THE HYPOGLYCAEMIC AND ANTI-DIABETIC EFFECTS OF *P. ANISUM*

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A Thesis submitted to the University of Khartoum in partial fulfillment of the requirements for Master of Science in Biochemistry

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December 2006
DEDICATION

This study is dedicated to
My father and mother, from whom I learned
responsibility,
To my family, friends
And to all
With respect

Mujahid
Acknowledgement

I would like to express my sincere appreciation and gratitude to my supervisor Dr. Suliman Mohammed El Hassan for his excellent and wise guidance and continued encouragement during the study period which facilitates the tough work.

Grateful to staff member of Biochemistry Department, Faculty of Veterinary Medicine, U. of K., especially to Mr. Abdel Kareem Syeed and Mr. Eltayeib Abbas for their kind supports and great technical assistance.

I extend my thanks to the staff members of Medicinal and Aromatic Plants Research Institute (MAPRI) especially Dr. Mohamed Galal.

I highly acknowledge the technician El Walid Issa who contributed to this work. Also, my thanks to Hafez Al hajj who assist during the collection of samples.

Special thanks to Khalid Ahmed for their assistance in the statistical analysis.

I am deeply indebted to my dear father and mother who donated me great patience and care.

Great acknowledgement extended to my family, friends, teachers and any one donated help and even hopes.
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Abstract

The anti-diabetic and hypoglycemic effects of *P.ansium*, which is used traditionally world wide for the treatment of several disease were studied. The effect of the plant on the plasma glucose, total protein, albumin, bilrubin, cholesterol, triglyceride, enzyme (GOT, GPT and alkaline phosphatase) and concentrations of N+ and K+ were evaluated in diabetic and normal rats. Adult male Wister albino rats were used in the present work. The diabetes was induced by injection of streptozotocin. The experiment extended over three weeks. Rats were divided into four groups each group consisted of 6 rats: Group A was control group and contained normal rats, fed rat diet only (63g/day), Group B contained normal rats fed *P.anisum* powder which replaced 15% of diet to examine the *P.anisum* effects on normal rats, Group C contained diabetic induced rats fed diet only (63g/day) and Group D contained diabetic induced rats fed *P.anisum* powder which replaced 15% of rat's diet to examine the effects of feeding *P.anisum* on diabetic rats. The addition of 15% of *P.anisum* to the diet lowered significantly the plasma glucose level of diabetic rats at 0,1,2,3 h after overnight fasting when compared with diabetic fed diet only. The feeding of *P.anisum* to normal rats, lowered the glucose level at 2h and elevated it significantly at 3h but the difference was small. The feeding *P.anisum* had no significant effect on plasma total protein, albumin, GOT, GPT, alkaline phosphatase, cholesterol, triglyceride, bilrubin, N+ and K+ concentrations in both normal and diabetic rats. Thus it could be concluded that *P.anisum* is unlikely to be toxic. It was noticed in this study, that the induction of diabetes in rats by
streptozotocin caused significant elevation of plasma glucose level, cholesterol and urea; and lowered significantly plasma albumin, triglycerides and the body weight but the induced diabetes had no significant effect in plasma total protein, GOT, GPT, alkaline phosphatase, bilirubin creatinine, Na$^+$ or K$^+$.

Therefore, it can be concluded *P. anisum* is promising antidiabetic agent and could be used to lower hyperglycemia in diabetic patient.
الأطروحة ملخص

المعالجات الأخرى والدم في الجلوكوز لتركيز الخافض الأجراة دراسة تم الأطروحة هذه في

كثير لعلاج العالم دول من كبر عدد في يستخدم والذي اليوس نبات لمرض السكري بواسطة

الفروبين,البليوبين, الكلي البروتين, الجلوكوز نسبة على تأثيره بمرابه ذلك و الأسراض من

للقرناء الدم بلازما في الصوديوم و اليوسوم, الأنزيمات بعض و, الترايجلسيريدات, الكولسترول

سليمة أخرى و السكري بمرض مصابة.

عن منها جزء إضرا증 تم التي و البيضاء وسر فنان من الذكور على البحث هذا في التجارب أجريت

قسمت و متصلة أسابيع ثلاثة لعدة التحري وستمرون الاستريبيتوزن مادة بواسطة حقنها طريق

وضبط مجموعة الأولى المجموعة فنان 6 من تتكون مجموعة كل مجموعات أربع إلى الفنان

غذائي من 15% استبدال التي و السليمة الفنان من ثانية المجموعة, السليمة للفنان مراقبة

الثانية المجموعة, السليمة الفنان في أثره لاختبار ذلك و اليوسون حيو بمسحوق (جرام 6) اليومي

أما الاستريبيتوزن مادة بواسطة السكري بدأ أمراضها تم التي للفنان مراقبة و ضبط مجموعة

(جرام 36) للفنان اليوس المغذى من 15% استبدال تم أيضا أمراضها تم التي و الرابعة المجموعة

السكي بمرض المصابه الفنان في أثره اختبار اليوسون حيو بمسحوق

الدم بلازما في الجلوكوز مستوى انشقاض إلى أدى باليوسون للفنان اليومي اليوس من 15% استبدال

في أما, اليوسون إعطائها يتم لم والتي المصابه بالفنان مقارة 3 و 2,0,1,1,1,0 ساعات في المصابه للفنان

في قليل ارتفاع و ثانية الساعة في الجلوكوز مستوى انشقاض إلى اليوسون أدى السليمة الفنان

الثالثة الساعة.

، الكولسترول, البليوبين, الكلي البروتين, ألبومين البلاستات تركز لمستوى تغير اى في اليوسون يسبب لم

الصوديوم و اليوسوم, GOT,GPT and alkaline phosphatase,

أنزيمات و, الترايجلسيريدات أثر السوين بأن الفانون هذا يمكننا ومن السليمة أو السكري بمرض المصابة للقرناء الدم بلازما في

سأم غير بأنه و السكري لمرض معالج.

الجلوكوز مستوى في ارتفاع سجل الاستريبيتوزن مادة بواسطة إمراضها التي الفنان

تغير أى يسجل لم و الجسم وزن و الترايجلسيريدات, في الاليومين, انخفاض اليوسوم و انخفاض اليوسوم

الكرياتين, البليوبين, الكلي البروتينات, GOT,GPT and alkaline phosphatase,
وخفض السكري مرضى لمعالجة واعد عامل الالنسون يكون أن يمكن لذا الصوديوم والبوتاسيوم.

السكرى مرضى عند مستوى
Introduction

The use of the herbs for therapeutic purposes in different parts of the world dates back to immemorial times. In developing countries, like Sudan, where people live in communities far away from each other, the inhabitants used to be short of or almost deprived of proper medical care. The most common disease around the world is diabetes mellitus according to World Health Organization (WHO) and many people had used herbs to treat it, some known herbs were used to treat diabetes mellitus. Some studies revealed valuable therapeutic activities and pharmacological nature as well as nutritional values of these herbs.

A recent study carried out by Ajit et al. (2000) showed the activity of Anise ash in lowering glucose blood level in glucose tolerance test for normal rats, also in a separate study carried out by Krediyych et al. (2003) exhibited the efficiency of Anise in increasing the activity of Na\(^+\),K\(^+\) ATPase and consequently the sodium gradient needed for sugar transportation. Therefore it is appropriate and justifiable to undertake an investigation on \textit{P. anisum} antidiabetic effect in normal and diabetic rats.

This investigation is intended to study the effect of feeding \textit{P. anisum} seed on diabetic and normal rats by monitoring biochemical parameters.

Objective:

This study is undertaken:

- To evaluate the hypoglycemic and anti-diabetic effect of \textit{P. anisum} on normal rats.
- To evaluate the hypoglycemic and anti-diabetic effect of \textit{P. anisum} on diabetic rats.
- To evaluate the toxicity of the \textit{P. anisum}.
Chapter One

1. Literature Review

1.1. Pancreas

1.1.1 Pancreas gross anatomy

In man, the pancreas is an elongated, tapered organ located across the back of abdomen, behind the stomach. The right side of the organ (called the head) is the widest part of the organ and lies in the curve of the duodenum (the first section of the small intestine). The tapered left side extends slightly upward (called the body of the pancreas) and ends near the spleen.

1.1.2 Pancreas histological structure

The pancreas consists of exocrine and endocrine cells. The former are acinar cells, which are grouped into lobules forming the ductal system, which eventually point into the main pancreatic duct. The endocrine pancreas consists of hormone-producing cells arranged in nets or islets – the islets of langerhans. They do not connect directly to the duct system (Kumar and Clark, 1988).

1.1.3 Pancreas function

Pancreatic acini synthesize digestive enzymes, which are stored in secretary granules and released by exocytosis in response to stimulation by several hormones. The islet of langerhans is composed of four types of cells each of which synthesizes and secreted a distinct poly-peptide hormones. Insulin in the β cell, glucagons in the A-cell, somatostatin in the D-cell and pancreatic poly-peptide in the PP or F-cell, makes up 60% to 80% of islet and form its central core. There are four main types of islet cells and these have different secretary granules in their cytoplasm:

β – cells, which are the most common cells, produce insulin
α – cells, produce glucagons
D –cells produce somatostatin
PP cells produce pancreatic polypeptide (PP)
A number of other hormones (e.g. bombestin, neuropeptide, Y and galanin) are present in pancreatic neurons and probably act as neurotransmitters (Kumar and Clark, 1988).

1.2. Insulin

1.2.1 Structure and secretion
Insulin is key hormone involved in the storage and controlled release within the body of chemical energy available from food. It is coded for on chromosome 11 and synthesized in the β cells of pancreatic islets. Insulin consists of A and B chains linked by disulphide bonds as shown in figure 1 (Kumar and Clark, 1988). The synthesis, intracellular processing and secretion of insulin by the β cell are typical to many peptide hormones. The manufacture and release of insulin from β cell is triggered by the metabolism of glucose entering the β cell, generates ATP; and ATP closes potassium channels in the cell membrane. The cell membrane depolarizes allowing calcium ions to enter the cell via calcium selective channels in the membrane. The rise in intracellular calcium triggers activation of calcium-dependent phospholipids protein kinase which, via intermediary phosphorylation steps, leads to fusion of insulin-containing granules with the cell membrane and exocytosis of the insulin-rich granule contents. Similar in many other endocrine cells secretion, insulin enters the portal circulation and is carried to the liver, its prime target organ. About 50% of secreted insulin is extracted and degrade in the liver, kidneys break down the residence, and c-peptide is only partially extracted by the liver and hence provides a useful index of the rate of insulin secretion, but is mainly degraded by kidneys (Kumar and Clark, 1988).
Figure 1: Insulin Structure, A and B chains linked by disulphide bonds
1.2.2 Insulin function

The actions of insulin have been known for quite sometime (Steiner, 1977). Insulin function include membrane transport of glucose, amino acids and certain ions; increased storage of glycogen; formation of triglycerides; stimulation of DNA, RNA and protein synthesis.

Once in the blood, insulin controls glucose homeostasis by stimulating the uptake of glucose into skeletal muscle and, to a lesser extent, into liver and adipose tissue. In muscle and adipocytes this uptake is mediated by the so-called insulin-sensitive glucose transporter GLUT-4, a process that is not yet understood. Other processes in the regulation of glucose homeostasis are: alterations in glycogen metabolism in muscle and liver and decreased gluconeogenesis in the liver. The enzymes involved in the insulin-regulated processes of glucose metabolism appear to be regulated by (de)phosphorylation of serine and/or threonine residues (Lee and Pilch, 1994).

1.2.3. Other pancreatic hormones function

Three other peptide hormones are produced in the islets of Langerhans in the pancreas:

1. glucagon, consisting of 29 amino acids, in the A cells;
2. somatostatin, a cyclic 14 amino acid polypeptide, in the D cells;
3. pancreatic polypeptide, 36 amino acids with an amide C terminus, in the PP cells.

Glucagon antagonises most of insulin's actions, while stimulating insulin secretion. Somatostatin inhibits the three other islet hormones and a range of hormones from different origins. Pancreatic polypeptide inhibits pancreatic secretion altogether (Johnston et al., 1988).
1.3 Diabetes mellitus

Diabetes mellitus is a syndrome characterized by chronic hyperglycemia that is due to relative insulin deficiency, or resistance, or both. It is affects more than 30 million people worldwide. Diabetes is usually irreversible and although patients can have a reasonably normal lifestyle, its late complications result in reduced life expectancy and considerable uptake of health resources. Macro-vascular disease caused by diabetes mellitus leads to an increased prevalence of coronary artery disease (Kumar and Clark, 1988).

1.3.1 Diabetes mellitus in domestic animals:

Diabetes mellitus is frequently found in the dog (1 in 152), and cats (1 in 800), although it has been reported in horse, cattle, sheep and pigs. The fundamental defect in domestic animals is an absolute or relative lack of insulin. As a result of this deficiency in insulin, the animal is unable to utilize glucose. Consequently hyperglycemia is constant findings in diabetic animal. Clinical signs typically associated with diabetes include polydipsia, polyurea, increased appetite and weight loss. These classic signs of ketosis including anorexia; vomiting, weakness, lethargy and increase respiratory rate (Coles, 1986).

1.3.2 Classification of diabetes mellitus

Most patients can be classified clinically as having either insulin–dependent diabetes mellitus (IDDM or Type 1) or non-insulin dependent diabetes mellitus (NIDDM or Type 1I). In 1979, the National Diabetes Data Group formally classified diabetes mellitus and other categories of glucose intolerance as follows:

1.3.2.1 Type I insulin-dependent-diabetes-mellitus (IDDM):

Since this type usually occurs in juveniles, it was previously called juvenile diabetes. It can, however, start at any age. Patients usually present with easily
recognizable symptoms, so diagnosis is not difficult. Genetic determinants seem important for the onset in most patients, with environmental factors a close second. Abnormal immune responses (e.g. in normal childhood diseases like mumps) and autoimmunity are thought to play an etiologic role.

1.3.2.2 Type II noninsulin dependent diabetes mellitus (NIDDM):
This type can become recognizable at any age, although it can be asymptomatic for years and thus usually presents in patients over 40 years of age. Often only the complications seen after years of having diabetes, like neuropathy and cataracts, cause a diagnosis to be made. Occasionally people are diagnosed as a direct result of population studies, e.g. Mooy et al. (2005), a study in Hoorn, the Netherlands. This research into the prevalence of NIDDM and predictors for the development of the disease resulted in 106 newly diagnosed diabetics out of 2,484 participants between age 50 and 74. Not only age is an important factor in NIDDM, the genetic basis of NIDDM seems even stronger than that of IDDM, and it is aggravated by environmental factors. Moreover, 60 to 90% of all NIDDM patients in the Western world are obese, which should be seen as an indicator for subclassification of the type II diabetes. Symptoms are usually at least partly alleviated by weight loss.

1.3.2.3 Gestational diabetes mellitus (GDM).
There are two ways in which diabetes mellitus and pregnancy can occur simultaneously. One is where a previously diagnosed diabetic woman becomes pregnant, which involves certain risks for both mother and child. The other is in gestational diabetes, when a pregnant women becomes diabetic at some stage during pregnancy, because of the pregnancy and most commonly only for the duration of the pregnancy. It is easy to see the origin of problems in both cases, if one considers the fact that even in a normal pregnancy the third trimester is a
permanent state of mild hypoglycaemia because all organs have to work harder than normal, some up to 50%. Sometimes the pancreas functions sub-optimally. This means that the pregnant woman does not produce enough insulin herself, and the fetus will start to produce insulin to attain an acceptable maternal insulin level. This means the production of more fetus urine into the amniotic fluid. At the same time the level of growth hormone in the fetus rises along with the fetus insulin level (Remmers, 1994). This causes macrosomia (birth weight >4,000 grams), which may cause problems at birth. A Glucose tolerance test usually points out the need for diet or insulin therapy. Oral hypoglycaemic treatment cannot be used by a pregnant diabetic (Drury, 1988). Even after insulin treatment for GDM, most pregnant women return to normal glucose tolerance after delivery. If this is not the case, they have acquired clinical diabetes. From the previously gestational diabetics, 40% acquire overt diabetes mellitus within 20 years (Coustan, 1993) so they should be kept under some sort of observation.

1.3.2.4. Other types of diabetes.

Diabetes forms part of certain other conditions and syndromes, whether obviously an etiologically related or not. This class can be subdivided according to known or suspected a etiological relationships, where diabetes may be secondary to pancreatic disease (neonatal or later on in life), hormonal abnormalities which may have either hypoinsulinaemia or hyperinsulinaemia as a consequence, the administration of certain hormones, drugs and chemical agents, of which oral contraceptives, tricycle antidepressants and marijuana are but a few, insulin receptor abnormalities, either in the number of receptors or their affinity for insulin, or even because of the presence of antibodies to receptors (with or without associated immune disorders), certain genetic syndromes, e.g. metabolism disorders, insulin resistance, hereditary muscle disorders and cytogenic disorders
like Down's syndrome and other types, of which diabetes associated with malnourished populations is the most prominent example.

It may also come secondary to drug administration or other disease or may occur during pregnancy (gestational diabetes mellitus). On rare occasions, diabetes may also result from point mutations in the insulin gene. In the diabetic patient with insulin deficiency or insulin resistance and hyperglucagonemia, there is an increase in gluconeogenesis and glycogenolysis, a decrease in peripheral glucose uptake, and a decrease in conversion of glucose to glycogen in the liver.

1.3.2.4.1. Impaired-glucose-tolerance (IGT).

For the diagnosis of impaired-glucose-tolerance (IGT), an oral glucose tolerance test is essential. The criteria for this classification lie between those for normal subjects and diabetics. Consequently, the seriousness of the disorder is intermediate between normal and diabetic, with some clinical complications being completely absent while others, especially cardiovascular abnormalities, commonly present. Thus, IGT may have prognostic implications that should not be overlooked, especially in seemingly healthy individuals. Like overt diabetes, IGT can be linked to numerous disorders and obesity. Patients with IGT do not necessarily proceed to develop clinical diabetes; many return to normal glucose tolerance for no apparent reason while others stay in the IGT class for many years. Apart from the clinical classes mentioned above, there are two so-called statistical risk classes.

1.3.2.4.2. Previous abnormality of glucose tolerance.

This class is restricted to those persons who have normal glucose tolerance but have previously demonstrated diabetic hyperglycemia or IGT either spontaneously or in response to an identifiable stimulus. Re-classification of gestational diabetics, former obese diabetics who have normal glucose tolerance after weight
loss and temporarily hyperglycaemic patients (due to trauma or injury) into this class is a useful tool for facilitation of follow-up of such patients. The likelihood of such persons developing clinical diabetes again should be considered to be increased.

1.3.2.4.3. Potential abnormality of glucose tolerance (Pot AGT).
Persons who have never exhibited abnormal glucose tolerance but who are at substantially increased risk for the development of diabetes should be classified as PotAGT. Certain risks for development of IDDM and NIDDM are well established, such as being a relative of an IDDM or NIDDM diabetic or belonging to certain ethnic or racial groups, although the degree of risk for any of the specific circumstances is much less clear.

1.3.3 Biochemical changes associated with diabetes mellitus:
Lack of insulin causes significant disturbance of carbohydrate, protein and fat metabolism (Kumar, 1988).

1.3.3.1 Disturbance in carbohydrate metabolism
The disturbance in carbohydrate metabolism is due to the fact that the liver and skeletal muscles can not store glycogen and the muscles are unable to utilize glucose. When the kidney threshold for glucose is exceeded, glucouria occurs with consequent increase of water excretions and disturbances of electrolyte and water balance.

1.3.3.2 Disturbance in the protein metabolism:
Protein metabolism in the liver is also deranged and an excessive amount of protein is transformed into carbohydrate (Wilson et al., 1975)

1.3.3.3 Disturbance in fat metabolism:
In addition, the amount of fat metabolized by the diabetic patient is excessive, and
since normal fat catabolism can only proceeds at a limited rate, Ketone bodies are present in the blood and the urine in much large amount than normally; these substances are excreted in the urine as $\beta$ – hydroxybutyric acid and acetoacetic acid and as acetone in the breath (Wilson et al., 1975). The liver produces ketone bodies by oxidation of the free fatty acids synthesis and this decreases the production of Ketone bodies ,conversely , glucagons stimulates ketone body production by stimulating lipolysis and increasing fatty acid oxidation .In the diabetic patient ,particularly with type 1 diabetes , the consequences of insulin deficiency and glucagons excess provide a hormonal milien that favors ketogenesis and in the absence of appropriate treatment , may lead to ketoneamia and acidosis .

Insulin also interacts with the capillary endothelium to activate lipoprotein lipase, the enzyme that hydrolyses the triglycerides present in very –low –density lipoprotein VLDL and chylomicrons ,resulting in release of intermediate –density lipoprotein IDL particles (Taskinen,1987) these ILD particles are converted by the liver to the more cholesterol –rich – density lipoprotein LDL .In the untreated or under treated diabetic patient , hypertriglycridemia may occur as a result of the decrease removal of VLDL secondary to decrease activity of lipoprotein lipase .In addition, deficiency of insulin may be associated with increased production of VLDL.

1.3.4 Treatment of diabetes mellitus

1.3.4.1 Earliest treatment of diabetes mellitus

Until the 1910s opium was the only widely used medicine in the treatment of diabetes mellitus. However, this could only dull the patients' despair, but did nothing to cure or treat. Further treatment consisted of more or less trendy diets. In
the late 1850s Piorry advised the use of extra sugar, to compensate for the loss of sugar into the urine. This 'eating a lot to compensate' was practised until the early 1900s. In Paris under German siege, in 1870, Bouchardat noticed that rationing of food caused the disappearance of glycosuria in diabetic patients, while exercise also seemed to have a positive effect. The idea settled that may be the body should be put under as little metabolic strain as possible by limited eating.

At the time of the earliest tests of pancreatic extracts in the treatment of diabetes mellitus, America had two leading diabetologists who did not believe in pancreas therapy. They were Allen and Joslin. They both practiced 'starvation treatment' where the patients are undernourished for a certain amount of time. They argued that apart from the carbohydrate metabolism, the protein and fat metabolisms in diabetic patients were also affected. By cutting down on food until the patient's body was relieved of all metabolic strain, and then slowly building up again until a reasonably healthy diet was achieved, many diabetics could live years longer. Some patients, however, did not even tolerate the minimum amount of food (the 'living diet'), and succumbed quickly.

1.3.4.2 Modern treatment of diabetes mellitus with insulin

Since 1922, a lot of research has gone into the improvement of insulin therapy, both in terms of improved insulin preparations and ease of use.

Achieving purity was the first challenge. Abel discovered insulin could be crystallised, which became standard procedure in insulin purification only after. Scott (1934) established that zinc was needed in order to crystallise insulin in rhombohedral form, a discovery inspired by his observation of zinc in the pancreas. Another crystallisation step reduced allergic reactions (Jorpes, 1949). Chromatographic techniques started to play a role in the 1960s, leading to the first
chromatographically purified insulin and monocomponent of insulin (Schlichtkrull et al., 1970). The search for new insulin preparations with various desired properties was approached in many different ways. At first it was thought the number of injections needed every day could be reduced by using insulins with a retarded uptake from the injection site, which could be achieved by introducing basic additives as in protamine and isophane insulin (Hagedorn et al., 1936). Addition of compounds like surfen or globin to acid insulin solutions, which produce heavily insoluble complexes upon neutralisation in tissue fluids, and complex formation of zinc with neutral insulin suspensions, had the same effect. However, the need for strict metabolic control in prevention of long-term complications called for the reinstatement of multiple injections, along with the development of rapid-acting insulin for the relief of the glucose-surge just after meal times, and mixtures of rapid-acting and intermediate-to-long-acting insulins. Developments in this field include Rapitard and Actrapid (Schlichtkrull, 1959; Schlichtkrull et al., 1961). The duration of action could also be influenced by the physical state and size of the insulin particles. Ultralente (Hallas et al., 1956) is an example of a long-acting crystalline insulin preparation. Initially, only bovine insulin was used for crystalline preparations, having a slightly longer action than porcine insulin in crystalline form. Gradually, longer acting porcine preparations became available. Then, in 1979, recombinant DNA techniques made it possible to produce human insulin in Escherichia coli (Goeddel et al., 1979). Amazingly, this was done without knowledge of the nucleotide sequence of the human insulin gene. Crea et al. (1978) had chemically synthesized two separate genes for chain A and B (77 and 104 base pairs for 21 and 30 amino acids, respectively, plus start and stop codons and restriction site bases following nucleotide sequences that had been designed from the amino acid sequences. By designing the nucleotide sequences the way they did, there was no need to produce every possible tri-
nucleotide separately. They produced 29 oligonucleotides, made from carefully chosen di-, tri- and tetra nucleotide building blocks. Goeddel et al. (1979) describe the actual assembly of the genes, the subsequent construction of the plasmid, and the expression and characterizations of the product. They showed that the amino acid content of the product is indistinguishable from that of porcine insulin. It took another year before the actual nucleotide sequence of the human insulin gene was published by Bell et al. (1980). Only 21 out of 51 of the codons used by Crea et al. (1978) turned out to be the same in the correct DNA-sequence.

Production of human insulin could also be achieved by conversion of porcine insulin (Markussen, 1982) or biosynthesis in *Saccharomyces cerevisiae* rather than *E. coli* (Markussen et al., 1986). Over the years, the production of many therapeutic insulins has involved chemical alteration of the molecule. Nowadays, modified insulins can be synthesized by mutation of the genes used in *E. coli* or *S. cerevisiae*, thus facilitating structural and functional studies. But apart from the scientific possibilities opened up by the availability of recombinant insulin, there was a pressing need for a new source of the protein, because the demand for insulin for therapy was outgrowing the supply of slaughter pancreases for the isolation of insulin. Even now (Kott, 1996) the major insulin manufacturers are not able to provide insulin for every area of the world, and cheaper beef and pork insulin is still produced, especially for third world countries.

Because of the varying needs of diabetic patients, insulin has to be available in multidose quantities. This requires the addition of antimicrobial preservatives. Banting and best used tricresol to that effect, and to this day phenol and derivatives like *m*-cresol and methylparaben are used throughout the range of therapeutic agents. Other additives include sodium chloride or glycerol as isotonic agents, and certain buffers (Brange, 1987).
Alongside research into better and purer preparations, came the development of techniques of administering them. At the time of Banting and Best it was already established that oral therapy did not have any desired effect, and that subcutaneous or intravenous injections were the only option. Many other routes of absorption were tested, including rectal administration and absorption by mucosae. Success was limited. Even with more modern technology of aerosol powder (Wigley et al., 1971), surfactants (Hirai et al., 1981), liposome-enclosure (Dapergolas and Gregoriadis, 1976) or polymer-crosslinking (Saffran et al., 1986) the desired efficiency and bioreactivity has not been achieved. The subcutaneous implantation of vinyl-ethylene copolymer pellets (Creque et al., 1980) or biodegradable insulin-albumin microbeads (Goosen et al., 1983), releasing insulin slowly and constantly over a longer period of time, seems more promising. Most recently, reports have appeared on glucose-responsive insulin release from certain polymeric systems (Shiino et al., 1995). However, the only techniques in actual clinical use are based on injections. Hospitals operate systems of continuous infusion, either according to continuously measured glucose concentrations (Albisser et al., 1974) or a pre-programmed schedule. These insulin pumps are not yet available to the general public, although additional research is done on implantable pumps (Buchwald et al., 1981). Today's most patient-friendly portable insulin delivery is the NovoPen, which has reduced injections to just pressing a button. Most diabetics seem to prefer this to other available options (Jefferson et al., 1985).

1.3.5 Inducing Diabetes Mellitus by Cytotoxic agents:

Specific cytotoxic agents such as alloxan or streptozotocin can selectively destroy the $\beta$-cells of pancreas, injection of these agents produces a form of "Chemical" diabetes which closely resembles diabetes due to surgical removeal of the
pancreas. Streptozotocin is a broad spectrum antibiotic with anti-tumor and oncogenic properties which is widely used as a diabetogenic agent in experimental animals (Herr et al., 1967). This action is mediated by beta-cell destruction, which results in the development of an insulin dependent syndrome.

1.4 Medicinal Plants

Recently, many experimental and clinical trials were made to detect the hypoglycemic and anti-diabetic effects of many medicinal plants used in the medicine for the treatment of diabetes mellitus.

Earlier studies on the leave of *Zizyphus lotus* carried by (Glombitza et al., 1994) reported that *Zizyphus lotus* caused a significant reduction in fasting serum glucose, and improved glucose utilization in alloxan diabetic rabbits without affecting serum insulin and peptide levels.

Streptozotocin induced diabetic and non-diabetic rats were given an oral 100 mg/kg dose of butanol fraction prepared from the methanolic extract of leaves of *Zizyphus spina christi*, or 10 mg/kg of christinin – A, the principle saponin glycoside contained in this butanol fraction, treatments were continued daily for period of 1 week or 1 month, whereas no significant effects were observed in non-diabetic (control) rats, both the butanol fraction and christinin-A (long treatments) significantly reduced serum glucose levels, inhibited liver phosphorylase and glucose-6-phosphates activities, significantly increased serum pyruvate level and line glycogen content, and improved glucose utilization in the diabetic rats. The butanol fraction was also found significantly increased serum insulin and pancreatic-c-AMP levels in diabetic rats.

The chloroform and methanolic extracts of seeds of *Citrullus colocynthis* and the ethanolic extracts of leaves and pulp were investigated for pharmacological effects in normal and diabetic (streptozotocin-induced) rats and in isolated organs of rats and rabbits. Neither seed extract had any effect on fasting glucose level in normal
or diabetic rats and they found no effect in the oral glucose tolerance test (Wasfi, 1994). investigated the potential hypoglycemia effect of the decoction of *Junibers communis* berries on normal and streptozotocin – diabetic fanals Wister rats, diabetic rats which were given the decoction at a dose of 125mg of total berries per kg body weight for 24 days, showed a significantly reduced blood glucose levels, lost less weight and had a significant lower mortality index compared with untreated diabetic rats, oral administration of decoction at single dose of 250 and 500 mg/kg had no effect. Studies on intestinal glucose absorption, perphenral glucose consumption and pancreatic action indicated that the hypoglycemic activity of *J.communis* berries is due to an increased consumption of peripheral glucose and or increased glucose – indeed insulin secretion.

1. 4.1 *Pimpinella anisum*

Family: Apiceae (Umbelliferae)
Genus: Pimpinella
Species: *Pimpinella anisum*
Synomons names: Anisum vulgare Gaertn and Anisum Officinarum Moench
English name: Anise or Aniseed
Arabic name: Al yansoon

The origin locations of Pimpinella are Egypt and Greece, and then it spread in different part of the world. In Sudan we found it in northern state and with possibility to grow in Khartoum state.

1.4.2 Plant description:

Anise or sweet Alice belongs to umbelliferae family. Anise is an annual herb that reaches heights of about two feets, leaves and seeds produced in large loose clusters. The upper leaves are very lacy while the lower leaves are broader up to one inch wide resembling parsley. Seeds are oblong about 1/6 inch long and curved, fresh leaves are used for flowering and garnishing, but the important
articles of commerce are seed and oil obtained from them.

1.4.3 Phytochemistry:
Phytochemistry screening of seeds and leaves of pimpinella revealed the presence of acetaldehyde, alpha pinene, alpha terpinean, alpha zingibaren, anisealdehyde anise-acid, anise-alcohol, ascorbic-acid, bergapter B.bisabolene, pinene, boron, caflicaeid, calcium, comphore, chlorogen-acid, choline, copper, carvone, dianethole, estrogole, egalal, fiber, limonen, livaloal, magnesium, manganese, mannitol and trans-a-nethole.

1.4.4 Biological activities
1.4.4.1 Anti-microbial activity
Pimpinella has antimicrobial activity against bacteria and fungi. Hexane and ethyl acetate extraction of pimpinella tuberous roots exhibit abroad spectrum of antimicrobial activity and were analysed for different photochemical.

1.4.4.2 Anti-Oxidant activity:
Antioxidant activities of Pimpinella was determined using the free radical alpha-alpha-diphenyl-beta-picrylhydrazyl(DPPH) and preformed radical monocation 2,2.-azino-bis(3-ethylbenzothiazoline-6-sulphonicacid).Pimpinella had antioxidant capacities in a range comparable to that of alpha tocophand, BHA, ascorbic and Trolox, which were used as reference antioxidants.

1.4.4.3 Acquisition and expression of morphine:
Pimpinella essential oil has effect on acquisition and expression of morphine indeed condition place preterene in mice, and it appeared that the essential oil of the pinpinella may reduce the morphine effects via a GABAergic mechanism.
Figure 2: *P. anisum* flower

Figure 3: *P. anisum* (flowering plants)

Figure 4: *P. anisum* Seeds
Chapter Two

2. Material and Methods

This work has been conducted in the department of Pharmacology in Medicinal and Aromatic Plants Research Institute (MAPRI) National Center for Research, and department of Biochemistry Faculty of Veterinary Medicine, University of Khartoum.

2-1 Materials:

2-1-1 Experimental animals:
Wistar albino rats of either sex were used. Twenty four rats were divided into four groups on the basis of body weights in such way that the average weight of each group was 91-100g.

2-1-2 Plant:

*Pimpinella anisum* was purchased from local market. It was dried in the shade. The seeds were the part used in experimentation.

2-1-3 Experimental design:
The groups were designated as A, B, C and D and six rats were included in each group.
- The rats of groups A and B were kept as normal control.
- The rats of groups C and D were injected with 60mg /kg body weight Streptozotocin dissolved in citrate buffer pH 4.5 to induced diabetes

2-1-4 Feeding Program:
Each group was kept in a cage and was supplied with feed composed of meat and flour plus salt. The feed was available at the rate of 36 g per day in each cage. The four groups were fed as followed as follows:
Group A: received flour and meat diet + salt
Group B: received flour and meat diet + salt + 15% of total diet was replaced by *P. anisum* seed powder

Group C: received flour and meat diet + salt

Group D: received flour and meat+ salt+15% of total diet was replaced by *P. anisum* seed powder.

Table No 1: Experimental design and feeding program

<table>
<thead>
<tr>
<th>Description</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numbers of rats</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Induction of diabetes</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Feeding <em>P. anisum</em> seeds</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

2-1-5 Reagents and chemicals

Reagents used for estimation of biochemical parameters were obtained from Crescent Diagnostics Co.

- Streptozotocin was purchased from Sigma Co.

2-1-6 Instruments:

The instruments used in the experimentation included:

- WTW – pH meter.
- CIBA - Corning colorimeter- Model 252
- I E G – Centra – 4 B centrifuge.
- SB Water bath for the incubation of samples
- FINN pipettes, digital micropipette
- Heparinzed capillary tubes
2-1-7 Samples collection
One to two ml of blood were drawn out from orbital plexus of rats by capillary tubes in fluorinated test tubes from each rat at day 2 to estimate glucose and checking whether the diabetes was induced or not. The rats were weighted every week to monitor its weight. At day 21st the blood was collected from the rats and plasma was separated to perform glucose tolerance test. At day 22\textsuperscript{nd} the rats were sacrificed and blood was collected and centrifuged at 3000 rpm for 5 minuets to separate plasma. The plasma was used to estimate some biochemical parameters.

2-2-Methods

2-2-1: Biochemical methods:
Rats were subjected to fasting for 18 hours period pro experiment. After that the level of plasma glucose was determined at 0 h, then they were injected by 50% glucose solution at dose of 2g/kg and plasma glucose level was monitored 1 h, 2h and 3 h after injection.

2-2-1-1 Determination of glucose
Glucose concentration in the plasma was determined by an enzymatic method as described by Trinder (1969)

Principle;
Glucose is determined after enzymatic oxidation in the presence of the enzyme glucose oxidase. The hydrogen peroxides formed react under catalysis of peroxidase, with phenol and 4. aminophenazone to from a red violet quinoneimine dye as indicator.

\[
\text{Glucose} + \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{GOD}} \text{gluconic acid} + \text{H}_2\text{O}_2 \\
2\text{H}_2\text{O}_2 + 4.\text{aminophenaone} + \text{phenol} \xrightarrow{\text{POD}} \text{quinoneimine} + 4\text{H}_2\text{O}
\]

Reagent:
Phosphate buffer 0.1mol /l pH 7
Phenol 11mmol/l
4. Aminopherazone 1.5 KU/I
Peroxidase 1.5 KU/I

**Standard:**
Glucose 100 mg / dl

**Procedure:**
Twenty µl of each standard and sample were pipetted into separated two test tubes. 2000 µl of the reagent were added to each tube and they were mixed and incubated for 5 minutes at 37°C in water bath. The absorbance of the standard A\(_{\text{(std)}}\) and of the sample A\(_{\text{(s)}}\) were measured against the reagent blank at the wavelength 490 nm by CIBA Corning Colorimeter- Model 252.

**Calculation:**
Glucose concentration (mg/dl) = \(A_{\text{(s)}} \div A_{\text{(std)}} \times 100\)

**2.2.1.2 Determination of total cholesterol:**
The cholesterol concentration was estimated by an enzymatic method, which measures the total cholesterol concentration in the serum or heparinized plasma as described by Richmond (1973)

**Principle:**
Cholesterol and its esters are released from lipoproteins by detergents. Cholesterol esterase hydrolyzes the esters and H\(_2\)O\(_2\) is formed in the subsequent enzymatic oxidation of cholesterol by cholesterol – oxidase according to the following reactions:

\[
\text{Cholesterol ester} + \text{H}_2\text{O} \xrightarrow{\text{cholesterol esterase}} \text{cholesterol} + \text{fatty acids}
\]
\[
\text{Cholesterol} + \text{O}_2 \xrightarrow{\text{Cholesterol oxidized}} \text{Cholesten -3-on} + \text{H}_2\text{O}_2
\]
\[
2\text{H}_2\text{O}_2 + \text{phenol} + 4.\text{aminophenazone} \xrightarrow{\text{peroxidase}} \text{quinoneimine-dye} + 4\text{H}_2\text{O}
\]
**Reagents:**

- **Reagent (buffer) R1:**
  - Pipes buffer pH: 6.9 \(90\text{mmol/l}\)
  - Phenol \(26\text{mmol/l}\)

- **Enzyme reagent R2:**
  - Cholesterol oxidase \(200\text{U/l}\)
  - Cholesterol esterase \(300\text{U/l}\)
  - Peroxidase \(1250\text{U/l}\)
  - 4. Aminophenazone \(0.4\text{mmol/l}\)

- **Standard Reagent R4:**
  - Cholesterol \(200\text{mg/l}\)

To prepare the working reagent, R2 were dissolved with the corresponding volume of buffer R1.

**Procedure:**

Twenty µl of the standard and sample were pipetted into two separated test tubes, 2000 µl of the working reagent were added, mixed and incubated for 5 minutes at 37°C in water bath. Absorbance of the sample \(A_{(s)}\) and of standard \(A_{(std)}\) were measured against a reagent blank at wavelength 550 nm by CIBA Corning Colorimeter-Model 252.

**Calculation:**

Cholesterol concentration \(= A_{(s)} ÷ A_{(std)} \times \text{standard concentration (200mg/l)}\)

2.2.1.3 **Determination of triglyceride**

Triglyceride level in plasma was measured by an enzymatic method in which triglyceride are enzymatic hydrolyzed to glycerol as reported by Richmond (1973) and according to the following reactions:

\[
\text{Triglyceride} \xrightarrow{\text{Lipoprotein Lipase}} \text{glycerol + free fatty acids}
\]
Glycerol +ATP $\xrightarrow{\text{Glycerol Kinase}}$ Glycerol-3-Phosphate +ADP

Glycerol-3-Phosphate+O$_2$ $\xrightarrow{\text{Glycerol-3-phosphatase}}$ Dihydroxy acetone phosphate +H$_2$O$_2$

H$_2$O$_2$ +4-amino antipyxine +p-chlorophenol $\xrightarrow{\text{Peroxidase}}$ 4H$_2$O +4 quinoneimine-dye

**Reagents:**

Buffer reagent (R1):
- Pipes buffer pH: 7.2 50mmol / l
- P-chlorophenol 2mmol/l

Enzymatic reagent (R2):
- Lipoprotein lipase 150,000 U/l
- Glycerol Kinase 800U/l
- Glycerol -3-p-oxidaese 4000 U/l
- Peroxidase 440 U/l
- 4-aminoamtipyrine 0.7mmol / l
- ATP 0.3mmol / l

Standard reagent (R3):
- Glycerol 200 mg /dl

Working reagent:
- R2 were dissolved with the corresponding volume of buffer R1

**Procedure:**

Twenty µl of sample and standard were pipetted into two separated test tubes. 2000 µl of the working reagent were added. They were mixed and incubated at 37°C for 5 minutes in water bath. The absorbance of the sample $A_{(s)}$ and standard $A_{(std)}$ were measured against reagent blank at wave length 546 nm by CIBA Corning Colorimeter- Model 252.

**Calculations:**

Triglyceride concentration mg/dl = $A_{(s)} \div A_{(std)} \times$ standard concentration (200mg/dl)
2.2.1.4 Determination of plasma total protein:
Photometric colorimetric method (Biuret method) was used to determine the total protein in the plasma.

Principle:
Protein in serum or plasma forms a blue violet complex when mixed with copper ions in alkaline solution. In Biuret reaction each copper ion binding with 5 or 6 peptide bonds. Tartarate is added as a stabilizer and iodide is used to prevent auto reduction of the alkaline copper complex. The absorbance of this complex measured at 546 nm is proportional to protein concentration.

Reagents:
Biuret reagent:
- Sodium hydroxide 200mmol /l
- Potassium sodium tartrate 32mmol /l
- Copper sulphate 18mmol /l
- Potassium iodide 30mmol /l

Standard
Protein 8g/dl

Procedure:
The standard (0.02 ml) and the sample (0.02 ml) were pipetted into two separated test tubes. One ml of the Biuret reagent was added to each tube then they were mixed and incubated at 37°C for 5 minutes in water bath. Absorbance of sample ($A_S$) and standard ($A_{std}$) were measured against the reagent blank within 30 minutes by CIBA Corning Colorimeter- Model 252 at wave length 546 nm.

Calculation:
Plasma total protein (g/dl) = $A_S \div A_{std} \times$ concentration of standard
2.2.1.5 Determination of plasma albumin:

Albumin concentration in plasma was determined by colorimetric test – BCG Method.

**Principle:**

Albumin binds selectively to dye bromocresol green at pH 4.2. The absorbance of this blue/green complex at 546 nm is proportional to albumin concentration.

**Reagents:**

- Citrate buffer (pH 4.2) 7.5 mmol/l
- Bromocresol green 180 mmol/l
- Sodium Azide 0.05%
- Standard Albumin 4 g/dl

**Procedure:**

The standard (0.01 ml) and the sample (0.01 ml) were pipetted into two separated test tubes. One ml of reagent was added to each tube. Then they were mixed and incubated in water bath for 8 minutes at 20-25°C. The absorbance of the sample ($A_S$) and standard ($A_{std}$) were measured against reagent blank by CIBA Corning Colorimeter- Model 252 at wave length 546 nm.

**Calculation:**

Albumin concentration (g/dl) = $A_S$/ $A_{std}$ X concentration of standard

2.2.1.6 Determination of creatinine

Kinetic method without deproteinisation – Jaffe reaction was used to estimate the creatinine concentration in the plasma in this study.

**Principle:**

In the Jaffe reaction, creatinine reacts with alkaline picrate to produce a reddish-orange colour, the intensity of which at 490 nm is directly proportional to...
creatinine concentration

**Reagent:**
- Picric acid 35mmol/l
- Sodium hydroxide 320mmol/l
- Standard Creatinine 2mg/l

**Procedure:**
The standard (0.1) ml and the sample (0.1) ml were pipetted into two separated test tubes. Then one ml of the reagent was added to each tube, and in other two separated test tubes 0.05ml of sample and standard were pipetted, and 0.5ml of the reagent was added to each tube. Then the tubes were mixed well in each case. Absorbance A1 was measured after 30 seconds for first two tubes, and after 2 minutes the absorbance A2 was measured for second two tubes by CIBA Corning Colorimeter- Model 252 at wave length 490 nm.

A = A2 - A1 for each tubes.

**Calculation:**
Creatinine mg/dl = $$A_{(s)} / A_{(std)} \times \text{concentration of Standard}$$.

### 2.2.1.7 Determination of urea:
Enzymatic colorimetric, endpoint- Berthelot method was used to measure the urea level in the plasma examined in this study.

**Principle**
Urease catalyses the conversion of urea to ammonia. In modified Berthelot reaction, the ammonium ions react with mixture of salicylate, hypochlorite and nitroprusside to yield a blue – green dye (indophenol) the intensity of this dye is proportional to urea in the sample.

$$\text{Urea} + \text{H}_2\text{O} \xrightarrow{\text{Urease}} 2\text{NH}_3 + \text{CO}_2$$
NH$_3$+Salicilate + hypochlorite $\xrightarrow{\text{nitroprusside}}$ 2-Dicarboxy-indophenol

**Reagents:**

1. Phosphate buffer 120mmol/l
   
   Sodium salicylate 60mmol/l
   
   Sodium nitroprusside 5mmol/l
   
   EDTA 1mmol/l
   
   Urease 5KU/l

2. Phosphate buffer 120mmol/l
   
   Sodium hydroxide 400mmol/l
   
   Sodium hypochlorite 10mmol/l

3. Standard
   
   Urea 80 mg/dl

**Procedure:**

The standard (0.01) ml and the sample (0.01) ml were pipetted into two separated tubes. Reagent (1.0) ml was added to each tube then they were mixed and incubated in water bath for 5 minutes at 25°C after that 1.0 ml of reagent 2 was added to each tube. They were mixed and incubated for two minutes at 25°C. The absorbance of sample ($A_S$) and standard ($A_{std}$) were measured against the reagent blank by CIBA Corning Colorimeter- Model 252 at wave length 578 nm.

**Calculation:**

Urea (mg/dL) = $A_S/A_{std}$ X concentration of standard.

**2.2.1.8 Determination of bilirubin**

Bilirubin concentration in plasma was determined according to the method.

**Principle:**

Bilirubin in the presence of a sulphanilic acid diazonium salt forms a red coloured azo compound in alkaline solutions. The total and direct Bilirubin in plasma is determined using the method on which we can coupling it with diazotized
sulphanilic acid after the addition of caffeine and sodium benzoate.

**Reagents:**
Reagents (R1)- Sulphanic acid solution
   Sulphanilic acid                   30mmol/l
Reagent (R2)- Sodium nitrite solution
   Sodium nitrite                      50mmol/l
Reagent (R3)- caffeine solution
   Caffeine                               100mmol/l

**Procedure:**
In three test tubes indicated as total, direct and blank, 200 µl of reagent (R₁) was pipetted in each tube. Then one drop of R₂, 2.0ml of R₃ and 200 µl of sample were added into total tube. In direct tube, one drop of R₂, 2.0ml of saline and 200 µl of sample were added. In blank tube 2.0ml of saline and 200 µl of sample were added. The tubes were mixed and incubated for 5 minutes at 28°C. The absorbance of total and direct were measured against reagent blank by CIBA Corning Colorimeter- Model 252 at wave length 549nm.

**Calculation:**

Total bilirubin (mg/dl) = Absorbance of total tube X17.5

Direct Bilirubin (mg/dl) = Absorbance of direct tube X17.5

Bilirubin (mg/dl) = Total – Direct

**2.2.1.9 Determination of alkaline phosphatase:**
The concentration of alkaline phosphatase in the plasma was determined by optimized standard method.
**Principle:**

\[ \text{p-nitrophenylphosphate} + \text{H}_2\text{O} \xrightarrow{\text{Alkaline phosphatase}} \text{phosphate} + \text{p-nitrophenol} \]

**Reagents:**

**Buffer**

- Diethanolamine buffer
  - 1 mol/l (pH 9.8)
- MgCl₂

**Substrate**

- p-nitrophenylphosphate
  - 10 mmol/l

**Procedure:**

Half µl of the sample was pipetted into a test tube, then 3 µl of reagents were added and mixed, the initial absorbance was read and timer was started simultaneously. Then the absorbance was read again after 1, 2, and 3 minutes at wavelength 405 nm by CIBA Corning Colorimeter- Model 252.

**Calculation**

The alkaline phosphatase activity was calculated by the following formula:

\[ \text{U/l} = 3300 \times \Delta \text{A} \text{ 405nm/min Macro} \]

2.2.1.1. Determination of glutamic-oxaloacetic transaminase

The activity of glutamic oxaloacetic transaminase was measured by Reitman methods.

**Principle**

Glutamic oxaloacetic transaminase is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine.

\[ \text{α- oxoglutarate} + \text{L-aspartate} \xrightarrow{\text{GPT}} \text{L-glutamate} + \text{oxaloacetate} \]
Reagents

1-Buffer

- Phosphate buffer 100 mmol/l, pH 7.4
- L-aspartate 100 mmol/l
- \(\alpha\) oxoglutarate 2 mmol/l

2- 2,4dinitrophenyl hydrazine 2 mmol/l

3- Sodium hydroxide solution 0.4 mol/l

Procedure:

0.5 ml of the Buffer and 0.1 ml distilled water were pipetted in a test tube and labeled as reagent blank, 0.1 ml sample and 0.5 ml of the buffer were pipetted in another test labeled as sample then they were mixed and incubated for exactly 30 min at 37 °C after that 0.5 ml of 2,4dinitrophenyl hydrazine was added to each tube. The absorbance of sample (A) against the reagent blank after 5 min was read at wave length 546 nm by CIBA Corning Colorimeter- Model 252.

Calculation:
The activity of GOT obtained from ideal Calibration curved for the absorbance against the activity.

2.2.1.11. Determination of glutamic pyruvic transaminase (GPT):
The activity of glutamic pyruvic transaminase (GPT) was measured by Reitman method.

Principle:
\[ \alpha- \text{oxoglutarate} + \text{L-alanine} \xrightarrow{\text{GPT}} \text{L-glutamate} + \text{pyruvate} \]
Glutamic pyruvic transaminase is measured by monitoring the concentration of pyruvate hydrazone formed with 2-4-dinitrophenythydrazine.
**Reagent**

**Buffer**

- Phosphate buffer: 100 mmol/l, pH 7.4
- L-alanine: 200 mmol/l
- α-oxoglutarate: 2.0 mmol/l
- 2,4-dinitrophenylhydrazine: 2.0 mmol/l
- 3- Sodium hydroxide solution: 0.4 mol/l

**Procedure:**

Half ml of the Buffer and 0.1 ml distilled water were pipetted in a test tube and labeled as reagent blank, 0.1 ml sample and 0.5 ml of the buffer were pipetted in another test labeled as sample then they were mixed and incubated for exactly 30 min at 37 °C after that 0.5 ml of 2,4-dinitrophenyl hydrazine was added to each tube. The absorbance of sample (A) against the reagent blank after 5 min was read at wave length 546 nm by CIBA Corning Colorimeter- Model 252.

**Calculation:**

The activity of GOT obtained from ideal Calibration curved for the absorbance against the activity.

### 2.2.1.12 Determination of sodium and potassium:

The concentration of sodium and potassium in the plasma were determined according to the Standard Official Method of Analysis (A.O.A.C,) using Coring 410, flame photometer.

**Principle:**

Using compressed air, diluted plasma is sprayed as a fine mist of droplets into a non-luminous gas flame which becomes colored by the characteristic emission of
the sodium or potassium metallic ions in the sample.

Light of wave length corresponding to the metal being measured is selected by a light filter or prism system and allowed to fall on a photosensitive detector system.

The amount of light emitted depend on the concentration of metabolic ions present in the sample.

**Procedure:**

One ml of the sample was taken and diluted in a 50ml conical flask with distilled water. The standard of the KCl and NaCl were prepared by dissolving 2.54g, 3.33g of KCl and NaCl respectively, each in 1000ml distilled water. Ten ml of this solution were taken and diluted with 1000 ml distilled water to give a 10 ppm concentration. The flame photometer was adjusted to zero degree using distilled water as blank and 100 degree using standard solution. Then the flame photometer reading was recorded for each sample.

**Calculation:**

Concentrations of the alkaline metals were calculated by the following equations:

\[
\text{Mineral K or Na (\%) = } \frac{[\text{F.R} \times \text{D.F} \times 100]}{106 \times \text{S} \times 10}
\]

**Where:**

FR=Flame photometer reading

DF=Dilution factor

S =Sample weight
2.3 Statistical Design:

According to completely randomized design the rats were divided into four groups (A, B, C and D). Each group contains 6 rat of similar body weight. The data were analyzed by one and two way ANOVA procedure according to SPS software. Each test was conducted at 5% level of significance. Means were separated by Duncan multiple range test.
Chapter three

Result

3.1 The effect of Streptozotocin injection on plasma glucose concentration of rats
When wistar albino rats were injected with streptozotocin, the plasma glucose concentration in day 2 increased to (321 and 293.1) for group (C&D) respectively when compared with glucose level in day Zero, while the glucose level at day 2 of control groups (A & B) remain the same when compared glucose level at day as shown in figure 3.

3.2 The effect of feeding P.anisum on plasma glucose concentration of normal and diabetic rats
When P.anisum seeds powder was added to the diet of normal and diabetic rats, plasma glucose level measured after overnight fasting and then after 1, 2 and 3 hours after break fasting are shown in table 2.
At 0h, there is no significant difference in glucose level between normal rats fed diet only (group A) and normal rats fed diet plus P.anisum powder (Group B), although the glucose level of P.anisum fed normal rats (Group B) was numerically lower than (group A) normal rat fed diet only (77.8 vs 71.8).
The glucose level of diabetic rats (group C) at 0h was significantly higher than glucose level of normal rats fed diet only (group A) and normal rats fed diet containing P.anisum (Group B). The glucose plasma concentration of diabetic rats fed diet containing P.anisum (group D) was significantly lower than glucose concentration of diabetic rats fed diet only (group C).
At 1 hour, the plasma glucose concentration of normal rats fed diet only (group A)
and normal rats fed diet containing *P. anisum* were similar (155.8 vs 153). While the plasma concentration of diabetic rats (group C) was significantly higher than normal rats (group A), normal rats fed *P. anisum* (group B) and diabetic rats fed diet containing *P. anisum* (group D).

The plasma glucose level of diabetic rats fed *P. anisum* after 1 hour was significantly higher than plasma glucose level of normal rats fed diet only (group A) and normal rat fed diet containing *P. anisum* (Group B).

Two hours after feeding of fasted rats, the plasma glucose level of normal rats fed diet and *P. anisum* (Group B) was significantly lower than normal rats fed diet only (group A), diabetic rats (group C) and diabetic rats fed diet containing *P. anisum* (group D).

While the plasma glucose concentration of diabetic rats fed diet only is significantly higher than normal rats fed diet only (group A). The plasma glucose concentration of diabetic rats fed diet containing *P. anisum* (group D) was significantly lower than diabetic rats fed diet only (group C), but significantly higher than normal rats fed diet only (group A) and normal rats fed diet containing *P. anisum* (Group B).

Three hours after feeding, the glucose level of normal fed diet and *P. anisum* (Group B) was significantly higher than normal rats feed diet only (group A) (88.7 vs 84.8). While plasma glucose concentration of diabetic rats fed diet only (group C) was significantly higher than normal rats (group A and Group B) and diabetic rats fed *P. anisum* (group D), when examined three hours after feeding.

### 3. 3 The effect of feeding *P. anisum* on plasma total protein and plasma albumin of normal and diabetic rats

When *P. anisum* fed to normal and diabetic rats, it was noticed, that the feeding of *P. anisum* had no effect on plasma total protein concentration as shown in table
The level of plasma albumin of normal rats (group A), and normal rats fed diet containing *P. anisum* (Group B) and diabetic rats fed diet only (group C) were not significantly different but the feeding of *P. anisum* significantly decreased the albumin concentration of diabetic rats (group D) when compared with normal rats fed *P. anisum* (Group B) (3.71 vs 4.31).

### 3.4 The effect of feeding *P. anisum* on GOT, GPT and alkaline phosphatase enzymes of normal and diabetic rats

The feeding of *P. anisum* had no significant effect on plasma concentration of GOT, GPT and alkaline phosphatase enzymes of normal and diabetic rats (p>0.05).

However, the feeding of *P. anisum* to normal rats (Group B) increased numerically the plasma concentration of GOT when compared with normal rats (Group A) (7.33 vs 5.66) and increased numerically the plasma concentration of diabetic rats (Group D) when compared with normal rats (Group A) (7.33 vs 5.66) and diabetic rats (Group C) (7.33 vs 6.16).

The feeding of *P. anisum* decreased numerically the plasma GPT concentration of normal rats (Group B) when compared with normal rats fed diet only (Group A) (4.66 vs 6.66), but feeding *P. anisum* increased slightly the concentration of GPT plasma concentration of diabetic rats (Group D) when compared with diabetic rats fed diet only (Group C) (4.66 vs 4.16).

The Alkaline phosphatase plasma concentration was almost the same in all experimental groups (p=0.106) as shown in table 4.
Figure 5: Level of the glucose at Zero and 48 hours after Streptozotocin injection
Where:
A Normal group.
B Normal group.
C Injected with Streptozotocin.
D Injected with Streptozotocin.
Table (2): The effect of feeding *P. anisum* on plasma glucose concentration of normal and diabetic rats

<table>
<thead>
<tr>
<th>Test</th>
<th>Treatment</th>
<th>SD</th>
<th>Prob</th>
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<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>0 h</td>
<td>77.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>220.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1 h</td>
<td>155.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15308&lt;sup&gt;a&lt;/sup&gt;</td>
<td>335.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 h</td>
<td>101.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>333.7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>3 h</td>
<td>84.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>88.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>330.7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean values have different superscript letters within each raw differ significantly at (P≤0.05)

Table (3) the effect of feeding *P. anisum* on normal and diabetic rats plasma total protein and albumin

<table>
<thead>
<tr>
<th>Test</th>
<th>Treatment</th>
<th>SD</th>
<th>Prob</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>Total Protein</td>
<td>7.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Albumin(g/dl)</td>
<td>4.15&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.00&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean values have different superscript letters within each raw differ significantly at (P≤0.05)
3. 5 The effect of feeding *P. anisum* on normal and diabetic rats plasma concentration of cholesterol, triglyceride and bilirubin

The feeding of *P. anisum* did not affect significantly the plasma cholesterol of normal rats (Group B) when compared with normal rats fed diet only (Group A) (185.2 vs 183.5) nor plasma cholesterol of diabetic rats (Group D) when compared with diabetic rats fed diet only (Group C) (198.0 vs 207.5). It was noticed the plasma cholesterol concentration of diabetic rats (Group C and Group D) was numerically higher than normal rats (Group A and Group B) (207.5 - 198.0 vs 183.5 - 185.2).

Also the feeding of *P. anisum* did not affect significantly the concentration of plasma triglyceride of normal rats (Group B) when compared with normal rats fed diet only (Group A) (182.8 vs 180.5), nor plasma triglyceride of diabetic rats (Group D) when compared with diabetic fed diet only (Group C) (172.8 vs 166.8). However, the plasma triglyceride concentration of diabetic rats (Group C) was significantly lower than normal rats fed diet alone (Group A) and normal rats fed *P. anisum* (Group B).

The plasma bilirubin concentration was not significantly affected by feeding *P. anisum* in normal rats (Group A vs. Group B) (0.866 vs. 0.780) nor in diabetic rats (Group C vs. Group D) (0.683 vs. 0.750). The plasma bilirubin concentration of diabetic rats (Group C) was numerically lower than that of normal rats fed diet alone (Group A) (0.683 vs. 0.866) as shown in table 5.

3. 6 The effect of feeding *P. anisum* on normal and diabetic rat's plasma urea and creatinine concentrations

The feeding of *P. anisum* did not affect significantly the plasma urea concentration of normal rats (Group A vs Group B) nor diabetic rats (Group C vs Group D), but numerically decreased the plasma urea of diabetic rats (27.83 vs 34) when
compared with diabetic rats.
However, the plasma urea concentration of diabetic rats (Group C) and diabetic rats fed *P. anisum* (Group D) was significantly higher than normal rats (Group A) and normal rats fed *P. anisum* (Group B) as shown in table 5.
The feeding of *P. anisum* did not affect significantly the plasma creatinine concentration when fed to normal rats (Group A vs Group B) nor diabetic rats (Group C vs Group D), but lowered numerically the plasma creatinine concentration of diabetic rats (Group C vs Group D) (0.816 vs 0.700) as shown in table 6.

### 3.7 The effect of feeding *P. anisum* on normal and diabetic rats plasma sodium (Na\(^+\)) and potassium (K\(^+\)) concentrations:
The plasma sodium concentration was not significantly affected when *P. anisum* was fed to normal rats (Group A vs Group B) (137.2 vs 143.2) nor when fed to diabetic rats (Group C vs Group D) (140.2 vs 138.8).
The feeding of *P. anisum* increased numerically the plasma potassium concentration of normal rats (Group A vs Group B) (3.75 vs 3.96) but increased significantly the plasma potassium concentration of diabetic rats (Group C vs Group D) (3.7 vs 4.10) as shown in table 7.

### 3.8 The effect of feeding *P. anisum* on body weight of normal and diabetic rats:
The feeding of *P. anisum* decreased significantly the body weight of normal rats (Group B vs. Group A) and diabetic rats (Group D vs. Group C) as shown in table 8. The body weight of diabetic rats whether fed diet only (group C) or fed diet containing *P. anisum* was significantly lower than the body weight of normal rats (group A), while the body weight of diabetic rats fed *P. anisum* was significantly lower than normal rats fed *P. anisum* (group B) and diabetic rats fed diet only (group C).
Table (4) the effect of feeding *P. anisum* on GOT, GPT and ALK phosphatase plasma concentration of diabetic and normal rat’s enzymes

<table>
<thead>
<tr>
<th>Test</th>
<th>Treatment</th>
<th>SD</th>
<th>Prob</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>GOT (U/I)</td>
<td>5.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GPT(U/I)</td>
<td>6.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.16&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALK phosphatase</td>
<td>170.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>167.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>169.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean values have different superscript letters within each raw differ significantly at (*P*≤0.05)

Table (5) the effect of feeding *P. anisum* on normal and diabetic rat's plasma cholesterol, triglyceride and bilirubin concentrations

<table>
<thead>
<tr>
<th>Test</th>
<th>Treatment</th>
<th>SD</th>
<th>Prob</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>183.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>185.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>207.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>180.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>182.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>166.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bilirubin (mg/l)</td>
<td>0.866&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.780&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.683&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean values have different superscript letters within each raw differ significantly at (*P*≤0.05)
Table (6) the effect of feeding *P. anisum* on normal and diabetic rat's plasma urea and creatinine concentrations

<table>
<thead>
<tr>
<th>Test</th>
<th>Treatment</th>
<th>SD</th>
<th>Prob</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>20.17b</td>
<td>20.33b</td>
<td>34.00a</td>
</tr>
<tr>
<td>Creatinin (mg/dl)</td>
<td>0.633a</td>
<td>0.650a</td>
<td>0.816a</td>
</tr>
</tbody>
</table>

Mean values have different superscript letters within each raw differ significantly at (P ≤ 0.05)

Table (7) the effect of feeding *P. anisum* on normal and diabetic rats’ plasma sodium (Na⁺) and potassium (K⁺) concentration.

<table>
<thead>
<tr>
<th>Test</th>
<th>Treatment</th>
<th>SD</th>
<th>Prob</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>Sodium (Na⁺) (%)</td>
<td>137.2 a</td>
<td>143.3 a</td>
<td>140.2 a</td>
</tr>
<tr>
<td>Potassium (K⁺) (%)</td>
<td>3.75 b</td>
<td>3.96 ab</td>
<td>3.70 b</td>
</tr>
</tbody>
</table>

Mean values have different superscript letters within each raw differ significantly at (P ≤ 0.05)
Table (8) the effect of feeding *P. anisum* on body weights of normal and diabetic rats

<table>
<thead>
<tr>
<th>Test</th>
<th>Treatment</th>
<th>SD</th>
<th>Prob</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (g)</td>
<td>A 125.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>95.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>98.40&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean values have different superscript letters within each raw differ significantly at (P ≤ 0.05)
Chapter Four
Discussion

Many experiments and clinical trials were made to detect the hypoglycemic and antidiabetic effects of some medicinal plants used for treatment of diabetes mellitus such as *Zizphus Lotus* (Glomitza *et al.*, 1994), *Citrullus colocynthis* (Wasfi, 1994), *Junibers communis*, and *cinnamon*. *P. anisum* is a medicinal plant, which has antimicrobial activity against bacteria and fungi, anti-oxidant activity and increase glucose absorption activity (Kreydiyyeh *et al.*, 2003).

The current study was carried out to investigate the antidiabetic effect of *P. anisum*. The effects of feeding *P. anisum* on normal rats and streptozotocin induced diabetic rats were examined. Glucose tolerance test was performed and plasma total protein, albumin, cholesterol, triglyceride, bilirubin, Na+, K+, enzymes and body weight were monitored.

In this experiment, diabetes mellitus was induced in rats by injection of streptozotocin and the glucose tolerance test confirmed, the injection of streptozotocin could induce diabetes mellitus as also shown by previous experiments. The diabetogenic effect of this antibiotic is mediated by B-cell destruction, which result in development of an insulin dependent syndrome. This condition is most commonly associated with hypoinsulinaemia and it may occur as primary entity or may be associated with acute pancreatic necrosis (Greene and Latimar, 1983). The fasting insulin decreased to about one third and fasting glucagons increases three fold (Admdal *et al.*, 1986). This result in raised glucose concentration in experimentally induced diabetic rats (Greene and Lattimar, 1983). Glucose tolerance test carried out in this experiment showed, the feeding of *P. anisum* to diabetic rats lowered significantly the plasma glucose level of diabetic rats (Group D vs Group C) when measured at 0, 1, 2, 3 h after fasting.
This indicates *P. anisum* feeding has significant effect on concentration of plasma glucose in diabetic rats. However, the feeding of *P. anisum* did not lower the plasma glucose level to normal concentration when diabetic rats fed *P. anisum* (Group D) were compared with normal rats whether fed *P. anisum* (Group B) or not (Group A).

In this study, it was noticed, the feeding of *P. anisum* to normal rats (Group B vs Group A) lowered numerically the plasma glucose level at 0h, and had no effect at 1h and increased the level significantly at 3h but the difference was small.

It seems, the *P. anisum* feeding has more pronounce effect on plasma glucose concentration in diabetic rats. This agrees with previous studies which showed medicinal plants, *Zizphus lotus* (Glombitza *et al*., 1994), and *Jumibers commuins* berries had antidiabetic effect and lowered the blood glucose level in induced diabetic rabbits and rats. Also it was found that, cinnamon reduced the mean fasting serum glucose (18-29%) in people with type 2 diabetes (Khan *et al*., 2003). *Zizyphus lotus* caused significant reduction in fasting serum glucose and improved glucose utilization in alloxan diabetic rabbits (Glombitaz *et al*., 1994).

The hypoglycemic activity of *J. communis* berries is due to an increases consumption of peripheral glucose and or increased insulin secretion. Thus, it can be concluded *P. anisum* has some antidiabetic effect. Further work is needed to investigate the mechanism of action and to identify the active ingredients of *P. anisum*.

The feeding of *P. anisum* had no effect on plasma total protein and albumin concentration in normal or diabetic rats in this study. This revealed the feeding of *P. anisum* has no deleterious effect on liver or kidney, since it was reported that drastic alteration in plasma proteins values may be observed in association with both kidney and liver diseases (Coles, 1986).

However, the albumin concentration in diabetic rats fed *P. anisum* (Group D) and
plasma albumin in diabetic rats fed diet only (Group C) was significantly low when compared with normal rats (Group A). This hypoalbuminemia may be attributed to diabetes, as it is known hypoalbuminemia develop as result of excessive breakdown which occurs in prolonged fever, and uncontrolled diabetes mellitus (Coles, 1986).

The feeding *P. anisum* had no significant effect on plasma GOT, GPT and alkaline phosphatase in both normal and diabetic rats in this experiment, but the feeding increased numerically the plasma GOT level in both normal and diabetic rats. This numerical increase in GOT might indicate slight pathological changes in the liver as GOT increased in serum when cellular degeneration or destruction in the liver occurs (Coles, 1986).

Also in this experiment there was no significant differences in plasma GOT and GPT between normal and diabetic rats this disagree with Awadallah and El-Dessouky (1977) who reported significant increase in GOT and GPT when normal rats were compared with diabetic rats. Also disagree with Mannipieri et al. (2005) who reported that higher GOT (ALT) was significantly associated with both impaired glucose tolerance and diabetes.

This study reveal significant changes in plasma alkaline phosphatase in diabetic rats when compared with normal rats. However, Mannipieri et al. (2005) reported significant association between higher alkaline phosphatase and diabetics.

In this experiment, the feeding of *P. anisum* did not affect significantly the plasma cholesterol level in normal or diabetic rats. It was also observed, the cholesterol level of diabetic rats (Group C and D) was significantly higher than that of normal rats (Group A and B). Blood cholesterol levels invariably increased in diabetic animals and high total cholesterol levels were observed in dogs with leptospirosis, diabetes mellitus or nephritis (Coles, 1986).

It was also noticed in the current experiment, the level of plasma triglyceride was
not affected by feeding *P. anisum* to normal or diabetic rats but triglyceride level in the diabetic rats fed diet only (Group C) was significantly lower than normal rats whether fed diet only (Group A) or diet with *P. anisum* (Group B). This disagree with findings of Khan *et al.* (2003) who reported high triglyceride level which was reduced by consumption of cinnamon by human patient with type 1 diabetes.

The plasma bilirubin level was not significantly different in normal and diabetic rats, however the bilirubin level in diabetic rats was numerically lower than normal and diabetic rats fed *P. anisum*. This reduction in bilirubin agree with that of Stone *et al.* (1997) who reported significant increase bile acid excretion and bile flow in streptozotocin induced diabetic rats and decrease in plasma bilirubin concentration. This explains by the fact that, the bilirubin increase is due to decreased removal of bilirubin by hepatocellular transport (Coles, 1986).

The plasma urea concentration in normal and diabetic rats was not affected significantly by *P. anisum* feeding. This indicates *P. anisum* probably has no deleterious effect on the liver or the kidney of normal and diabetic rats.

Elevation of plasma urea in diabetic rats was observed in this investigation. This agree with that of Almdal *et al.* (1986) who found in streptozotocin induced diabetic rats, urea nitrogen synthesis capacity was doubled. They suggested the increase of urea synthesis to be due to enzyme induction by glucagon enzyme which increases in diabetic rats. Cole (1986) stated that ureamia is frequently seen with diabetes mellitus, although it is not a consistent finding.

The plasma creatinine level was not affected significantly in normal rats by *P. anisum* feeding but the feeding decreased numerically the creatinine concentration in diabetic rats (Group D and Group C).

The level of plasma creatinine in diabetic rats was not significantly different from that of normal rats but it was numerically higher as the rate of secretion of
creatinine is influenced by GFR and abnormality that decreases GFR will result in an increase in the concentration of serum creatinine (Coles, 1986). It seems, induced diabetes influenced slightly the GFR in rats and thus increases numerically the creatinine level in diabetic rats. Also, it seems, *P. anisum* feeding relieved to some extent the diabetogenic effect and lowered numerically the level of plasma creatinine of diabetic rats (Group D vs Group C).

The concentrations of Na+ in plasma in normal and diabetic rats were not significantly different. This showed the feeding of *P. anisum* has no effect on plasma Na+ concentration in normal and diabetic rats and also diabetes had no effect on plasma Na+ concentration in diabetic rats. Coles (1986) stated that, there may be occasional hyponatremia and sodium may be lost as consequence of impaired kidney reabsorption associated with osmotic diuresis. It seems *P. anisum* or diabetes had no deleterious effect that impaired kidney tubular reabsorption. The *P. anisum* feeding in this study did not increased significantly the level of K+ in normal rats, but increased significantly K+ in diabetic rats (Group D vs Group C). This experiment also showed there was significant increase in plasma K+ concentration in diabetic rats fed *P. anisum* when compared with normal rats fed diet only. Hyperkalemia may develop when a patient with renal disease become oliguria or anuric, as K+ is retained (Coles, 1986). The increase plasma K+ concentration in diabetic rats fed *P. anisum* might be a result of combined effect of *P. anisum* feeding and diabetes on the kidney which result in some renal damage or other cause.

The *P. anisum* feeding in this study decreased significantly the body weight of normal rats (125.1 vs 95.27) and diabetic rats (98.4 vs 92.8). This may be attributed to replacement of 30% of the diet by *P. anisum* seeds which may have less nutritive value than the diet. Also, it was observed in this study, the body weight
of diabetic rats was significantly lowered than the body weight of normal rats (98.4 vs 125.9). The reduction in the body weight of diabetic rats confirms the previous work of Greene and Lattimer (1983) which showed experimental diabetes significantly lowered the final body weight by about 48%. Weight loss is a clinical sign associated with diabetes (Coles, 1986).

It can be concluded, *P. anisum* is a promising antidiabetic agent and could be used to lower hyperglycemia in diabetic patients.
Conclusions and Recommendations

Conclusions:

From the observation and results of this investigation, it can be concluded that:

1. *P. anisum* seeds decreased significantly plasma glucose level in streptozotocin induced diabetic rats.

2. Therefore, *P. anisum* could be considered a promising antidiabetic agent to reduce blood glucose in diabetic patients.

3. Inclusion of *P. anisum* in rat diet decreased significantly the body weight this revealed *P. anisum* do not contribute to caloric intake. Hence *P. anisum* could be included in the diet or regularly intake by diabetic patients or those have elevated blood glucose.

4. *P. anisum* is safe as it showed no or little signs of toxicity.
Recommendations:

Since *P. anisum* is promising antidiabetic agent it is recommended that:

1. Further research should be carried out to determine the mechanism of action of *P. anisum* on blood glucose.
2. The active ingredient in *P. anisum* should be identified and purified.
3. To exclude the possibility of human poisoning, further research should be carried out to examine the safety and toxicity of *P. anisum* and also to determine the proper dosage and potential interactions with other medicinal plant and synthetic drugs.
4. If *P. anisum* proved safe, limited treatment trial to treat both type 1 and type 2 diabetic human patients is recommended.
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company U.S.A.


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