephrotoxic rats through the antioxidant effect[82,83]. The diabetic nephropathy has been considered an important cause of mortality and morbidity and many of the end stage renal failure results are due to diabetic nephropathy[83]. Streptozotocin and Alloxan-induced diabetic rodents develop nephropathy similar to the early stage of human diabetic nephropathy[84]. In the diabetic animals, a significant increase in the kidney weight was observed[85,86]. The factors like glucose over-utilization and subsequent increased uptake, glycogen accumulation, lipogenesis and protein

synthesis in the kidney tissue are may be attributing in kidney enlargement in diabetes. Nigella sativa extract administered to the diabetic rats successfully prevented the enlargement of the kidney. Histological studies investigated on the induced diabetic rat kidneys showed the damage to the glomerulus as it is degenerated or necrotized, thickened basement membrane and edematous renal convoluted tubules with increase in mucopolysaccharide deposits which were found to be absent or reduced in the diabetic kidneys treated with Nigella sativa extract. The treated diabetic rats however showed healing features which resembled that of a normal kidney[87,88]. Advanced histopathological studies on human diabetic nephropathy showed arteriolar hyalinosis and tubular atrophy coupled with an increase in the interstitial volume. These features are largely absent in normal and Nigella sativa treated animals. The tubular changes noted in the kidney of diabetic rats primarily consist of vacuolization of tubular cells with evidence of tubular atrophy, tubulointerstitial fibrosis and alteration of the medullary structure [89,90].

According to the study of Samad A et al[91] effect of Nigella sativa on gluconeogenesis and liver glucose production helps to clarify part of the hypoglycemic mechanism since hepatic glucose production through gluconeogenesis is known to contribute to hyperglycemia in diabetic patients. Research on isolated hepatic cells showed a significant decrease in glucose production from gluconeogenic elements like glycerol, alanine and lactate in Nigella sativa treated animals as compared to the untreated animals[92]. This significant decrease in liver glucose output and ameliorative effect on regeneration of pancreatic islets suggests that the observed antidiabetic action of Nigella sativa is at least partially mediated through an effect on hepatic gluconeogenesis.

EL-Kholy WM et al[93] observed no toxicity and no histopathology of liver tissue of Nigella sativa treated rats. Mohammad A D et al[94] observed that oral administration of Nigella sativa has no toxic effect on liver to the parameters used, ALT and aspartate aminotransferase AST. Histopathology examination showed no hepatic vacuolization, degeneration, inflammation and necrosis in liver tissue. Almost all the studies suggested a hepatoprotective effects of Nigella sativa due to some component such thymoquinone and monoterpenes or tocopherols, phytosterols and phenols[95]. Antioxidant and anti-inflammatory properties of Nigella sativa are the main features of preventing and protecting liver from injury. Several studies have shown the protective effects of Nigella sativa against liver injury produced by ROS with its free radical scavenger properties and enhanced antioxidant defences in body. Thymoquinone is the main active ingredient of Nigella sativa seed responsible for it. The effect of Nigella sativa to postpone progression in chronic liver diseases should be considered as preventive medicine in patients with hepatic disorders[96,97,98,99,100]. Rakesh Jadhav[101] observed that administration of anti-tubercular resulted in degeneration, necrosis and fibrosis. Administration of Nigella sativa showed significant reduction in the scores in degeneration and necrosis.

Previous studies showed the repairing ability of CCl4 induced centrilobular hepatocellular vacuolar degeneration and necrosis by Nigella sativa. Treatment with Nigella sativa seeds significantly declined the effects of CCl4 induced damage and it was evidenced by the decreased level of liver enzymes and lipid profile and restoration of hepatocellular architecture[102,103]. Al-Razzuqi et al[104] reported protective effect of oil extract of Nigella sativa seeds against carbon tetrachloride induced acute liver injury in experimental rabbit models. Protective effect of Nigella sativa on lead

acetate-induced hepatic tissue damage in mice was investigated by Alarifi et al[105]. Thymoquinone, the active constituent of Nigella sativa, has been well documented as a potent antioxidant, particularly against the CCl4-induced free radical species[106]. Thymoquinone restores liver cellular architecture and prevents leakage of its enzymes through preventing the formation of toxic stable complex by a combination of CCl3, O2 free radical and the glycolipid component of cell membrane[107]. Amina E.E et al[108] in their bio-chemical and histological study proved that Nigella sativa seeds possess potential to protect the liver tissue against oxidative damages and could be used as an effective protector against CCl4 induced liver damages. There are very few studies on histopathology of liver of streptozotocine induced diabetic rats treated with Nigella sativa and thymoquinone.

In a histopathological study of Kanter M[109] studied on the sciatic nerves of streptozotocin induced diabetic rats and they evaluated the possible beneficial effect of Nigella sativa and thymoquinone on STZ induced diabetic changes in sciatic nerve. Histological evaluation of the tissues in diabetic animals treated with thymoquinone and Nigella sativa showed fewer morphologic alterations. Myelin breakdown decreased significantly after treatment with Nigella sativa and thymoquinone. The ultra-structural features of axons also showed remarkable improvement suggesting the utility of Nigella sativa and thymoquinone as a potential treatment for peripheral neuropathy in streptozotocine induced diabetic rat. Improvement of the diabetic peripheral nerve injury in the rat by administration of Nigella sativa was reported by Kazim et al[110]. Beuch etal[111] reported the regeneration of injured peripheral nerves after three months. Many previous studies are showing

the beneficial effect of Nigella sativa in biochemical parameters and histopathological changes in induced diabetes and other induced injuries.

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CHAPTER 3

HYPOTHESIS

AIMS & OBJECTIVES

HYPOTHESIS

Both Nigella sativa seed powder and thymoquinone can reduce diabetes induced alterations in various biochemical parameters and histopathological changes of metabolic organs in albino rats.

AIMS & OBJECTIVES

a. To evaluate the effect of Nigella sativa seed powder and thymoquinone in normal and streptozotocin(STZ) induced diabetic rats on various biochemical parameters and morphological effect on metabolic organs.

   i. Biochemical parameters (Serum)

      Glucose, Insulin, Oral Glucose Tolerance Test,

      MDA,

      Endogenous antioxidant -SOD,

      Exogenous antioxidants –Vitamin C and E.

      Lipid Profile

      Liver function tests

   ii. Histopathological changes – Pancreas, Liver, Kidney and Nerve.

b. Purpose of the present study was to address the issues to support to the existing information pertaining to the effect of Nigella sativa seed powder and thymoquinone in treating diabetes.

CHAPTER 4

MATERIALS & METHODS

4.1. Procurement and Rearing of Experimental Animal

Laboratory inbred adult albino Wister rats fed with laboratory stock diet and water *ad libitum*, and weighing 175- 250g were used in the study. Rats are acclimatized a week to the laboratory conditions at 22- 24^{o} C and a 12 h light: dark (circadian) cycle. The acclimatized rats were housed in polypropylene cages (32x40x18 cm) three animals in each. The experimental protocols were approved by the Institutional Animal Ethical Committee (IAEC) Shri B M Patil Medical College, Vijayapura and the experiments were performed as per norms of Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA).

4.2. Preparation of Nigella sativa seed powder

Seed of Nigella sativa were procured from Safa Honey co Ltd, Bangalore (recognized Nigella sativa seed seller), authenticated by botanist from DRM Science college, Davangere. The Nigella sativa seeds were grained in to the fine powder with help of the department of pharmacognigence, Bapuji College of Pharmacy, Davangere. The powder was stored in to vacuum tight containers. Nigella sativa seed powder administrated orally[1,2] (300mg / kg body weight).

4.3. Thymoquinone

Thymoquinone is a phytochemical compound found in the Nigella sativa seed. Thymoquinone procured from Sigma – Aldrich, Bangalore. Its stock solution was prepared by dissolving 1g in 10mL ethanol. Diluted solution of thymoquinone at

concentration of 4mg mL^{-1} was prepared by dissolving 400 µL of stock solution in 10mL sterile distilled water. This freshly prepared solution was administrated to rats through intraperitoneal injection [3] (4mg / kg body weight).

4.4. Streptozotocin Induced Diabetes

Streptozotocin

Streptozotocin is a naturally occurring chemical that is particularly toxic to the insulin-producing beta cells of the pancreas. In medicine streptozotocin is used for treating certain cancers of Islets of pancreas and used in medical research to produce an animal model for diabetes. Streptozotocin is a glucosamine-nitrosourea compound. Like other alkylating agents it is in class of nitrosourea, it is toxic to cells by causing damage to the DNA, though other mechanisms may also contribute. DNA damage of pancreas beta cells induces activation of poly ADP-ribosylation, which is very important role for diabetes induction than DNA damage itself. Streptozotocin is similar enough to glucose to be transported into the cell by the GLUT2, but is not recognized by the other glucose transporters. This explains its relative toxicity to beta cells, since these cells have relatively high levels of GLUT2[4, 5, 6].

Citrate Buffer

- Citric acid – Dissolve 2.101gm of citric acid in 100ml of distilled water.
- Sodium citrate solution 0.1M: Dissolved 2.941gm of sodium citrate in 100ml distilled water.

- Preparation of Citrate Buffer with 4.4 PH – 49.5ml of citric acid solution add with 50.5 ml of sodium citrate solution.

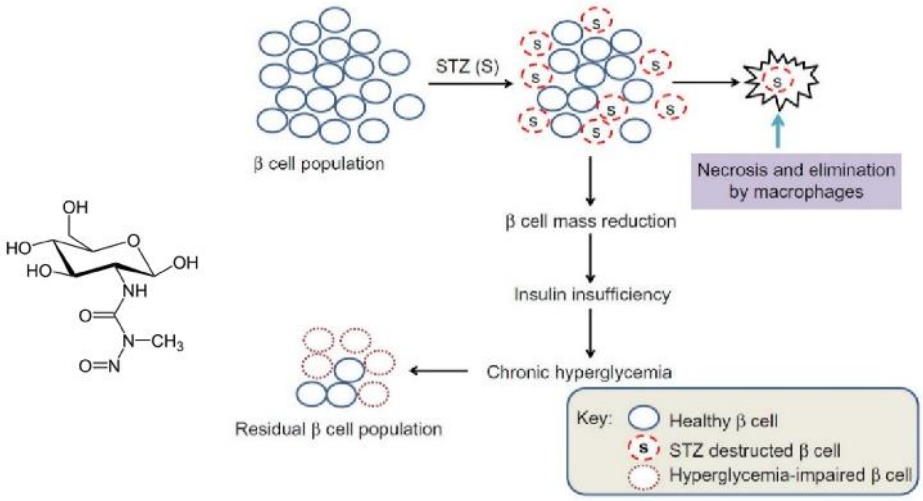


Figure 2: Mechanism of Streptozotocin[7]

Streptozotocin induced diabetes

- Streptozotocin procured from SRL lab, Hyderabad. 50mg/kg body weight streptozotocin used for inducing diabetes into albino rats with single rapid intraperitoneal injection.
- Preparation – 15mg of streptozotocin dissolved in 1ml of citrate buffer

  = 50mg/1000 X body weight (gm) = Result / 1.5 = result in ml
- Confirmed Diabetes after 24 hrs >180mg/dl[1] , repeated fasting glucose checkup first 7 days, after 1 week of induction, confirmed diabetic(>180mg/dl) rats only taken for study[8].

4.5. Gravimetry

Effect on body weight, metabolic organs (Liver, kidney and pancreas) of all the groups was evaluated. Animals are anesthetized by euthanasia where in animals are placed in sealed chambers where high levels of aesthetic gas are introduced by soaking cotton in 40 ml of diethyl ether. Once after loss of righting reflex approximately after 60 sec rats will be removed from the chambers and continued for further process. Liver, kidney and pancreas were carefully removed and weighted using digital electronic balance. The organosomatic index was calculated.

$$\text{Organosomatic index} = \frac{\text{Weight of organ (One side/both sides) X 100}}{\text{Body weight of rats}}$$

4.6 Method of euthanasia

Euthanasia is the act of humanely putting an animal to death or allowing it to die. Euthanasia methods are designed to cause minimal pain and distress. Euthanasia is distinct from animal slaughter and pest control, which are performed mainly for the purpose other than an act of mercy, although in some cases the procedure is the same. Gas anaesthetic such as diethyl ether can be used for euthanasia of rats. The animals are placed in sealed chambers where high levels of aesthetic gas introduced by soaking cotton in 40ml of diethyl ether. Death may also be caused using carbon dioxide once unconsciousness has been achieved by inhaled anaesthetics. Once after loss of righting reflex approximately after 60 sec will be removed from the chamber and continued for further process.

4.7. Blood & Tissue collection

4.7.1. Blood collection

Blood samples were collected by the retro-orbital route on 46th day and processed for biochemical assay.

Procedure for retro orbital venous blood collection[9]

Also referred to as periorbital, posterior-orbital and orbital venous plexus bleeding

- The animal is anaesthetized and at an appropriate depth of anesthesia prior to commencement or even can be euthanize, signs that indicate a satisfactory plane of anesthesia include a lack of response to a toe pinch and respiration that are regular and relaxed.
- The anaesthetized rat made to lie on its back, the head of the rat toward the edge of table.
- Fix the head with thumb and forefinger, tightening the skin over the sides of the face. This leads to retraction of the eyelids and protracts the eye ball.
- Insert a micro hematocrit (capillary) tube into the medical canthus and twist, it leads to break through the bulbar conjunctiva. Capillary tube should pass toward the medial aspect of the bony orbit.
- Collect the blood into test tubes and seal. Continue until unable to collect. If difficulty arise changes to other eye.

- Blood flow can be controlled or stopped by applying finger pressure gently to the soft tissue. A finger should be placed over the closed eyelid for approximately 30 seconds. The rat should be checked for post-operative pre-orbital lesions approximately 30 minutes after blood sampling and on at least one more occasion with two hours of the sampling.

Advantages of retro orbital venous blood collection

- Rapid – large number of animals can be bled within short period of time.
- Obtained volume: medium to large.
- Good sample quality. Potential contamination with topical anesthetic, if used should be taken into account.
- A minimum of 10 days should be allowed for tissue repair before repeat sampling from the same orbit. Otherwise it may interfere in the healing process with blood flow.
- Alternating orbit should not be attempted until the phlebotomist is proficient in obtaining samples from the orbit accessed most readily by the dominant hand that is a right handed person should withdraw samples from the right orbit before attempting to obtain samples from the left orbit.

4.7.2. Tissue collection

After euthanasia rats were kept in supine position and dissected. Metabolic organs liver, kidney, pancreas and nerve were collected and washed in normal saline. Histopathology of all the collected organs was done. For the evolution of histopathology

specimens were kept in 10% formalin. By following routine histological techniques, the samples were put into paraffin and serial sections of 5µm were taken from tissue blocks.

4.7.3. Analysis of Biochemical assay

Glucose

GOD-POD Method[10]

Principle:

The reaction sequence employed in this assay is as follows:

Glucose is oxidized by glucose oxidase and produces gluconate and hydrogen peroxide. The hydrogen peroxide is oxidatively coupled with 4aminoantipyrine and phenol. The intensity of the coloured complex (quinoneimine) is proportional to the glucose concentration in the sample and can be measured photometrically at 505nm (500-540nm).

Reagent composition:

Active ingredients	**Concentration**
Phosphate buffer	100 mmol/L
Glucose oxidase	7000 U/L
Peroxidase	6700 U/L
4-Aminoantipyrine	0.7 mmol/L
Phenol	0.1mmol/L

Procedure:

Pipette into test tubes labelled Blank (B), Standard (S) and Test (T) as follows:

	B	S	T
ERBA Glucose reagent	1.0ml	1.0ml	1.0ml
Glucose Standard	-------	1.0ml	-------
Specimen	-------	--------	1.0ml

Table 1: Procedure of Glucose estimation

Mix and incubate for 10 minutes at 37^0 C (or) 15 minutes at R.T.

Mix and read absorbance of Standard (S) and Test (T) against Blank (B) 505 nm or with green filter (500-540nm). The final colour is stable for 1 hour at R.T

Calculations:

$$\text{Glucose Conc. in mg/dl} = \frac{\text{Abs. of T} \times 100}{\text{Abs. of S}}$$

Estimation of Serum Malondialdehyde (MDA) (Nadiger et al method 1986)[11]

Principle - Auto oxidation of unsaturated fatty acids lead to the information of semi stable peroxides which then undergo a series of reaction to form short chain aldehydes like malondehyde(MDA). Two molecules of thiobarbituric acid (TBA) react with one molecule of MDA with the elimination of 2 molecule of water to yield pink crystalline pigment with an absorption maximum at 530nm.

Reagents

1. 10% tricholoacetic acid(TCA)

   10gm of trichloroacetic acid in 100ml distilled water

2. 0.67% thibarbituric acid in 100ml distilled water

Reagents	**Blank(ml)**	**Test(ml)**
Distilled water	0.5	----------
Serum	----------	0.5
0.67% Thiobarbituric acid(TBA)	1.5	1.5
10% Trichloroacetic acid	3.6	3.6
Keep in water bath and boil for 10-15 min and cool. Centrifuge for 10-15 min		
Absorbance of supernatant at 530nm		

Table 2: Procedure of MDA estimation

Calculation:

Concentration of serum MDA

$$= \frac{\text{Absorbance of test}}{\text{Nanomolar extin non co- efficient}} \times \frac{\text{Total Volume}}{\text{Sample Volume}}$$

$$= \frac{\text{Absorbance of test X } 5.6}{1.5 \times 10^5 \times 0.5} \times \frac{10^9}{1000}$$

= Absorbance of test X 73.33

=……nmol/ml

Estimation of serum Superoxide Dismutase (SOD) (Marklund and Marklund method 1974)[12]

Principle – Superoxide anion is involved in auto oxidation of pyrogallol at alkaline pH(8.5). The superoxide dismutase(SOD) inhibits auto-oxidation of phyrogallol, which can be determined as an increase in absorbance at 420nm.

Reagents –

1. Tris buffer, 0.05M, pH 8.5 containing 1mM EDTA

   50ml of tris buffer(50mM of Tris buffer and 1mM of EDTA) was prepared to this 50ml HCL was adjust the pH 8.5 and volume was made upto 100ml.

2. Pyrogallol(20mM): 25mg pyrogallol dissolved in 10ml distilled water.

Procedure

	Tris Buffer(ml)	**Serum(ml)**	**Pyrogallol(ml)**	**OD at 420 nm after 1 min, 30 sec**	**OD at 420 nm after 3 min, 30 sec**
Control	2.9	0	0.1		
Test	2.8	0.1	0.1		

The contents of the above tube were mixed and the absorbance at 420nm was measured

$$\text{A/min} = \frac{\text{Absorbance at 3 min 30sec – Absorbance at 420nm Was measured}}{2}$$

Table 3: Procedure of SOD estimation

Calculation:

Serum SOD activity

$$= \frac{\text{A/min of control - A /min of Test}}{\text{A/min of control x 50}} \times 100 \times \frac{1}{\text{Sample Volume}}$$

$$= \frac{C - T}{C \times 50} \times 1000$$

=……U/ml

one unit of SOD is defined as the amount of enzyme required to cause 50% inhibition of phyrogallol auto oxidation.

<u>Vitamin C Estimation</u>

By Roe & Kuether Method[13]

Vitamin C includes standardization of chemicals & analysis vitamin C in sample. Standardization of vitamin C (Ascorbic acid)

Principle

The ascorbic acid is oxidized to diketogulonic acid in presence of strong acid solution reacts with 2,4, dinitrophenyl hydrazine to form diphenylhydrazone which dissolves in strong H_2 So_4 solution to produce red colour which can be measure at 505nm(range of vitamin C 500-520nm) spectrophotometrically.

Reagents:

1. **TCA (10%):** 10 gm TCA dissolved in distilled water(DW) and make volume up to 100ml.
2. **2,4-DNPH:** 2gm 2,4-DNPH dissolved in $9NH_2SO_4$ & make volume up to 100ML. 9N H_2SO_4 preparation : MW: 98(2+32+64); Density:1.83gm/cc (i.e.1830gm in 1000cc) Molarity= density/MW; 1830/98=18.67M, but here each mole contribute $2H^+$ so conc. H_2SO_4 is approx.. 37 Normal. To make 9N H2SO4 solution in 100ml DW: V1N1=V2N2; 100x9=V2x37; V2=24.324ml; i.e. 24.32ml H_2SO_4 added in WD up to 100ml.
3. **Thiourea :** 10gm thiourea dissolved in 100ml of 50% ethanol (store in 4^oC).
4. **1.5% $CuSO_4$:** 1.5 gm $CuSO_4$ dissolved in DW upto 100ml.
5. **Combined C. R. (freshly):** 5ml 2,4-DNPH+0.1 ml $Cu(SO_4)$+ 1ml thiourea.
6. **85%H_2SO_4 :**85 ml H_2SO_4added in DW upto 100ml.
7. **1% Stock Solution of Vitamin C :** 1gm Vit. C makes it 100 ml with DW.
8. **Working standard:** 1ml stock diluted upto100ml DW to 500DW

Standard Curve Preparation:

Standardization: 100ml DW = 1gm vit. C

1ml = 0.01gm, 0.1=0.001gm.

Stock(ml)	DW(ml)	TCA(ml)	Colour Reagent(ml)	Mixed & kept at 56^0C in warm water bath for 1hr then cooled in ice bath for 5 min.	85% H_2SO_4 (cold)(ml)
Blank	--	0.5	0.4		2
0.1	0.5	0.5	0.4		2
0.2	0.4	0.5	0.4		2
0.3	0.3	0.5	0.4		2
0.4	0.2	0.5	0.4		2
0.5	0.1	0.5	0.4		2

All test tubes are mixed and after 20min OD of Stock Std was read against B at 500nm.

Stock(ml)	Concentration(g/ml)	OD
0.1	0.001	
0.2	0.002	
0.3	0.003	
0.4	0.004	
0.5	0.005	

Table 4: Procedure for standardization of vitamin -C

Estimation

1. Principle

Ascorbic Acid is oxidized to diketoglutoxic acid in presence of strong acid solution, which reacts with 2, 4-DNPH to form diphenylhydrazine which dissolves in strong H_2SO_4 solution to produce red colour which can be measure at 500 nm (range of vit. C 500-520 nm) spectophotometrically.

2. Depolarization:

For plasma / serum sample: 1ml sample+ 1ml 10% TCA (1.1 v/v)

1ml plasma / serum in dry centrifuge + 1ml 10% TCA

Mix well (10-15 sec.)

Centrifuge (10min at 3500 rpm)

Take supernatant

Sl.no	Reagents	T(ml)	S(ml)	B(ml)
1	Supernatant	1	--	--
2	Working standard(g/ml)	--	(Stock: DDW)	--
3	10% TCA	--	0.5	0.5
4	DDW	--	----	0.5
5	Colour reagent (Fresh)	0.4	0.4	0.4
Mix & all test kept in warm water bath at 56 degree C for 1hr then cooled in ice bath for 5min.				
6	85% cold H2SO4	2	2	2

All test tubes are mixed and after 20min OD of T& S was read against B at 500nm. T= test, S= standard, B= blank

Table 5: Vitamin C estimation Procedure

Vitamin E estimation

***By Das et al (2012)*[14]**

Reagents

Absolute ethanol (aldehyde free), N-propanol, Xylene (extra pure), Ferric chloride (%), Distilled water, DL α- tocopherol acetate, & 2, 2` - Bipyridyl.

Preparation of reagents

Stock standard of α- tocopherol (0.27% w/v): 270mg of α- tocopherol acetate dilute in 100ml absolute ethanol and mix thoroughly.

2, 2` - Bipyridyl (0.12% w/v): 120 mg 2, 2` - bipyridyl dissolved and made volume up to 100ml of n-propanol & kept in brown bottle.

Ferric chloride (0.12% w/v): 120mg $FeCl_3.6H_2O$ dissolved in 100ml ethanol & kept in brown bottle. All the solutions are stable at room temperature.

Preparation of standard curve

Working standard of α- tocopherol (27µg/mL): Dilute 1ml of stock standard to 100mL absolute ethanol to obtain concentration 27 µg/mL (2.7 µg/100mL). This solution is stable at room temperature. In six centrifuged tubes labelled as (blank) B, S_1, S_2, S_3, S_4 and S_5 place 00, 150, 300, 450, 600 and 750 µL of working standard α-tocopherol (27µg/mL) in respectively and add absolute ethanol to make the volume of each tube equal as 750µL. These solutions (S_1-S_5) are equivalent to 4µg/mL, 8µg/mL, 12µg/mL, 14µg/mL, 16µg/mL and 20µg/mL of α- tocopherol respectively. Use these solutions in the routine procedure shown in following table(Table 6). Read the absorbance by using 200µl of solutions prepared above including blank putting on plain ELISA micro plate (non-antibody coated) and read in ELISA reader at 492nm. Plot standard curve absorbance *vs* α- tocopherol (µg/mL).

S. no	Working standard		DW (µL)	Xylene (µL)	Centrifuge Xylene at 3000 rpm. 500 µL Xylene layer taken out	2,2` - Bipyridyl (µL)	$FeCl_3$ (µL)	After 2 min read OD at 492 nm
	α- tocopherol (µL)	Ethanol (µL)						
B	0	750	750	750		500	100	
S_1	150	600	750	750		500	100	
S_2	300	450	750	750		500	100	
S_3	450	300	750	750		500	100	
S_4	600	150	750	750		500	100	
S_5	750	0	750	750		500	100	

Table 6: Procedure for standardization of vitamin E

The curve is drawn to determine the extent of adherence to the Beer-Lambert law with various photoelectric instruments.

Analysis of serum α- tocopherol

Sample preparation: Allow the 3ml blood to clot in centrifuge tube for hrs at room temperature and centrifuge at 3000rpm for 15 min to get serum. Serum for the analysis of α- tocopherol should be protected from sunlight and undue agitation. α- tocopherol darkens on exposure to light and slowly oxidized by atmospheric oxygen. α- tocopherol was found stable in separated serum at 25^{o} C for 1day, at 4^{o}C for 2 weeks and at -20^{o}C for 2 months.

Procedure Step -1: two centrifuge tubes label as T and B (i.e. sample and blank). To the sample tube add 750µL absolute ethanol and 750µL serum. Add the serum slowly with shaking to obtain a finely divided protein precipitate. To the blank tube add 750µL distilled water and 750µL absolute ethanol. Stopper the tubes tightly by wrap paper and shake vigorously for at least 30sec. To all these tubes add 750µL xylene. Again stopper the tubes tightly by wrap paper and shake vigorously for at least 30sec and centrifuge all tubes for 10 min at 3000rpm (Table 7).

Step-2: Transfer 500µL of the xylene layer (supernatant) into properly ladled clean small size test tubes. To each tube add 500µL of 2, 2- bipyridyl solution and 100 µL ferric chloride solution and wait for 2 min (Table 8).

Step-3: Transfer 200 µL solutions from these tubes to uncoated micro wells respectively. Readings are made in ELISA Reader (Erba, Lisa Scan II), with the rapid measure mode. Set the primary wavelength as 492 and measure the absorbance within 4 min (Table 9).

	T(µL)	**B(µL)**	
Serum	750	---	
DW	---	750	
Ethanol	750	750	
Mix for 30 sec			
Xylene	750	750	Mix for 30 sec & centrifuge for 10 min at 3000 rpm

Table 7: Analysis of α- tocopherol procedure

	T(µL)	B(µL)	
Supernatant	500	500	
2,2-bipyridyl	500	500	Wait for 2 min
$FeCl_3$	100	100	

Table 8: Analysis of α- tocopherol procedure

	T(µL)	B(µL)
ELISA	200	200
Read the absorbance at 492nm within 2min		

Table 9: Analysis of α- tocopherol procedure

Note: we can take the serum 350µL (in case less availability) by reducing all other reagents by half.

Calculation:

$$\text{Concentration of Vitamin E µg/ml} = \frac{\text{OD of test} - \text{OD of Blank}}{\text{Slope}} \times \text{Dilution factor}$$

$$\text{Where Slope} = \frac{Y_2 - Y_1}{X_2 - X_1}$$

X & Y are concentration and absorbance of standards respectively.

Oral glucose tolerance test (OGTT) [15, 16]

Oral glucose tolerance test (OGTT) for rats were performed according to the standard method. Group I served as untreated normal control and the oral 2 h glucose tolerance test was carried out via estimating glucose of blood sample collected at 0, 30, 60, 90 and 120 min. An oral glucose load of 3.5 g/kg body weight was administered for the test[2]. Group II rats were normal rats treated with Nigella sativa seed powder (300mg/kg BW) for 45days and similar OGTT was conducted on these animals. Group III normal rats treated with thymoquinone(4mg/ kg BW) for 45days followed by OGTT. Group IV served as untreated diabetic control rats followed by OGTT. Group V diabetic rats received a dose of 300 mg/kg BW of Nigella sativa seed powder for 45days and followed by OGTT and Group VI diabetic rats received a dose of 4mg/kg BW of thymoquinone for 45days and followed by OGTT.

Insulin

By Chemiluminescence immunoassay (CLIA) [17, 18, 19]

Principle of the Assay

The insulin CLIA Kit is based on a solid phase enzyme-linked immunosorbent assay. One anti-Insulin antibody utilizes by the assay system for solid phase (microliter wells) immobilization and another anti-Insulin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test specimen (serum) and standards are added to the insulin antibody coated microliter wells. Then anti-Insulin antibody labeled with horseradish peroxidase (conjugate) is added. If Insulin present in the

specimen, it will combine with the antibody on the well and the enzyme conjugate resulting in the insulin molecules being sandwiched between the enzyme-linked antibodies and solid phase. After incubation of one hour at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of chemiluminescent substrate is then added and read relative light units (RLU) in an illuminometers. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of Insulin in the serum. By reference to a series of insulin standards assayed in the same way, the concentration of Insulin in the unknown sample is quantified.

Assay Procedure

Reagent Preparation

- All reagents should be at room temperature (18-25°C) and mixed by gently inverting or swirling prior to use.
- To prepare substrate solution, make a 1:1 mixing of reagent A with reagent B before using of it. Mix gently till complete mixing. Discard excess after use.
- Dilute 1 volume of wash buffer concentrate (50x) with 49 volumes of distilled water. 750 ml of washing buffer (1x) can be prepared by diluting of 15 ml of wash buffer concentrate (50x) with 735 ml of distilled water, mix well before use.
- Reconstitute each lyophilized standard with 0.5 ml distilled water. Allow the material of reconstituted to stand for at least 20 minutes. Reconstituted standards should be stored sealed at 2-8°C.

Procedure

- Secure the desired number of coated well in the holder. Dispense 50 µl of insulin standards, specimens, and controls into the appropriate wells. Gently but thoroughly mix for 10 seconds.
- Dispense 100 µl enzyme conjugate reagent into each well. Mix well for 30 seconds and incubate at room temperature for 60 minutes.
- Remove the mixture of incubation by emptying the plate content into a waste container. Rinse and empty the microliter plate 5 times with 1 x wash buffer (300 µl each well). Strike the microliter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
- Dispense 100 µl of Chemiluminescence substrate reagent into each well. Gently mix for 10 seconds. Incubate at room temperature, in the dark, for 20 minutes.
- Stop the reaction by adding 100 µl of stock solution to each well. Gently mix for 10 seconds until the blue color completely changes to yellow.
- Read the optical density at 450 nm with a microliter plate reader within 15 minutes.

LIPID PROFILE

Total Cholesterol

by modified Roeschau`s Method[20, 21]

Principle

The estimation of cholesterol involves the following enzyme catalysed reactions.

1. Cholesterol ester $\xrightarrow{CE}$ cholesterol + Fatty acid

2. Cholesterol + O_2 $\xrightarrow{CHOD}$ cholest-4-en-one+H_2O_2

3. $2H_2O_2$ + 4AAP + phenol $\xrightarrow{POD}$ $4H_2O$+ Quinineimine

Procedure

Reagents	**Blank**	**Standard**	**Test**
Working reagent	1000µl	1000µl	1000µl
Distilled water	20µl	-----	-------
Standard	-------	20µl	
Test	-------	------	20µl

Table 10: Procedure for Cholesterol estimation

<u>Triglycerides</u>

This reagent is based on the method of *Wako* and the modified by *McGowan et a*l [22, 23,24,25]

Principle

The triglycerides are determined after enzymatic hydrolysis with lipases. The indicator for this is a quinoneimine formed from hydrogen-peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

Triglycerides + H_2O $\xrightarrow{LPL}$ Glycerol + Free fatty acids

Glycerol + ATP $\xrightarrow{GK}$ Glycerol-3-phospate + ADP

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{DAP} + H_2O_2$$

$$H_2O_2 + \text{4AAp} + \text{3,5-DHB} \xrightarrow{\text{Peroxidase}} \text{Quinone mine dye}$$

Reagents	**Blank**	**Standard**	**Test**
Working reagent	1000µl	1000µl	1000µl
Distilled water	10µl	-----	-------
Standard	-------	10µl	-----
Test	-------	------	10µl

Table 11: Procedure for Triglycerides

Mix and incubate for 10 min at 37^{o}. Read the absorbance of standard and each test at 505 nm (500-540nm) against reagent blank.

Calculation

$$\text{Triglycerides (mg/dl)} = \frac{\text{Abs of test}}{\text{Abs. of standard}} \mathbf{X} \text{ Conc. Of standard}$$

HDL Cholesterol

By Phospotungastic Acid Method, End point[26,27.]

Principle

Chylomicrons, LDL and VLDL (low and very low density lipoprotein) are precipitated from serum by phoshotungastate in the presence of divalent captions such as

magnesium. The HDL cholesterol remains unaffected in the supernatant and is estimated using ERBA cholesterol reagent. Serum / plasma phosphotungstate (HDL + LDL + chylomicrons).

$$\text{Serum/Plasma} \xrightarrow[Mg2+]{\text{Phosphotungstate}} \text{HDL} + (\text{LDL+VLDL+Chylomicrons})$$

$$\text{HDL Cholesterol} \xrightarrow[\text{CHOD}]{\text{CHE}} \text{Cholest-4-en-3-one} + H_2O_2$$

$$2H\,O + 4AAP + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine dye} + 4H\,O$$

Absorbance of quinoneimine is directly proportional to the HDL cholesterol concentration, when measured at 505nm.

Procedure

Reagents	Blank	Standard	Test
Cholesterol Working reagent	1000µl	1000µl	1000µl
Distilled water	50µl	-----	-------
Standard	-------	50µl	-----
Test	-------	------	50µl

Table 12: Procedure for HDL Cholesterol

Mix well; incubate for 10min. at 37°C, or 12 min. at 30° C. Read the absorbance of the standard and each test at 505nm.

Calculation

$$\text{HDL cholesterol} = \frac{\text{Abs of test}}{\text{Abs. of standard}} \times \text{Conc. Of standard} \times \text{dilution factor Abs. of Test}$$

$$\text{HDL cholesterol} = \frac{\text{Abs of test}}{\text{Abs. of standard}} \times 25 \times 3$$

$$\text{HDL cholesterol} = \frac{\text{Abs of test}}{\text{Abs. of standard}} \times 75$$

LDL Cholesterol[28]

LDL-C, usually called as 'bad cholesterol' transports cholesterol to the tissues and is linked to the development of cardiovascular diseases. Accurate measurement of LDL-C is therefore of very importance in therapies which focus on reduction of lipid content to reduce or prevent the progress of atherosclerosis and to avoid plaque rupture.

Calculation

$$\text{LDL} = \text{Total Cholesterol} - \text{HDL Cholesterol} - \frac{\text{Triglycerides}}{5}$$

Where [TG] / 5 is an estimate of VLDL-cholesterol.

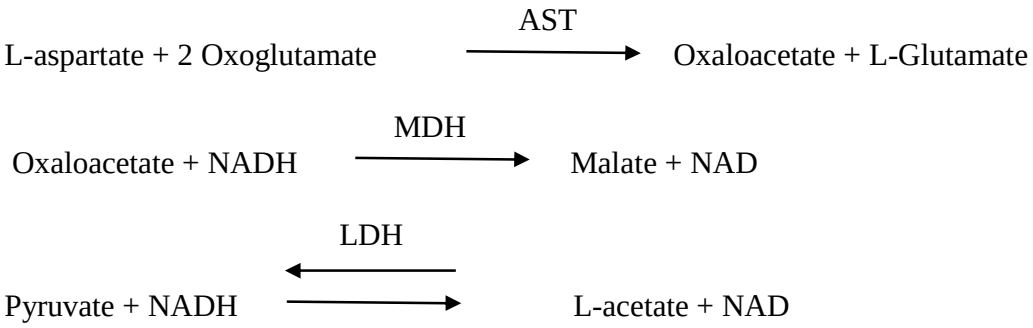
LIVER FUNCTION TESTS

Aspartate aminotransferase (AST) / Serum glutamate oxaloacetate transferase (SGOT)

By IFCC method [29,30.]

Aspartate aminotransferase (AST) also referred to as serum glutamate oxaloacetate transferase (SGOT) is an enzyme involved in amino acid metabolism. AST is widely distributed in liver, red blood cells, heart, muscles tissues, and pancreas and kidney. It is released into blood when cells that contain it are damaged. Both AST and ALT measurements are useful in the diagnosis of hepatocellular disease.

Principle



Specimen – Serum

Procedure

Dispense in tube as follows: working reagent 1000µl

Sample 50µl

Mix and incubate 60 seconds at 37^{o}C, then record of absorbance.

Perform other 3 readings at 60 seconds interval. Calculate the delta A/minute.

Calculation- Perform calculations in units per litre. Multiplying the delta A/min by the factor.

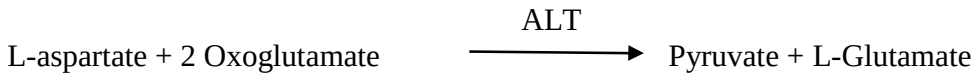
Activity in U/L = delta A/min X 3376

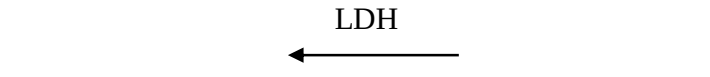
SI conversion factor: 1U/L X 0.017 = 1µ Kat /L

A**alanine aminotransferase (ALT) / Serum Glutamate pyruvate transaminase (SGPT)**

By IFCC Kinetic method[29, 31]

The enzyme alanine aminotransferase (ALT) also commonly called as serum Glutamate pyruvate transaminase (SGPT) is widely reported in a variety of tissue sources. The major source of ALT is of hepatic origin and has led to the application of ALT determinations to the study of hepatic diseases.





Specimen serum

Procedure

Dispense in tube as follows: working reagent 1000µl

Sample 50µl

Mix and incubate 60 seconds at 37°C, then record of absorbance.

Perform other 3 readings at 60 seconds interval. Calculate the delta A/minute.

Calculation

Perform calculations in units per litre. Multiplying the delta A/min by the factor.

Activity in U/L = delta A/min X 3376

SI conversion factor: 1U/L X 0.017 = 1µ Kat /L

Alkaline Phosphatase (ALP)

By King & King's Method[27, 32]

Alkaline phosphatase (ALP) catalyses the hydrolysis of a wide variety of non-physiological and physiological phosphoric acid esters in alkaline medium). The liver and biliary tract is the source of normal sera.

Principle:

Serum ALP hydrolyzes phenyl phosphate into phenol and disodium hydrogen phosphate at pH 10.0 the phenol so formed reacts with 4-Aminoantipyrine in presence of oxidizing agent potassium ferricyanide in alkaline medium to form a red colored complex whose absorbance is proportional to the enzyme activity.

$$\text{Phenyl phosphate} \xrightarrow[\text{pH 10}]{\text{ALP}} \text{Phenol + Phosphate}$$

Specimen serum

Procedure:

Pipette into clean dry tubes labeled Blank (B), Standard (S), Control (C) and Test (T) and add the reagents in the following order.

	B	S	C	T
Working Buffered Substrate	1.0ml	1.0ml	1.0ml	1.0ml
Deionized water	3.1ml	3.0ml	3.0ml	3.0ml
Incubate at 3 min at 37^{o}C				
Serum	--------	--------	----------	0.1ml
Phenol Standard	--------	0.1ml	----------	--------
Incubate for 15 minutes at 37°C				
Color Reagent	2.0ml	2.0ml	2.0ml	2.0ml
Serum	--------	--------	0.1ml	-------

Table 13: Procedure for ALP estimation

Mix well after each addition of reagent and measure absorbance (A) for Blank (B), Standard (S), Control (C) and Test (T) against deionized water on photocolorimeter using a green filter or on spectrophotometer at 510 nm.

Calculations:

$$\text{Serum ALP in KA units/dl} = \frac{A(T)-A(C)}{A(S)-A(B)} \times 10 \text{ (Std. Conc.)}$$

1KA unit/dl =7.1 U/l.

4.7.4. Histopathology

Manual tissue processing

Procedure is followed as per the Bancroft procedure[33]

Fixation: Fixation is the first step towards the preparation of a histological section from a dead biological specimen. The substances used for fixation are called as fixatives. Tissue will be kept in fixative for 24hrs.

Common fixatives - Formalin, Zenker`s fluid.

Washing: After fixation tissue is washed under running tap water one to two hours. It removes fixative from tissue.

Dehydration, clearing and Impregnation: Our aim is imbibe tissue with paraffin; this cannot be done directly because paraffin is not mixable with water of tissue. Dehydration is done in stages, first the tissue is immersed in alcohol which replaces water of tissue (dehydration). Paraffin is not mixable with alcohol also; Xylene benzene, chloroform and toluene are substances which get mixed both the alcohol and paraffin therefore after alcohol the tissue is treated with one of these liquids (called clearing agents) and then tissue is impregnated in melted paraffin. Following is the list of time for manual tissue processing.

Step	Treatment	Hours
Fixation	Formalin (10%)	24
Dehydration	30%	1
	50%	1
	80%	1
	90%	1
	100%	1
	100%	1
	100%	1
Clearing	Xylene	1
	Xylene	1
Infiltration	Paraffin at 50-56^{o}C	1
	Paraffin at 50-56^{o}C	1
	Paraffin at 50-56^{o}C	1

Table 14: Procedure for manual tissue processing

Embedding: The process of embedding enables specimens too small and / or too delicate to the surrounded with some suitable material example paraffin that will support them on all sides with firmness but without producing any injurious effect on the specimen the embedded tissue may then be section into sufficiently thin slices without distortion.

Preparation of Blocks: It is done with the help of L-blocks; L-blocks are L-shaped metallic pieces. Two L-blocks are placed on glass plate, so that enclose a rectangular

space. The tissue is put in it over which the melted paraffin is poured which solidifies slowly. The L-blocks are removed.

Section cutting: For histological preparation tissue cut (sectioned) into thin slices trim the upper or cutting surface of the block parallel to the knife edge until the surface of the tissue get exposed. Attach the block holder to the microtome. Clamp the knife firmly in position in microtome. Adjust the desired thickness scale in the microtome. Move the microtome fairly constant and fast. Pick up the end of short ribbon with a soft brush and gently pull it away from the knife as you continue sectioning.

Hematoxylin and Eosin Staining

1. Xylene 2 Minutes
2. Xylene 2 Minutes
3. Absolute alcohol 1 Minutes
4. Absolute alcohol 1 Minutes
5. 90% Alcohol 2 Minutes
6. 70% Alcohol 2 Minutes
7. 50% Alcohol 2 Minutes
8. Distilled water 5 Minutes
9. Hematoxylin 2 to 5minutes depending on which of the above types of Hematoxylin Solution is used (Stain for 20 minutes with Erlich`s and for 2-5 minutes with Harris` Hematoxylin.
10. Wash well in running tap water for 2-3 minutes section may be examined with microscope to confirm a sufficient degree of staining.

11. Remove excess stain by differentiation in acid alcohol (1% HCL in 70% alcohol) for a few seconds. Blue staining of hematoxylin stained section is changed to red by action of acid.
12. Immediately wash in alkaline tap water for at least 5 minutes to regain the blue colour (`bluing section`) (a few drops of saturated lithium carbonate may be added to the tap water in a large beaker and bluing of section done in this. After bluing, the slides should be thoroughly washed in running tap water to remove excess lithium carbonate). If necessary, examine microscopically.
13. 1 % aqueous Eosin 1 to 3 minutes
14. Wash the surplus eosin water
15. Examine the sections microscopically. Cytoplasm and muscle cell should be deep pink, collagen fibers a lighter pink, erythrocytes and eosinophil granules should be bright orange red.
16. 90% alcohol for 10 to 15 min
17. Absolute alcohol I for 10 to 15 seconds
18. Absolute alcohol II for 10 to 15 seconds
19. Xylene I – 1 to 2 min
20. Xylene I – 1 to 2 min or until completely clear
21. Mount in D.P.X. or any other synthetic resin medium.

Results

Muscle cell – deep pink, Collagen fibres – lighter pink, erythrocytes and Eosinophil granules – bright orange red, Cytoplasm – pink, Nuclei – blue of blue –back.

4.8. Grouping of Animals

Laboratory bred adult albino Wister rats weighing between 175 - 250gm were used in the study. The acclimatized animals were divided into six groups of six rats each. Group I rats were normal control rats; group II were normal rats treated with Nigella sativa seed powder (300mg/kg body weight); group III were normal rats treated with thymoquionone(4mg/kg body weight); group IV were streptozotocin induced diabetic control rats; group V were streptozotocin induced diabetic rats treated with Nigella sativa seed powder(300mg/kg body weight) and group VI were streptozotocine induced diabetic rats treated with thymoquinone(4mg/kg body weight).

4.9. Statistical Analysis

Data obtained from all control and experimental samples has been subjected to statistical analysis for evaluating the range of significance. After collecting all data was filled in Microsoft excel, data was presented with tables and diagrams. Mean $\pm$ SD (standard deviation) values were calculated for each group.

To determine the significance of inter-group differences, each parameter will be analysed separately. All the parameters were compared by using proper statistical tests using software SPSS V 20 32bit, values were analysed by one-way analysis of variance (ANOVA) using post hoc Turkey HSD test to study the differences between the groups. The level of statistical significance was set at $P<0.05$.

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CHAPTER 5

RESULTS

5.1. Gravimetry

5.1.1. Weight gain

Regular weight of the rat was recorded during dosage period. Initial and final weights were considered to calculate the weight gain. All the rats in group I, II and III remained healthy and active with normal behaviour. However, rats of group IV acted as diabetic control, where the diabetic induced without any treatment were found to be lethargic and their body weight gain was found to be negative among all groups. This was statistically significant when compared with normal control rats, normal rats treated with Nigella sativa seed powder, normal rats treated with thymoquinone, diabetic induced rats treated with Nigella sativa seed powder and diabetic induced rats treated with thymoquinone. However, in case of group V(diabetic induced rats + Nigella sativa seed powder) and group VI (diabetic induced rats + thymoquinone) an improvement in body weight was observed, as compared with the group IV (diabetic induced control) rats (Table 15).

5.1.2. Organosomatic Index

Organosomatic index was calculated by weight of organ multiplied by 100 and divided by total body weight.

Pancreas – Pancreaticosomatic index was increased significantly in diabetic untreated rats compared with normal control rats, in diabetic treated rat groups it was decreased, but no statistical significance change was found between diabetic untreated and diabetic treated groups (Table 16).

Liver - Hepatosomatic index was increased significantly in diabetic untreated rats compared with normal control rats, in diabetic treated rat groups it was decreased and it was statistically significance change (Table 16).

Kidney – Renalsomatic index was increased significantly in diabetic untreated rats compared with normal control group, in diabetic treated rat groups it was decreased, but no statistically significance change was found between diabetic untreated and diabetic treated groups (Table 16).

Groups ⇨	**I**	**II**	**III**	**IV**	**V**	**VI**		
Parameter ⇩	**Normal Rats– Control**	**Normal Rats – Nigella sativa seed powder**	**Normal Rats – Thymo quinone**	**Diabetic Rats– Control**	**Diabetic Rats – Nigella sativa seed powder**	**Diabetic Rats – Thymo quinone**	**F - Value**	**P- Value**
Initial Body weight (g)	234.67± 11.55[a]	225.67± 16.62[a]	229.17± 13.75[a]	173.17± 8.18[b]	164.67± 10.95[b]	168.83± 14.54[b]	40.79	0.05
Final Body weight (g)	345.50± 15.83[a]	339.17± 11.02[a]	332.82± 20.35[a]	144.17± 9.17[b]	225.17± 13.08[c]	241.33± 20.52[c]	162.89	0.001
Change in Body Weight(%)	47.72± 12.97[a]	51.15± 14.62[a]	45.66± 12.00[a]	-16.65± 5.49[b]	37.28± 12.40[a,c]	43.23± 9.73[a,c]	29.32	0.003
Values with superscripts in each row among various groups are statistically significant with each other(P<0.05)								

Table 15: Results of initial, final body weight & weight gain in each group

Groups ⇨	I	II	III	IV	V	VI		
Parameter ⇩	Normal Rats– Control	Normal Rats – Nigella sativa seed powder	Normal Rats – Thymo quinone	Diabetic Rats– Control	Diabetic Rats – Nigella sativa seed powder	Diabetic Rats – Thymo quinone	F - Value	P- Value
Pancreaticosomatic index (%)	0.18± 0.02[a]	0.21± 0.03[a]	0.20± 0.04[a]	0.34± 0.07[b]	0.27± 0.04[b]	0.26± 0.08[b]	7.55	0.04
Hepatosomatic index (%)	2.14± 0.14[a]	2.08± 0.16[a]	2.25± 0.21[a]	3.81± 0.27[b]	3.15± 0.32[c]	2.86± 0.23[c]	54.3	0.002
Renalsomatic index (%)	0.43± 0.06[a]	0.39± 0.05[a]	0.41± 0.07[a]	0.58± 0.04[b]	0.49± 0.12[b]	0.46± 0.07[b]	5.07	0.02
Values with superscripts in each row among various groups are statistically significant with each other (P<0.05).								

Table 16: Results of organosomatic index of Pancreas, Liver and Kidney.

5.2. Biochemistry

5.2.1. Glucose - Levels of glucose were increased significantly in diabetic control (group IV) rats compared with normal control (group I) rats, normal rats treated with Nigella sativa seed powder (group II) and thymoquinone (group III). The levels of glucose were reduced significantly in diabetic rats treated with Nigella sativa seed powder (group V) and thymoquinone (group VI). There was no significant change between normal control (group I) rats and normal rats treated with Nigella sativa seed powder (group II) and thymoquinone (group III) (Table 17).

Groups ⇨	I	II	III	IV	V	VI		
Parameter ⇩	Normal Rats– Control	Normal Rats – Nigella sativa seed powder	Normal Rats – Thymo quinone	Diabetic Rats– Control	Diabetic Rats – Nigella sativa seed powder	Diabetic Rats– Thymo quinone	F - Value	P- Value
Initial Glucose (mg/dl)	88.83± 11.72[a]	79.83± 15.11[a]	84.33± 18.61[a]	215.50± 32.04[b]	211.83± 14.84[b]	220.17± 28.27[b]	67.93	0.05
Final Glucose (mg/dl)	86.33± 14.61[a]	83.17± 18.48[a]	79.67± 13.22 [a]	241.33± 40.73[b]	177.67± 33.79[c]	162.33± 25.41[c]	38.02	0.003
Values with superscripts in each row among various groups are statistically significant with each other(P<0.05)								

Table 17: Results of Glucose(mg/dl)

5.2.2. Insulin

Levels of insulin were decreased significantly in diabetic control (group IV) rats compared with normal control (group I) rats, normal rats treated with Nigella sativa seed powder (group II) and thymoquinone (group III). The levels of insulin were increased significantly in diabetic rats treated with Nigella sativa seed powder (group V) and thymoquinone (group VI). There was no significant change between normal control (group I) rats and normal rats treated with Nigella sativa seed powder (group II) and thymoquinone (group III) (Table 18).

Groups ⇨	I	II	III	IV	V	VI		
Parameter ⇩	Normal Rats– Control	Normal Rats – Nigella sativa seed powder	Normal Rats – Thymo quinone	Diabetic Rats– Control	Diabetic Rats – Nigella sativa seed powder	Diabetic Rats – Thymo quinone	F - Value	P- Value
Insulin (mu/L)	0.44± 0.14[a]	0.41± 0.06[a]	0.48± 0.11[a]	0.16± 0.05[b]	0.30± 0.04[c]	0.33± 0.06[c]	12.13	0.001
Values with superscripts in each row among various groups are statistically significant with each other(P<0.05)								

Table 18: Results of Insulin (mu/L)

5.2.3. Oral Glucose Tolerance Test (OGTT)

Diabetic control rats (group IV) did not show tolerance to Oral Glucose Tolerance Test compared with normal control (group I) rats, normal rats treated with Nigella sativa seed powder (group II) and thymoquinone (group III). Diabetic rats treated with Nigella sativa seed powder (group V) and thymoquinone (group VI) showed significant improvement in tolerance to Oral Glucose Tolerance Test. There was no significant change in tolerance to Oral Glucose Tolerance Test between normal control (group I) rats and normal rats treated with Nigella sativa seed powder (group II) and thymoquinone (group III) (Figure 3).

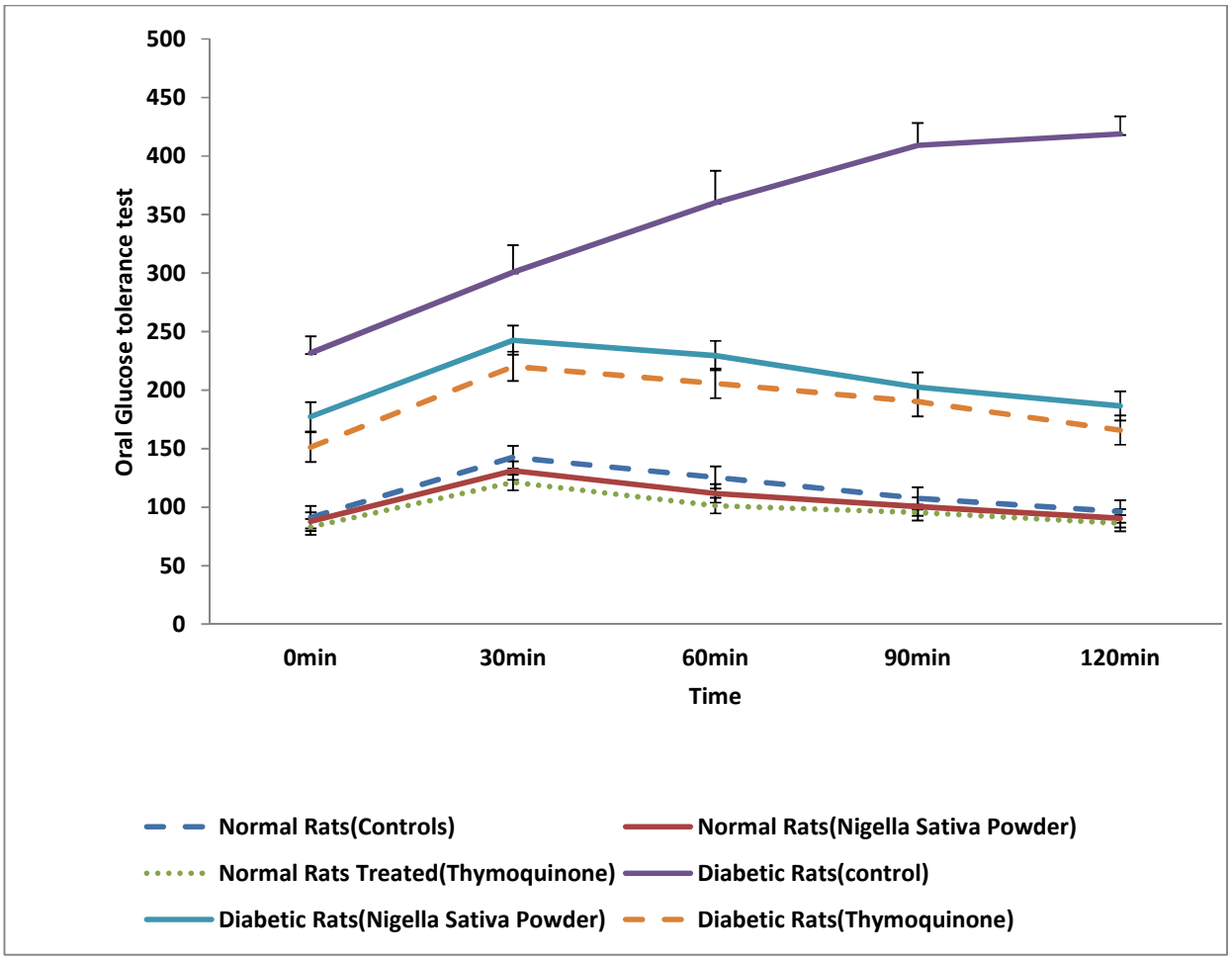

Figure 3: Graph – OGTT

[a]FBS: Fasting blood suger

[b,c,d,e] Horizontal values are the mean±SD of six observations in each group in every 0.5h interval till 2.0h in each row. Values with different superscription (a,b,c,d) are different significantly from each other ($p<0.05$). One way ANOVA of different groups in every 0.5h interval till 2.0h is referred below vertically.

5.2.4. Serum Malondialdehyde (MDA)

The MDA levels were increased significantly in diabetic control (group IV) rats compared with normal control (group I) rats, normal rats treated with Nigella sativa seed powder (group II) and thymoquinone (group III). The levels of MDA were reduced significantly in diabetic rats treated with Nigella sativa seed powder (group V) and thymoquinone (group VI). There was no significant change between normal control (group I) rats and normal rats treated with Nigella sativa seed powder (group II) and thymoquinone (group III) (Figure 4).

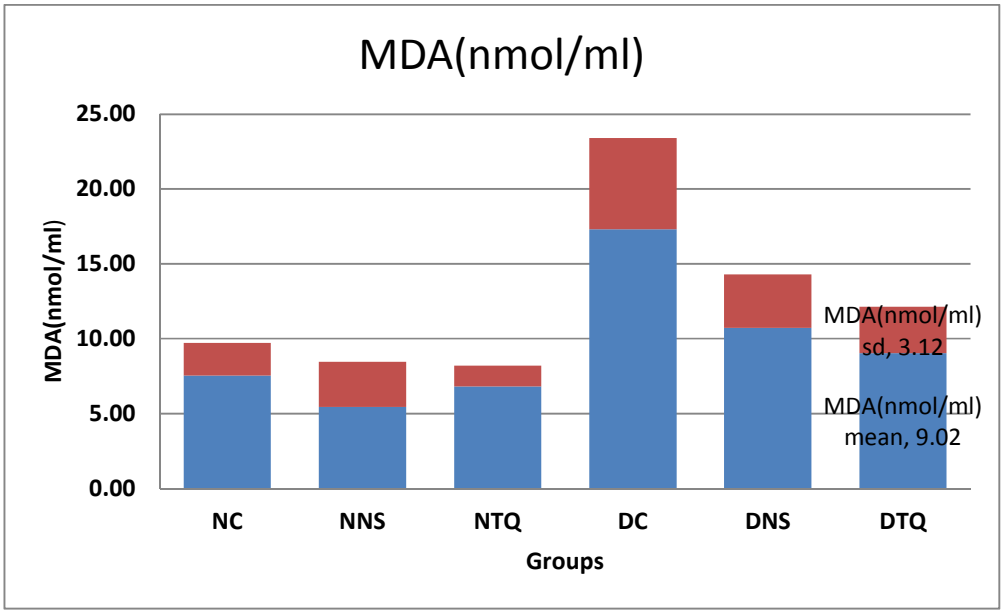

Figure 4: Graph – MDA (nmol/ml)

NC- Normal Control Rats, NNS- Normal rats treated with Nigella sativa, NTQ- Normal rats treated with Thymoquinone, DC- Diabetic Control Rats, DNS- Diabetic rats treated with Nigella sativa, DTQ- Diabetic rats treated with Thymoquinone.

5.2.5. Endogenous antioxidant - Serum Superoxide dismutase (SOD),

Exogenous antioxidants – Serum Vitamin C and E

The levels of Superoxide dismutase (SOD), Vitamin C and E were decreased significantly in diabetic control (group IV) rats compared with normal control (group I) rats, normal rats treated with Nigella sativa seed powder (group II) and thymoquinone (group III). The levels of Superoxide dismutase (SOD), Vitamin C and E increased significantly in diabetic rats treated with Nigella sativa seed powder (group V) and thymoquinone (group VI). There was no significant change between normal control (group I) rats and normal rats treated with Nigella sativa seed powder (group II) and thymoquinone (group III) (Figure 5,6,7).

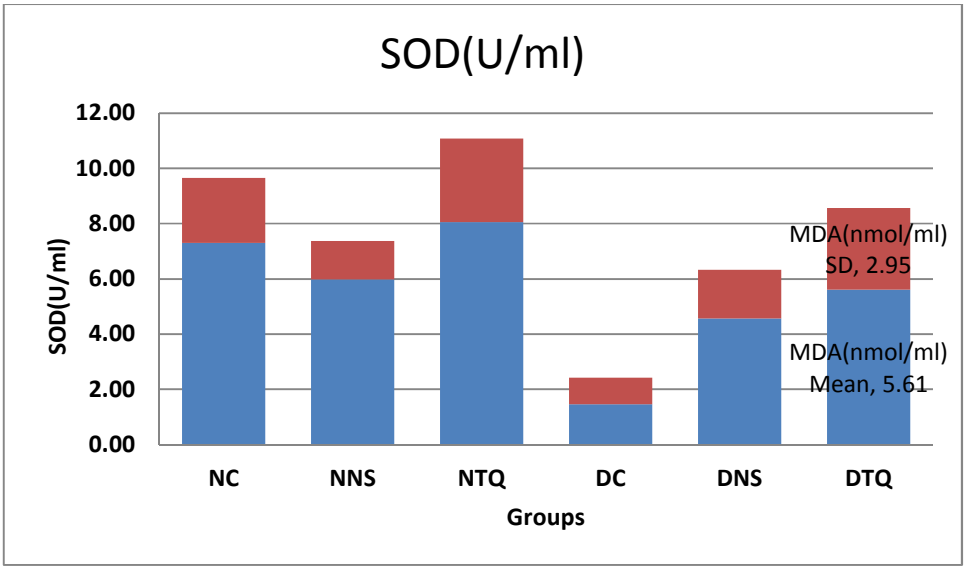

Figure 5: Graph – SOD(U/ml)

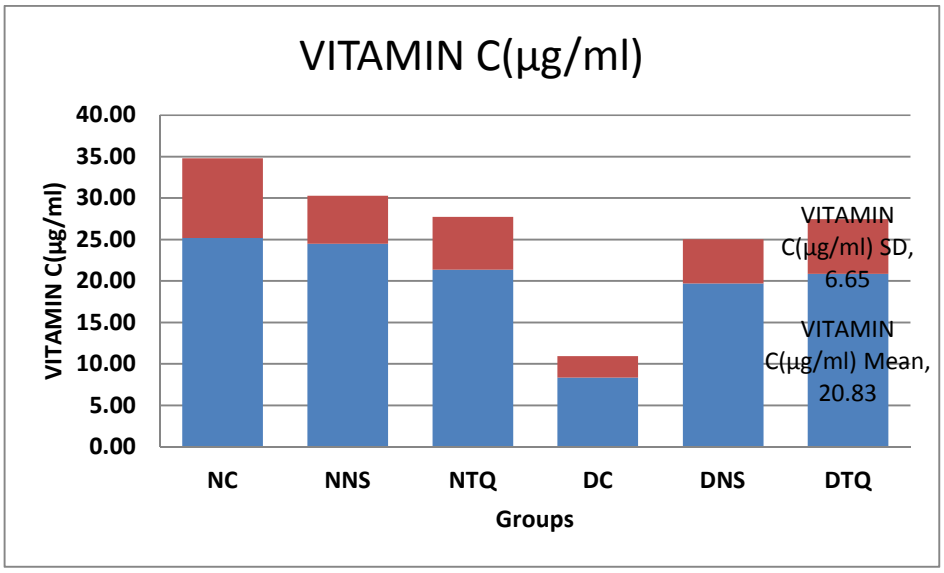

Figure 6: Graph – Vitamin C (µg/ml)

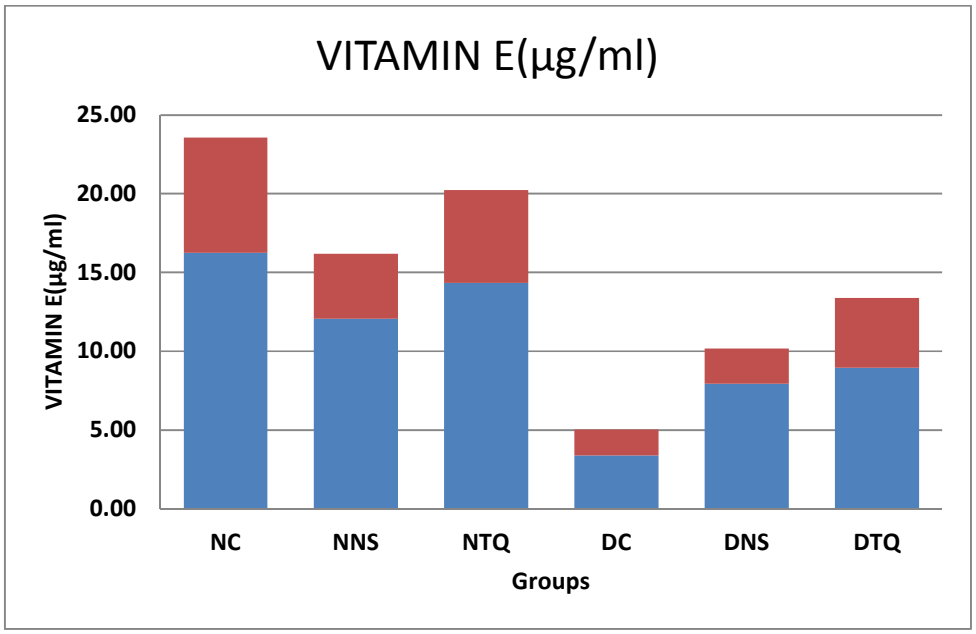

Figure 7: Graph – Vitamin E (µg/ml)

5.2.6. Lipid profile

In case of Lipid profile parameters the levels of HDL –C was decreased significantly in diabetic control (group IV) rats, compared with normal control (group I) rats, normal rats treated with Nigella sativa seed powder (group II) and thymoquinone (group III). The levels of HDL –C were increased significantly in diabetic rats treated with Nigella sativa seed powder (group V) and thymoquinone (group VI). There was no significant change between normal control (group I) rats and normal rats treated Nigella sativa seed powder (group II) and thymoquinone (group III). The levels of TG, TC, LDL –C and VLDL –C were increased significantly in diabetic control rats compared with normal control (group I) rats, normal rats treated with Nigella sativa seed powder(group II) and thymoquinone (group III). The levels of TG, TC, LDL –C and VLDL –C were decreased significantly in diabetic rats treated with Nigella sativa seed powder (group V) and thymoquinone (group VI). There was no significant change between normal control (group I) rats and normal rats treated Nigella sativa seed powder (group II) and thymoquinone (group III) (Table 19).

Groups ⇨	I	II	III	IV	V	VI		
Parameter ⇩	Normal Rats– Control	Normal Rats – Nigella sativa seed powder	Normal Rats – Thymo quinone	Diabetic Rats– Control	Diabetic Rats – Nigella sativa seed powder	Diabetic Rats – Thymo quinone	F - Value	P- Value
HDL (mg/dl)	36.83± 8.95[a]	38.17± 17.36[a]	41.17± 13.92[a]	14.17± 5.38[b]	28.50± 8.34[c]	30.67± 11.33[c]	7.19	0.002
TG (mg/dl)	128.83± 25.89[a]	106.83± 13.59[a]	122.7± 20.91[a]	204.33± 36.97[b]	162.17± 30.97[c]	151.83± 16.55[c]	12.85	0.001
TC (mg/dl)	139.17± 29.18[a]	126.67± 25.91[a]	118.50± 19.60[a]	255.33± 43.36[b]	197.17± 21.96[c]	173.67± 23.24[c]	18.38	0.003
LDL (mg/dl)	76.57± 27.70[a]	67.13± 24.12[a]	52.90± 17.86[a]	199.63± 42.99[b]	136.57± 36.30[c]	112.63± 23.45[c]	20.24	0.005
VLDL (mg/dl)	25.77± 5.18[a]	21.37± 2.72[a]	24.43± 4.18[a]	40.87± 7.39[b]	32.43± 4.39[c]	30.37± 3.31[c]	12.85	0.001
Values with superscripts in each row among various groups are statistically significant with each other(P<0.05)								

Table 19: Results of HDL, TG, TC, LDL and VLDL Cholesterol (mg/dl).

5.2.7. Liver function tests

In case of Liver function tests the levels of serum AST, ALT and ALP were increased significantly in diabetic control rats (group IV), compared with normal control (group I) rats, normal rats treated with Nigella sativa seed powder (group II) and thymoquinone (group III). The levels of AST, ALT and ALP were decreased significantly in diabetic rats treated with Nigella sativa seed powder (group V) and thymoquinone (group VI). There was no significant change between normal control (group I) rats and normal rats treated with Nigella sativa seed powder (group II) and thymoquinone (group III). The levels of total protein were decreased significantly in diabetic control rats (group IV), compared with normal control (group I) rats, normal rats treated with Nigella sativa seed powder (group II) and thymoquinone (group III). The levels of total protein were increased significantly in diabetic rats treated with Nigella sativa seed powder (group V) and thymoquinone (group VI). There was no significant change between normal control (group I) rats and normal rats treated Nigella sativa seed powder (group II) and thymoquinone (group III) (Table 20).

Groups ⇨	I	II	III	IV	V	VI		
Parameter ⇩	**Normal Rats– Control**	**Normal Rats – Nigella sativa seed powder**	**Normal Rats – Thymo quinone**	**Diabetic Rats– Control**	**Diabetic Rats – Nigella sativa seed powder**	**Diabetic Rats – Thymo quinone**	**F - Value**	**P-Value**
Total Protein(g/dl)	10.03± 2.07 [a]	8.45± 0.79[a]	9.30± 1.58[a]	3.80± 0.74[b]	6.47± 0.97[c]	7.02± 1.55[c]	16.24	0.01
AST (IU/L)	83.83± 11.05[a]	79.17± 7.99[a]	74.67± 9.24[a]	153.33± 13.71[b]	125.67± 9.71[c]	106.50± 15.69[c]	44.16	0.003
ALT (IU/L)	53.67± 10.61[a]	45.33± 6.28[a]	48.67± 9.44[a]	96.50± 15.62[b]	73.33± 10.46[c]	65.33± 8.02[c]	20.23	0.006
ALP (IU/L)	69.50± 13.41[a]	56.67± 7.50[a]	62.67± 10.32[a]	129.67± 17.37[b]	97.67± 11.69[c]	85.17± 13.67[c]	27.37	0.002
Values with superscripts in each row among various groups are statistically significant with each other(P<0.05)								

Table 20: Results of Liver Function Tests (Total Protein, AST, ALT, ASP)

5.3. Histopathology

5.3.1. Histopathology of Pancreas

Normal control rats (group I): Sections studied under H&E stain showed the normal islet cells with classical histological features. Cells of the pancreas were all present in their usual proportions. The acinar cells with prominent nuclei strongly

stained were arranged in lobules. The islet cells were seen surrounded by a fine capsule. The islet of Langerhans showed the clear β- cells with nuclei (Figure 8,14).

Normal rats treated with Nigella sativa seed powder (group II): Sections studied under H&E stain showed the same architecture as normal control rats. There were no pathological changes (Figure 9).

Normal rats treated with thymoquinone (group III): Sections studied under H&E stain showed the same architecture as normal control rats. There were no pathological changes(Figure 10).

Diabetic control rats (Group IV): Sections studied under H&E stain showed the degenerative and necrotic changes and shrinking of the islets of Langerhans. The nucleus showed either marginal hyperchromasia or pyknosis. There was mostly hydropic degeneration and degranulation in the cytoplasm, while some of the cells had a dark eosinophilic cytoplasm (Figure 11,15).

Diabetic rats treated with Nigella sativa seed powder (Group V): Section studied under H&E stain showed the decreased severity of necrotic and degenerative changes in the islets of pancreas. The parenchyma was more in amount than in the diabetic group. A few cells were with pyknotic nuclei, but the majority of cells showed significantly light hydropic degeneration, partly indicating regranulation of islet cells. The islets of Langerhans were distinctly increased in size (Figure 12,16).

Diabetic rats treated with thymoquinone (group VI): Sections studied under H&E stain showed the same features as diabetic rats treated with Nigella sativa seed powder (Group V) (Figure 13,17).

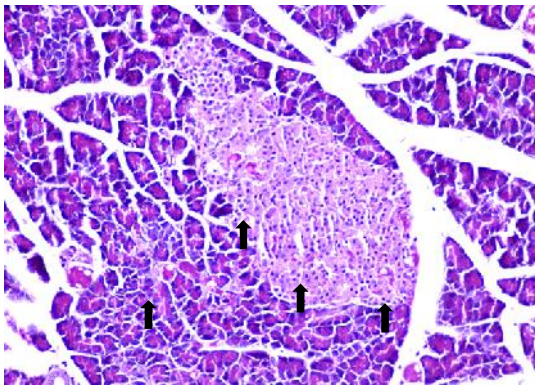

Figure 8: Pancreas of normal control rats showing normal acinar cells and Islet of Langerhans (H&E10X)

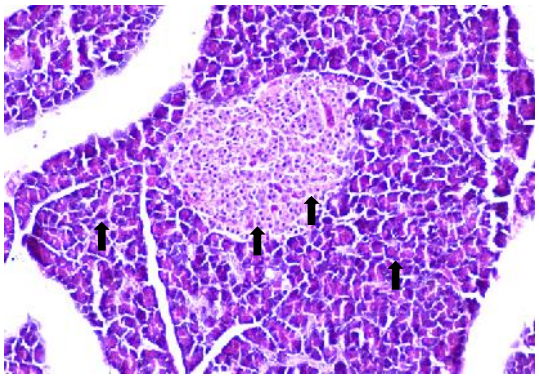

Figure 9: Pancreas of normal rats treated with Nigella sativa seed powder showing normal acinar cells and Islet of Langerhans (H&E -10X)

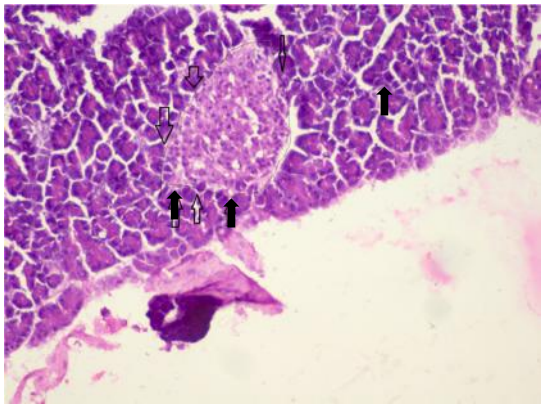

Figure 10: Pancreas of normal rats treated with thymoquinone seed powder showing normal acinar cells and Islet of Langerhans (H&E -10X)

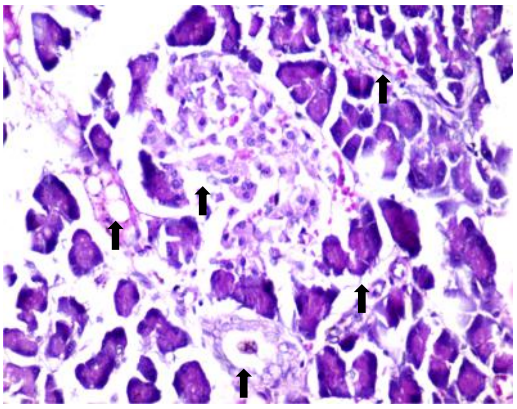

Figure 11: Pancreas of STZ diabetic control rats showing the degenerative changes in islet of Langerhans (H&E -10X)

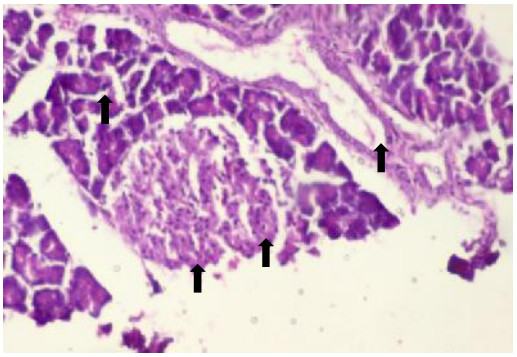

Figure 12: Pancreas of STZ diabetic rats treated with Nigella sativa seed powder showing few diabetic changes and improved architecture of islet of Langerhans (H&E-10X).

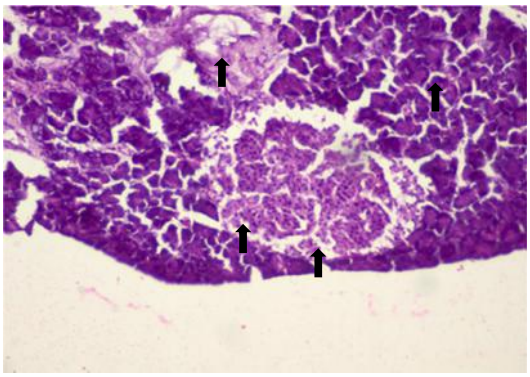

Figure 13: Pancreas of STZ diabetic rats treated with thymoquinone showing few diabetic changes and improved architecture of islet of Langerhans (H&E-10X).

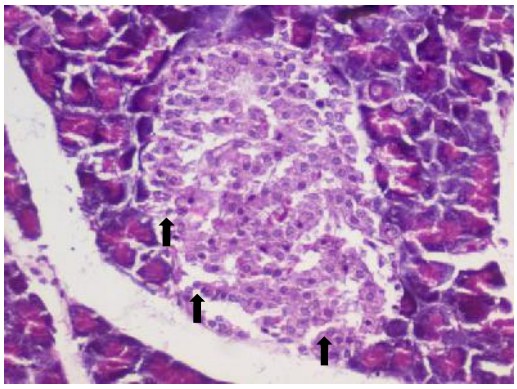

Figure 14: Pancreas of normal control rats showing normal acinar cells and Islet of Langerhans (H&E-20X)

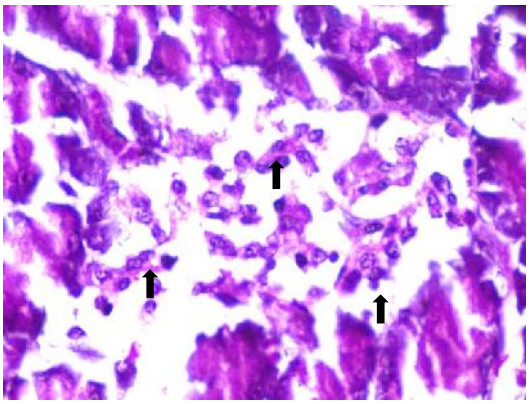

Figure 15: Pancreas of STZ diabetic control rats showing the degenerative changes in islet of Langerhans (H&E -20X).

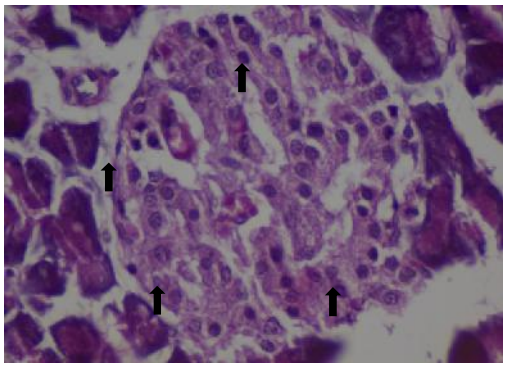

Figure 16: Pancreas STZ diabetic rats treated with Nigella sativa seed powder showing few diabetic changes and improved architecture of islet of Langerhans (H&E -20X)

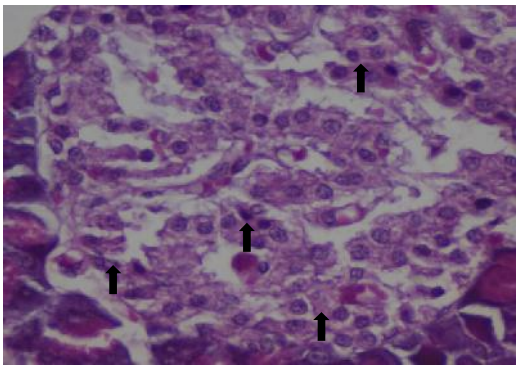

Figure 17: Pancreas of STZ diabetic rats treated with thymoquinone showing few diabetic changes and improved architecture of islet of Langerhans (H&E -20X).

5.3.2. Histopathology of Liver

Normal control rats (group I): Sections studied under H&E stain showed normal parenchymal tissue composed of numerous hexagonal to pyramidal lobules. Each lobule consisted of central vein from which the hepatic plates radiated outwards. 3 to 5 portal triads were located at the periphery of the lobule, containing branches of bile duct, portal vein and hepatic artery and occasional mononuclear cells. The sinusoids were lined by both endothelial cells and Kupffer cells both of which with inconspicuous

flattened nuclei and ill-defined cytoplasmic margins. The central veins were lined by endothelial cells surrounded by ring of collagen fibres. The hepatocytes were polygonal in shape with well-defined borders. The nucleus was single, round and has a fine chromatin pattern with 1 to 2 clearly defined amphiphilic prominent nucleoli. The cytoplasm was eosinophilic and finely granular (Figure 18,24).

Normal control rats treated with Nigella sativa seed powder (group II): Section studied under H&E stain showed the same architecture as normal control rats. There were no pathological changes (Figure 19).

Normal control rats treated with thymoquinone (group III): Section studied under H&E stain showed the same architecture as normal control rats. There were no pathological observations (Figure 20).

Diabetic control rats (Group IV): Section studied under H&E stain showed distortion in the arrangement of cells around the central vein, periportal fatty infiltration (fatty steatosis) with focal necrosis of hepatocytes, hydropic changes and aggregation and infiltration of lymphocytes between hepatocytes (Figure 21,25).

Diabetic rats treated with Nigella sativa seed powder (Group V): Sections studied under H&E stain showed the normal cellular arrangement around the central vein and reduced necrotic changes and the blood vessels in normal condition (Figure 22,26).

Diabetic rats treated with thymoquinone (group VI): Section studied under H&E stain showed the same features as diabetic rats treated with Nigella sativa seed powder (Group V) (Figure 23,27).

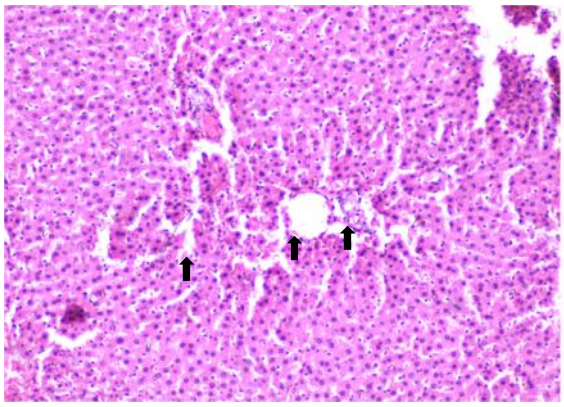

Figure 18: Liver of normal control rat showing normal architecture with central vein, hepatocytes and hepatic sinusoids (H&E-10X).

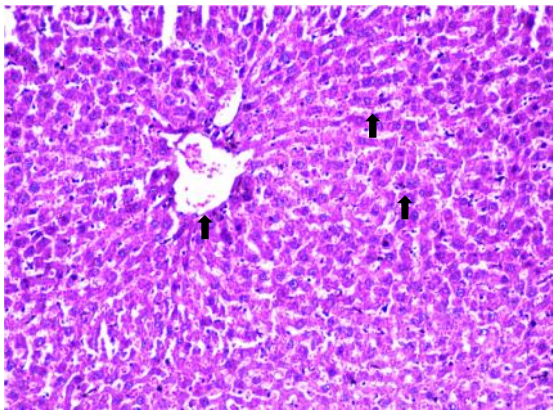

Figure 19: Liver of normal rats treated with Nigella sativa seed powder showing normal architecture with central vein, hepatocytes and hepatic sinusoids (H&E 10X).

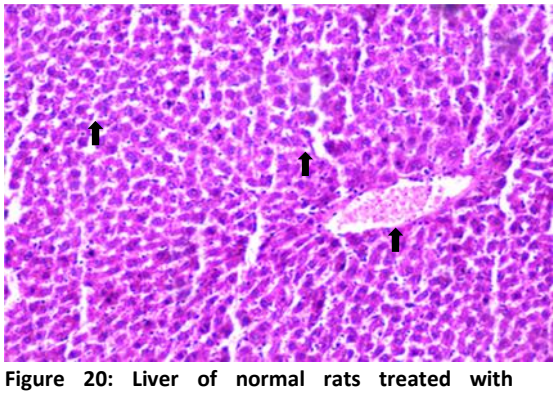

Figure 20: Liver of normal rats treated with thymoquinone showing normal architecture with central vein, hepatocytes and hepatic sinusoids (H&E -10X).

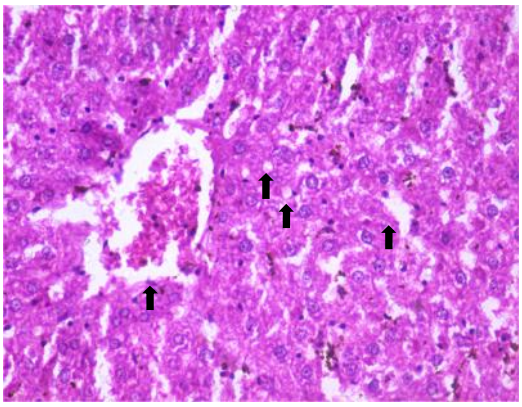

Figure 21: Liver of STZ diabetic control rat showing distortion in the arrangement of cells around the central vein, and fatty steatosis (H&E -10X).

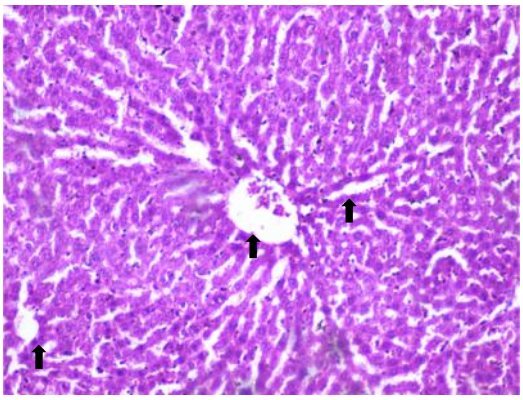

Figure 22: Liver sections of diabetic rats treated with Nigella sativa seed powder showing normal cellular arrangement around the central vein and reduced necrotic changes (H&E10X).

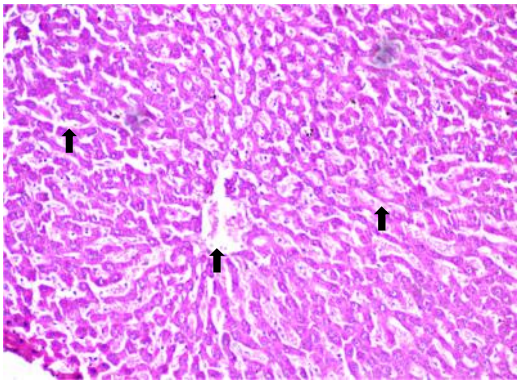

Figure 23: Liver sections of diabetic rats treated with thymoquinone showing normal cellular arrangement around the central vein and reduced necrotic changes (H&E 10X).

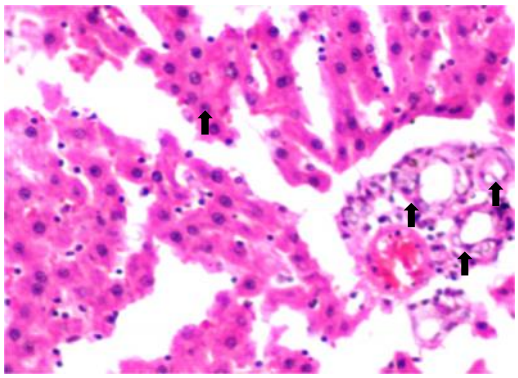

Figure 24: Liver section of normal control rat showing normal architecture of portal triad (H&E-20X).

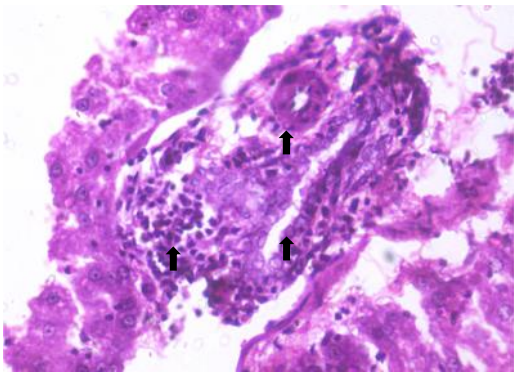

Figure 25: Liver section of STZ diabetic control rat showing destructed architecture of portal triad with monocyte infiltration (H&E-20X).

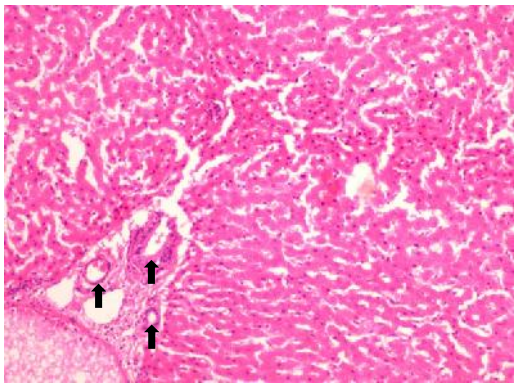

Figure 26: Liver section of STZ diabetic rat treated with Nigella sativa showing the portal triad as near to normal (H&E 20X).

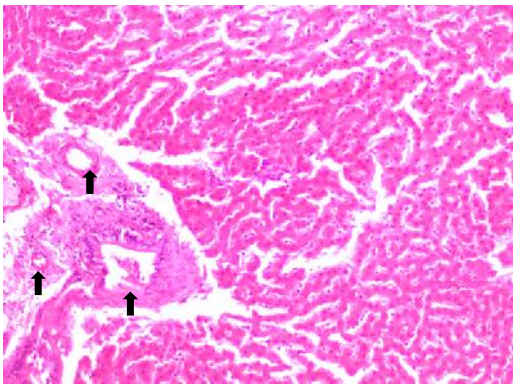

Figure 27: Liver section of STZ diabetic rat treated with thymoquinone showing the portal triad as near to normal (H&E 20X).

5.3.3. Histopathology of Kidney

Normal control rats (group I): Sections studied under H&E stain showed normal renal parenchymal tissue composed of glomeruli and tubules separated by small amount of interstitial connective tissue containing peritubular capillaries. Each glomerulus was a spherical collection of interconnected capillaries within Bowman`s space lined by flattened parietal cells. The outer aspects of the glomerular capillaries

were covered by a layer of visceral epithelial cells (podocytes). The capillary tufts were supported by the mesangial cells. Tubules and glomeruli appear normal in morphology and in cellularity, interstitium appears normal, vessel appear normal (Figure 28,34).

Normal control rats treated with Nigella sativa seed powder and thymoquinone (group II and III): Sections studied under H&E stain showed the same architecture as normal control rats. There were no pathological observations (Figure 29,30,35,36).

Diabetic control rats (Group IV): Sections studied under H&E stain showed deposits of hyaline material diffuse and evenly spread in the mesengium of the glomeruli. There was a diffuse infiltration of the glomerular tuft with eosinophilic material and also heavy focal deposition. The diffuse infiltrate appeared to be in the basement membranes of the capillaries and the capillary bed had been obliterated and this deposition caused complete hyalinization of many glomeruli. In many of the glomeruli, lesions characteristic of diabetic glomerulosclerosis were present and these glomeruli consisted of round, practically acellular, hyalinized nodule in their tuft. There was a considerable deposit of hyaline material thickening in Bowman's capsule. Both afferent and efferent arterioles appeared as narrow ovals and also showed strong eosinophilic hyaline thickening. In most of the sections, areas of lymphocytic infiltrate were seen in the interstitium (Figure 31,37).

Diabetic rats treated with Nigella sativa seed powder (Group V) and thymoquinone (group VI): Sections showed features of normal glomerulus, very less number of inflammatory cells, normal basement membrane and capillaries and decrease in the hyaline deposit (Figure 32,33,38,39).

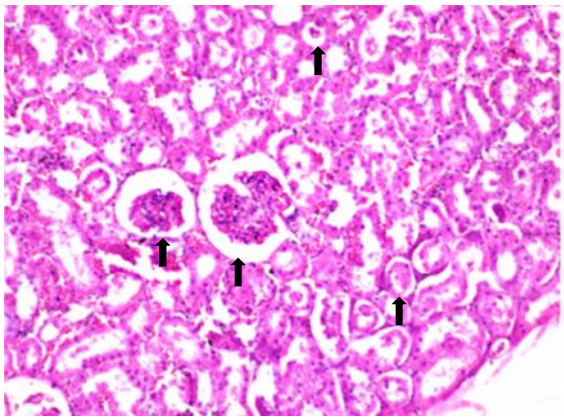

Figure 28: Kidney section of normal control rat showing normal renal parenchymal tissue which is composed of glomeruli and tubules (H&E-10X).

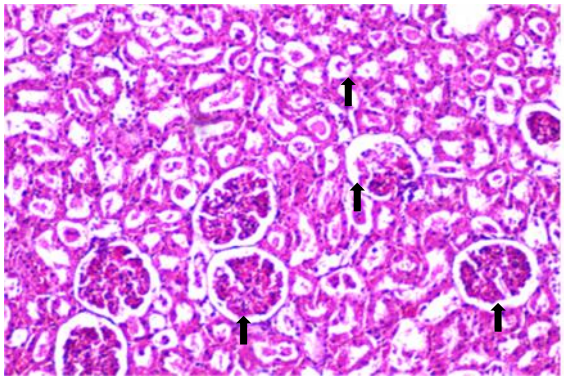

Figure 29: Kidney section of normal rat treated with Nigella sativa seed powder showing normal renal parenchymal tissue which is composed of glomeruli and tubules (H&E-10X).

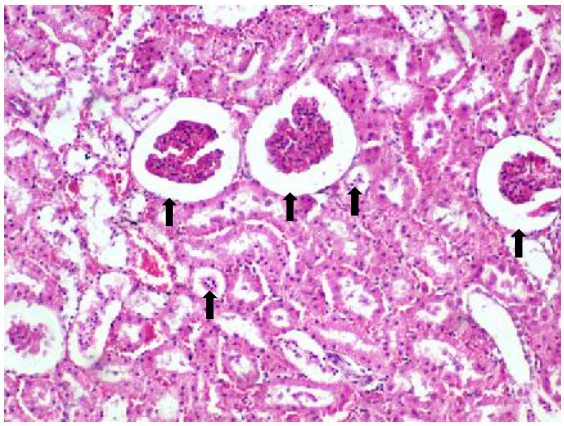

Figure 30: Kidney section of normal rat treated with thymoquinone showing normal renal parenchymal tissue which is composed of glomeruli and tubules (H&E-10X).

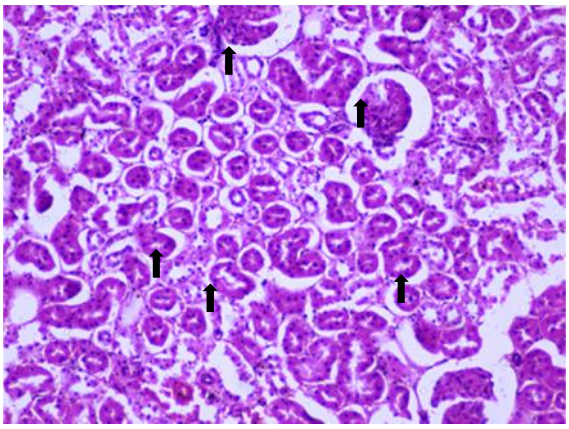

Figure 31: Kidney section of STZ diabetic control rat showing deposits of hyaline material and diffuse infiltration in the mesengium of the glomerulus (H&E-10X).

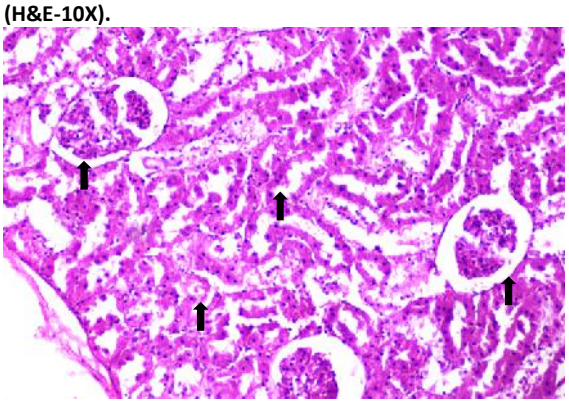

Figure32: Kidney section of STZ diabetic rats treated with Nigella sativa seed powder showing normal glomerulus, very less number of inflammatory cells, normal basement membrane and capillaries, decrease in the hyaline deposit (H&E-10X).

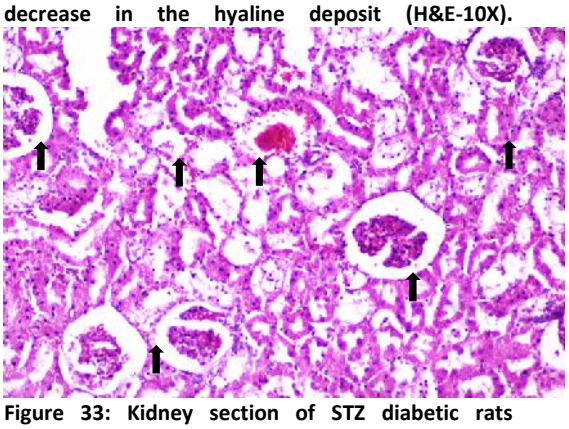

Figure 33: Kidney section of STZ diabetic rats treated with thymoquinone showing normal glomerulus, very less number of inflammatory cells, normal basement membrane and capillaries, decrease in the hyaline deposit (H&E-10X).

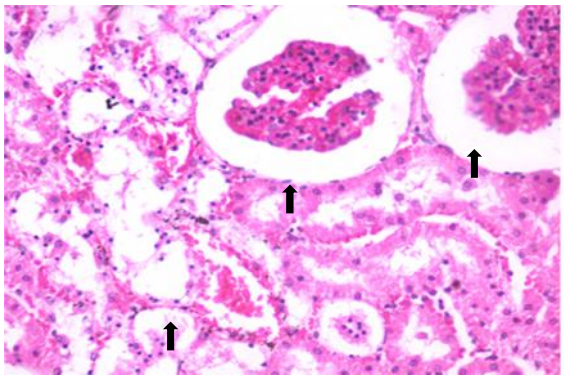

Figure 34: Kidney section of normal control rat showing normal renal parenchymal tissue which is composed of glomeruli and tubules (H&E-20X).

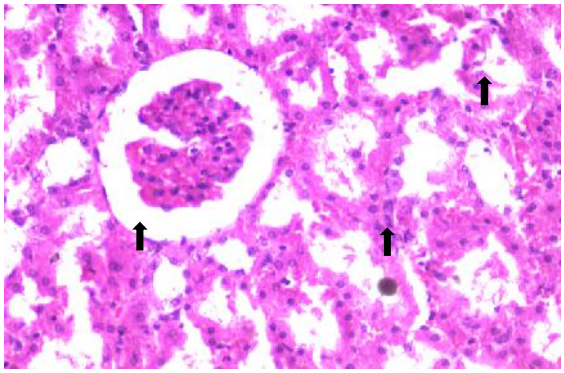

Figure 35: Kidney section of normal rat treated with Nigella sativa seed powder showing normal renal parenchymal tissue which is composed of glomeruli and tubules (H&E-20X)

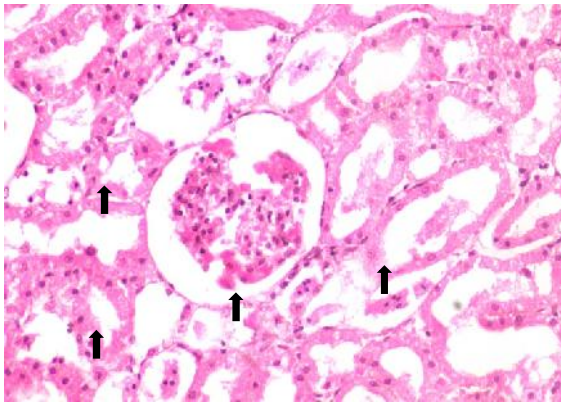

Figure 36: Kidney section of normal rat treated with thymoquinone showing normal renal parenchymal tissue which is composed of glomeruli and tubules (H&E-20X).

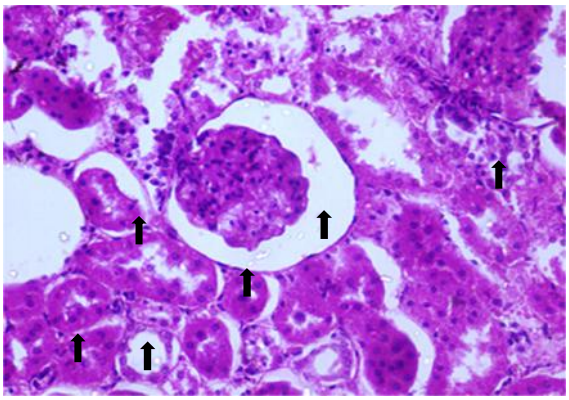

Figure 37: Kidney section of STZ diabetic control rat showing deposits of hyaline material and diffuse infiltration in the mesengium of the glomerulus (H&E-20X).

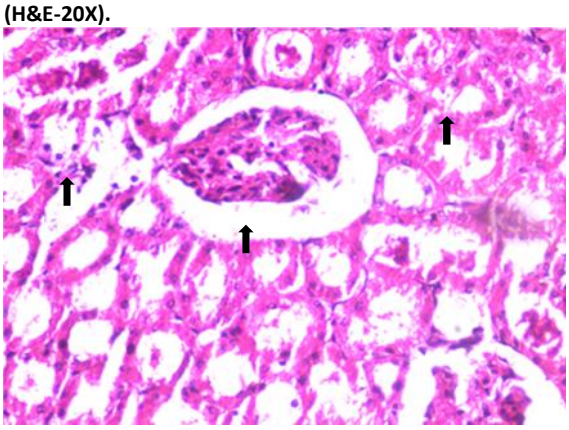

Figure 38: Kidney section of STZ diabetic rats treated with Nigella sativa seed powder showing normal glomerulus, very less number of inflammatory cells and decrease in the hyaline deposit(H&E-20X).

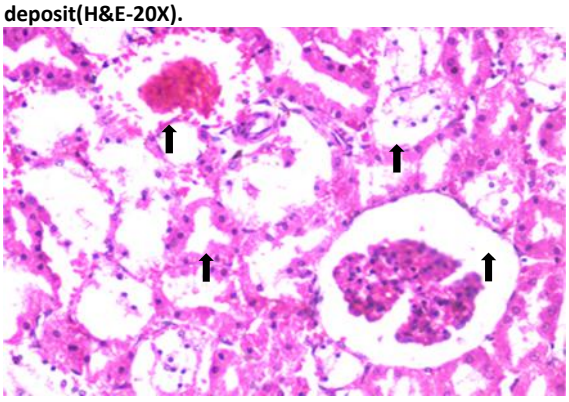

Figure 39: Kidney section of STZ diabetic rats treated with thymoquinone showing normal glomerulus, very less number of inflammatory cells and decrease in the hyaline deposit (H&E-20X).

5.3.4. Histopathology of Nerve

Sections tibial nerve of normal control rats showed normal nerve architecture. Fine myelinated nerve fibres were found with nuclei of Schwann cells and connective tissue coverings(endoneurium, perineurium, and epineurium). There was no pathological observations in normal rats treated with Nigella sativa seed powder (group II) and thymoquinone (group III). The diabetic control (group IV), diabetic rats treated with Nigella sativa seed powder (group V) and thymoquinone (group VI) also showed architecture similar to normal control rats (Figure 40,41,42,43,44,45).

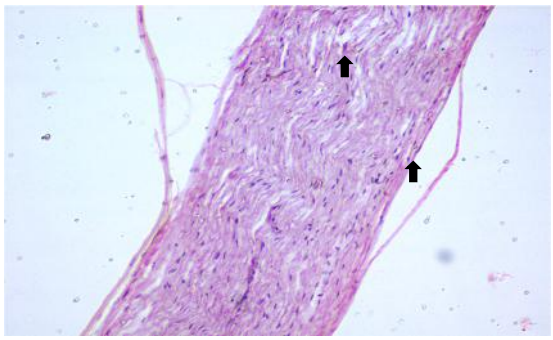

Figure 40: Nerve section of normal control rat showing normal architecture, fine myelinated nerve fibres are found with nucleus of Schwann cells and connective tissue coverings (H&E-10X).

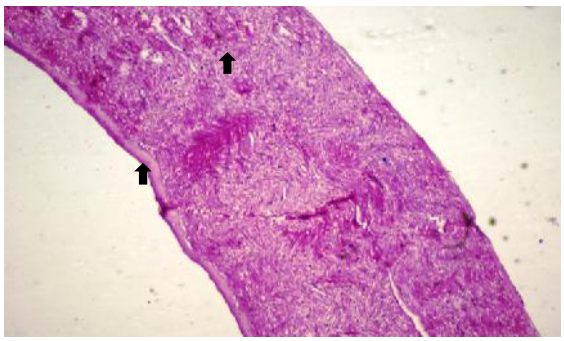

Figure 41: Nerve section of normal rat treated with Nigella sativa seed powder showing normal architecture, fine myelinated nerve fibres are found with nucleus of Schwann cells and connective tissue coverings (H&E-10X).

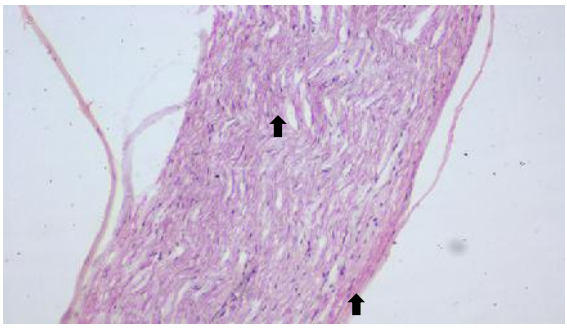

Figure 42: Nerve section of normal rat treated with thymoquinone showing normal architecture, fine myelinated nerve fibres are found with nucleus of chwann cells and connective tissue coverings (H&E-10X).

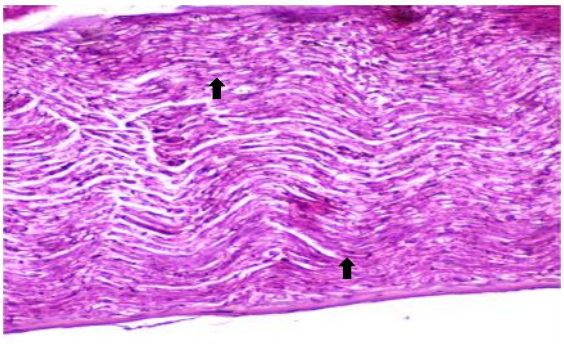

Figure 43: Nerve section of STZ diabetic control rat showing normal architecture, fine myelinated nerve fibres are found with nucleus of Schwann cells and connective tissue coverings (H&E 10X) .

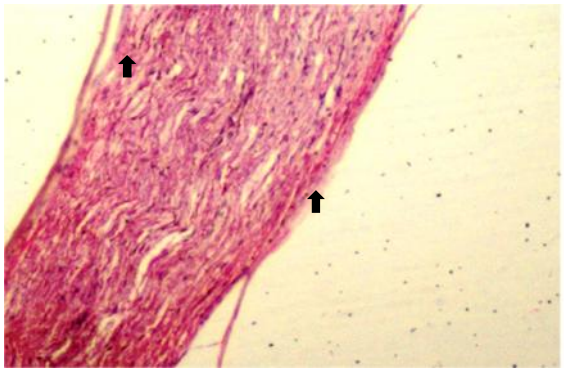

Figure 44: Nerve section of STZ diabetic rat treated with Nigella sativa seed powder showing normal architecture, fine myelinated nerve fibres are found with nucleus of schwann cells and connective tissue coverings (H&E-10X).

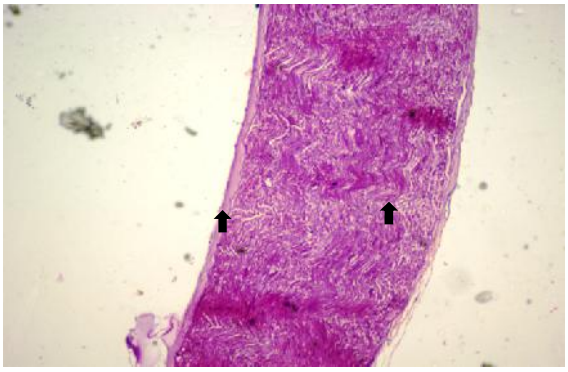

Figure 45: Nerve section of STZ diabetic rat treated with thymoquinone showing normal architecture, fine myelinated nerve fibres are found with nucleus of schwann cells and connective tissue coverings (H&E-10X).

CHAPTER 6

DISCUSSION

6.1. Gravimetry

6.1.1. Weight gain

Results of the present study showed negative weight gain in case of streptozotocin induced diabetic rats. It clearly indicates diabetes interfering with metabolic pattern of experimental animals. This may be due to increased hyperglycaemia leads protein wasting due to inaccessibility of carbohydrate[1]**.** A significant improvement of body weight gain in case of Nigella sativa seed and thymoquinone treated induced diabetic rat groups indicates a beneficial role of both the compounds to overcome the effect of diabetes[2]**.** A possible explanation for this might be that Nigella sativa seed and thymoquinone reduce hyperglycaemia and therefore protein wasting due to inaccessibility of carbohydrate[3]**.** There was no significant change in normal rats treated with Nigella sativa seed powder and thymoquinone compared with normal control rats. Our results are in agreement with the findings of Samad Alimohammadi et al[2] study. A significant weight loss was observed in the diabetic untreated group, while Nigella sativa seed powder and thymoquinone treated rat groups exhibited significant increase in the body weight in comparison with diabetic untreated group but lower than in the normal controls. In the study of Kanter et al[3] Nigella sativa markedly improved body weight gain in STZ-induced diabetic rats.

6.1.2. Organosomatic index

Increase in organosomatic index of pancreas, liver and kidney of streptozotocin induced diabetic group may be due to decreased bodyweight. Similarly, significant

decrease in pancreas, liver and kidney weight in induced diabetic group rats indicates an alteration of metabolism in those organs. The improvement of organ weight in pancreas, liver and kidney in groups of Nigella sativa seed powder and thymoquinone treated induced diabetic rats indicates a beneficial effect in this regard, possibly by resorted altered metabolism of those organs[4]. There was no significant change in normal rat groups treated with Nigella sativa seed powder and thymoquinone compared with normal control rat group. Our results are in agreement with findings of Kaleem M et al[4] that streptozotocin induced diabetic rats showed significant decrease in body, kidney and liver weights and after treatment with Nigella sativa significant improvement in the body, kidney and liver weight was observed.

6.2. Biochemistry

6.2.1.Glucose hemostatisis

Glucose, Insulin and Oral Glucose Tolerance Test

The level of glucose was increased significantly in induced diabetic rats. The level of glucose was reduced significantly in diabetic rat groups treated with Nigella sativa seed powder and thymoquinone. The level of insulin was decreased significantly in induced diabetic rats. This may be due to toxic effect of streptozotocin to β-cells of islets of Langerhans which leads to insufficient secretion of insulin[1]. The treated groups of induced diabetic rats with Nigella sativa seed and thymoquinone showed significant increase in insulin levels. There was no significant change in normal rats treated with Nigella sativa seed powder and thymoquinone compared with normal control rats. The

anti-hyperglycaemic effect of Nigella sativa seed and its active component, thymoquinone may be due to reduction of oxidative stress, thus preserving pancreatic beta cell integrity leading to insulin level increase[2,5]. This is supported by our histopathological observations of pancreas. In the study of Kaleem M et al[4] treatment of Nigella sativa seed resulted in elevation of glutathione(GSH) level which is present in the beta cells of islets and protects the membranes against oxidative damage by regulating the redox status of protein in the membrane[6]. In the study of Kanter et al[3] Nigella sativa caused a sharp decrease in the elevated serum glucose and increase in serum insulin concentration in streptozotocin diabetic rats and in addition Nigella sativa treatment protected the majority of the Langerhans islet beta cells. In another study of same researchers where the Nigella sativa in streptozotocin diabetic albino rats caused a decrease in the elevated serum glucose and an increase in the lowered serum insulin concentrations as well as an improvement in histological appearance of islets of Langerhans of diabetic rats. They stated that hypoglycemic effect of Nigella sativa could be partly due to amelioration in beta cells of pancreatic islets causing an increase in insulin secretion[7].

According to Alsaif MA[8] the other possible reason of blood glucose lowering effect of Nigella sativa may be due to improved insulin insensitivity in diabetic rats. Furthermore, the Nigella sativa contains many bioactive constituents such as thymoquinone, p-cymene, pinene, dithymoquinone and thymohydroquinone, which act as antioxidants[9].

In the present study streptozotocin induced diabetic rats showed impaired oral glucose tolerance. The treated groups of diabetic rats with Nigella sativa seed powder and thymoquinone showed significant improvement in glucose tolerance. The normal control rats, normal rats treated with Nigella sativa seed powder and normal rats treated with thymoquinone showed tolerance to OGTT and there was no significant change between groups of normal control rats and normal rats treated. In the study of Labhal et al[10] Nigella sativa alone was very effective at restoring glucose homeostasis**.** They showed in another study that Nigella sativa seed given by intragastric gavage was able to correct diabetes. The Nigella sativa reduced blood glucose within one month[11]**.** Le et al[12] confirmed the reduction of intestinal transport in vivo and are increased insulin sensitivity. They are in agreement with Al-Awadi et al[13], who obtained an improved OGTT response in streptozotocin rats treated with a mixture of plants containing Nigella sativa. Bouchra M et al[14] showed that Nigella sativa aqueous extract directly affected intestinal absorption of glucose. Moreover inhibition of electrogenic sodium dependent glucose transport occurred. Thus inhibition of intestinal glucose absorption by Nigella sativa is significant and may participate in the recognized hypoglycemic effect of the plant. Most of the previous studies showed that Nigella sativa hypoglycemic activity in streptozotocine induced diabetic rats because of its rich components mainly thymoquinone and nigellone antioxidant nature helping in preserving integrity of beta cells of islets[1,2,3,4,5]. The study of El-Dakhakhny et al[15] proved that hypoglycemic activity of Nigella sativa and thymoquionone. Nigella sativa and thymoquinone may

also be acting to increase peripheral insulin sensitivity or glucose utilization or to decrease intestinal glucose absorption.

6.2.2. Oxidative stress (MDA) and Anti-oxidative (SOD, Vitamin C & E) markers

The level of malondialdehyde(MDA) was increased significantly in induced diabetic rats. The level of malondialdehyde(MDA) was reduced significantly in diabetic rat groups treated with Nigella sativa seed powder and thymoquinoe. The levels of superoxide dismutase(SOD), vitamin C and E were decreased significantly in induced diabetic rats. The treated groups of induced diabetic rats with Nigella sativa seed and thymoquinone showed significant increase in SOD, vitamin C and E levels. There was no significant change in normal rats treated with Nigella sativa seed powder and thymoquinone compared with normal control rats. Lipid peroxidation may bring about protein damage and inactivation of membrane-bound enzymes either through chemical modification by its end products or through direct attack by free radicals, MDA and 4-hydroxynonenal[16]**.** Our results are in agreement with the findings of Wolf[17] , El-Missiry etal[18] and Mahmood et al[19]. These studies reported an increase in lipid peroxides and a decrease in antioxidant enzymes in diabetes. These studies also have results as ours showing improvement in reduced antioxidants after treatment with Nigella sativa. In the study of Bahram Pourghassem-Gargari et al[20], Nigella sativa seed powder showed a significant decrease in serum MDA level. The antioxidant effect of black seed seems to be due to its oil, thymoquinone, flavonoids and also antioxidant vitamins like ascorbic acid and tocopherols. It has been shown that Nigella sativa and thymoquinone inhibit

non-enzymatic lipid peroxidation in liposomes and both work as a scavenger of various reactive oxygen species including superoxide anion and hydroxyl radicals[21]**.** In addition flavonoids present in Nigella sativa are a class of polyphenolic compounds that seem to have antioxidant properties by suppressing reactive oxygen and nitrogen species formation, scavenging reactive oxygen and nitrogen species and protecting the antioxidant defense system[22,23]. In the study of A A Sayed[24] it was observed that thymoquinone has antioxidative properties as evidenced by the significant increase in glutathione and SOD activity and reduction of lipid peroxidation and NO levels in the diabetic treated groups. So it might improve the function of beta cells in the diabetic rats and influence insulin effects by directly acting on specific components of the insulin-signaling transduction pathway. Kanter et al[25] studied the effect of black seed on lipid peroxidation and antioxidant defense system and found that treatment with Nigella sativa decreased MDA level and increased the antioxidant defense system activity in carbon tetrachloride treated rats. The study of Kanter et al[26] showed that Nigella sativa treatment reduced the spinal cord tissue MDA and prevented inhibition of SOD, GPX and catalase enzyme activities, following the experimental spinal cord injury in rats. Schettler et al[27] suggested that the reduced antioxidant production was due to increased oxygen metabolites causing a decrease in the activity of the antioxidant defence system**.** Kennedy and Baynes[28] stated that non-enzymatic glycosylation of the enzymes leading to decreased antioxidant enzyme activity in diabetes. In diabetic rats decreased vitamin C and E was observed. Because of more oxidative stress the utilization of Vitamin C and E is more and when treated with Nigella sativa and thymoquinone the utilization of

vitamin C and E is becoming less which leads to increase in their levels in diabetic rat groups treated with Nigella sativa seed powder and thymoquinone. In addition there are many fatty acids in Nigella sativa, the most important of which are linoleic acid (55.6%), oleic acid (23.4%) and palmitic acid (12.5%)[29], tocopherols (α, β, and γ) and vitamin C[30] which lead to increase in vitamin C and E levels. Hence Nigella sativa may have antioxidant properties that will be useful for therapeutic purposes. The results of the present study indicate that the preventive effects of Nigella sativa may be due to inhibition of lipid peroxidation as a result of its antioxidant nature. These findings suggest that Nigella sativa treatment has a therapeutic protective effect against diabetes by preserving pancreatic beta cell integrity through decreasing oxidative stress. Consequently Nigella sativa may be clinically useful for protecting β-cells against oxidative stress.

6.2.3. Lipid Profile

The level of HDL-cholesterol was decreased significantly in induced diabetic rats. The level of HDL-cholesterol was increased significantly in diabetic rat groups treated with Nigella sativa seed powder and thymoquinone. The levels of total cholesterol(TC), triglycerides(TG), LDL-cholesterol and VLDL- cholesterol were increased significantly in induced diabetic rats. The treated groups of induced diabetic rats with Nigella sativa seed and thymoquinone showed significant decrease of all these parameters. There was no significant change in normal rats treated with Nigella sativa seed powder and thymoquinone compared with normal control rats. The results of our

study showed a promising hypolipidemic effect produced Nigella sativa and thymoquinone supplementation separately in induced diabetic rats. It has been reported that LDL-C positively and HDL-C negatively correlate with cardiovascular disease[31]**.** Circulating LDL-C can pass into the arterial wall from the blood where it may be oxidized and engulfed by macrophages forming foam cells. Thereafter a complex interplay of cell necrosis, collagen deposition and smooth muscle recruitment lead to the forming of atherosclerotic plaques. However HDL-C possesses antioxidant and anti-inflammatory activities and promotes the efflux of cholesterol to liver from the peripheral tissue, thereby reducing the uptake of cholesterol by macrophages and providing a protective effect against atherosclerosis[32]**.**

The present study showed a decrease in TC, TG and LDL-C in diabetic treated groups with Nigella sativa seed powder and thymoquinone. The changes in lipid profile monitor an improvement in the dyslipidemia in diabetic rats. The decrease in LDL-C is sufficiently enough predictor for the beneficial effect of Nigella sativa on lipid pattern. American Diabetic Association has identified LDL-C as the primary target of lipid lowering in diabetic dysfunction[33]**.** Baharam Pourghassem-Gargari et al[20] showed significant decrease in the concentration of TC and TG. The study of Zaoui et al[34] indicated that treatment with Nigella sativa, decreased serum cholesterol and TG levels. El-Dakhakhny et al[15] observed that Nigella sativa administration to rats significantly decreased serum TC, LDL-C and TG and increased HDL-C. Le et al[12] reported a significant decrease in plasma TG and an increase in HDL-C level in Nigella sativa seed treated rats, compared to the control group.

The results of Bamosa et al[35] demonstrated a decrease in serum TC, LDL-C, TG and increased HDL-C during intraperitoneal injection of thymoquinone in rats. Morikawa et al[36] studied the hypotriglyceridemic effect of Nigella sativa seed and diterpene alkaloids nigellamines was reported. Nigellamines are equivalent to the hypolipidemic agent, clofibrate. The hypolipidemic effect of Nigella sativa seed was due to the synergistic action of its different constituents including thymoquinone and nigellamine, soluble fiber like mucilage, sterols, flavonoids and high content of polyunsaturated fatty acids (PUFAs)[37].

Mechanism of hypolipidemic action of thymoquinone may help in decreased cholesterol synthesis because of its antioxidant role. Lipid lowering effects of dietary soluble fibers[38,39] are probably related to decreased dietary cholesterol absorption, increased primary bile acid synthesis and its fecal losses. Flavonoids may act by making liver cells more efficient to remove LDL-C from blood. To do this, flavonoids increase LDL receptor densities in liver and by binding to apolipoprotein B[40,41].

Hamdy NM et al[42] reported that thymoquinone, the active constituent of Nigella sativa has attenuate oxidative stress in streptozotocin induced diabetic rats. Ismail M[43] reported thymoquinone hypolipidimic effect in hypercholesterolemic rats. Kaleem et al[4] confirmed the antidiabetic activity of Nigella sativa and its efficiency in controlling the dyslipidemia due to its antioxidant effects. Therefore the antioxidant activity of Nigella sativa may also be implicated in ameliorating the dyslipidemia associated with diabetes through decreasing insulin resistance.

6.2.4. Liver function tests

In present study the level of total protein was reduced significantly in diabetic control rats. The level of total proteins was increased in groups of diabetic rats treated with Nigella sativa and thymoquinone. The levels of AST, ALT and ALP were increased significantly in diabetic control rats. The treatment of Nigella sativa and thymoquinone separately helped in lowering of AST, ALT and ALP levels. The decrease in serum total protein observed in diabetic rats is coinciding with the findings of Ayed Al-Logmani[44], Wanke et al 1991[45], Tragl et al[46]. This decline may be due to the inhibited oxidative phosphorylation processes which lead to decrease of protein synthesis, increase in the catabolic processes and reduction of protein absorption. Ayed Al-Logmani et al[44] found Nigella sativa treatment improved the lowered levels of total protein in diabetic rats. This effect of the Nigella sativa is presumably due to its ability to increase insulin secretion[47].

In streptozotocin induced diabetic rats the activities of blood AST, ALT and ALP were significantly elevated compared to their normal levels. These results indicated that liver dysfunction may be inducing the diabetes [48] and liver was necrotized in STZ induced diabetic rats. Therefore increase in the activities of liver enzymes in blood may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream[49] which gives an indication on the hepatotoxic effect of streptozotocin. These findings are supported by histopathological observations of liver sections. On the other hand, treatment of the diabetic rats with Nigella sativa seed caused reduction in the activity of these enzymes in blood compared with the mean values of diabetic group and

consequently may alleviate liver damage caused by streptozotocin induced diabetes. Thymoquinone treated diabetic rats also produced similar results and histopathological observations are in support of these findings. Findings of Ayed Al-Logmani et al[44] and Ayed Sh. AL-logmani[47] are similar to our results. A study was performed on rat model to evaluate the hepato protective effect of Nigella sativa alcoholic extract against D-Galactosamine (D-GalN) / Lipo polysaccharide induced hepatotoxicity and found that D-GalN/LPS showed significant rise in AST, ALT and ALP. Nigella sativa maintained the levels of AST, ALT and ALP close to normal[50]**.** Another study based on carbon tetrachloride induced rats has shown that CCl4 treatment increased the lipid peroxidation and liver enzymes and decreased the antioxidant enzyme level and further Nigella sativa treatment decreased the elevated lipid peroxidation, AST, ALT and ALP levels and increased the reduced antioxidant enzyme levels[25]**.** Mansour MA[51] reported that thymoquinone, chief constituent of Nigella sativa seed showed a vital role as antioxidant and may efficiently act as a protective agent against chemically induced hepatic damage**.** Studies in vitro using isolated rat hepatocytes have shown that preincubation of hepatocytes with thymoquinone showed protection of isolated hepatocytes against TBHP induced toxicity evidenced by decreased leakage of ALT and AST[52]**.** Another study showed that thymoquinone is efficient cytoprotective agent against CCl4-induced hepatotoxicity, possibly via inhibition of the production of oxygen free radicals that cause lipid peroxidation[53]**.**

Thymoquinone treatment showed similar significant improvement in liver function parameters in diabetic rats of our study. These results are similar to the results

of Adel Abdel Moneim et al[54]. Bashandy et al[55] observed that AST and ALT levels were significantly decreased in the thymoquinone treated diabetic group as compared to diabetic control group. Thymoquinone is associated with beneficial changes in hepatic enzyme activities and thereby exerts potential antihyperglycemic effect[56]. In the study of Abdel-Moneim A[57] Nigella sativa administration to rats with aflatoxin induced toxicity caused a significant amelioration of the activities of AST and ALT. In another study Abdel Moneim A[58] Nigella sativa administration to diabetic rats exhibited amelioration effect in serum ALT and AST. This study showed that in control diabetic group there was significant decrease in serum total protein which may be due to structural distortion and functional impairment of the hepatic cells which associated with low serum protein and albumin levels[59]**.** A possible explanation for the differential effects of Nigella sativa and thymoquinone on the activities of AST and ALT in blood is that these treatments may inhibit the liver damage induced by streptozotocin[48]. In the present study there was no significant change in normal rats treated with Nigella sativa seed powder and thymoquinone compared with normal control rats, indicating that Nigella sativa seed powder or its major biochemical component, thymoquinone do not show any toxic effect. Our histopathological observation also confirmed this.

6.3. Histopathology

6.3.1. Pancreas

H&E stained sections of pancreas of normal control rats showed normal islet cells with classical histological features. Cells were present in their normal proportions. The pancreatic acinar cells were strongly stained and arranged in lobules with prominent nuclei. The islet cells were seen embedded within the acinar cells and surrounded by a fine capsule. The islet of Langerhans showed clear β- cells with nuclei. H&E sections of pancreas of normal rat groups treated with Nigella sativa seed powder and thymoquinone showed the same architecture as normal control rats. There were no pathological observations. These findings indicate non-toxic effect of Nigella sativa seed and thymoquinone.

H&E stained of pancreas of diabetic control rats sections showed degenerative and necrotic changes and shrinking of the islets of Langerhans. The nuclei of necrotic beta cells showed either marginal hyperchromasia or pyknosis. There was mostly degranulation in the cytoplasm of the degenerative and necrotic cells and also hydropic degeneration. Our findings are similar to the results of studies of Aughsteen A.A[60] and Azza Attia A[61]. H&E sections of pancreas of diabetic rat groups treated with Nigella sativa seed powder and thymoquinone showed the severity of degenerative and necrotic changes in the islet of Langerhans. Parenchyma was less than those in the diabetic control group. In treated groups few cells were with pyknotic nuclei but the majority of cells showed significantly light hydropic degeneration as compared to islet cells of

untreated diabetic rats, indicating regranulation of islet cells. The islets of Langerhans were distinctly increased in size.

The study of Afaf Jamal[62] clearly indicated that in diabetic rats many histological changes were observed in the pancreatic islets as compared with the normal control. The islets were small and atrophied. Degenerative, necrotic changes and shrinking of the islets of Langerhans vaculation of cytoplasm and degranulation were observed. Our findings are similar to this study.

Results of current study are similar to the findings of Kanter et al[3] who showed that the use of Nigella sativa reduced the severity of degenerative and necrotic changes in islets of streptozotocin induced diabetic rats. Sections of pancreatic tissues of treated rats with Nigella sativa showed that the majority of cells had slight degeneration compared to the diabetic group with no treatment. Sections of pancreatic tissue of diabetic treated rats with thymoquinone showed similar findings. Findings from the other study suggested that the active ingredient of Nigella sativa seed thymoquinone is the most effective against streptozotocin diabetes as its administration ameliorated most of the pathological changes[5]. The study of A. A. Albajali1[63] showed the histological abnormalities caused by diabetic induction and improvement after treatment with Nigella sativa. In the study of Kanter M[7] immunohistochemical labeling of the pancreatic tissue of control rats revealed strong insulin antigen positivity in the beta cells of the islets. In untreated diabetic rat, the cells were negative for insulin-immunoreactivity and only a few beta cells in some islets displayed slight insulin immunopositivity in small granules. In diabetic rats with thymoquinone treatment, both

the number of insulin-immunoreactive beta cells and the immunopositivity of their granules increased in comparison with those seen in diabetic control rats. The same study suggested that thymoquinone treatment has a therapeutic protective effect against diabetes by preserving pancreatic beta cell integrity and decreasing oxidative stress. It is concluded that all our histological findings support the antioxidant nature of Nigella sativa seed and thymoquinone which help in preserving pancreatic beta cell integrity.

6.3.2. Liver

Sections of liver of normal control rats studied under H&E stain showed normal liver parenchymal tissue composed of hexagonal lobules. Each lobule consists of central vein from which the hepatocytes radiate outwards. The portal triads 3 to 5 number are located at the periphery of the lobule, containing branches of bile duct, portal vein and hepatic artery. Cords of hepatocytes and sinusoids radiate from central vein to the peripheral portal triads. The sinusoids are lined by both Kupffer cells and endothelial cells, both of which have ill-defined cytoplasmic margins and inconspicuous flattened nuclei. The central veins are lined by endothelial cells surrounded by ring of collagen fibres. The hepatocytes are polygonal in shape with well-defined borders. Sections of liver of normal control rats treated with Nigella sativa seed powder showed the same architecture as normal control rats. There were no pathological observations. Normal control rats treated with thymoquinone also showed same features as normal control rats. These findings supports nontoxic effect of Nigella sativa seed powder and thymoquinone.

Section of liver of diabetic control rats studied under H&E stain showed the distortion in the arrangement of hepatocytes around the central vein, focal necrosis of hepatocytes with periportal fatty infiltration, hydropic changes, infiltration and aggregation of lymphocytes between hepatocytes. Our findings are similar to the findings of Morfologia etal[64], Das et al[65] and Degirmenchi et al[66], who showed dilatation of veins, loss of usual concentric arrangement of hepatocytes and liver fibrosis in their studies. Sections of liver of diabetic rats treated with Nigella sativa seed powder studied under H&E stain showed the normal hepatocyte arrangement around the central vein, reduced necrotic changes near to normal cellular architecture and the blood vessels in some places coming near to normal appearance. Diabetic rats treated with thymoquinone also showed the same beneficial effect as Nigella sativa seed treatment. These beneficial findings of Nigella sativa and thymoquinone may be due to antioxidant effect and hepatorepairing ability. These histological findings are in support to our biochemical parameters, that is lowered liver enzymes in diabetic rats by Nigella sativa seed powder and thymoquinone treatment.

Studies of Das AV [65], Elmarakby[67], Evelson P[68] and Badary OA[69] also reported the similar histopathological finding of present study, in their study showed congestion in blood sinusoids as well as the central and the portal veins in the liver sections of diabetic untreated rats. Dilatation of the hepatic veins in diabetic rats as well as hepatic sinusoids also observed. In their studies, an increase in mononuclear cellular infiltration was observed in the periportal areas. In study of Elmarakby[67], Badary OA[69] and El-Tawil O[70] studies showed that diabetic rats treated with Nigella sativa treatment had

preserved hepatic tissues compared with diabetic untreated group. The hepatocytes were relatively normal, with vesicular nuclei. The portal areas were not apparently infiltrated with inflammatory cells in Nigella sativa treated group, the present study also observed similar findings. The present study shows Nigella sativa seed and thymouinone having hepatoprotective nature against liver damaged by STZ induced diabetes.

6.3.3. Kidney

The H&E stained sections of kidney of normal control rats showed classical histological features of normal renal parenchymal tissue. The renal sections of normal rat groups treated with Nigella sativa and thymoquinone showed all classical features of renal tissue as normal control rats. This indicates nontoxic effect of Nigella sativa seed powder and its phytochemical component thymoquione on renal tissue. Our observations are similar to the observations of Le et al.[12] in normal rats treated with Nigella sativa. Mohammad Aziz Dollah[71] showed that oral administration of Nigella sativa has no toxicity by different doses used. These results are similar to the observations of EL-Kholy et al[72] and AL Ameen et al[73].

The renal H& E stained sections of streptozotocin induced diabetic untreated rats observed the mesengium of the lobules of the glomerulus deposited with hyaline material. There was a diffuse infiltration of the glomerular tuft and also heavy focal deposition. The diffuse infiltrate appeared to be in the basement membrane of the capillaries and the capillary bed had been obliterated in places. In many of the glomeruli, lesions characteristic of diabetic glomerulosclerosis were present. In diabetic control rats renal sections showed that the nodules scattered among some non-affected normal

glomeruli and it is considered as a characteristic of diabetes mellitus. In most of the diabetic control rats renal sections showed areas of lymphocytic infiltrate in the interstitium. The S. L. Teoh et al[74] observed the damage in the glomerulus, thickened basement membrane and mucopolysaccharide deposits in proximal convoluted tubule of diabetic untreated kidneys. Sugano M et al[75] also observed similar findings in diabetic rats. The groups that were treated with Nigella sativa seed powder and thymoquinone showed features of normal glomerulus, normal basement membrane and capillaries, very less number of inflammatory cells and decrease in the hyaline deposit. Overall the kidney of treated diabetic rats appears to have normal histological architecture. The beneficial effect of Nigella sativa seed and thymoquinone may be due to antioxidant effect of these. In the study of Abdul Karim Salim Mahood[76] similar results were found in streptozotocin diabetic rats treated with Nigella sativa showed features of healing that are normal glomerulus with normal basement membrane and capillaries, decrease in the mucopolysaccharides, hyaline deposit and absence of inflammatory cells. The tissue necrosis was also observed to decrease. In the study of Kanter M[7] treatment of thymoquinone repaired degenerated glomerular size, glomerular and tubular basement membranes, thickening of capsule, increased amounts of mesangial matrix and tubular dilatation and renal function as compared with diabetics untreated. This study concluded that thymoquinone therapy causes renal morphologic and functional improvement after streptozotocin induced diabetes in rats. The study of Ola Omran[77] found similar results and concluded that thymoquinone has protective effect on experimental diabetic nephropathy. All the above mentioned studies concluded that the antioxidant properties

of Nigella sativa seed and thymoquinone are the main reason to repairing ability of diabetic nephropathy.

6.3.4. Nerve

The nerve sections of normal control rats showed normal nerve architecture. Fine myelinated nerve fibres are found with nucleus of Schwann cells and connective tissue coverings. There was no pathological observations in normal rat groups treated with Nigella sativa seed powder and thymoquinone. The diabetic control rat nerve sections showed similar architecture as normal control rats. The diabetic rats treated with Nigella sativa seed powder and thymoquinone also showed similar architecture as normal control rats. There was no pathological changes between normal rats and streptozotocin induced diabetic rats. Our results similar to the findings of Guven A et al[78]**.**

Histopatholological architecture of pancreas, liver and kidney of diabetic rat groups treated with Nigella sativa and thymoquinone supports beneficial effect of Nigella sativa and its major bioactive component thymoquinone. This may be due to antioxidant properties of both phytochemical components.

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CHAPTER 7

Summary and Conclusion

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Based on the results of the present study it may be concluded that Nigella sativa seed and thymoquinone have beneficial effect in treating the streptozotocin induced diabetic rats. It may be due to their antioxidant nature which helps to repair the damaged metabolic organs tissue like pancreas and liver.

- Glucose levels of induced diabetic rats were increased significantly and were lowered after treatment with Nigella sativa seed and thymoquinone. Insulin levels of induced diabetic control rats were decreased significantly and increased significantly in diabetic treated rat groups with Nigella sativa seed and thymoquinone. This may be due to preservation of integrity of β-cells by their antioxidant properties, which leads to increased secretion of insulin. Increased level of insulin in diabetic treated rat groups leading to lowering of higher glucose levels.
- Tolerance in oral glucose tolerance test is increased significantly in diabetic rat groups treated with Nigella sativa seed and thymoquinone compared with untreated diabetic rats. It may be due to increased insulin secretion or Nigella sativa seed and thymoquione helping to increase peripheral glucose utilization or insulin sensitivity or decreased intestinal glucose absorption through extrapancreatic action.
- Streptozotocin caused shrinkage of islets of Langerhans and necrosis of β-cells in pancreas and increased oxidative stress due to toxic effect of streptozotocin on β-cells. This is supported by increased levels of MDA in streptozotocin induced diabetic rats and histopathological observations of pancreatic tissue.

- SOD levels of Nigella sativa seed and thymoquinone treated diabetic rat groups revealed that these components have antioxidant nature. This is also supported by histopathological observations of pancreatic tissue which showed reduced severity of degenerative and necrotic changes in islets of diabetic treated rat groups.
- In diabetic rats decreased levels of vitamin C and E were observed. It may be because of more oxidative stress and utilization of vitamin C and E. Increased levels of vitamin C and E in diabetic treated rat groups with Nigella sativa seed and thymoquinone indicating the antioxidant nature of both components. In addition there are many fatty acids in Nigella sativa, the most important of which are linoleic acid, oleic acid, palmitic acid, tocopherols and vitamin C which lead to increase in vitamin C and E levels.
- The levels of HDL-cholesterol were decreased significantly in induced diabetic untreated rats. The levels of HDL-cholesterol were improved significantly in diabetic treated rat groups with Nigella sativa seed and thymoquinone. The levels of total cholesterol(TC), triglycerides(TG), LDL-cholesterol and VLDL- cholesterol were elevated significantly in induced diabetic untreated rats. The treated groups of induced diabetic rats with Nigella sativa seed and thymoquinone showed significant decrease of all these parameters. The results of our study showed a promising hypolipidemic effect produced Nigella sativa and thymoquinone supplementation in induced diabetic treated rat groups. Flavonoids of Nigella sativa seed may be one of the reasons for hypolipidimic effect of it, as flavonoids helps liver cells to make more efficient to remove LDL-C from blood. To do this, flavonoids are acting to increase LDL receptor densities in liver by binding to apolipoprotein B.

- The level of total protein was reduced significantly in diabetic control rats. The levels of total proteins were improved in diabetic rat groups treated with Nigella sativa seed and thymoquinone. This effect is presumably due to its ability to increase insulin secretion.
- The levels of AST, ALT and ALP were increased significantly in diabetic control rats. Treatment with Nigella sativa seed and thymoquinone helped in lowering of AST, ALT and ALP levels. A possible explanation for the beneficial effects of Nigella sativa and thymoquinone on the activities of AST, ALT and ALP in blood is that these components may inhibit the liver damage induced by streptozotocin. The histopathological observation of liver sections of diabetic treated groups supporting this.
- There was no difference in any parameters between normal control rat group and normal rat groups treated with Nigella sativa and thymoquinone. This indicates non-toxic effect of Nigella sativa and thymoquinone. Our histopathological findings further support this observation as there were no pathological observations in normal rats groups treated with Nigella sativa seed and thymoquinone.
- Nigella sativa seed and thymoquinone give the same results. This suggests that the active ingredient thymoquinone is the most effective against streptozotocin diabetes as its administration ameliorated most of the pathological changes.
- All the results clearly indicate that there is beneficial effect in altered parameters which are affected by streptozotocin induced diabetes. As the beneficial effect of Nigella sativa seed and thymoquinone are seen in the present study, these phytochemical components may be considered as antidiabetic.

- The mechanism of beneficial effect of Nigella sativa seed and thymoquinone, probably through acted as antioxidant agents, which keep to rectify oxidant and antioxidant balance in tissues like liver, kidney and pancreas. Also it may be due to a cellular protective mechanism through cell signal pathways in pancreas with resultant amelioration of glucose homeostasis. Perhaps both Nigella sativa seed and thymoquinone improve cellular integrity especially in hepatocytes and β cells of islets, this modulates pathophysiology of metabolically active tissue.

Limitations of the present study

- We could not evaluate the exact active compound of Nigella sativa seed which is acting as antidiabetic compound.
- Crude of seed was used directly.

Scope for future study

- More refine screening of Nigella sativa seed may be done for further conclusion.
- Different extractions of Nigella sativa seed may be used to evaluate its medicinal properties.
- Further evaluation of molecular factors, especially transcriptional factors need to be evaluated.

ANNEXURE - I

PUBLICATIONS

- Hepatoprotective effect of nigella sativa seed in sterptozotocin induced diabetic albino rats: histological observations. Int J Anat Res 2016,4(2):2459-63.
- Effect of Nigella sativa seed on streptozotocin induced diabetic reno toxicity: histological observations. Int J Anat Res 2016;4(3):2566-70.
- Antioxidant Effect of Nigella Sativa Seed Powder and Thymoquinone in Normal and Sterptozotocin Induced Diabetic Albino Rats. Int J Intg Med Sci 2016;3(3):242-47.
- Effect of Nigella Sativa Seed Powder on MDA and SOD levels in Sterptozotocin Induced Diabetis Albino Rats. J. Pharm. Sci. & Res. Vol. 7(4), 2015, 206-209.
- Effect of Thymoquinone on MDA and SOD levels in Sterptozotocin Induced Diabetic Albino Rats. J. Pharm. Sci. & Res. Vol. 7(4), 2015, 234-37.

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ETHICAL CLEARANCE CERTIFICATE

The Institutional Animal Ethics Committee (IAEC) of this College met on 26.07.2013 at 10.30am to scrutinize the Research Project submitted by Ph.D) candidate of this College.

The queries raised by the Ethics Committee have been satisfactorily answered by you, hence the Ethical Clearance is accorded for your Research project.

Title: "Effect of Nigella Sativa seed extract on glucose, lipid profile, liver function tests, oxidative stress and histological changes in pancreas, kidney, liver and tibial nerve in normal and streptozotocin induced diabetic rats".

Principal investigator: Mr. Shaik Hussain Saheb, PhD (Anatomy) Candidate.

11.09.2013

11/9/2013
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Annexure-I

PLAGIARISM VERIFICATION CERTIFICATE

1. Name of Student: SHAIK HUSSAIN SAHEB Reg. No. 11PHD0014
2. Title of the Thesis: EFFECT OF NIGELLA SATIVA SEED EXTRCT ON GLUCOSE, LIPID PROFILE, LIVER FUNCTION TESTS, OXIDATIVE STRESS AND HISTOPATHOLOGI CHANGES IN PANCREAS, KIDNEY, LIVER AND TIBIAL NERVE IN NORMAL AND STREPTOZOTOCI INDUCED DIABETIC RATS
3. Department: ANATOMY
4. Name of Guide & Designation: DR. S.D. DESAI, PRINCIPAL, SIMS&RC, TUMKUR.
5. Name of Co Guide & Designation: DR. G.F. MAVISHETTAR, PROFESSOR OF ANATOMY, JJMMC, DAVANAGERE

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