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Effect of Some Medicinal Plants in U.A.E. on Glucose Homeostasis in Rats

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United Arab Emirates University
Deanship of Graduate Studies

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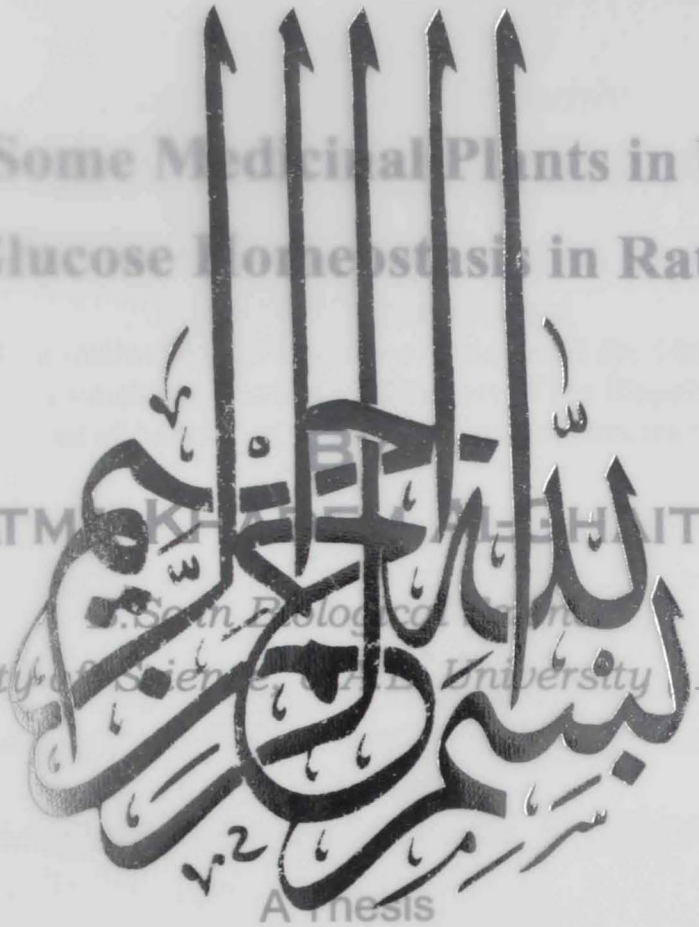

By
FATMA KHALFAN ALGHAITHI
(Biology, Faculty of Science, UAE University, 1997)

A Thesis

Submitted to

United Arab Emirates University

in Partial Fulfillment of the Requirements for the

Degree of Master of Environmental Sciences

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DECLARATION

The work presented in this thesis has not been accepted in substance for any other degree and is not concurrently being submitted in candidature for any other degree.

Signed----------

(Candidate)

Date ----------

This is to certify that the work here submitted was carried out by the candidate. Due acknowledgment has been made for any assistance received.

Signed ----------
(Supervisor)

Signed ----------
(Supervisor)

The thesis of: Effect of some medicinal plants in UAE on glucose homeostasis in rats

for the degree of Master of Environmental Science is approved.

(Chair of Committee, Dr. Mohamed H. Amiri.....)

(Examining Committee Member Dr. .....)

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(Dean of the Faculty of Science, ...)

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2002

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List of abbereviations

Abbreviation	Meaning
mRNA	messenger ribonucleic acid
cDNA	complementary deoxyribonucleic acid
b-cell or βcell	Beta cell
GLUT	glucose transporter
ATP	adenosine triphosphate
ADP	adenosine diphosphate
NADH	nicotinamide adenine dinucleotide
FADH2	flavin adenine dinucleotide
cAMP	cyclic adenosine monophosphate
NIDDM	non-insulin dependent diabetes mellitus
D cell	delta cell
SRIF	somatotrophin releasing inhibiting factor
PP cell	pancreatic polypeptide cell
AST	aspartate aminotransferase
ALT	alanine aminotransferase
ALP	alkaline phosphatase
GGT	gamma glutamyl transpeptidase
WHO	World Health Organization
OGTT	oral glucose tolerance test
STZ	streptozotocin
BUN	blood urea nitrogen

ABSTRACT

ABSTRACT

Diabetes mellitus is one of the most common endocrine diseases in the world that affects almost 6% of the world population. The treatment of diabetes mellitus takes three main forms. First, diet and exercise. Secondly, insulin therapy, and thirdly, using hypoglycaemic agents (sulphonylureas and biguanides). Many of these agents are not very effective in lowering glucose level in diabetic patients and are either too expensive or have undesirable side effects and contraindications. As a result of this, the search for effective drugs for the treatment of diabetes mellitus continues

Before the introduction of insulin in 1922, the treatment of diabetes mellitus relied heavily on initiatives derived from folk medicine. These initiatives include the use of traditional plants therapies. Many traditional plants treatment for diabetes exist but few have received scientific or medical scrutiny.

The use of traditional plants for the treatment of diabetes mellitus is widely practiced in the Middle East. In UAE many traditional plants are used as anti-diabetic remedies, such as the leaves of *Rhaza stricta* (Al Harmal), *Tephrosia apollinea* (Dhafra), *Zygophyllum simplex* (Al hirm), *Moringa peregrina* (Shoo), *Ziziphus spina-cristi* (Al seder), *Citrullus colocynthis* (Handal) and *Teucrium mascatense* (Jaadah). In spite of the wide use of these herbs to treat diabetes, no laboratory investigations have been conducted to determine the accuracy of the claims about their anti-diabetic effects.

The aim of this study was to examine the efficacy of these plants in the treatment of diabetes mellitus. The aqueous extracts of these plants were incubated with pancreatic fragments to determine whether they have the ability to stimulate insulin secretion. Aqueous extracts of *Citrullus colocynthis, Zygophyllum simplex and Tephrosia apollinea* stimulated insulin secretion significantly *in vitro*. However, only *Citrullus colocynthis* was able to stimulate insulin release in a consistent manner.

The oral administration of aqueous extract of *Citrullus colocynthis* seeds was investigated for its effects on biochemical and metabolic parameters of normal and streptozotocin (STZ)-induced diabetes rats. The results of this study revealed that oral administration of the aqueous extract of the seed significantly reduced blood glucose levels in normal and diabetic rats in a dose-correlate manner. Moreover, aqueous extract of *Citrullus colocynthis* seeds increased plasma insulin level. In addition, *C. colocynthis* appeared to induce a significant increase in the number of insulin-secreting cells after induction of diabetes islets. There is strong indication that aqueous *Citrullus colocynthis* significantly decreased the percentage distribution of glucagon secreting cells. However, the treatments of aqueous extract of *C. colocynthis* did not completely normalize these parameters as the values were still significantly different from those of controls.

LITERATURE REVIEW

I. THE PANCREAS

The pancreas is a pinkish-white organ; located retroperitoneally on the posterior wall of the abdominal cavity (Fig.1), at the level of the second and third lumbar vertebrae (Bockman, 1986). The pancreas of the adult human lies below the stomach and extend from the loop of the duodenum to the spleen (Kloppel, 1998). The gland is highly lobulated and the outlines of the larger lobules can be seen by the naked eye (Bockman, 1986). It is invested by connective tissue that provides the septation to produce these macroscopic lobules. Each one of these lobules is composed of many microscopic lobules, called acini, which are considered as functional units of the exocrine pancreas (Bockman, 1986). The pancreas contains about 10^5 to 10^6 islets that are scattered throughout the parenchymal tissue. These islets are not uniform in size (Kloppel, 1998).

The pancreas is commonly described as having a head, neck, body and tail. The head remains to some extent divisible structurally and functionally. The neck is thinned front-to-back; the splenic and superior mesenteric veins unite posteriorly to the neck of the pancreas forming the portal vein. The body continues as the main part of the gland towards the left and blends without a distinct boundary into the tapering tail, which terminates bluntly adjacent to the spleen. The substance of the gland is penetrated by numerous vascular structures, both by those supplying the gland proper and those passing to other locations (Bockman, 1986).

The pancreas is an organ of both exocrine and endocrine functions. The exocrine pancreas secretes enzymes and bicarbonate, which affect digestion and absorption of foods and the endocrine pancreas secrete hormones, which regulate the glucose metabolism. Because of these endocrine and exocrine functions, the pancreas is one of the most important and complex organs involved in the assimilation of food (Owang, 1993).

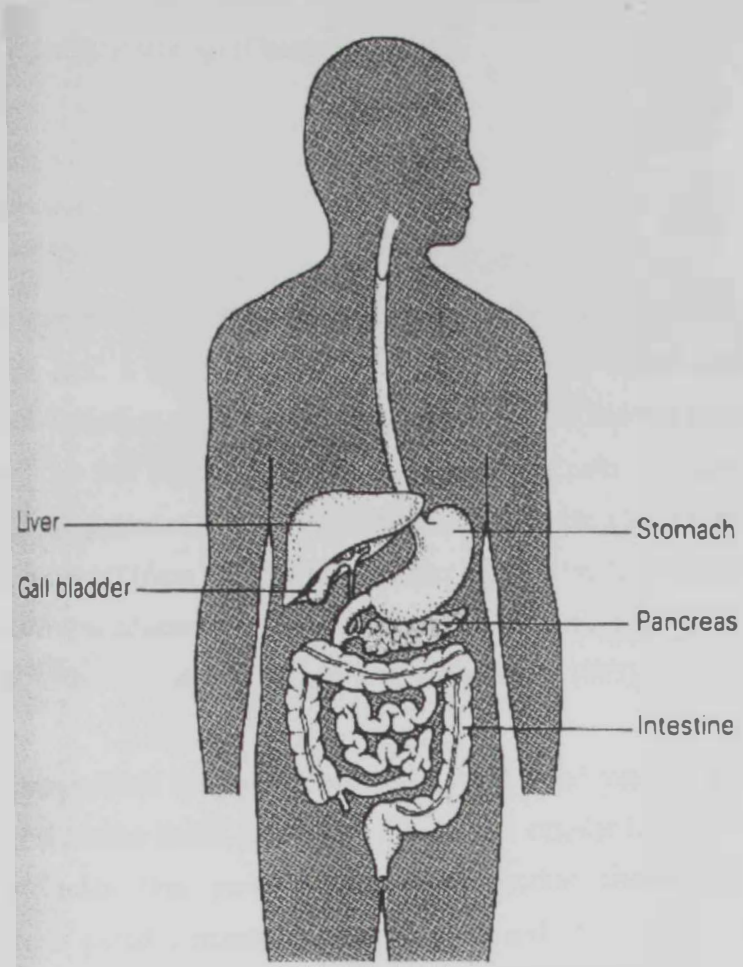


Figure 1. Shows the location of the pancreas in the abdominal cavity (Rhoades and Pflanzer, 1992).

The major role of digestive enzymes produced by exocrine pancreas is the processing of ingested foodstuffs and then it will be ready for absorption. Whereas hormones of the endocrine pancreas play important role in the metabolism of every other aspects of cellular nutrition from rate of absorption of foodstuffs to cellular storage (Greenspan, 1997).

I.1. Endocrine pancreas

In all mammalian species, the islets account for 1 to 2 % of the volume of the pancreas, and a typical islet is composed of about 5,000 endocrine cells (Williams & Goldfine, 1993). The islets of Langerhans are not uniform in size and may contain few dozens, hundreds or thousands of cells. The islets constitute only 2% of the pancreas, but 20% of the intrapancreatic blood flow is directed toward the islets (Lifson et al., 1980, Lifson et al., 1985). Because of that the islets are well vascularized. In the presence of high glucose levels, blood flow to the islets is further increased (Jansson and Hellerstrom, 1983).

The larger islets are located near the larger blood vessels that bypass the surrounding exocrine tissue. On the other hand, the smaller islets are located near smaller arterioles that pass through the exocrine tissue and forms an intrapancreatic portal circulation (Bonner Wier and Orci, 1982). This type of circulatory arrangement exposes many periinsular acini to extremely high level of insulin. Therefore, the pancreatic acinar cells are the first target cells subjected to insulin action after release from the islets. The priority is for the microcirculation of the islets, through which insulin is carried to alpha, delta and polypeptide-cells to exert direct effects on these cells (Bonner Wier, 1988). In the islets of Langerhans there are four different hormone-secreting cells containing insulin, glucagon, somatostatin and pancreatic polypeptide.

I.1.a. Alpha cells

Glucagon secreting cells or alpha cells form 15-20% of the total endocrine mass and contain typical secretory granules with an electron-dense core and pale peripheral mantle (Orci et al., 1983). These cells contain glucagon, a 29-amino acid peptide, and its hyperglycemic action was discovered in 1923 (Murlin et al., 1923). Glucagon stimulates the breakdown of glycogen in the liver resulting primarily in an increase in the level of sugar in the blood via a cyclic adenosine monophosphate (cAMP) mediated signal. The signal is initiated by the binding of the glucagon with its receptor on liver parenchymal cells (Unger and Orci, 1981a, 1981b; Unger, 1983).

I.1.b. Beta cells

Beta cells (B) of the pancreas form 60-80% of the total endocrine cell mass. These cells contain insulin. The name of insulin is derived form the Latin word 'insula', which means island. The hormone was first isolated in 1920 to 1921, and its crude form was used in the treatment of diabetics (Banting and Best, 1922; Bliss, 1982).

Beta cells store up to 13000 of secretory granules, some fuse with the plasma membrane and after appropriate secretory stimulation and releases their content into the extracellular space (Howell, 1984; Orci et al., 1986). Beta cells contain two types of secretory granules as revealed by electron microscopy. The more abundant type of these granules consists of a crystalline core surrounded by a halo and a clearly defined limiting membrane. They are scattered within the cytoplasm.

The other type of granules is less abundant and is found close to the Golgi apparatus. These granules have a homogeneous core, surrounded by a membrane coated externally with bristles consisting of the protein, clathrin. The techniques of electron microscope show that the coated granules contain high levels of

proinsulin, with small amount of cleaved insulin. Because of this it is considered immature granules. In contrast, the non-coated granules contain mostly insulin and therefore considered as mature granules (Orci et al., 1987)

I.1.c. Delta cells

The Delta or somatostatin-secreting cell forms 5-10% of the endocrine cell mass. The cell forms a long, slender cell processes rich in secretory granules which end with a knob-like structure in the pericapillary space (Grube and Bohn, 1983). Somatostatin suppresses the secretion of both effects on the secretion of both insulin and glucagon. Somatostatin is released by delta cell in response to nutrients and hormones (Bloom and Plolak, 1987; Arimura and Fishback, 1981). The first isolation of somatostatin was from the hypothalamus, but now it is known that it is produced by neurons and endocrine cells in many sites in the body (Pickup and Williams, 1997). Delta cells of pancreas, like that of the gut produces somatostatin, but can be distinguished morphologically by apical or basal elongations, which project on to adjacent cells or capillaries and may release somatostatin at their terminals (Larsson et al., 1979).

I.1.d. Pancreatic polypeptide cells

Pancreatic polypeptide-secreting cells (PP) form 3-6% of the endocrine cell mass. They contain the polypeptide of 36-amino acid. The peptide was first discovered as a contaminant of insulin preparation. There are two morphologically different types of PP-cells. The ventrally cells are derived from pancreatic head contain round to angular secretory granules (formerly called F cells). The second cells derived form the dorsal part contains small granules (Pickup and Williams, 1997; Kovacs and Asa, 1998).

I.2. Insulin

I.2.a. Insulin structure

Insulin is a big protein that has a molecular weight of 5,808 KD (Montague, 1993). The protein is formed from two polypeptide chains, A and B. The two chains are linked together by two disulphide bonds. Also, A chain contains an intrachain disulphide bond which links between amino acid residues 6 and 11. The A chain consists of 21 amino-acid residues, while B chain consists of 30 amino-acid residues (Pickup and Williams, 1997).

I.2.b. Insulin synthesis

In the human, the control of the energy metabolism and glucose is highly dependent on the hormones secreted by islets of Langerhans. The most important hormone is insulin (Owerbach et al., 1980). The encoded insulin mRNA is translated into preproinsulin, a 110 amino acid peptide, which contains a signal sequence made of 24 amino acids. In the endoplasmic reticulum, the polypeptide is cleaved into proinsulin (Orci et al., 1987; Davidson et al., 1988). In the Golgi bodies, proinsulin is packaged, processed into mature insulin and stored in coated secretory vesicles. The secretory vesicles are released from the trans-face of the Golgi apparatus (Orci et al., 1987; Davidson et al., 1988), by two enzymes whose cDNA were recently cloned (Smeekens and Stiener, 1990; Seidah et al., 1991). One is trypsin-like enzyme which removes the intervening C peptide by cutting at two dibasic amino acid sequences, and the other enzyme is a carboxypeptidase B-like enzyme which removes the dibasic peptide from the carboxyl terminal end (Smeekens and Stiener, 1990; Seidah et al., 1991). The mature insulin is a polypeptide of 51-amino acids. The peptide consists of A and B polypeptide chains that are linked by two disulfide bonds at positions A7 to B7 and A20 to B19 (Orci et al., 1987).

The biosynthesis of insulin is regulated by several hormones and nutrients, most notably by glucose. The synthesis of insulin increases after exposure of the

β cell to glucose *in vitro* (Welsh et al., 1986; German et al., 1990). In order for glucose to exert its effects on insulin secretion and synthesis, it must first get into the cell. This accomplished through a process of facilitated diffusion, which is mediated by a glucose transporter (Bell et al., 1990).

I.2.c. Insulin secretion

In normal adults, the human pancreas secretes about 40-50 unit of insulin per day. The basal level of insulin concentration in fasting blood sample is about 10 μU/mL. After standard meals, insulin will arise above 100 μU/mL. The peripheral insulin concentration begins to increase (8-10 min) after ingestion of food. Then insulin will reach the peak concentration within 30-45 min. This is followed by decline in post-prandial plasma glucose level (Greenspan and Gardner, 2001).

The beta cells are poised to sense glucose to accomplish the moment-to-moment adaptation of insulin secretion to blood glucose fluctuations. This is possible through particular expression profiles of carbohydrate transporters and enzymes in the beta cell (Newgard and McGary, 1995; Matschinsky, 1996). There are three main important molecular characteristics of glucose metabolism by the beta cells. In the first one, glucose equilibrates across the plasma membrane, as observed in hepatocytes, because both cell types express high capacity, low affinity glucose transporter (Newgard and McGary, 1995).

There are five types of facilitative glucose transporters. These transporters have been termed GLUT1, GLUT2, GLUT3, GLUT4, and GLUT5. GLUT1 is present in all tissue of human. This transporter has a very high affinity for glucose. Because of this, it can transport glucose at relatively low concentrations. On other hand, GLUT2 has low affinity for glucose and transports when plasma glucose is high. This transporter is the major transporter of glucose in pancreatic and hepatic cells (Greenspan and Gardner, 2001). In human beta cells, GLUT1 dominates and GLUT2 is moderately expressed (De Vos et al., 1995).

Secondly, phosphorylation of glucose to glucose-6-phosphate is catalysed by high K_M hexokinase IV called glucokinase (GK) that constitutes the flux-determining step for glycolysis (Iynedjian, 1993; De Vos et al., 1995). Thirdly, the pyruvate generated by glycolysis is channelled to the mitochondria. In fact, more than 90% of glucose carbons that enter beta cell are converted to CO_2 in the mitochondria (Schuit et al., 1997). The concentration of lactate dehydrogenase (LDH) is extremely low in the beta cell. This enzyme catalyzes the conversion of pyruvate to lactate. In the native beta cells, pyruvate and lactate are not insulin secretagogues; this is due to the low activity of monocarboxylate in the plasma membrane (Sekine et al., 1994; Ishihara et al., 1999). Pyruvate enters mitochondria and provides substrate to the Krebs cycle or tricarboxylic acid (TCA) cycle. The cycle generates ATP and other mitochondrial factors that promote insulin secretion (Maechler et al., 1998; Kennedy et al., 1998).

The intracellular second messenger Ca^{2+} is the crucial trigger for the exocytosis of insulin (Wollheim and Sharp, 1981; Wollheim et al., 1996). In the cytosol, concentration of ionised Ca^{2+} is raised by glucose. The increased TCA cycle activity leads to the production of coenzymes in form of NADH+H and $FADH_2$ in the matrix of mitochondria (Pralong et al., 1990, 1994; Duchen et al., 1993). The reduced coenzymes transfer their hydrogen ions in form of electrons and proton to the electron transport chain, which also receives electrons from the glycerol phosphate shuttle (Newgard and McGary, 1995; Eto et al., 1999).

There are two consequences, firstly, ATP is generated and transferred to the cytoplasm (Maechler et al., 1998). Secondly, the potential of the membrane across the inner mitochondrial membrane is hyperpolarised, becoming more negative inside (Duchen et al., 1993; Maechler et al., 1997). The above coenzymes lead to an increase in the level of cytosolic ATP, or rather the ATP: ADP ratio (Matschinsky, 1996; Detimary et al., 1998). The high level of cytosolic ATP causes closure of the ATP-sensitive K^+ (K_{ATP}) channels. The closure of K^+ channels leads to depolarisation the plasma membrane and initiation of typical electrical activity (Dean and Mtthews, 1968). The depolarization evokes the opening of voltage-sensitive Ca^{2+} channels (Horvath et al., 1998; Ligon et al., 1998).

I.2.d. Insulin action

The carbohydrate metabolism is regulated by several hormones and by the activity of the sympathetic and parasympathetic divisions of the autonomic nervous system (McGuinness et al., 1993; Cersosimo et al., 1994). The tight homeostatic control of the glucose range is governed by the balance between the glucose absorption from the intestine, and production of glucose by the liver and their uptake and metabolism by peripheral tissues (Saltiel and Kahn, 2001). Insulin is the primary regulator of blood glucose concentration. The regulatory action of insulin is manifested through enhancing effects on glucose uptake by muscle and adipose tissues, and through its inhibiting effects on hepatic glucose production (Saltiel and Khan, 2001).

Also, insulin promotes the storage of glucose in fat, liver and muscle by stimulating lipogenesis, glycogen and protein synthesis, and inhibiting lipolysis, glycogenolysis and protein breakdown. Insulin also stimulates cell growth and differentiation (Michael et al., 2000). Therefore, insulin deficiency of resistance results in dysregulation of these processes, and enhance in fasting and postprandial glucose and lipid levels elevation (McGuinness et al., 1993; Cersosimo et al., 1994).

I.3. Glucagon

Pancreatic glucagon is a 29 amino-acid peptide produced in A-cells of the islet of Langerhans from proglucagon which is 160 amino-acids peptide and larger than glucagon by five to six times. This precursor of glucagons undergoes different post-translational processing in the gut, giving rise to glucagon-like peptide 1(GLP-1) and other products (Habener et al., 1991; Holst et al., 1991; Kreyman and Bloom, 1991).

The secretion of glucagon is regulated by nutrients, autonomic nerves and by both islet and gastrointestinal hormones (Pipeleers et al., 1985). There are some factors that induce glucagon release. These factors include hypoglycaemia

and stress-induced sympathetic nerve activation. Feeding also enhance glucagon secretion. The feeding effect is either direct through stimulatory actions of amino acids and free acids on the A-cell, or indirect through the activation of meal-parasympathetic nerve and hence the release of gastrointestinal hormones including GIP (Schuit and Pipeleers, 1986; Berggren et al., 1992).

In contrast to its effect on insulin, glucose inhibits glucagon secretion. However, glucagon by itself is a powerful stimulus to insulin secretion. The stimulatory effect of glucagon on β cells is mediated by a specific plasma-membrane receptor of glucagon that is linked to adenylate cyclase system that upon proper activation generates cAMP within the β cell (Van Schravendijk et al., 1985, Schuit and Pipeleers, 1986, Berggren et al., 1992; Kawai et al., 1995). The generation of cAMP will activate PKA, which activates the secretory machinery of the β cells (McDermott Sharp, 1994).

Glucagon is the major factor that increases glucose output from the liver. Through its stimulating hepatic glycogenolysis and gluconeogenesis, glucagon increases the level of glucagon in the plasma. Therefore, glucagon is useful in the treatment of hypoglycaemia (McGuinness et al., 1993; Cersosimo et al., 1994). During fasting, glucagon plays important role in sustaining proper glucose concentration level (Cherrington et al., 1987).

Glucagon plays an important role in inducing hepatic glucose output, which largely accounts for basal hyperglycaemia in NIDDM. It also promotes hepatic output of ketone bodies from fatty acid precursors (ketogenesis). Furthermore, through its stimulating effects on adenylate cyclase system and generation of cAMP, glucagon enhances glycogen breakdown (Cherrington et al., 1987).

I.4. Somatostatin

Somatostatin is present in nerve terminals and other tissues such as the endocrine cells of the islets of Langerhans and gastrointestinal tract. Within the islets, somatostatin is released from D-cells. It consists of 14 amino acids, and it

is considered to be a local inhibitor of insulin and glucagon secretion from adjacent A and B cells (Bonner Weir, 1991; Marks, 1992).

The pre-pro-stomatostatin is converted to prostomatostatin and then by alternative pathways to either somatotrophin-releasing inhibiting factor (SRIF)-14 in nerve and islet or SRIF-28 in the gut (Holst et al., 1991). The secretion of smatostatin is regulated by nutrients, endocrine and neural factors (Reichlin, 1983; Williams and Bloom, 1992). The inhibitory effect of somatostatin on insulin secretion is associated with a decrease in the formation of cAMP, coupled with G-protein mediated actions on ion channels which result in membrane hyperpolarization and hence a decrease in the level cytosolic Ca^{2+} concentration (Nilsson et al., 1989; Kakei et al., 1994).

I.5. Pancreatic polypeptide

Pancreatic polypeptide (PP) is a 36 amino-acid peptide produced by PP cells. These cells are located in the head of the pancreas and also scattered throughout the exocrine parenchyma. Secretion of these peptides is under autonomic control, and it is released after feeding or during hypoglycaemia (Williams and Bloom, 1992). PP does not affect insulin, glucagon or somatostatin secretion (Degano et al., 1992). PP inhibits pancreatic bicarbonate and protein secretion (Mulvihill and Debas, 1997). This is considered to be the major physiological action of the PP. Its minor action includes relaxation of the gallbladder (Mulvihill and Debas, 1997).

1.6. Biochemical parameters

Diabetes mellitus causes shifts of potassium from cells into the extracellular space. Most of body's potassium is intracellular. For that, serum potassium is usually normal to slightly elevated prior to institution of treatment (Greenspan and Strewler, 1997). Serum potassium levels are controlled by uptake of potassium into cells, renal excretion and external losses. The uptake of potassium is governed by the activity of the Na^+-K^+-ATPase in the cell membrane and by H^+

concentration. Uptake of potassium is stimulated by insulin, β-adrenergic stimulation and by theophyllines (Kumar and Clark, 1999, Greenspan and Strewler, 1997). Sodium salts are the major solutes of the extracellular fluid Phosphate forms an essential part of most biochemical systems, from nucleic acids downwards. Approximately, 80% of phosphate is within bone, plasma phosphate normally ranges from 0.8 to 1.4 $mmolL^{-1}$. Phosphate reabsorption from the kidney is decreased by parathyroid hormones therefore that hyperparathyroidism is associated with low plasma levels of phosphate. The regulation of plasma phosphate is linked to calcium (Kumar and Clark, 1999, Greenspan and Strewler, 1997). Aminotransferases are enzymes present in hepatocytes and leak into blood with liver cell damage. Usually two enzymes measured aspartate aminotransferas (AST), and alanine aminotransferase (ALT). AST is a mitochondrial enzyme. Also AST is present in heart, brain, kidney and muscle. High levels are seen in hepatic necrosis, myocardial infarction muscle injury and congestive cardiac failure. ALT is a cytosol enzyme, more specific to the liver. So a rise in ALT level only occurs within the liver. Alkaline phosphatase (ALP) is an enzyme present in canalicular and sinusoidal membranes of the liver. Also, ALP is present in many other tissues, like bone, intestine and placenta. Any cause whether intrahepatic or extrahepatic disease can cause a rise in serum ALP. Gamma-Glutamyl transferase or γ-Glutamyl transpeptidase (GGT) is a microsomal enzyme. GGT is present in many tissues as well as the liver. Activity of GGT can be induced by drugs, such as, phenytoin and alcohol. ALP and GGT are excreted by a similar way. Because of that GGT rises in parallel with the ALP in cholestasis (Kumar and Clark, 1999). The normal range of calcium levels is 2.2- 2.6 $mmolL^{-1}$. 40% of this amount is ionized and physiologically relevant. The reminder is protein-bound, especially albumin, or complexed and thus unavailable to the tissues (Kumar and Clark, 1999).

II. DIABETES MELLITUS

Diabetes mellitus is one of the most common endocrine diseases in the world affecting almost 6% of the world population (Adeghate, 2001). In 1993, the World Health Organization (WHO) published a standardized global estimate based on data from 75 communities in 32 countries for the prevalence of diabetes and impaired glucose tolerance in adults (King and Rewers, 1993). In developed countries diabetes complications is considered to be one of the major causes of death, and available evidence indicates that diabetes has reached epidemic proportions in rapidly developing countries (Wellborn et al., 1995).

Between 1995 and 2025 the worldwide prevalence of diabetes will increase by 35%, from 4.0 to 5.4 %. The prevalence in developed countries is currently higher than the prevalence in developing countries but it will be same in 2025. The number of adult diabetic in the world is estimated to increase by 122%, from 135 million in 1993 to 300 million in 2025. In the developed countries, the increase will be about 42%, from 51 million to 72 million. But in developing countries, the increase will be about 170%, from 84 million to 228 millions (King, et al, 1998).

Non-insulin diabetes mellitus (NIDDM) in Arabian Gulf countries accounts for about 90% of all cases of diabetes. More than 10% of the adult population has diabetes. This disease is twice more common in females than in males (Taha et al., 1983; Mahroos, 1986; Fatani et al., 1987; Asfour et al., 1991). In UAE, the prevalence of NIDDM based on hospital records that show that the magnitude of the problem is at least as great as that in other Gulf States (Garner et al., 1994).

The prevalence of NIDDM among UAE adult population is perceived as being raised when compared with western societies (Papworth and Farjou, 1998). NIDDM is an important cause of disability in UAE. Recent, small survey in UAE reported an overall prevalence of 25% (El-mugamer et al., 1995; Papworth and Farjou, 1998). The prevalence of NIDDM in UAE depends on the location; in the rural, isolated areas it is about 7%, and about 14% in the sub-urban areas and 44% of the urban group (Papworth and Farjou, 1998).

Diabetes mellitus is the commonest cause of blindness after the age of 40 years, kidney failure requiring dialysis and kidney transplantation, damaging of foot and leg tissues (Albert et al., 1992). Also, it increases the incidence of defects in babies born to diabetic mothers. As a result it reduces life expectancy by up to a third. The cost of the disease worldwide in terms of lost working hours, treatment cost, approach billions of dollars (Sharma, 1993).

II.1. Definition

Diabetes is derived from Greek word "diabainein" which means to pass through, this, according to the early generation of physician's means the large quantity of water taken orally (polydypsia) pass through the body to give excessive amount of urine (polyuria). Diabetes simply means excessive urination. This urine has a sweet taste, which enable physician to distinguish it from diabetes insipidus (Adeghate, 2001).

Diabetes mellitus is a heterogeneous group of disorders characterized by high blood glucose levels (Pickup and Williams, 1997). Diabetes mellitus can also be defined as a chronic disease characterized by sustained hyperglycaemia because of absolute or relative deficiency of insulin. Absolute insulin deficiency caused by loss of beta cell and relative deficiency of insulin action in concert with inappropriate insulin release (WHO, Geneva 1980).

II.2. Classification

Diabetes mellitus can be divided into five types. Either fasting hyperglycaemia or elevated plasma levels of plasma glucose during an oral glucose tolerance test (OGTT) can characterize all these types. These types are Type I (insulin dependent diabetes mellitus; IDDM), Type II (non-insulin dependent diabetes mellitus, NIDDM), gestational diabetes mellitus, malnutrition related diabetes, and other types (American diabetes Association, 1997).

II.2.a. Type I diabetes mellitus

Type I diabetes was formally referred to as insulin-dependent diabetes, or juvenile-onset diabetes. This type of diabetes results from a cellular-mediated autoimmune destruction of the β-cells of the pancreas (Atkinson and Maclaren, 1994; Tuttle et al , 2001). The causes of autoimmune destruction of pancreatic β-cells are still not clear. Also, the environmental factors which trigger the destruction of β-cells in genetically susceptible children, adolescents and even adults still not clear (Bell and Polonsky, 2001).

The rate of β-cells destruction is variable. In some individuals it is rapid while in others it is slow (Zimmet et al., 1994). The rapid progressive form is observed commonly in children, but also it can occur in adults (Humphrey et al , 1998). On the other hand, the slowly progressive form also occurs in adults. In this type of diabetes, children and adolescents present with ketoacidosis as the first manifestation of the disease (Japan and Pittsburgh Childhood Diabetes Research Groups, 1985).

Other patients have modest fasting hyperglycaemia that rapidly changes to severe hypergylcaemia and/ or ketoacidosis in the presence of infection or other stress. Some adults may retain residual beta-cell function, which is sufficient enough to prevent ketoacidosis for many years (Zimmet, 1995). In Type I diabetes, patient depend on insulin for survival and they are at risk for ketoacidosis (Willis et al., 1996). At this stage of the disease, the secretion of insulin is low or no insulin secretion is manifested by low or undetectable levels of plasma C-peptide (Hother-Nielsen et al., 1988).

The majority of patients in Type I diabetes are children and adolescent, but it can also occur in any age, ranging from childhood to the ninth decade of life (Verge et al., 1996). Patients with Type I diabetes are usually not obese, but the presence of obesity is not incompatible with the diagnosis. Also, patients may have other autoimmune disorders such as Graves' disease, Hashimoto's thyroiditis, and Addisson's disease (Betterle et al., 1983).

II.2.b.Type II diabetes

In type II, formally referred to as (non-insulin-dependent diabetes mellitus), fat and muscle cells are resistance to the action of the insulin and this leads to activation of β-cells to secrete more insulin. This amount of insulin is not sufficient to maintain normal blood sugar level (Cavaghan et al., 2000; Kahn, 2001). Type II diabetes mellitus has different subtypes, and each type is characterized by variable degrees of insulin resistance and β-cells dysfunction, which all leads to hyperglycaemia (Bell and Polonsky, 2001).

This maturity onset diabetes mellitus is a complex disorder. It is characterized by insulin resistance and glucose intolerance (Garner et al., 1994). This disease is generally associated with obesity and it is common in adults of more than 40 years of age (Tuomilehto et al., 1992; Nathan, 1993).

Type II diabetes is the most common form of diabetes and one of the most frequent metabolic disorders worldwide (Nyomba, 1993). Individual with type II diabetes do not need insulin to survive. Type II diabetes mellitus is frequently undiagnosed for many years. This may be due to fact that the hyperglycaemia is not severe enough to cause noticeable symptoms of diabetes. Still, these patients are at increased risk of developing macrovascular and microvascular complications (Harris, 1993; Mooy et al., 1995).

The majority of type II patients are obese. Obesity is one of the causes of insulin resistance (Bogardus et al., 1985; Campbell and Carlson, 1993). Others who are not obese by traditional weight criteria may have increased percentage of body fat distributed predominantly in the abdominal region (Kissebah et al., 1982). In type II diabetes, ketoacidosis is infrequent, when seen it usually arises in association with stress such as infection (Banerji et al., 1994; Umpierrez et al., 1995).

In type II, insulin levels appear normal or elevated, and high level of glucose is expected with normal beta-cell function (Polonsky et al., 1996). For that, insulin secretion is defective and insufficient to compensate for the insulin resistance. Insulin sensitivity can be increased by increased physical activity, weight reduction, and / or physiological treatment of hyperglycaemia, but will not be restored to normal (Simonson et al., 1984; Wing et al., 1994). The risk of the disease increases with age, obesity and lack of physical activity. It may also occur with prior gestational diabetes mellitus and in patients with hypertension or dyslipidaemia (Zimmet, 1992; Harris et al., 1995).

II.3. Symptoms and diagnosis

The diagnosis of diabetes is promoted by symptoms such as increased thirst, passage of large amount of urine, mostly at night (nocturia) and sometimes sufficient to cause children to wet the bed (enuresis), overwhelming tiredness, unexplained weight loss in severe cases, recurrent infections, drowsiness and coma; high levels of glycosuria are present. There are minor symptoms such as muscular cramps especially in the legs, skin infections, nausea, and vomiting (World Health Organization, 1985; Pickup and Williams, 1997).

In diabetes mellitus, severe hyperglycaemia detected under condition of trauma, acute infection, circulatory or other stress should not in itself be regarded as diagnostic of diabetes. In symptomatic subjects of diabetes the diagnosis should never be made on the basis of a single abnormal blood sugar value. For that, one additional plasma/ blood glucose test result with a value in diabetic range is essential. The blood sample can be a fasting sample, a random sample, or a sample from the oral glucose tolerance test (OGTT). If these tests fail to confirm the diagnosis, it will usually be advisable to maintain surveillance with periodic re-testing until the diagnosis becomes clear (World Health Organization, 1999).

In children, diabetes mellitus manifest severe symptoms such as very high blood sugar levels, marked ketonuria, and glycosuria. In most cases of children the diagnosis is confirmed without delay by blood glucose measurements, and immediately the treatment including insulin injection is initiated (McCance et al., 1994).

II.4. Complications of diabetes mellitus

Many chronic metabolic disorders are associated with diabetes mellitus. These disorders affect the metabolism of carbohydrates, protein, fat, water and electrolytes. These disorders lead to structural changes in the tissues of many systems of the body, especially the vascular system (Adeghate, 2001).

All forms of diabetes are characterized by chronic hyperglycaemia and development of diabetes specific microvascular pathology in the retina, renal glomerulus and the neuropathy of peripheral nerve. As a consequence of microvascular pathology, diabetes will lead to blindness, end stage renal disease and a variety of debilitating neuropathies (Brownlee, 2001).

Also, diabetes causes accelerating atherosclerotic macrovascular disease affecting arteries, supplying the heart, brain and lower extremities. Because of this, diabetic patients have a higher risk of having myocardial infarction, stroke and limb amputation. Large number of studies shows a strong relationship between glycaemia and diabetic microvascular complications in both type-I and type-II diabetes (The diabetes control and complications trial research group, 1993; UK prospective diabetes study, 1998). Both insulin resistance and hyperglycaemia have important roles in the pathogenesis of macrovascular complications (Wei et al., 1998).

II.5. Treatment and management

Patients with IDDM require insulin. The usual method of treatment by insulin is subcutaneous injection (Pickup and Williams, 1997). NIDDM is difficult to manage and often poorly or insufficiently treated (Williams, 1994). It is important to treat coexisting cardiovascular risk factors such as dyslipidaemia, hypertension and smoking. Patients must start change their lifestyle, especially diet and increase physical exercise (Pickup and Williams, 1997).

The most commonly used drugs in the treatment of NIDDM are sulphonylureas (insulin secretagogues) and biguanide, metformin. These drugs act via enhancing insulin action in skeletal muscle and the liver (UK prospective diabetes study group, 1995). Sulphonylureas is more useful for non-obese patient with insulin deficiency as main pathologic cause, but metformin is more appropriate for obese patient with marked insulin resistance (Lean et al., 1989). Many of the patients fail to response to monotherapy and hence, they are treated with a combination of drugs. If this combination fails, then insulin will be prescribed (Gatling et al., 1985; Lean et al., 1989). Acarbose is (inhibitor of α-glucosidase in the gut wall), used for patients who are not controlled by sulphonylureas or metformin (Reaven et al., 1990).

New oral hypoglycemic agents have been introduced to assist in the treatment of diabetic patients. The most recent are rosiglitazone (a peroxisome proliferator-activated receptor- PPAR agonist) and rapid-acting insulin secretagogues such as repaglinide (Townsend, 2000).

Diabetes mellitus is a medical condition known for thousands of years and has been treated by many traditional herbs. Most of these herbal remedies are still awaiting intensive investigation and trial to confirm their efficacy (Pinn, 2001).

III. MEDICINAL PLANTS

A medicinal plant contains one or more chemical material in low or high concentration that can treat one or more of diseases, or decrease the symptoms of disease (Evans, 1996; El-Ghonemy, 1993).

III.1. History

Medicines derived from plants have been a part of the human evolution, and healthcare for thousands of years. Today, about 88% of the global populations turn to the medicines derived from plants. These plants are considered as the first line of defense for maintaining health and combating diseases. Secondary metabolites of one hundred and nineteen plants derived from plants are used as drugs globally (Perumal Samy, 1999).

Considerable attention has been placed on new drug discoveries. These discoveries are based on traditional medicinal plant used throughout the world. Several hundred plants in medical category, used in modern medicine and pharmacy (Lewis, 1994).

III.2. Classification

Medicinal plants are arranged in different ways according to:

1. Alphabetical classification: Either Latin or vernacular names can be used. This arrangement is used for dictionaries and pharmacopoeias. Also, it is suitable for quick reference, but it gives no indication o inter-relationships between drugs (Evans, 1996; El-Ghonemy, 1993).

2. Taxonomic: The drugs are arranged according to the plants, from which they are obtained, in classes, orders, families, genera and species. This arrangement allows of a precise and orderly arrangement. It accommodates any drug without

ambiguity. This system has becomes less popular for teaching purpose in pharmaceutical science (Evans, 1996).

3. **Morphological:** In this arrangement, drugs are divided into groups such as leaves, fruits, flowers, herbs, entire organisms, woods, barks, rhizomes and roots. This is known as organized drugs. On the other hand, dried lattices, extracts, gums, resins, oils, fats and waxes are known as unorganized drugs. This type of grouping is used for studding crude drugs, identification of powdered drugs that are based on macro-morphological properties (Evans, 1996; El-Ghonemy, 1993).

4. **Pharmacological or therapeutic:** In this classification arrange drugs are grouped according to the pharmacological action of their most important constituent or their therapeutic use. Despite this, it is important to appreciate that the constituents of any drug may fall into different pharmacological groups (Evans, 1996).

5.**Chemical or Biogenetic:** The most important composition such as alkaloids, glycosides, volatile oils, and others, or their biosynthesis pathways, form the basis of classification of the drugs. This type is the most popular arrangement used for teaching of pharmacognosy. Ambiguities arise when one drug possess a number of active compounds and thus belong to different phytochemical groups (Evans, 1996).

III.3. Medicinal plants and the treatment of diabetes mellitus

There is great scope for understanding the pathophysiology of diabetes mellitus due to its importance in human health. Historically, the studding of the pathogenesis of diabetes was in the traditional pattern of endocrinology (Jouad, 2000). The treatment of diabetes mellitus takes three main forms. First, diet and exercise. Secondly, insulin therapy, and thirdly, using hypoglycaemic agents (sulphonylureas and biguanides) (Ivorra et al., 1989). Many of these agents are not very effective in lowering glucose in diabetic patients. Also, these agents are either too expensive or have undesirable side effects and contraindications

(Jaouhari et al., 2000). Because of this, the search for effective drugs for treatment of diabetes mellitus continues (Subramoniam et al., 1996).

Since time immemorial, patients with diabetes have been treated orally with different plant extracts. The logical way of searching for new drugs to treat diabetes is to evaluate these plants, and especially their active natural principles (Ivorra et al., 1989). Treatment of diabetes by medicinal plants goes back to BC time when Papyrus Ebes (1550 BC) recommended a high-fibre diet of wheat, ochre and grains. Today, more than 400 medicinal plants have been described in the diabetes treatment (Wasfi et al., 1994).

Many products of herbs including several metals and minerals have been described for the care of diabetes mellitus in ancient literature. The use of traditional medicinal plants for diabetes mellitus is widely practiced in the Middle East. The World Health Organization has recommended that the use of herbal medicine should be encouraged (WHO, 1980). In folk medicine of different cultures, many of plants species are used for their hypoglycaemic properties. And therefore, it's appropriate used for the treatment of diabetes mellitus (Abdel-Barry et al., 1997).

There is a renewed interest in the use of traditional plant for the treatment of diabetes mellitus, fuelled by a growing public interest and awareness of the so-called complementary and natural type medicine. Before discovering insulin therapy in 1922, traditional plant treatments were the cornerstone of antidiabetic therapies. Traditional herbal preparations continue to form the predominant therapeutic approach in many deprived regions of the world. But, in Asian societies, insulin was soon recognized as the miracle life-saver and traditional plant treatment were forgotten (Day and Bailey, 1988).

The severity of diabetic complications correlates well with the duration and extent of hyperglycaemia. Improvement in the glycaemic control defers the late onset complication and slows their progression. Insulin injection and oral antidiabetic drugs (sulphonylureas, metformin, and acarbose) do not reinstate a completely normal pattern of glycaemic control, whether used alone or in

combination (UKPDS Group, 1995). A search for additional agents to combat hyperglycaemia and its complications reported an opening to revisit traditional antidiabetic plants (Gray and Flatt, 1998). Many plants have hypoglycaemic action in animals and humans (Prince et al., 1998).

Many traditional plants act at least in part via their fibre, vitamin or mineral content. Mineral deficiencies are common with diabetes. Supplements of mineral can benefit patients with mineral deficiencies, as demonstrated by the administration of magnesium and zinc. Plants rich in minerals can control glycaemia in diabetic patients (Day, 1990). Some inorganic elements like potassium, zinc, calcium, manganese and traces of chromium have role in the improvement of impaired glucose tolerance and their indirect a role in the control of diabetes mellitus are being increasingly recognized (Gurson and Saner, 1971; Mertz, 1981; Kinlaw et al., 1983; Niewoehner et al., 1986).

Furthermore, zinc ions are necessary for crystallization of insulin and insulin is stored as a zinc complex. Also, normal concentration of potassium is necessary for optimal secretion of insulin. Depletion of potassium or hypokalaemia can result in reduced glucose tolerance. Calcium, potassium and traces of chromium play important role in the release of insulin from cells of the islets of Langerhans (Underwood and Mertz, 1986).

Several plants have provided entirely new hypoglycaemic compounds. Many of these compounds are alkaloids, flavonoids and glycosides that do not lend themselves readily to pharmaceutical development. Also, antidiabetic medicinal plants are sources of agents that can benefit the co-morbid conditions of dyslipidaemia, hypertension or atherosclerosis (Day, 1995).

III.4. Local medicinal plants used in this study

1. *Zygophyllum simplex:*

Scientific name: *Zygophyllum simplex.*

Family name: Zygophyllaceae.

Local name: Hirm, Girm, Kharmeel (El-Ghonemy, 1993).

Description: Annual herb spreading with prostrate branches (Fig. 2). Leaves are cylindrical, succulent, 6-18 mm. Flowers are yellow, axillary, about 5 mm across; pedicel elongating in fruit, becoming deflexed. Capsule obovoid, with 5 narrow-winged carpels, wrinkled (Ghazanfar, 1994; Wester, 1989).

Distribution: Common in limestone along the Arabian Gulf littoral, in central desert area and along the east coast as well as foothills. Rare in deep sand; rapidly colonises gypseos soils and locally dominant annual west of Jabal Dhanna (Ghazanfar, 1994). Fairly widespread in small groups at upper elevations; also plants found in desert plantations at Shwayb (Wester, 1989).

Medicinal uses: This plant is used for infected swollen eyes, tumours, diabetes mellitus, eczema. Also used for camel diseases. The leaves of the plant are used as an antibiotic and laxative (El-Ghonemy, 1993; Saleh et al., 1993; Rizk and El-Ghazaly, 1995).

Chemical composition: This plant contains alkaloids, sterols and /or terpenes, tannins and flavonoids that have been identified as kaempferol 3-glucoside and quercetin 3, 7-diglucoside (Hassanean and Desoky, 1992; Ghazanfar, 1994; Rizk and El-Ghazaly, 1995).

Flowering and fruiting: March to June (Ghazanfar, 1994).

Figure 2: *Zygophyllum simplex:* (A) Branches of *Zygophyllum simplex.*

(B) Branches with flowers (Rizk and El-Ghazaly, 1995).

2. Tephrosia apollinea:

Scientific name: *Tephrosia apollinea*

Family name: Leguminosae.

Local name: Dhafra (El-Ghonemy, 1993)

Description: Perennial shrub, up to 50 cm tall. Stem is branched. Leaves are imparipinnate; leaflets 2-4 * 1.5-2 cm, oblong to oblanceolate. Flowers are purple, in axillary racemes (Fig. 3). Fruit a pod, 30-40 mm wide, flattened, 6-9 seeded, valves splitting spirally when mature (Ghazanfar, 1994).

Distribution: Extremely common in all foothills up to 3000 ft plus, along mountainous wadis and on rough alluvial plains on both sides of the Hajjar Mountains and locally dominant (Wester, 1989).

Medicinal uses: It is used for the treatment of bronchitis, cough, earache, wounds, piles antidotal, and for diabetes mellitus, dyspepsia, inflammation, tympanitis, calculous and dry eczema. It is useful in the treatment of disease of the heart and blood, liver, gonorrhea, tumours, piles and ulcers. The plant is also used as a tonic, laxative, blood purifier, deobstruent (Mossa et al., 1987; El-Ghonemy, 1993; Ghazanfar, 1994).

Chemical composition: Tephrosin, delguelin and quercetin, glucoside have been isolated in related species of *Tephrosia*. Also, the plant contains alkaloids, flavonol, tannins, volatile oils, anthraquinones (Mossa et al., 1987; El-Ghonemy, 1993; Ghazanfar, 1994).

Flowering and fruiting: Throughout the year (Ghazanfar, 1994)

Figure 3: *Tephrosia apollinea.*

(A) Whole plant (El-Ghonemy, 1993).

(B) Dried branches.

3. Rhazya stricta:

Scientific name: *Rhazya stricta.*

Family name: Apocynaceae.

Local name: Harmal (El-Ghonemy, 1993).

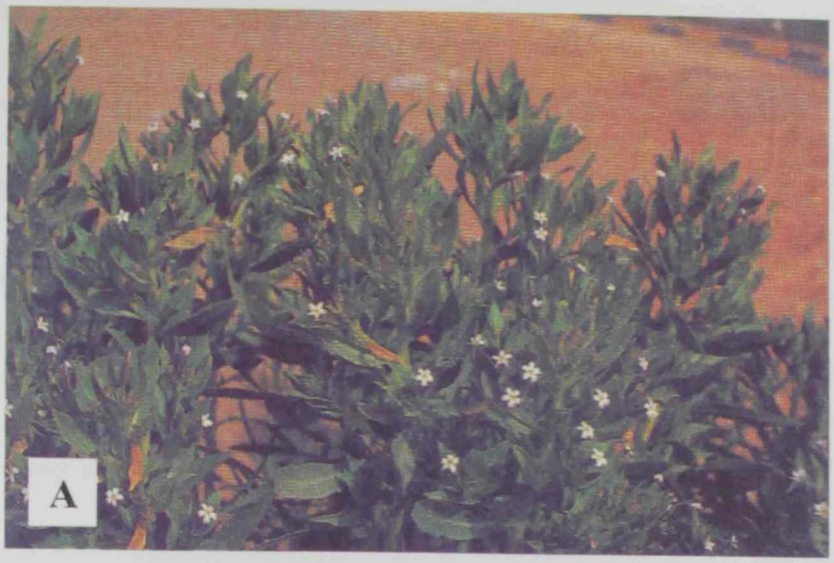
Description: Perennial shrub, 30-70cm tall, often the base of the stems is woody and covered with scars of old leaves (Fig. 4). Flowers are in terminal corymbose cluster, arising in the axils of the leaves, white. Seeds are compressed, brown, surrounded with a narrow pale brown wing (Wester, 1989; Ghazanfar, 1994).

Distribition: Very common alluvial plains around Al-Ain and on gravels east and west of Hajjar Mountains; sometimes in extinsive clumps in wide lower wadies.(3) Widespread south of Al-Ain towards Al Liwa on gravels, where is it locally dominant.

Medicinal uses: The whole plant including the seeds is used for medicinal purposes. It is used to improve bad breathing, chest pain, conjunctivitis, constipation, diabetes, lowering fevers, skin rash, abdominal pains, colic, to remove stomach worms, increase lactation and antihelminthic (Aynehdhi et al., 1982; Ghazanfar, 1994).

Chemical composition: The leaves, roots and seeds contain 4-indole alkaloids such as polyneuridine, sewarine and tetrahydrosescamine (Mukhopadhay et al., 1981; Ghazanfar, 1994).

Flowering and fruiting: February to April (Ghazanfar, 1994).



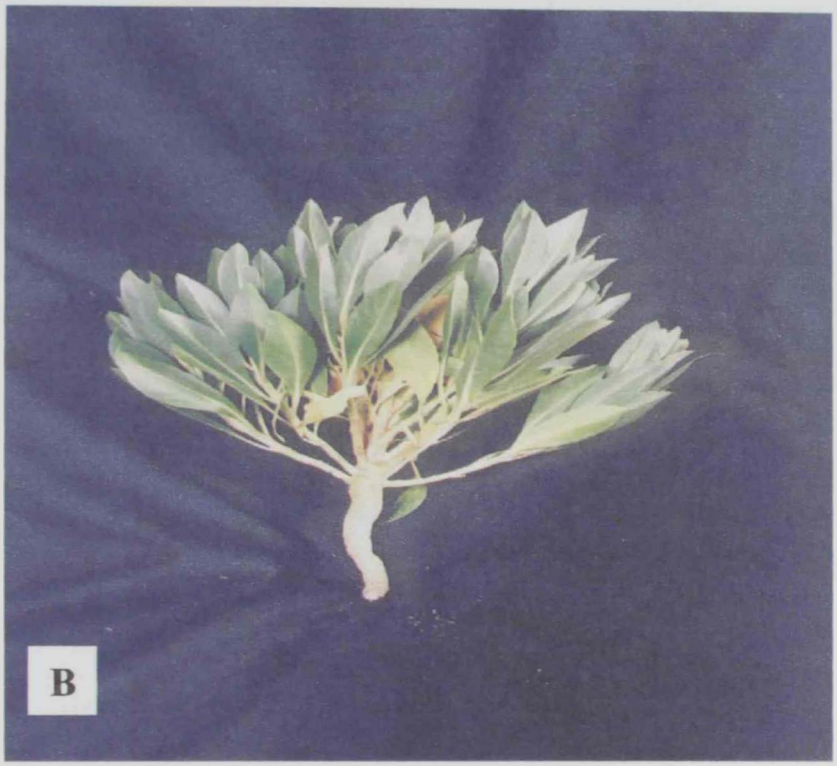


Figure 4: *Rhaza stricta*

(A) Whole plant (El-Ghonemy, 1993).

(B) Fresh branches.

4. *Teucrium mascatense:*

Scientific name: *Teucrium mascatense.*

Family name: Labiatae.

Local name: Jaada, Jaadah (El-Ghonemy, 1993).

Description: Perenial shrub, 10-25cm tall, stems have a woody base, erect or ascending (Fig. 5). Flowers sessile, congested in terminal heads and purple to pale-violet in colour (Wester, 1989).

Distribution: Fairly widespread in small groups at upper elevations; also plants are found in desert plantations at Shwayb (Wester, 1989).

Medicinal uses: The leaves and flowers-heads are used for the treatment of colic, diabetes, stomach pain, fever, renal disinfectant and malaria fever. The whole plant is used as an analgesic, antibiotic, purgative and antipyretic agent (Arnold et al., 1991; Saleh et al., 1993). In Egypt, the plant is used as a hypoglycaemic agent, appetizer, and expectorant (Mahmoud, 1980).

Chemical composition: The plant contains diterpenoids, iridoid glycosides, volatile oils and various flavonoids (Rizk, 1986).

Flowering and fruiting: March to May (Ghazanfar, 1994).

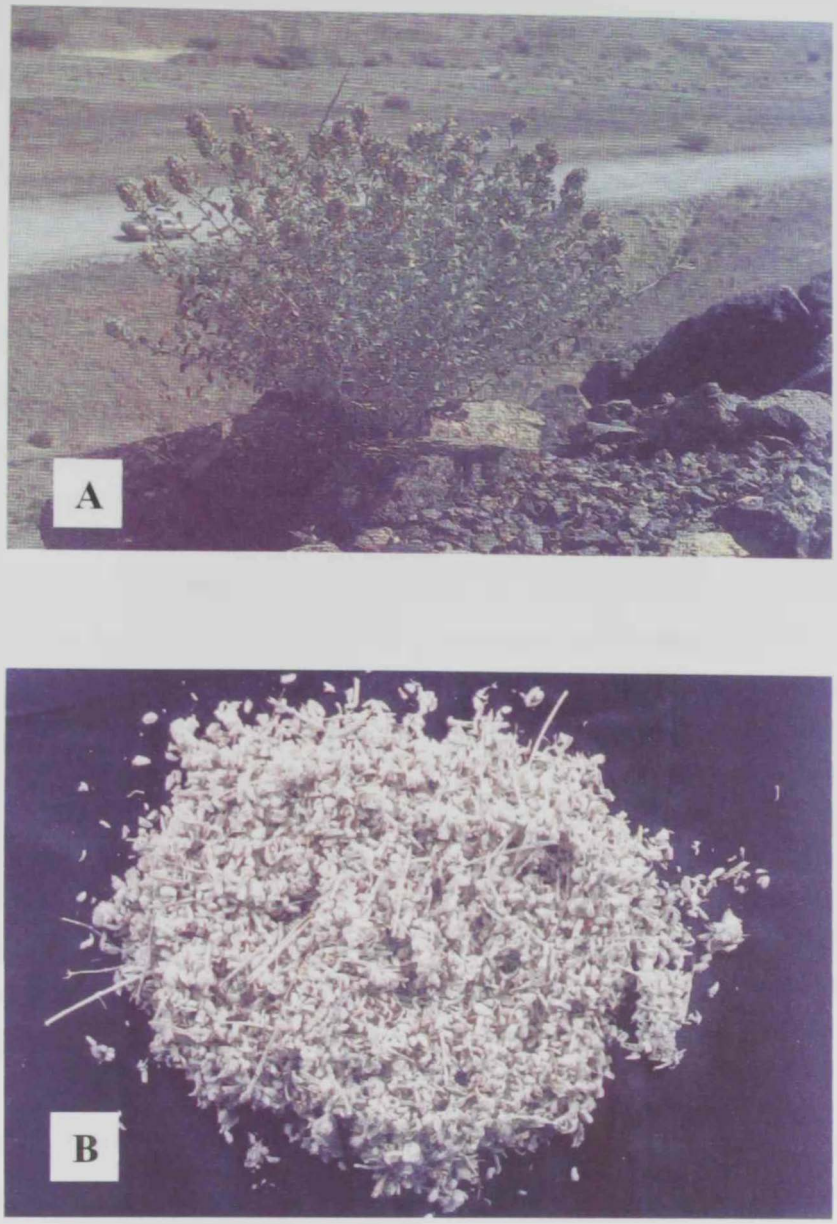


Figure 5: *Teucrium mascatense*

(A) Whole plant (El-Ghonemy, 1993).

(B) Dried branches.

5. *Moringa peregrina:*

Scientific name: *Moringa peregrina.*

Family name: Moringaceae.

Local name: shoo, ban, ysar (El-Ghonemy, 1993)

Description: Tree up to 12m tall but often shorter, with branches and willowy appearance. Leaves are few, tiny, and oblong on outer branches that droop in needle-like fronds (Fig. 6). Flowers tend to appear before leaves, with white petals with purple near centre (Wester, 1989).

Distribution: This tree is very common at low to intermediate elevations especially in Russ Al Jibal where it is a dominant tree on steep slopes. Also, the plant is scattered throughout Fujeirah at higher altitudes, and upper flanks of Jabal Hafit (Wester, 1989; El-Ghonemy, 1993).

Medicinal uses: It is used in the UAE as a very popular herbal medicine for several diseases such as infection of the respiratory tract, cardiac stimulant in asthma, diabetes, counter irritant, neuropathy, headache, earache, intermittent fever, epilepsy, hysteria, paralysis, dropsy, hepatomegaly and splenomegaly and tetanus (El-Ghonemy, 1993).

Chemical composition: Except for linolenic acid, the seed oil contains all the fatty acids that are found in olive oil (Ghazanfar, 1994).

Flowering and fruiting: April to May (Ghazanfar, 1994).

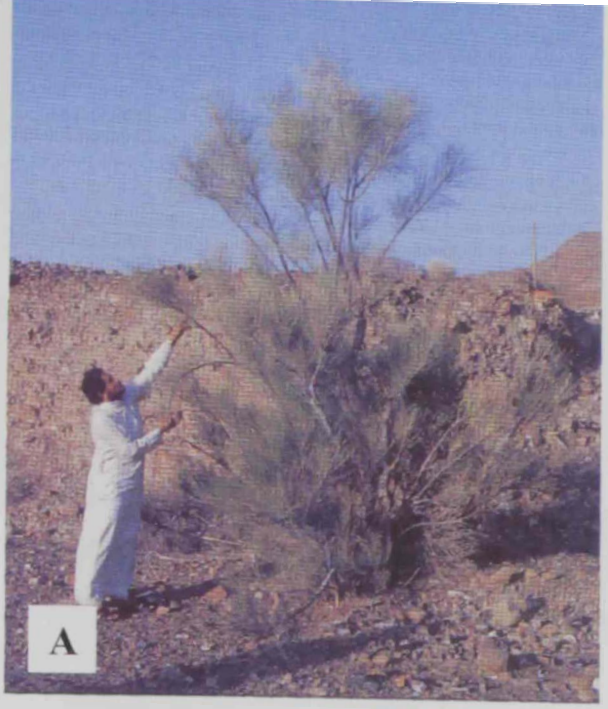


Figure 6: *Moringa peregrina*

(A) Whole plant (El-Ghonemy, 1993).

(B) Dried branches.

6. *Ziziphus spina-christi:*

Scientific name: *Ziziphus spina-christi.*

Family name: Rhamnaceae.

Local name: Seder, Sider (El-Ghonemy, 1993).

Description: Tree up to 12m tall, with spiny or unarmed branches (Fig. 7) Leaves are ovate with obscurely crenate margins. Fruit is globose drupe, fleshly, orange-yellow when ripe (Ghazanfar, 1994). Flowers are small, green, in axillary cymes (Rizk and El-Ghazaly, 1995).

Distribution: The tree is common in mountains. It is also common in lower wadis and rocky foothills. The tree can also be found along the coasts (Wester, 1989).

Medicinal uses: The plant is used for liver diseases, eyewash, antiseptic, demulcent, astringent, depurative, emollient, laxative, pectoral, refrigerant, venereal diseases (Duke, 1987). The plant is also used for the treatment of blisters, bruises, chest pains, dandruff, fractures, headache, mouth and gum problems (Ghazanfar, 1994). Extract of plants leaves have hypoglycaemic effect (Anand et al., 1989).

Chemical composition: The plant contains alkaloids, betulic acid, ceanothic acid. It also contains tannins, flavonoids, rutin, hyperin, quercitrin and glucoside (Rizk and Al-Nowaihi, 1989). The bark of the plant contains ziziphine-F, jubanine-A, and sapinanine (Abdel-Galil and El-Jissry, 1991).

Flowering and fruiting: September to November (Ghazanfar, 1994).

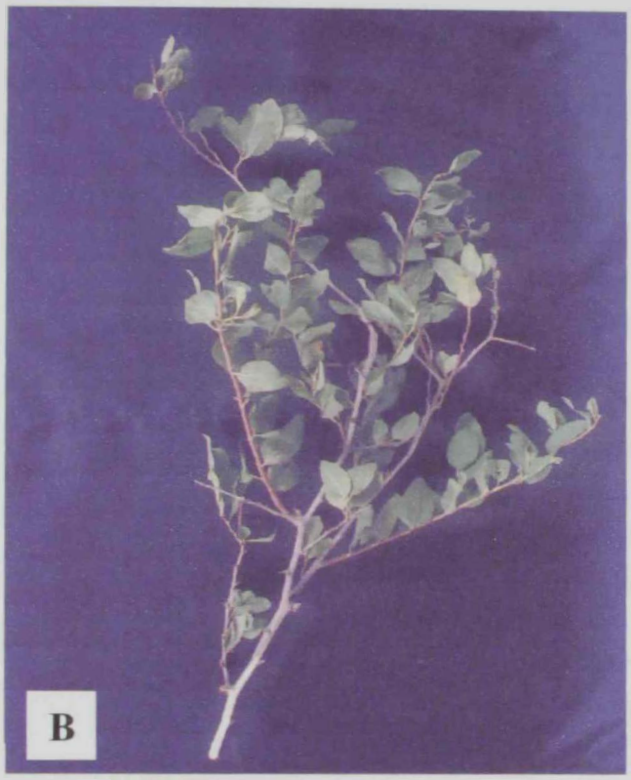


Figure 7: *Ziziphus* ***spina-christi***

(A) Whole plant and fruit (El-Ghonemy, 1993).

(B) Fresh branches.

7. *Citrullus colocynthis:*

Scientific name: *Citrullus colocynthis.*

Family name: Cucurbitaceae.

Local name: Handal (El-Ghonemy, 1993).

Description: Perennial herb with postrate, creeping stems with tendrils; whole plant is scrabid. Leaves alternate, 3-5 lobed, ovate to cordate in outline. Flowers are solitary and yellow (Fig. 8). Fruit is globlse gourd, 5-9cm in diameter, yellow or variously green and yellow striped, smooth. Seeds are embedded in white flesh bitter (El-Ghonemy, 1993).

Distribution: Common in central desert and in alluvial plains after rain; also along the East Coasts. Occasional in towns (Wester, 1989). Citrullus colocynthis is a member of the family Cucurbitaciae. This plant grows widely in the Arabian and Sahara deserts and in Sudan and introduced by Arabs in the middle age to Spain and Cyprus (Abdel-Hassan et al., 2000).

Medicinal uses: Seeds of this plant are used for the treatment of diabetes. The leaves of this plant are used for the treatment of jaundice and asthma. The root is used in inflammation of the breast, amenorrhoea, jaundice, joint pains, and used externally in ophthalmia and in uterine pains (Kirtikar and Basu, 1984; Baquar and Tasnif, 1984).

The fruit is used as a cooling purgative, antihelminthic, antipyretic, pungent, and carminative. Also, it cures ascites, tumours, ulcers, leucoderma, asthma, bronchitis, jaundice, urinary discharges, tuberculous glands of the neck, enlargement of the spleen, dyspepsia, constipation, anaemias, throat diseases, elephantiasis and is useful in abnormal presentations of the foetus and in atrophy of foetus. It is also used in the treatment of amenorrhea (El-Ghonemy, 1993; Kakarani and Saluja, 1993).

In addition, this plant has hypoglycaemic effect (Bellakhdar et al., 1991; Ziyyat et al., 1997). The plant is used to treat dog, and snake bites, diabetes, colic, lung problems, anemia, stomach pains, intestine problems, worms, and for head and tooth pains (Ghazanfar, 1994).

Chemical composition: Components of the dried pulp of fruit include cucurbitancin B and E, and B,I and L in the stems. The glycoside, colocynthis, consists of elatrin. It also contains sterols, alkanes, aliphatic alcohol and alkaloids. The peel contains a volatile oil consisting of hexanoic acid, heptanoic acid, limonene, anethol, benzyl alcohol, octanoic acid, citral, geranyl acetate, anisaldehyde, decanoic acid, methyl heptanone, phenylethyl alcohol, methyl eugenol and citmellol (El-Ghonemy, 1993; Ghazanfar, 1994; Rizk and El-Ghazaly; Rizk, 1987).

Flowering and fruiting: February to April (Ghazanfar, 1994).

C. colocynthis is a member of the family Cucurbitaciae. *C. colocynthis* is commonly known as the bitter cucumber or bitter apple. The fruit and dried pulp of *C. colocynthis* was used as a traditional medicine mostly for constipation (Arena and Drew, 1980). The seed tar was used in East Africa by nomads in traditional medications applied to skin (Habs et al, 1984). Many investigators used the fruits of the *C. colocynthis* as antitumour drug (Faust et al, 1989).

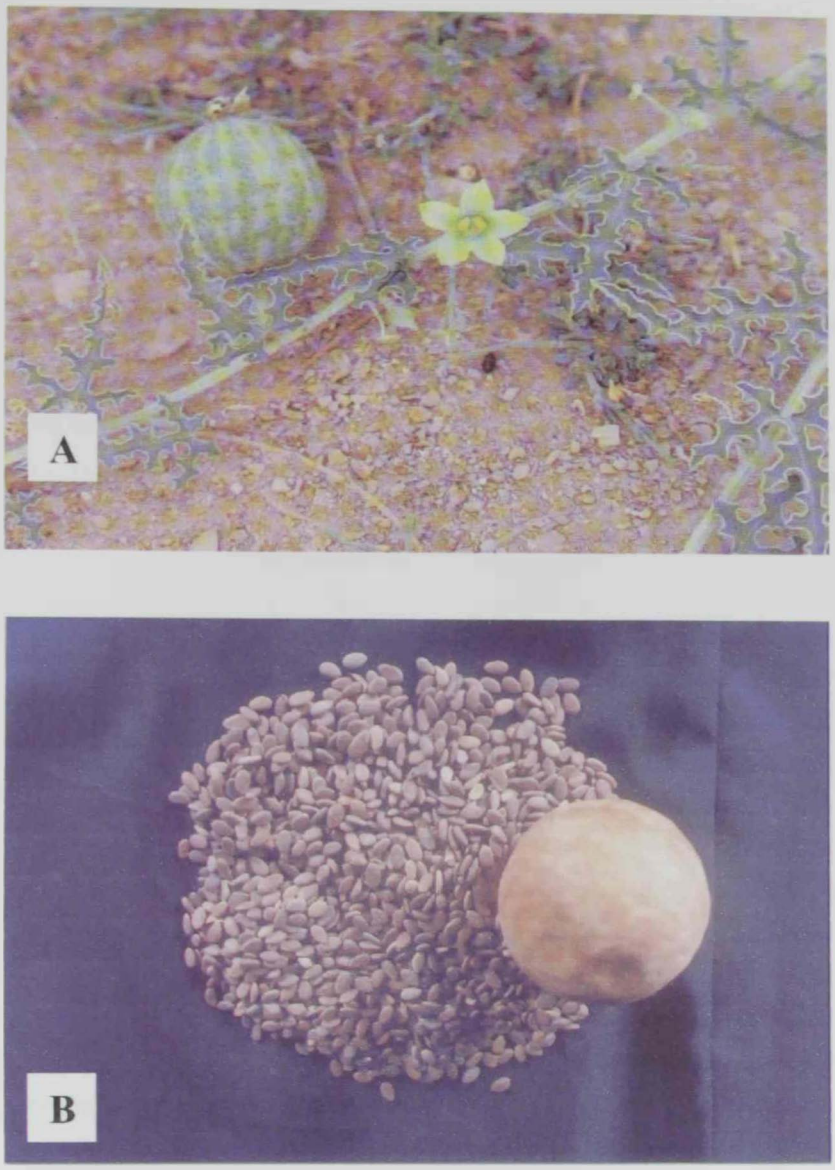


Figure 8: *Citrullus colocynthis:*

(A) Fruit and flower of *Citrullus colocynthis* (Rizk El-Ghazaly, 1995).

(B) Dried fruit and seed of *Citrullus colocynthis.*

Objectives of this Study

The main objective of this study is to screen some local UAE medicinal plants for their effects of insulin secretion *in vitro*. In addition, to select the most effective plant to further study its effect on glucose homeostasis *in vivo*. Depending on the results of the *in vitro* experiment, the most effective plant (*C. colocynthis*) with the most consistent effect in evoking insulin release was chosen to complete the *in vivo* experiment.

Specific aims of this study:

1. To elucidate the hypoglycaemic effect of aqueous extract of *C. colocynthis* seeds in normal and STZ-induced diabetic rats.

2. To examine the role of aqueous extract of *C. colocynthis* seeds on plasma insulin levels on normal and diabetic rats.

3. To investigate the effect of aqueous extract of *C. colocynthis* seeds on kidney and liver functions.

4. To investigate the effect of aqueous extract of *C. colocynthis* seeds on the morphology of pancreatic islets of Langerhans in normal and diabetic rats.

MATERIALS AND METHODS

I. CHEMICALS USED AND THEIR SOURCES

Dulbcco's Modified Essential Medium (DMEM), essential and non-essential amino acid solution, newborn calf serum, penicillin-streptomycin solution, HEPES buffer, Bio-Rad Protein Assay (Bio-Rad Laboratories, NY, USA) and streptozotocin were purchased from Sigma Chemicals (St Louis, MO, USA). Insulin kits were obtained from DRG Instruments GmbH (Germany). Standard laboratory chemicals used for processing of tissues and other laboratory techniques were acquired from either BDH (Poole, UK) or Sigma (St Louis, MO, USA). The immunohistochemical staining was performed by the Labelled Streptavidin Biotin method using (DAKO LSAB ® peroxidase kit) that purchased from DAKO Corp. (Carpenteria, CA, USA).

II. *IN VITRO* EXPERIMENT AND MEDICINAL PLANT SCREENING

II.1. Animal

Male Wistar rats weighing 170-220 g were used in this experiment. Rats were obtained from the Faculty of Medicine and Health Sciences (FMHS), United Arab Emirates University. The animals were raised and maintained in plastic cages and fed on a standard laboratory chew and tap water *ad libitum*.

II.2. Collection of the plants and preparation of aqueous extracts

Leaves of *Rhaza stricta* (Al Harmal) and *Tephrosia apollinea* (Dhafra) were collected from Al-Hajjar Mountains, Al-Ain district in February, 2000, and were shade dried. *Zygophyllum simplex* (Al hirm) leaves were collected from Hafit Mountains in March, 2000. *Moringa peregrina* (Shoo) leaves and *Ziziphus spina-cristi* (Al seder) leaves were collected from Al-Ain district, in January, 2001. Al Herm, Al Shoo and Al Seder aerial parts were dried in direct sun. *Citrullus*

colocynthis (Handal) seeds and *Teucrium mascatense* (Jaadah) aerial parts were bought from local market in March, 2001.

Table 1: Plants used in the study and their dry weight yields per 1 ml of extract.

No.	Plant name	Arabic name	Family	Yields in mg
1	*Citrullus colocynthis*	Handal	Cucurbitaceae	7.9
2	*Zygophyllum simplex*	Hirm	Zygophyllaceae	17.2
3	*Tephrosia apollinea*	Dhafra	Leguminosae	12.5
4	*Teucrium mascatense*	Jaada	Labiatae	12.4
5	*Rhazya stricta*	Harmal	Apocynaceae	19.4
6	*Ziziphus spina-cristi*	Seder	Savadoraceae	23.6
7	*Moringa peregrina*	Shoo	Moringaceae	12.8

Herbs were botanically authenticated and voucher specimen of each plant was deposited at the herbarium of the UAE University. All the dried plants were powdered in a grinder. Powdered plants (5 g each) were macerated in 100 ml of distilled water with continuous stirring at room temperature for 16 hours. Thereafter, the suspension was filtered using sterile gauze and filter paper (Wattman No. 2). The filtrates of most plants were further sterilized using millipore filter (0.22 μm) in order to make it suitable for tissue culture experiments (Subramoniam et al., 1996).

II.3. Tissue culture

Pancreatic tissue explants were prepared as described by Rouff and Hay, 1979 and Hay, (1979), and as modified in our laboratory. Briefly, animals were decapitated by cervical dislocation. Rat abdominal skin was sterilized with 70% alcohol; skin dissected, and the pancreas lifted with a pair of forceps. Pancreas adhesion to the spleen was removed with a pair of fine scissors. The gland was placed in a sterile petri dish containing media and transported to a horizontal

laminar flow hood. Under completely sterile condition, the pancreas was washed with sterile media and placed in a new petri dish. Further dissection was performed to remove associated fat and connective tissue. Pancreas was chopped into small (2 mm^3) pieces for tissue explants. Tissue was plated into 24-well culture plates containing 1 ml plating media (Appendix I). Tissue explants were incubated in CO_2-incubator at 37°C and 95% humidity for 48 hours. Thereafter media and tissues fragments were collected separately and kept frozen until assayed for insulin and protein analysis.

II.4. Stimulation of pancreatic tissue with plant extracts

Tissue explants were incubated with and without different concentrations (0.2, 1, 2, 4, 10µl) of the plant aqueous extracts. Each experimental group was represented by 8 individual wells (4 per plate). Media and plant aqueous extracts were added to the culture plates and were placed in the incubator for reconditioning before the tissue explants were plated. Pancreatic tissue explants (2 mm^3) were then placed in the (one piece per well) well. Since tissue explants were relatively different in size and total protein concentration in each well corresponds to the relative size of each piece, the total concentration of protein in each well was determined and insulin concentration in the supernatant was expressed per mg protein of pancreas.

II.5. Insulin ELISA

Insulin was determined using a DRG immunoenzymetric assay kit (Marburg, Germany) for the quantitative measurement of insulin in serum and plasma. The insulin EIA is a solid phase enzyme amplified sensitivity immunoassay performed on microtiter plate. A microtiteration plates with 96 anti-insulin coated wells were used. A standard curve was constructed using appropriate standard points (3.5, 15, 45, 133, 435 µlU/ml). The procedure was conducted as described in the kit manual. Briefly, 50 µl of standard, control, or sample were dispensed into the appropriate wells followed by addition of 50 µl of anti-insulin-HRP conjugate to each well. Thereafter, the plate was incubated for

30 min at room temperature using horizontal shaker set at 700 ± 100 RPM. After the incubation period, the liquid reagents were aspirated from each well. At the end of the incubation period, washing solution was dispensed into each well (0.4ml/ well) and was removed by aspiration. Washing step was repeated twice. Subsequently 200 µl of the freshly prepared revelation solution (tetramethylbenzdine, TMB-H202) was dispensed into each well and incubated for 15 min on a horizontal shaker (700 ± 100 RPM) at room temperature. At the end of incubation period, 50 µl of stopping reagent (H_2SO_4) was added to each well. Finally, the absorbances were read and the results were calculated (Flier et al., 1979; Frier et al., 1981; Judzewitsch et al., 1982; Kosaka et al., 1977; Starr et al., 1978)

II.6. Protein assay

Protein assay was determined using Bio-Rad determination kit (Bio-Rad, USA). The assay was based on the reaction of protein with an alkaline copper tartarte solution and Folin reagent. The diluted dye reagent was prepared by diluting 1 part dye reagent concentrate with 4 parts distilled water. Then, the diluted dye reagent was filtered through Whatman #1 filter to remove particulates. A protein standard curve was constructed using ten dilutions of the provided protein standard. Exactly 100 µl of each standard and unknown sample solution was pipetted into a clean, dry test tube. Diluted dye reagent (5.0 ml) was added to each tube and vortex. Then, tubes were incubated at room temperature for 30 min, absorbance was measured at 595nm and protein concentrations were calculated, (Bradford, 1976; Reisner et al., 1975; Sedmack and Grossberg, 1977; Compton and Jones, 1985; Spector, 1978; Duhamel et al., 1981; Macart and Gerbaut, 1982).

III. *IN VIVO* EXPERIMENT

III.1. Preparation of aqueous extract of *C. colocynthis*

Citrullus colocynthis seeds were shade dried and powdered in a grinder. A suspension of 200 g of the powdered plant in 400 ml of distilled water was

macerated in distilled water for 1 h at room temperature with continuous shaking. The mixture was filtered using sterile gauze followed by glasswool filtration. A fresh filtrate was used for the *in vivo* experiments (Subramoniam, 1996).

III.2. Animals

Male Wistar rats (170-250g) used in this study were raised and housed in the animal facility of the Faculty of Medicine and Health Sciences (FMHS), United Arab Emirates University. Animals were maintained at 22 ± 2°C and 12-h light /dark cycle. The animals were maintained on standard laboratory animal chew with food and tap water *ad libitum*.

III.3. Induction of diabetes mellitus

Streptozotocin (STZ) is a derivative of the mould *Streptomyces grieus*. This material can induce severe insulin-deficient diabetes in rats and other rodents, either by giving a single large dose (50-100 mg/kg in rats) or multiple smaller doses, which can induce autoimmune diabetes rather than islet toxicity. The most widely used method for inducing diabetes (IDDM) in animal models is a single intravenous or intraperitoneal injection of STZ. With a dose of 50-60 mg/kg of STZ, insulin will fall to 10-30% of the normal, leading to hyperglycaemia (20-30 mmol/l), polydipsia, polyuria, and body weight loss. STZ causes B-cell necrosis and diabetes supervenes within 24 h, by damaging B-cell membrane and inducing DNA strand breaks (Pickup and Williams, 1997).

In this experiment diabetes was induced by a single intraperitoneal injection of a buffered solution of STZ (0.5 M sodium citrate, pH 4.5) at a dosage of 60 mg/kg of body weight (Adeghate, 1999a). The animals were considered diabetic if fasting blood glucose level values were more than 126 mg/dl.

III.4. *In Vivo* Experimental Design

Eighty rats were randomly assigned into eight experimental groups. The first group served as normal non-diabetic control group that consisted of normal rats

with food and water only (normal non-diabetic group). The second group was positive control (diabetic group), which was injected with streptozotocin with food and water. The third, fourth and fifth (diabetic treated) groups were injected with streptozotocin and feed with three different doses of the water extract of the *C. colocynthis*; low dose (1 ml/kg), moderate dose (2 ml/kg) and high dose (4ml/kg). The sixth, seventh and eighth (non-diabetic treated) groups were normal rats fed with the same three doses of the plant extract that were given to the diabetic treated groups.

For diabetic treated groups, the plant extract was administrated daily by oral intubation for two weeks after one week from induction of diabetes. However, for other normal groups, the plant extract was administrated daily from the first day by oral intubation for two weeks.

At the end of the experimental period, all animals (diabetic and non-diabetic groups) were sacrificed, under anaesthesia, a mid-line abdominal incision was made and the pancreas was rapidly removed and cut into small fragments, and fixed overnight in freshly prepared Zamboni's fixative for immunohistochemical studies (Zamboni and de Martino, 1967).

III.5. Measurement of blood glucose levels and body weight

The fasting blood sugar level was evaluated twice a week for each rat. Blood was collected from the tail tip between 10:00 h and 12:00 h after and overnight fasting (no food) and blood glucose was determined using Sure Step glucometer (Life Scan, Johnson and Johnson, USA). The body weight was determined twice a week for each rat. Measurement of body weight was taken between 10:00 h and 12:00 h.

III.6. Biochemical Analysis

Insulin measurement was performed as described previously by using a DRG immunoenzymetric assay kit for the quantitative measurement of insulin in plasma (Germany).

Blood urea nitrogen (BUN), creatinine, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, lactic dehydrogenase, gamma-glutamyl transferase, calcium, phosphorus, sodium and potassium were performed in Al-Qattara Lab on Dimension ® clinical chemistry system (Dimension AR, Delware, USA). All kits for these tests (Urea nitrogen Flex™ reagent cartridge, Creatinine Flex™ reagent cartridge, Alkaline phosphatase Flex™ reagent cartridge, Alanine aminotransferase Flex™ reagent cartridge, Aspartate aminotransferase Flex™ reagent cartridge, Lactic dehydrogenase Flex™ reagent cartridge, Gamma-glutamyl transferase Flex™ reagent cartridge, Calcium Flex™ reagent cartridge, phosphorus Flex™ reagent cartridge, Sodium Flex™ reagent cartridge, and Potassium Flex™ reagent cartridge) were purchased from DADE BEHRING (DADE Behring, USA).

III.7. Histological and immunohistochemical techniques

III. 7. 1. a. Processing of tissue samples

At the end of the experiment, normal and treated rats were anaesthetized with chloroform. A long abdominal incision was made and the pancreas was quickly removed, trimmed of connective tissue and fixed for 72 h in Zamboni's solution. After the fixation process, the samples were dehydrated in ascending concentration of ethanol, cleared in xylene and embedded in paraffin wax. Sections of 6 µm thickness were cut and processed for haematoxylin eosin staining and for immunohistochemistry.

III.7. 1.b. Staining with haematoxylin and eosin stain for paraffin sections

Sections were dewaxed in xylene (twice, 2 min each), then hydrated through graded alcohols (100% twice (1 min for each), 95% (1 min), 70% (1 min) to water (1 min). Thereafter, the sections were stained in alum haematoxylin of choice for 4 min, followed by few dips in water. After that the sections were

differentiated in 1% acid alcohol. Next, slides were washed in running tap water (5 min), and stained in eosin (30 s). Finally, sections were dehydrated through graded ethanol 70% (15 s), ethanol 95% (15 s), ethanol 100% (twice, 2 min each) then cleared in xylene (twice, 2 min each); and mounted in Cytoseal (Stephens Scientific, Riverdale, USA).

III.7. 2. a. Immunohistochemistry

Three rats from each group were used for this experiment. The isolated pancreas was trimmed free of adherent fat and connective tissue and then cut into small pieces (2 mm^3) and fixed overnight in freshly prepared Zamboni 's fixative (Zamboni and de Martino, 1967). The tissues were later dehydrated in graded concentrations of ethanol. Tissues were embedded in paraffin wax as describe in the previous section.

Sections of 6 μm thickness were deparaffinised with xylene (twice, 5 min each) and then transferred into absolute ethanol (twice, 5 min each). The sections were then incubated for 30 min in 0.3% hydrogen peroxide solution in methanol to block endogenous peroxidase activity. The sections were hydrated in decreasing concentration of ethanol and brought to Tris buffered saline (TBS). The slides were washed 3 times (5 min each) in TBS. After washing in TBS, the tissues were marked around with a Dako pen to prevent solution draining away from the tissue sections. The staining procedure was started by incubating the sections with blocking reagent. After 30 min the blocking reagent was drained off and appropriate dilution of primary antibodies (anti insulin and anti glucagon) and negative control reagents were applied. The sections were incubated in primary antibodies for 24 hour at 4° C temperature. The slides were then washed (3 times 5 each) with TBS and incubate for 30 min with pre-diluted biotinylated anti-rabbit or anti-mouse IgG for 30 min, washed in TBS (3 times, 5 min each) and subsequently incubated in streptavidin peroxidase conjugate for 45 min. After a final wash in TBS (3 times, 5 min each), the peroxidase activity was revealed by incubating the specimens for 3 min in 3,3- diaminobenzidine tetrahydrochloride

containing 0.03% hydrogen peroxide in TBS. The slides were later washed for 10 min under running tap water, and counterstained with haematoxylin for 30 s. They were then differentiated in acid ethanol and washed for 10 min under running tap water, dehydrated in ascending grades of ethanol, and subsequently cleared in xylene for longer time to dissolve the Dako pen mark. The tissues were subsequently mounted in Cytoseal 60 (Stephens Scientific, USA). Slides were examined under Zeiss Axiophot microscope and immunopositive area of the tissue sections were photographed (Adeghate, 1999a).

III.7. 2. b. Morphometric analysis

Morphometric analysis of insulin and glucagon immunoreactive cells. The total number of cells in the islets of the pancreas of normal and diabetic rats was counted using a VID V Image Analysis System (Synoptics, Cambridge CB4 1TF, UK) attached to a light microscope. In addition, insulin and glucagon positive cells within a given islet were also counted. Immunoreactive cells were counted in the sections at X 40 magnification. A total of 5 random fields per slide were examined from a total of 6 slides for each group. The mean of the percentages of insulin and glucagon positive cells was calculated for each islet.

Statistical analysis

Data were analyzed by ANOVA (analysis of variance) and student *t* test was used where appropriate to detect differences among individual means. The criterion for significant was $p < 0.05$. The results are presented as the mean ± SEM.

RESULTS

I. *IN VITRO* EXPERIMENT

I.1. Screening of medicinal plants aqueous extract for their effect of insulin secretion

Citrullus colocynthis increased insulin secretion by 108 ± 5.1, 121 ± 9.8, 137 ± 12.6, 135 ± 16.1 and 134 ± 12.4 when pancreatic tissue fragments were incubated with 0.2 µl, 1µ, 2µl, 4 µl and 10 µl of the extract, respectively. Furthermore, aqueous extract of *Zygophyllum simplex* caused an initial decrease in insulin release with 0.2 µl of the extract and subsequent increases when 1 µl, 2 µl and 4 µl of the extract were added to the medium. This increase was followed by a decrease in insulin secretion when 10 µl of the extract was incubated with pancreatic tissue fragments. Aqueous extract of *Tephrosia apollinea* was able to increase insulin release only at a concentration of 2 µl (Table 2). Extracts of other plants, including *Rhaza stricta*, *Moringa peregrina*, *Ziziphus spina-cristi* and *Teucrium mascatense* failed to evoke significant increases in insulin release after incubation with pancreatic tissue fragments.

II. *IN VIVO* EXPERIMENT

II.1.Effect of aqueous extract of *C. colocynthis* on insulin secretion

Figure 9 shows the effect of aqueous extract of *C. colocynthis* on insulin secretion in non-diabetic groups. 1 ml of the extract had the most significant ($p < 0.0001$) effect on secretion of insulin. The level of plasma insulin increased from 2.87 to 3.22 (µlU/ml) when 1ml of the extract of the plant was given. Moderate doses (0.5 ml) did not induce any significant release in insulin secretion. The basal level only increased from 2.72 to 2.82. The effect of a lower dose (0.25 ml) was even less significant and a change in the basal level was only from 2.61 to 2.62. These results clearly indicate that the effect of *C. colocynthis* extract on insulin release is dose-correlate.

As far as the effect of *C. colocynthis* on diabetic animals is concerned, all concentrations of the plant extract had a stimulatory effect on insulin secretion *in vivo*. Low concentrations caused high increases in insulin release from normal rats. However, higher concentrations of the plant extract induced an even higher ($p < 0.0005$) increases in insulin secretion (Fig. 10).

Table 2: Effects of aqueous extracts of different plants used in the *in vitro* study on insulin release.

	Plant extract	Dilutions of extract in media				
		0.2µL	**1 µL**	**2µL**	**4 µL**	**10 µL**
1	***Citrullus colocynthis***	108 ± 5.1	121 ± 9.8	* 137 ± 12.6	* 135 ± 16.1	* 134 ± 12.4
2	***Zygophyllum simplex***	88 ± 10.1	* 133 ± 16	* 139 ± 12.9	* 142 ± 17.3	98 ± 8.2
3	***Tephrosia apollinea***	118 ± 12.3	107 ± 7.9	* 132 ± 14.1	115 ± 9.9	119 ± 12.8
4	***Teucrium mascatense***	79 ± 8.1	85 ± 5.9	83 ± 7.9	86 ± 10.7	84 ± 6.8
5	***Rhazya stricta***	80 ± 8.5	78 ± 8.1	76 ± 10.6	64 ± 5.4	57 ± 5.4
6	***Ziziphus spina-cristi***	62 ± 6.7	56 ± 5.8	57 ± 7.4	51 ± 5.8	36 ± 2.5
7	***Moringa peregrina***	70 ± 7.7	54 ± 4.7	57 ± 4.4	53 ± 6.1	57 ± 8.2

Data are shown as mean ± S.E of insulin release in relation to the blank. Each mean is calculated from the percentage increase in insulin release compared to the blank (n= 8 wells).
* = Significant increase in insulin release compared to the control (blank) at $p < 0.05$.

There is a significant increase in the plasma insulin level in rats treated with low, moderate and high doses of the plant extract compared to that of control rats.

II.2. Effect of aqueous extract of *C. colocynthis* on blood glucose level

Plasma glucose levels were significantly elevated in STZ-diabetic rats. Oral administration of aqueous extract of *C. colocynthis* seeds given over 2-week period significantly reduced plasma glucose level in non-diabetic and diabetic rats. The mean blood glucose levels of fasted normal (non-diabetics groups) animals treated by various concentration of the aqueous extract of *C. colocynthis* are shown in Figure 11. Low dose (0.25ml) has no effect on fasting blood glucose. The basal level changed from 78.4 to 79.4 (mg/dl). There was a significant decrease in glucose level from 76.2 to 67.3 (mg/dl) when moderate dose (0.5 ml) of the plant extract was given. The high dose has the maximum hypoglycaemic effect ($p < 0.0005$). The basal level changed from 79.6 to 62.7 (mg/dl) after the administration of 1 ml of *C. colocynthis*. These results showed that *C. colocynthis* reduced blood glucose in a dose-correlated manner.

The effect of aqueous extract of *C. colocynthis* on blood glucose of fasted diabetic animals treated by various doses of the extract is shown in Figure 12. There was a minimum hypoglycaemic effect (from 326.8 to 301.7) when low dose (0.25ml) of the seeds extract was given orally to hyperglycaemic rats. Significant hypoglycaemic effect (the mean change from 323.2 to 210.3 mg/dl) was observed when diabetic rats were given 0.5 ml of the aqueous extract of *C. colocynthis*. High doses (1 ml) have the maximum hypoglycaemic effect. The mean blood glucose level decreased from 340.1to 118.2 (mg/dl).

II.3. Effect of aqueous extract of *C. colocynthis* on body weight

As shown in Figure 13, the body weight of non-diabetic treated rats was not significantly different from those of normal control rats. In figure 14, the effect of aqueous extract of *C. colocynthis* on body weight of diabetic groups is shown.

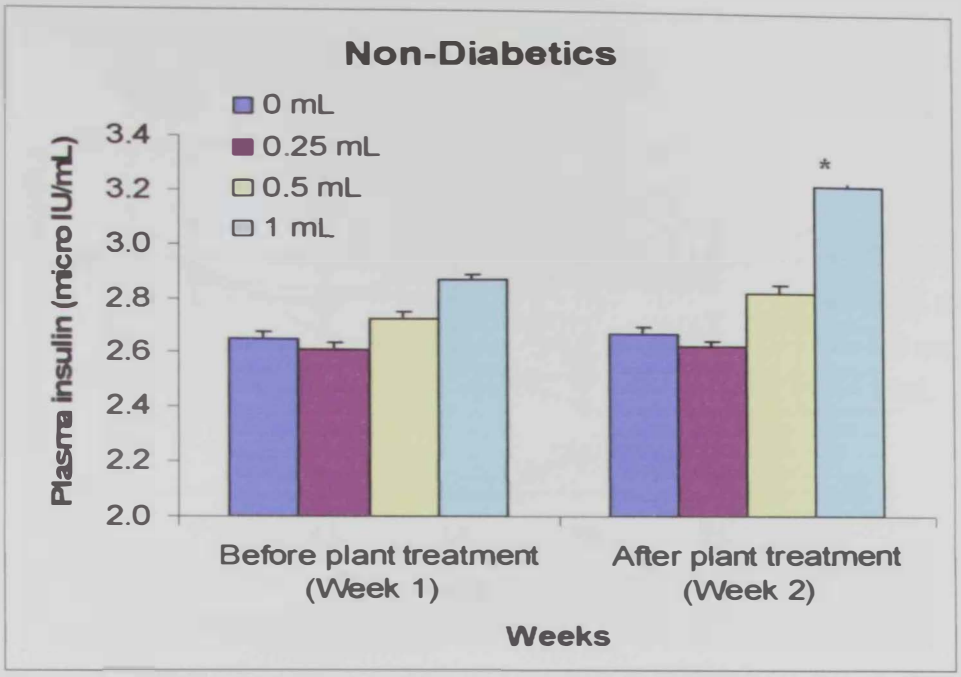


Figure 9: Effect of aqueous extract of *Citrullus colocynthis* on plasma insulin secretion in fasted non-diabetic rats (Data are mean ± SD, n=9). 1 ml of the plant extract induced significant (* = p <0.0001) quantities of insulin secretion compared to control.

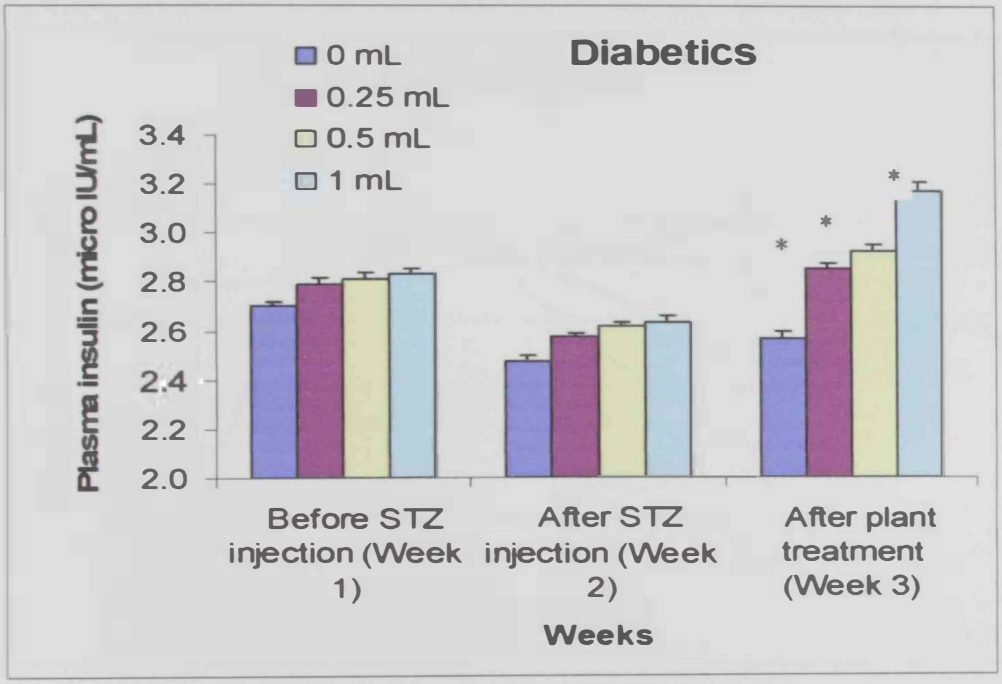


Figure 10: Effect of aqueous extract of *Citrullus colocynthis* on plasma insulin secretion in fasted diabetic rats (Data are mean ± SD, n=9). 0.25ml, 0.5ml, 1 ml of the plant extract induced very significant (* = p <0.0001) quantities in secretion release compared to control.

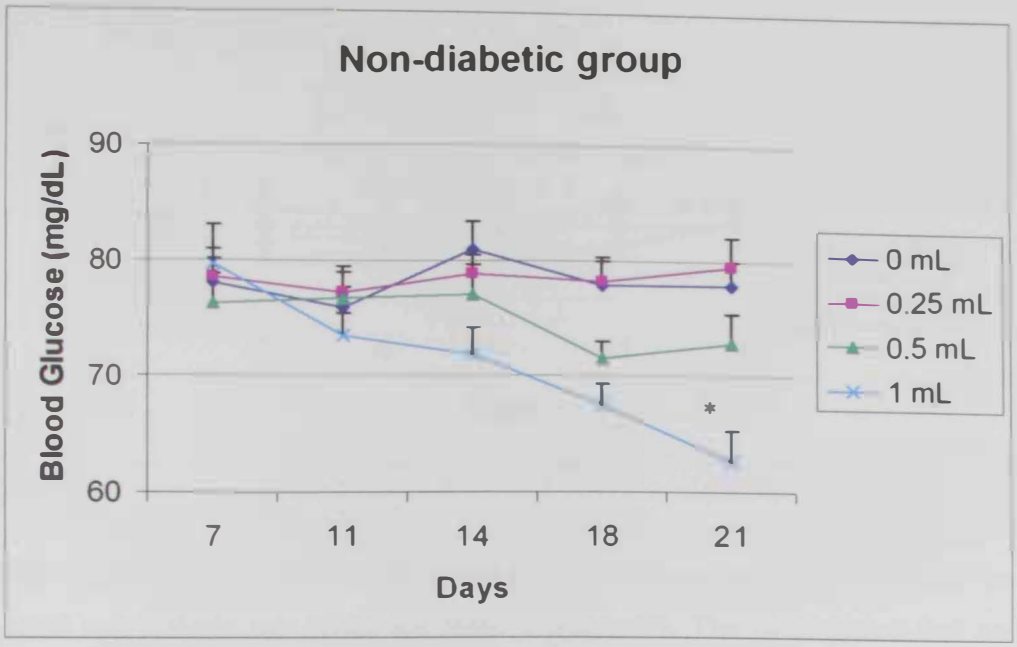


Figure 11: Effect of aqueous extract of *Citrullus colocynthis* on blood glucose level in fasted non-diabetic rats (Data are mean ± SD, n=9). 1 ml of the plant extract induced very significant (* = p <0.0005) decrease in blood glucose level compared to control.

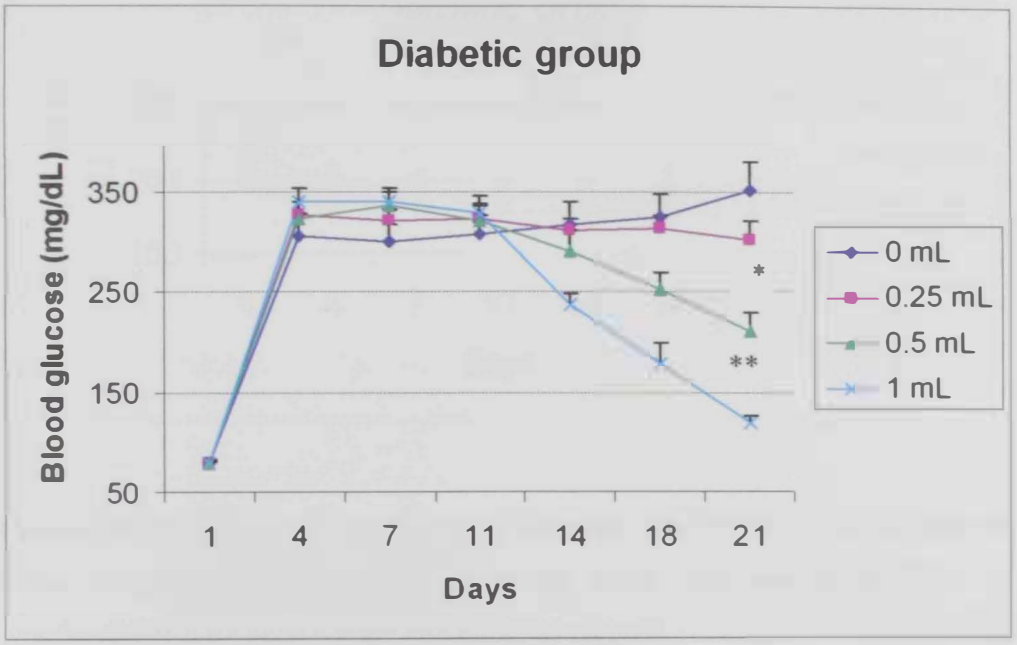


Figure 12: Effect of aqueous extract of *Citrullus colocynthis* on blood glucose in fasted diabetic rats (Data are mean ± SD, n=9). 0.5ml, 1 ml of the plant extract induced significant (* = p <0.001, ** = p < 0.000001) decrease in blood glucose level compared to control.

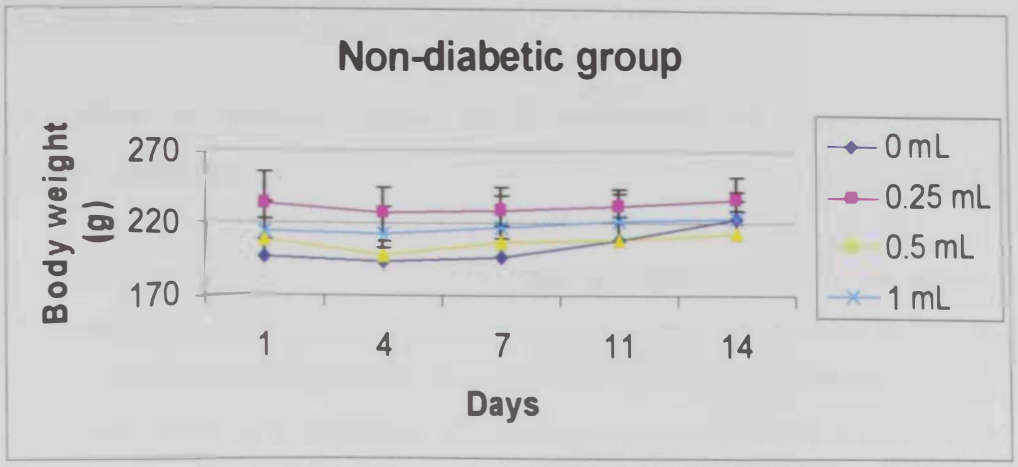


Figure 13: Effect of aqueous extract of *Citrullus colocynthis* on body weight in fasted non-diabetic rats (Data are mean ± SD, n=9). The plant extract had no significant effect compared to control.

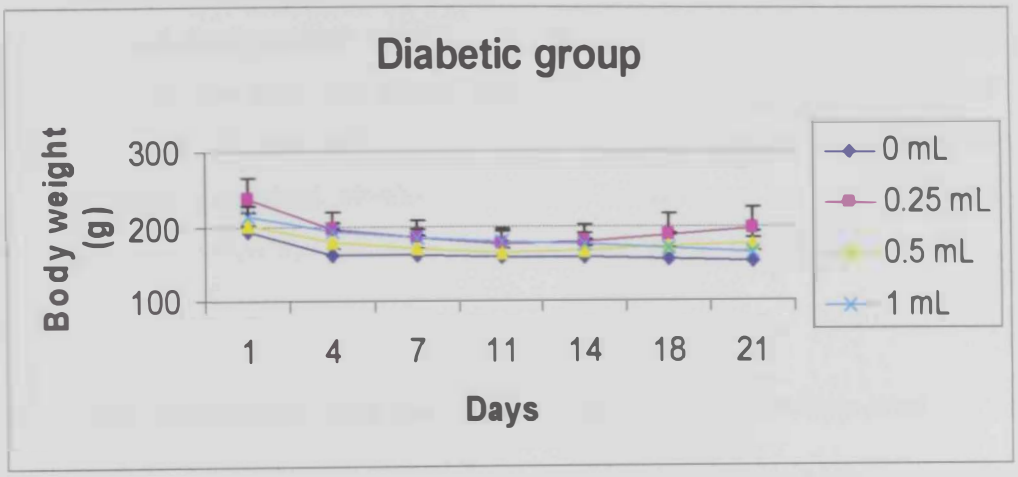


Figure 14: Effect of aqueous extract of *Citrullus colocynthis* on body weight in fasted diabetic rats (Data are mean ± SD, n=9). The plant extract had no significant effect on body weight compared to control.

II.4. Biochemical effects of *C. colocynthis*

II.4.a.Effect of aqueous extract of *C. colocynthis* on plasma alanine aminotransferase level

Aqueous extract of *C. colocynthis* had no significant effect on alanine aminotransferase level in non-diabetic rats. On the other hand, aqueous extract of *C. colocynthis* caused significant changes on alanine aminotransferase level in diabetic rats. There is no significant effect of *C. colocynthis* extract of fasted non-diabetic groups on alanine aminotrasferase in all doses. Moreover, aqueous *C. colocynthis* extract had a significant effect on alanine aminotransferase in fasted diabetic rats (Fig. 16).

II.4.b.Effect of aqueous extract of *C. colocynthis* on plasma potassium level

There is no significant effect of aqueous *C. colocynthis* extract on potassium level in non-diabetic normal rats (Fig.17). The mean potassium level of fasted hyperglycaemic rats after oral administration of aqueous *C. colocynthis* extract is shown in Figure 18. Low, and moderate doses caused no significant change in the level of plasma potassium. However, there was a significant increase in plasma potassium level when the rats were fed with 1 ml of the aqueous extract of *C. colocynthis*.

II.4.c.Effect of aqueous extract of *C. colocynthis* on plasma sodium level

The results presented in Figure 19 demonstrate that the aqueous *C. colocynthis* extract had no effect on plasma sodium level in non-diabetic normal rats. However, in diabetic animals, low dose (0.25ml) of the extract caused a significant decrease in plasma sodium level (Fig. 20). Moderate dose of the plant group had no effect on plasma sodium level. High dose (1ml) induced a significant increase in plasma sodium level.

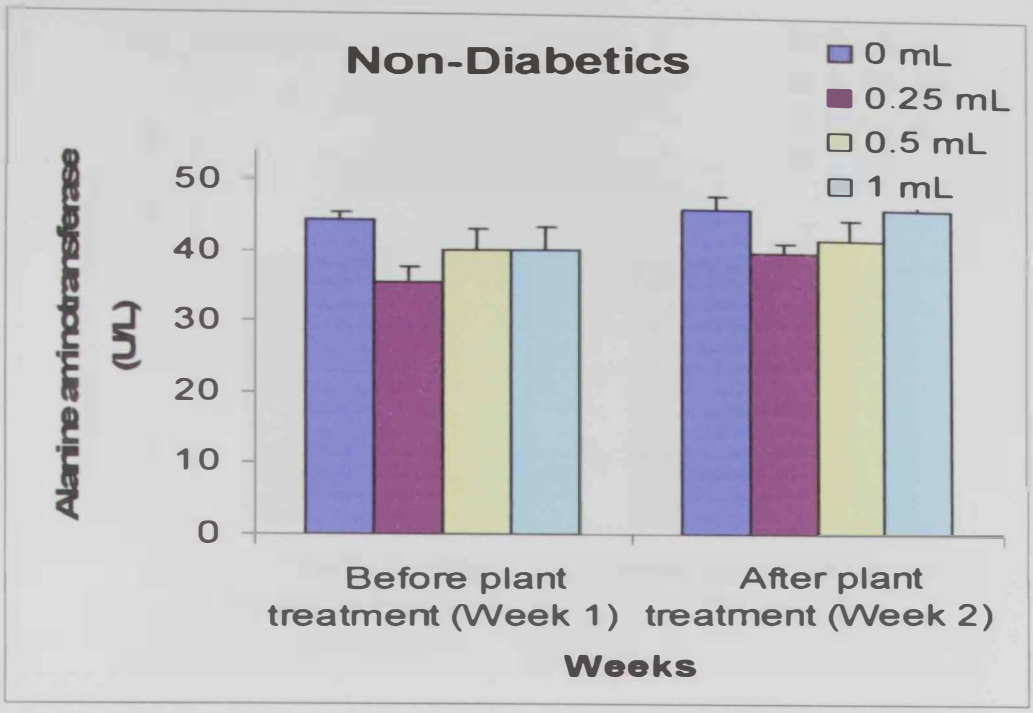


Figure 15: Effect of aqueous extract of *Citrullus colocynthis* on plasma alanine aminotransferase in fasted non-diabetic rats (Data are mean ± SD, n=9). No significant effect was induced by the plant extract on alanine aminotransferase level compared to control.

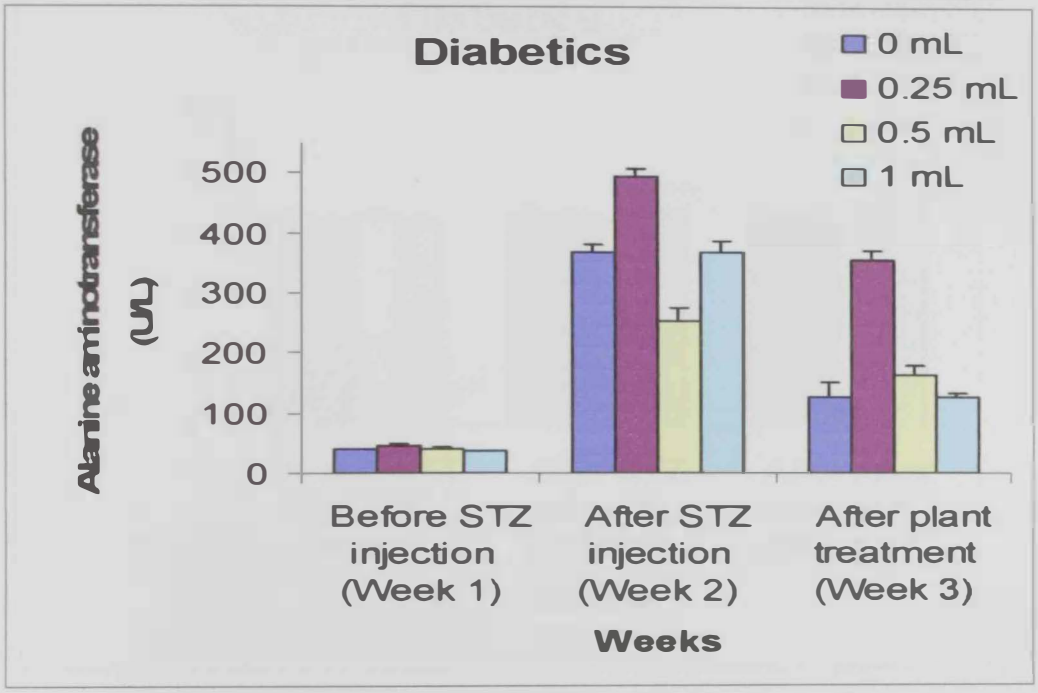


Figure 16: Effect of aqueous extract of *Citrullus colocynthis* on plasma alanine aminotransferase in fasted diabetic rats (Data are mean ± SD, n=9) The plant extract had no significant effect compared to control.

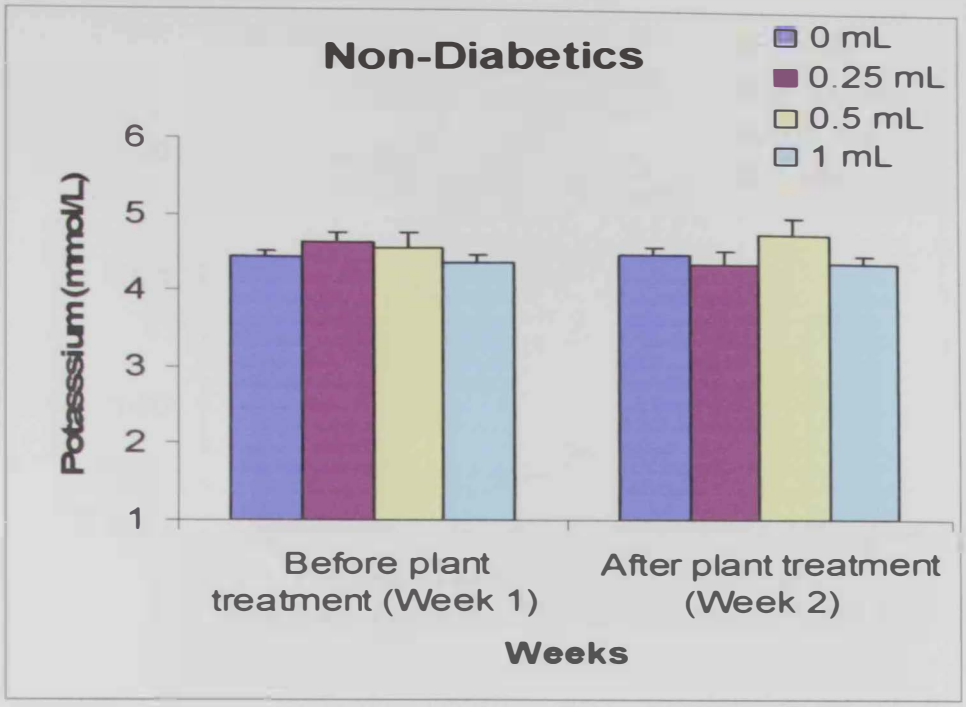


Figure 17: Effect of aqueous extract of *Citrullus colocynthis* on plasma potassium level in fasted non-diabetic rats (Data are mean ± SD, n=9). The plant extract had no significant effect on plasma potassium level compared to control.

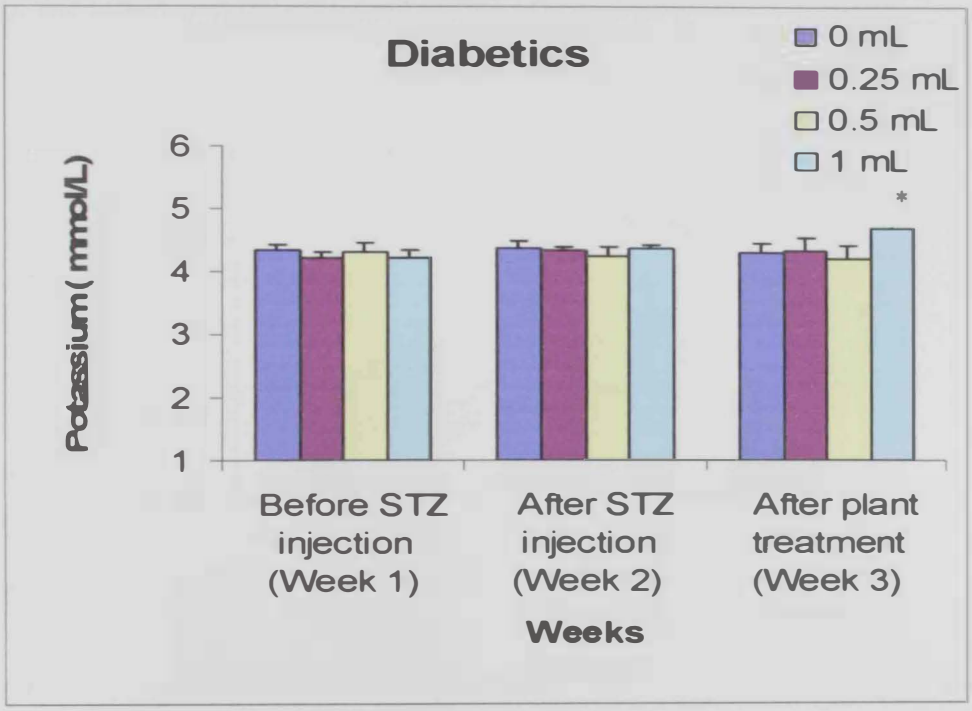


Figure 18: Effect of aqueous extract of *Citrullus colocynthis* on plasma potassium level in fasted diabetic rats (Data are mean ± SD, n=9). 1 ml of the plant extract induced significant (* = $p < 0.04$) increase in plasma potassium level compared to control.

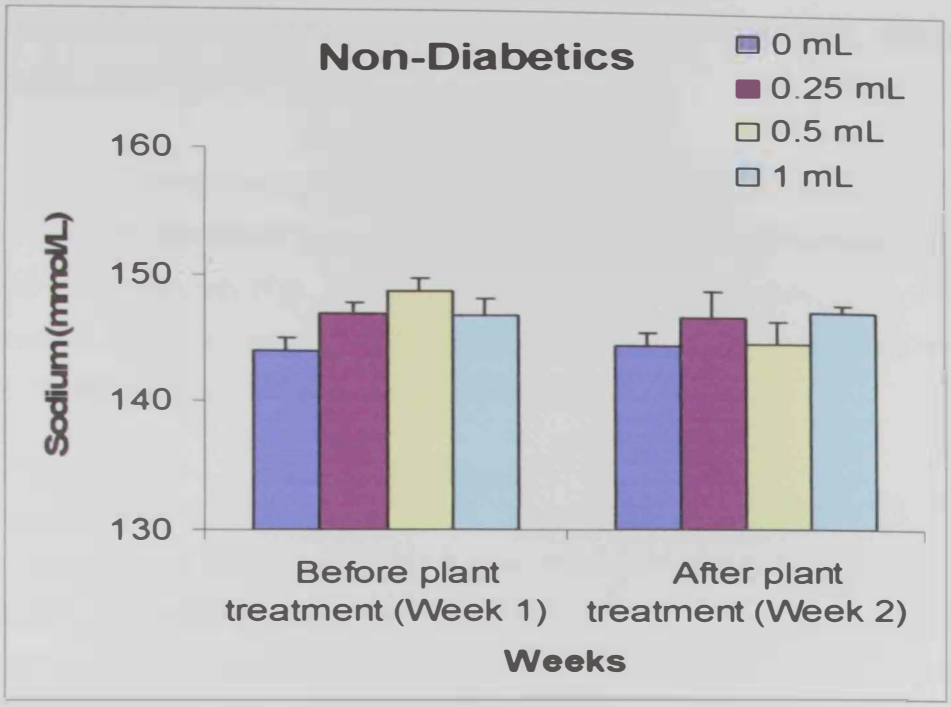


Figure 19: Effect of aqueous extract of *Citrullus colocynthis* on plasma sodium in fasted non-diabetic rats (Data are mean ± SD, n=9). Plant extract had no significant effect on plasma sodium level compared to control.

Figure 20: Effect of aqueous extract of *Citrullus colocynthis* on plasma sodium in fasted diabetic rats (Data are mean ±SD, n=9). 0.25ml of the plant extract induced significant (* = $p < 0.005$) decrease, while 1 ml of the plant extract induced significant (** = $p < 0.0001$) increases in plasma sodium level compared to control.

II.4.d. Effect of aqueous extract of *C. colocynthis* on plasma alkaline phosphatase level

A low concentration (0.25ml) of the aqueous extract of *C. colocynthis* did not cause any significant change in the plasma level of alkaline phosphatase when given to normal rats (Fig. 21). However, moderate and high doses (0.5, 1 ml) caused a significant ($p < 0.0001$) increase in alkaline phosphatase levels from (96.78, 102.11 up to 112.33, 120.56 U/L), respectively.

Plasma alkaline-phosphatase levels were significantly elevated in all STZ-diabetic rats from 104 up to 257 U/L (Fig. 22). After treatment with plant extract, alkaline phosphatase level remained high in all groups. This indicate that, all doses of the aqueous extract of *C. colocynthis* did not have significant beneficial effect on plasma alkaline-phosphatase level

II.4.e. Effect of aqueous extract of *C. colocynthis* on plasma gamma-glutamyl transferase level

The aqueous extract of *C. colocynthis* failed to induce significant increases in plasma gamma-glutamyl transferase level when given orally to normal rats (Fig. 23). However, there was an increase in plasma gamma-glutamyl transferase level after the onset of diabetes (3.4 to 7.7U/L). After treatment with low and moderate doses of the plant extract, a significant increase in the plasma level of gamma-glutamyl transferase level (7.9 to 11.9, 7.8 11.8 U/L) was observed. High doses of the plant extract caused a higher but not significant increase in gamma-glutamyl transferase level (7.9 to 8.4U/L) (Fig. 24).

II.4.f. Effect of aqueous extract of *C. colocynthis* on plasma lactic dehydrogenase level

The plasma level of lactic dehydrogenase in non-diabetic rats treated with low, moderate, high doses of the plant extract decreased significantly (0.25ml: 289 8 to 239.7, 0.5 ml: 281.9 to 238.4, 1ml: 253.1 to 235 U/L), respectively. In diabetic rats, plasma lactic dehydrogenase level increased from 250 to 400 U/L for all groups (Fig. 26). The plasma level of lactic dehydrogenase was still high after oral administration low and moderate doses of the plant extract of *C. colocynthis*. However, high (1ml) of the plant extract did not cause any significant changes in the plasma level of lactic dehydrogenase when compared to control (334.2 to 365.6 U/L)

II.4.g. Effect of aqueous extract of *C. colocynthis* on blood urea nitrogen level

Effect of aqueous *C. colocynthis* extract on blood urea nitrogen level in fasted non-diabetic rats is shown in Figure 27. Treatment of normal rats with different concentration of the plant extract had no significant effect on blood urea nitrogen level compared to control. Blood urea nitrogen level was elevated in all diabetic groups (Fig. 28). The level of blood urea nitrogen in diabetic rats did not change significantly after the administration of different doses of the aqueous extract of *C. colocynthis*.

II.4.h. Effect of aqueous extract of *C. colocynthis* on calcium level

Figure 29 shows the effect of aqueous *C. colocynthis* extract on calcium level in non-diabetic rats. Oral administration of moderate (0.5ml) and high (1ml) doses of the plant extract has no effect on plasma calcium level in normal rats. Low dose (0.25ml) of the plant extract induced significant (* = $p < 0.003$) increase in calcium level compared to control. All doses of plant extract caused no significant change in plasma calcium concentration in diabetic rats (Fig. 30).

II.4.i. Effect of aqueous extract of *C. colocynthis* on plasma creatinine level

Creatinine level was measured to determine if there was damage to the kidney. Aqueous extracts of the plant failed to cause significant changes in the creatinine levels of normal (Fig. 31) and diabetic (Fig. 32) rats. There was no significant effect on creatinine level in all non-diabetic groups Also, for diabetic groups there was no significant effect on creatinine level

II.4.j. Effect of aqueous extract of *C. colocynthis* on plasma phosphorus level

Figure 33 shows the effect of aqueous *C. colocynthis* extract on phosphorus level of non-diabetics rats. There was no significant change in plasma phosphorus level after normal rats were treated with different doses of the plant extract. There was no significant change in phosphorus level after treatment of diabetic rats with different concentrations of the plant extract (Fig. 34).

II.4.k. Effect of aqueous extract of *C. colocynthis* on plasma aspartate aminotransferase level

The effect of aqueous *C. colocynthis* extract on aspartate aminotransferase level in non-diabetics rats is shown in Figure 35. Oral administration of low and moderate doses of the plant extract failed to cause significant changes in the plasma aspartate aminotransferase level in normal rats compared to control.

Figure 36 shows the effect of aqueous *C. colocynthis* extract on aspartate aminotransferase level in diabetic rats. The plasma aspartate aminotransferase level increased more than two-fold after the onset of diabetes (100 to 220 U/L). Low and moderate doses of the plant extract failed to bring the plasma aspartate aminotransferase level down to the pre-treatment levels. Low and moderate doses (0.25ml, and 0.5 ml) of the plant extract caused a significant increase in aspartate aminotransferase level compared to untreated control. However, high dose (1 ml) of the plant extract induced a decrease in aspartate aminotransferase level when compared to untreated rats.

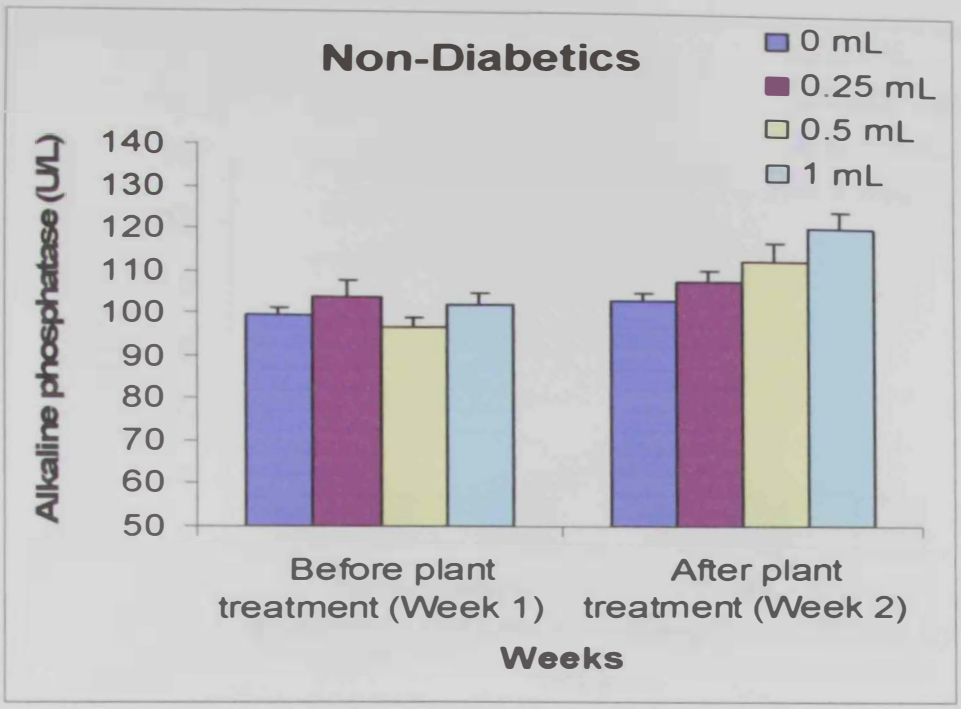


Figure 21: Effect of aqueous extract of *Citrullus colocynthis* on plasma alkaline phosphatase in fasted non-diabetic rats (Data are means ± SD, n=9). No significant effect was induced by plant extract on plasma alkaline phosphatase level compared to control.

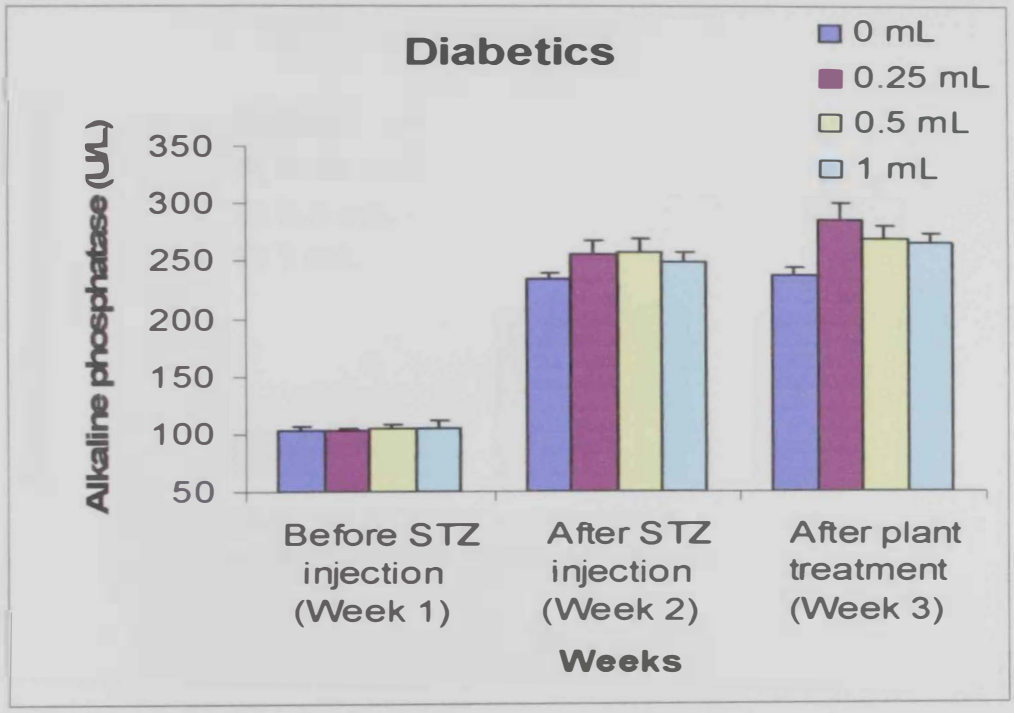


Figure 22: Effect of aqueous extract of *Citrullus colocynthis* on plasma alkaline phosphatase in fasted diabetic rats (Data are mean ± SD, n=9). Plant extract had no significant effect on plasma sodium level compared to control.

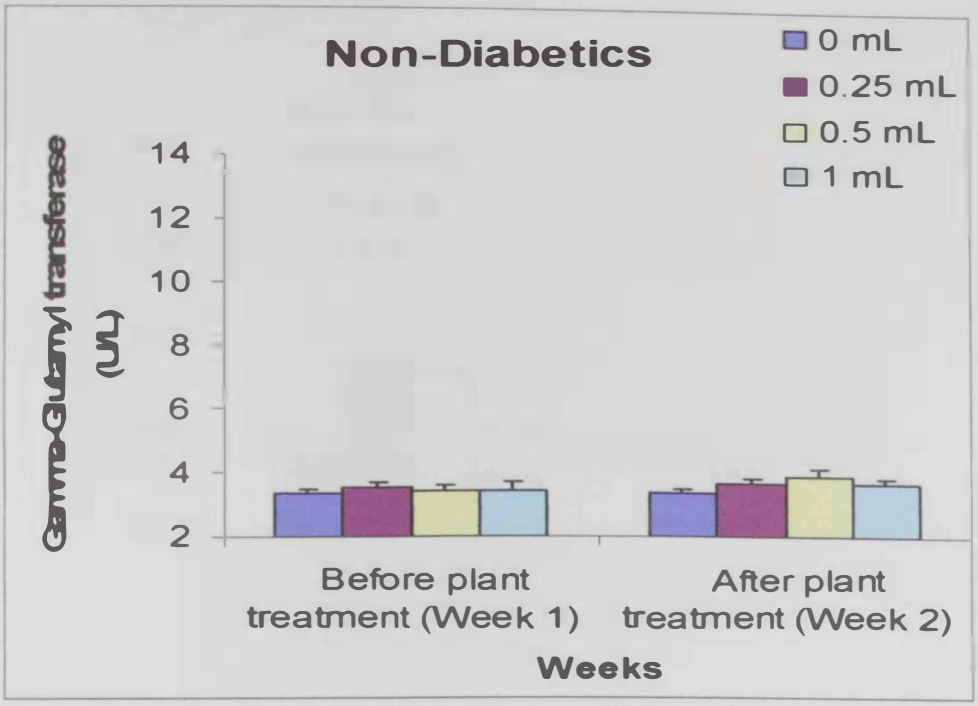


Figure 23: Effect of aqueous extract of *Citrullus colocynthis* on gamma-glutamyl transferase in fasted non-diabetic rats (Data are mean ± SD, n=9). The plant extract had no significant effect on gamma-glutamyl transferase level compared to control.

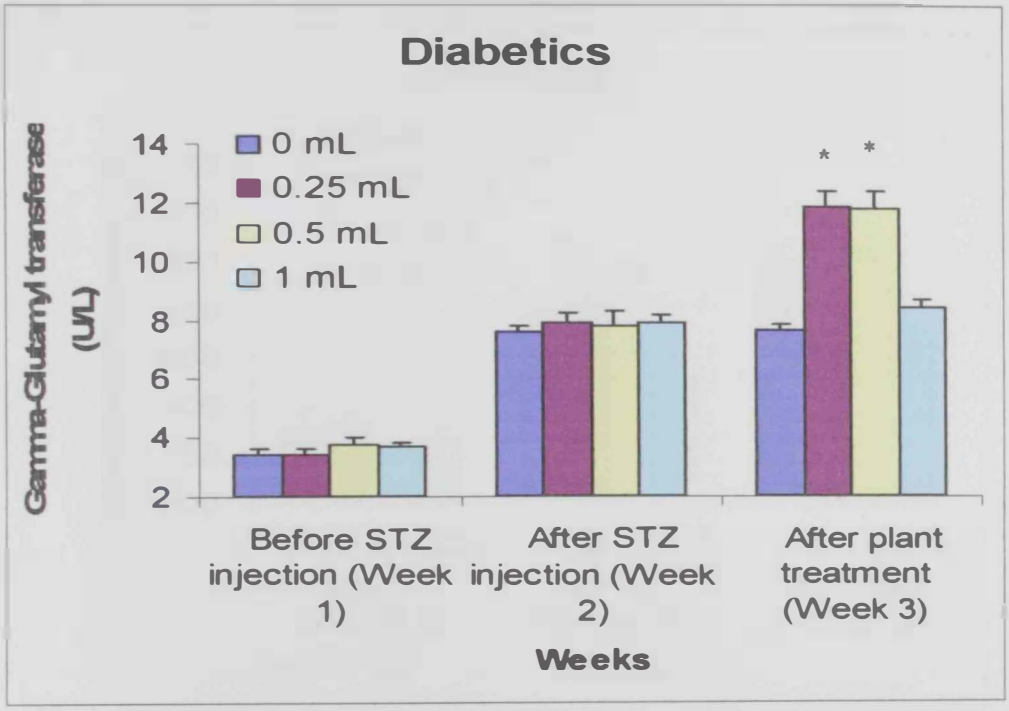


Figure 24: Effect of aqueous extract of *Citrullus colocynthis* on gamma-glutamyl transferase in fasted non-diabetic rats (Data are mean ± SD, n=9). 0.25ml, 0.5ml of the plant extract induced significant increase (*= $p < 0.0001$) increase in plasma gamma-glutamyl transferase compared to control.

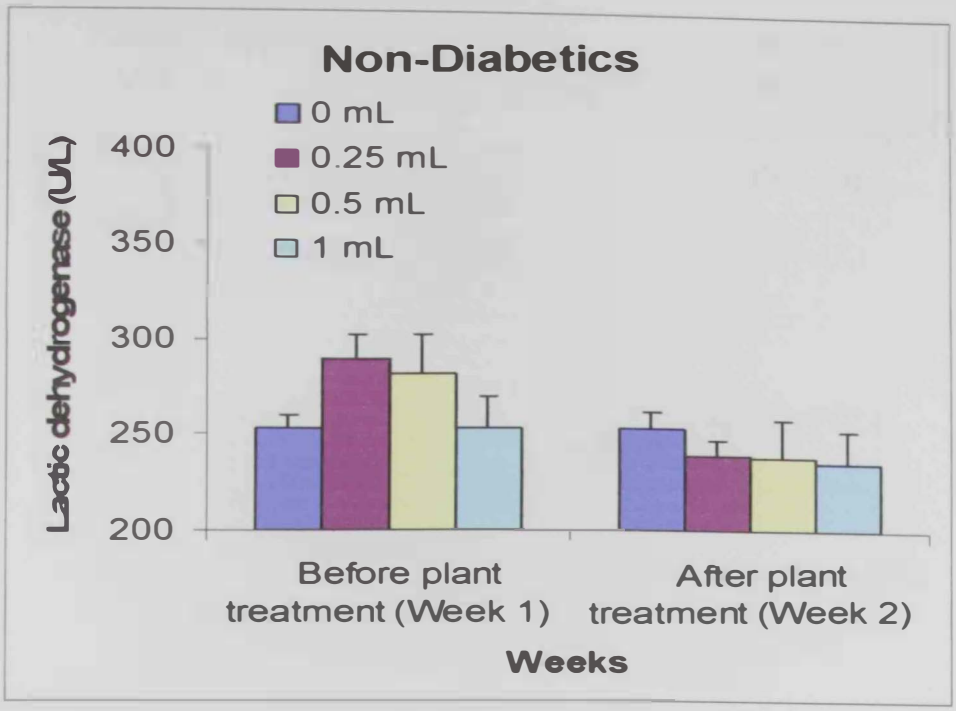


Figure 25: Effect of aqueous extract of *Citrullus colocynthis* on lactic dehydrogenase in fasted non-diabetic rats (Data are mean ± SD, n=9). The plant extract had no significant effect on plasma lactic dehydrogenase level compared to control.

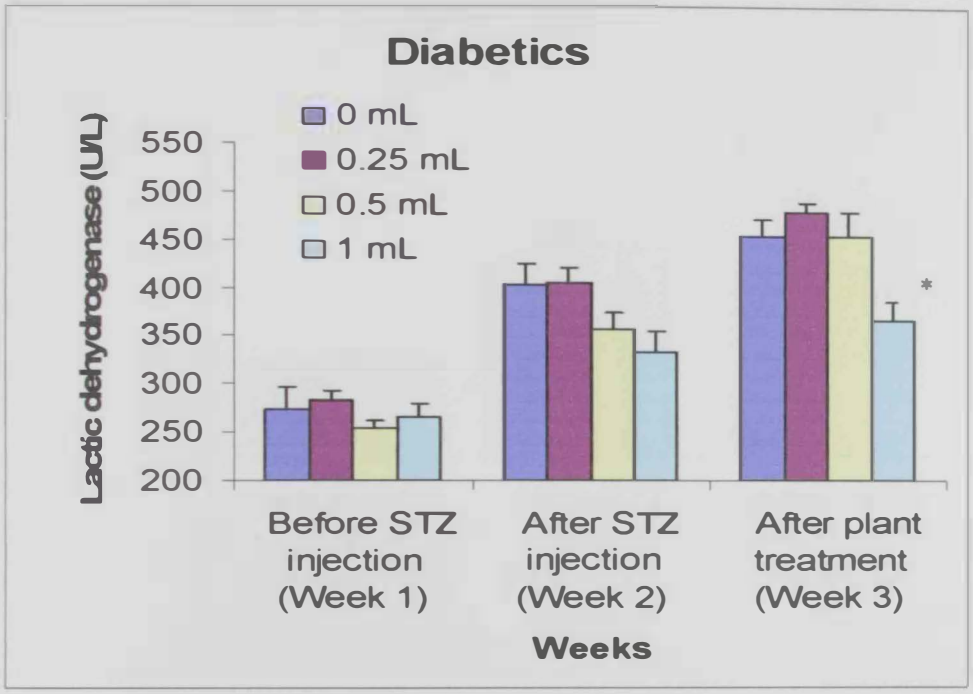


Figure 26: Effect of aqueous extract of *Citrullus colocynthis* on lactic dehydrogenase in fasted diabetic rats (Data are mean ± SD, n=9). 1 ml of the plant extract induced significant (* = p <0.0003) decrease in plasma lactic dehydrogenase level compared to control.

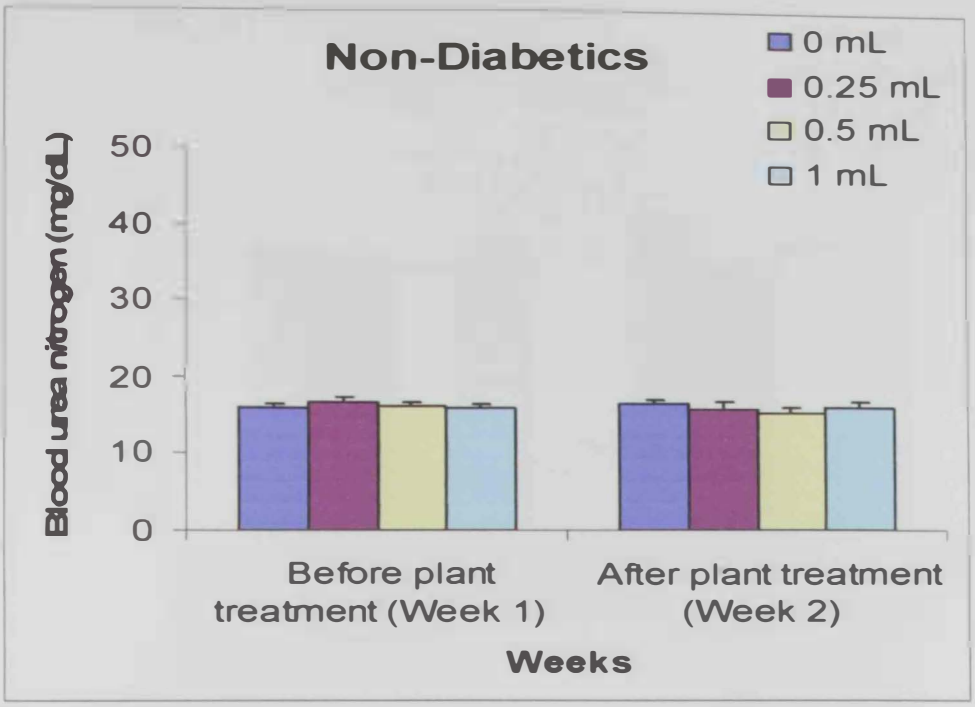


Figure 27: Effect of aqueous extract of *Citrullus colocynthis* on blood urea nitrogen in fasted non-diabetic rats (Data are mean ± SD, n=9). The plant extract caused no changes in the level of blood urea nitrogen compared to control.

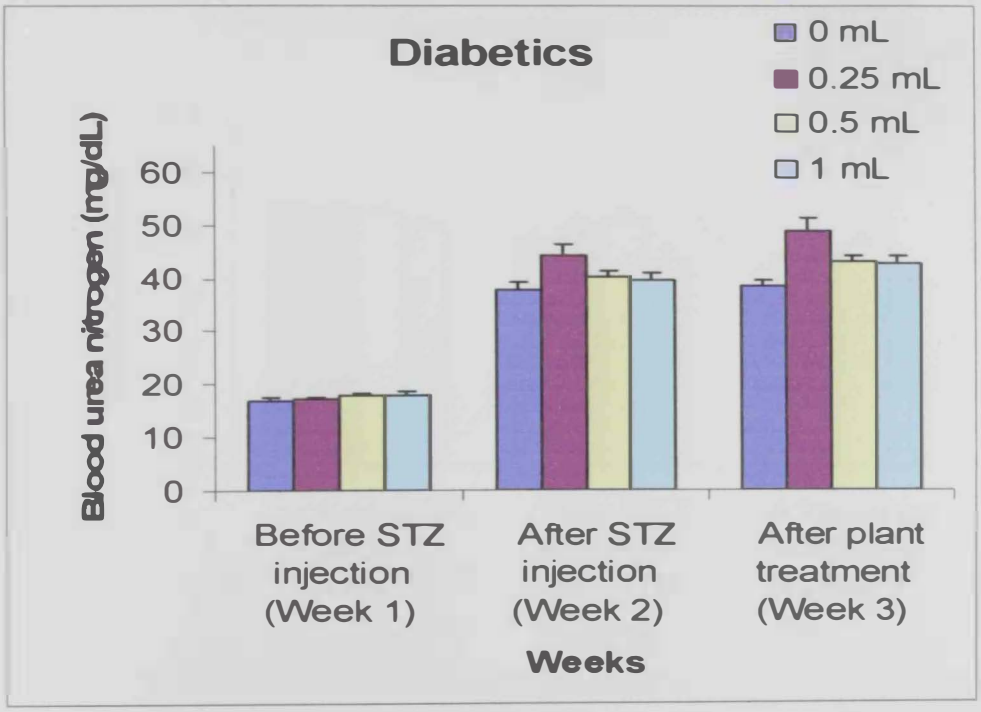


Figure 28: Effect of aqueous extract of *Citrullus colocynthis* on blood urea nitrogen in fasted diabetic rats (Data are mean ± SD, n=9). The plant extract caused no changes in the level of blood urea nitrogen compared to control.

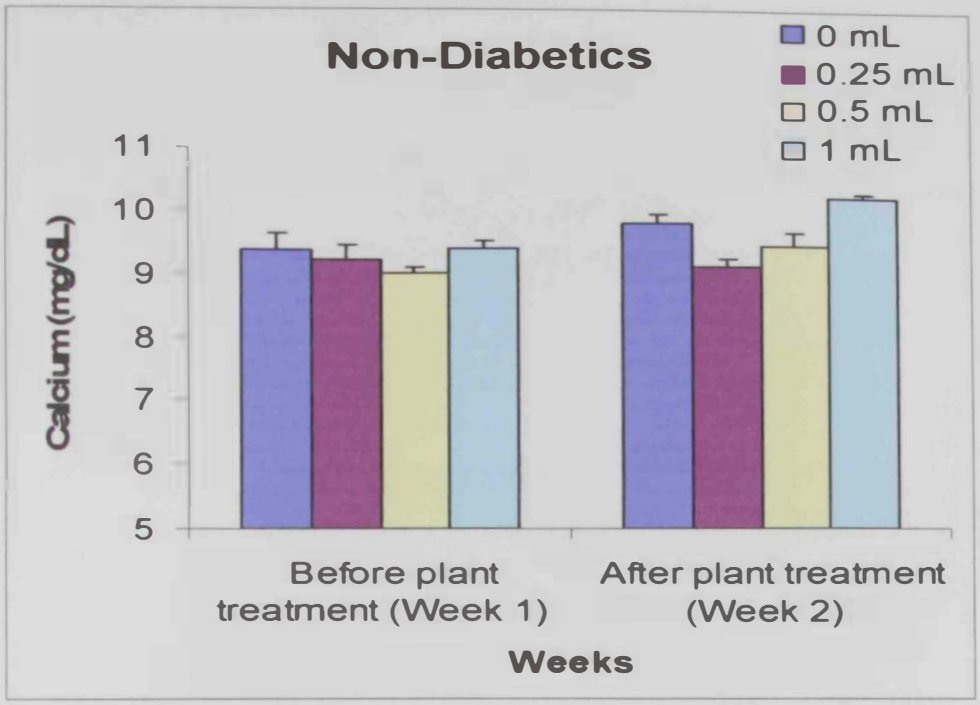


Figure 29: Effect of aqueous extract of *Citrullus colocynthis* on plasma calcium level in fasted non-diabetic rats (Data are mean ± SD, n=9). The plant extract caused no changes in the level of plasma calcium compared to control.

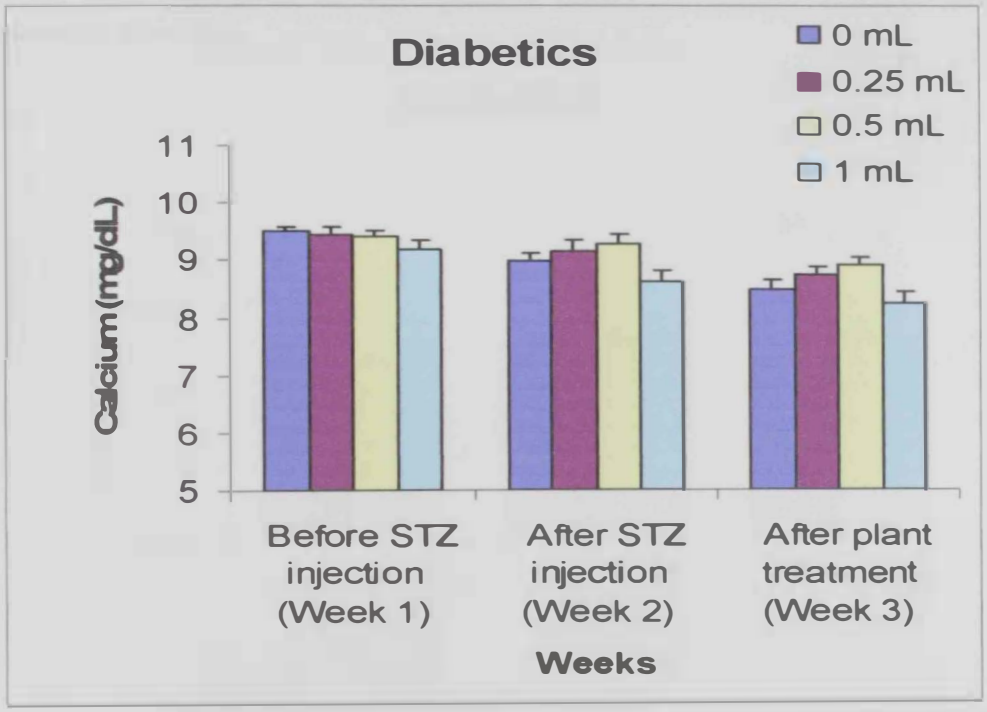


Figure 30: Effect of aqueous extract of *Citrullus colocynthis* on calcium level in fasted diabetic rats (Data are mean ± SD, n=9). The plant extract did not cause significant changes in the plasma calcium level compared to control.

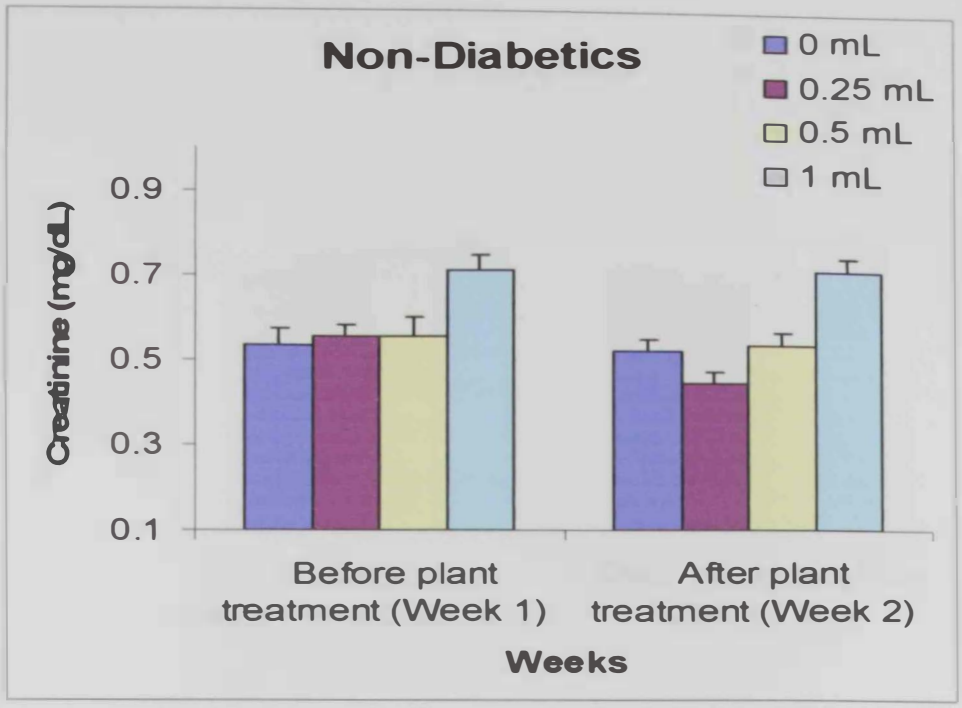


Figure 31: Effect of aqueous extract of *Citrullus colocynthis* on plasma creatinine level in fasted non-diabetic rats (Data are mean ± SD, n=9). The aqueous extract of the plant did not cause any significant changes in plasma creatinine level compared to control.

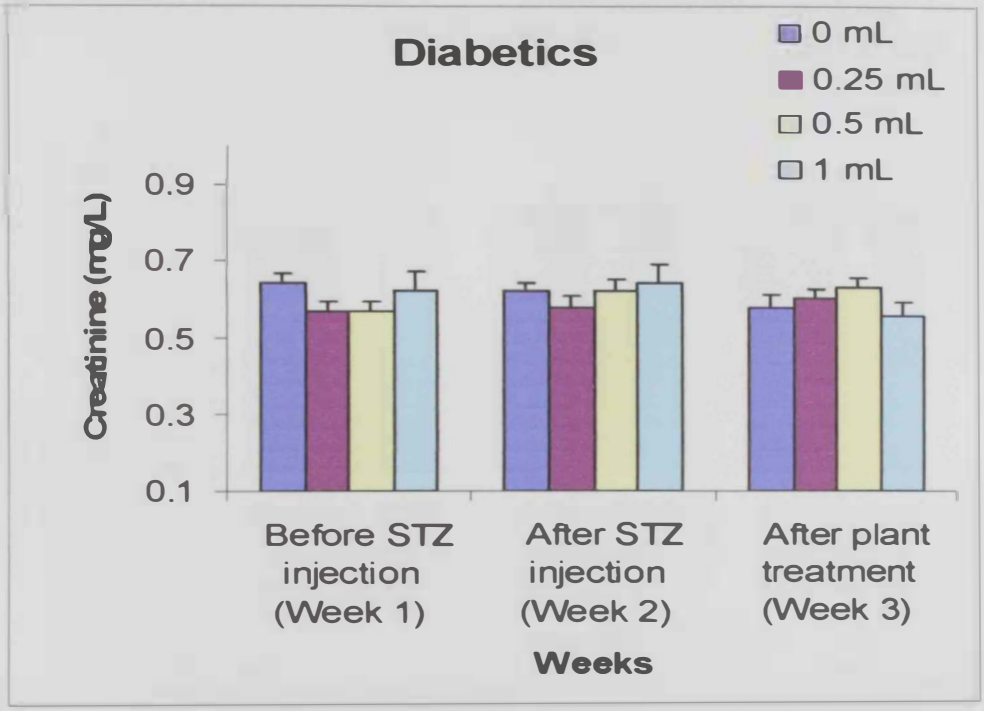


Figure 32: Effect of aqueous extract of *Citrullus colocynthis* on plasma creatinine level in fasted diabetic rats (Data are mean ± SD, n=9). Plasma creatinine level did not change significantly after oral administration of the plant extract compared to control.

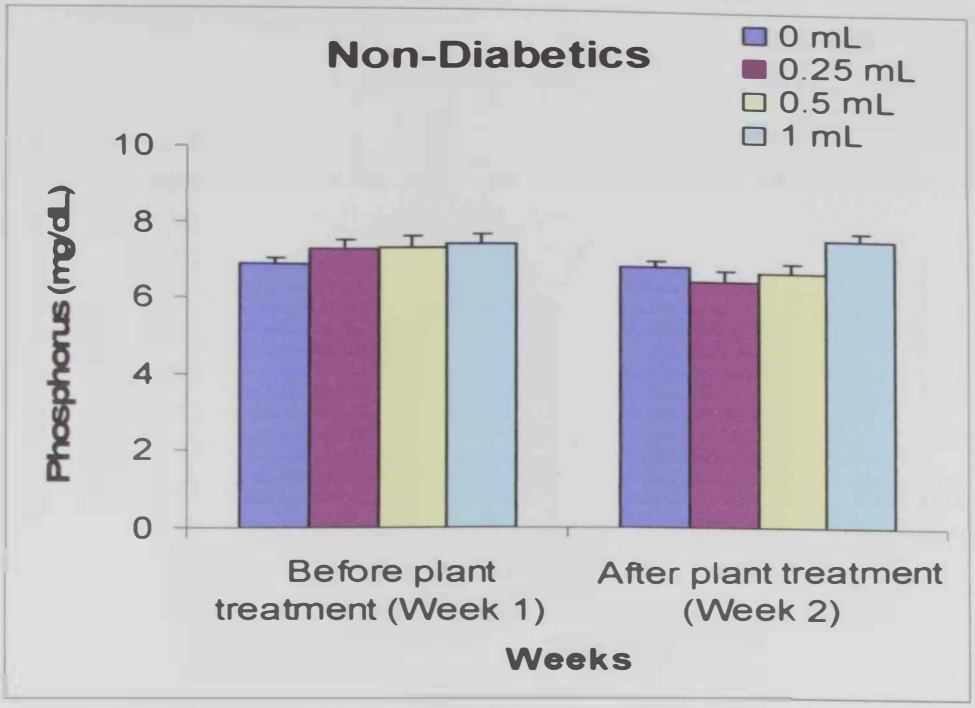


Figure 33: Effect of aqueous extract of *Citrullus colocynthis* on plasma phosphorus level in fasted non-diabetic rats (Data are mean ± SD, n=9). The plant extract did not induce significant differences in plasma phosphorus level compared to control.

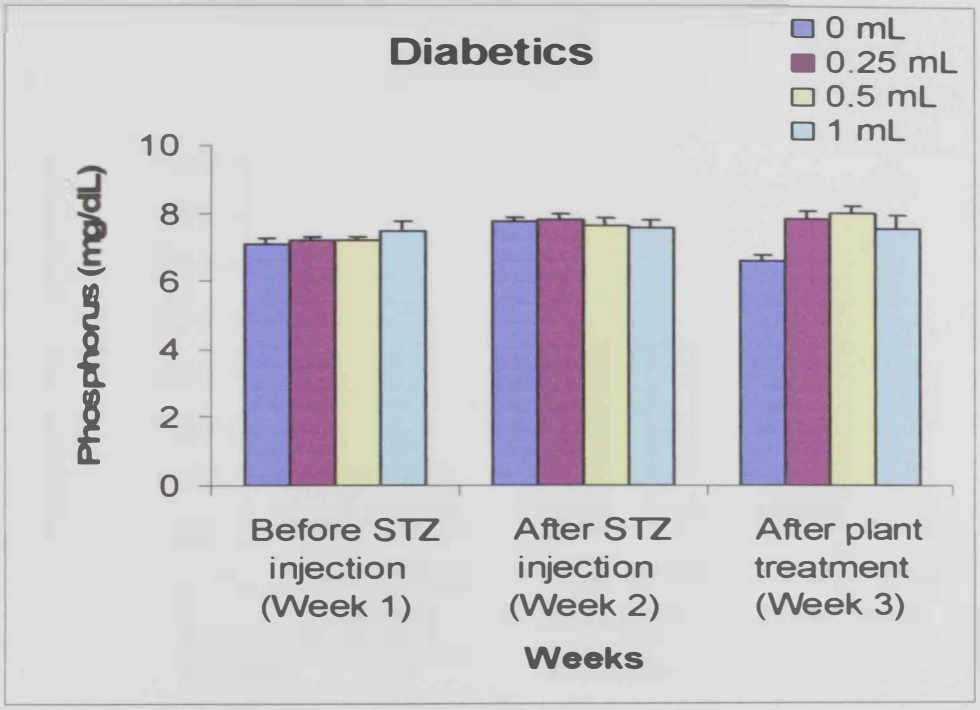


Figure 34: Effect of aqueous extract of *Citrullus colocynthis* on plasma phosphorus in fasted diabetic rats (Data are mean ± SD, n=9). The plant extract did not induce significant differences in plasma phosphorus level compared to control.

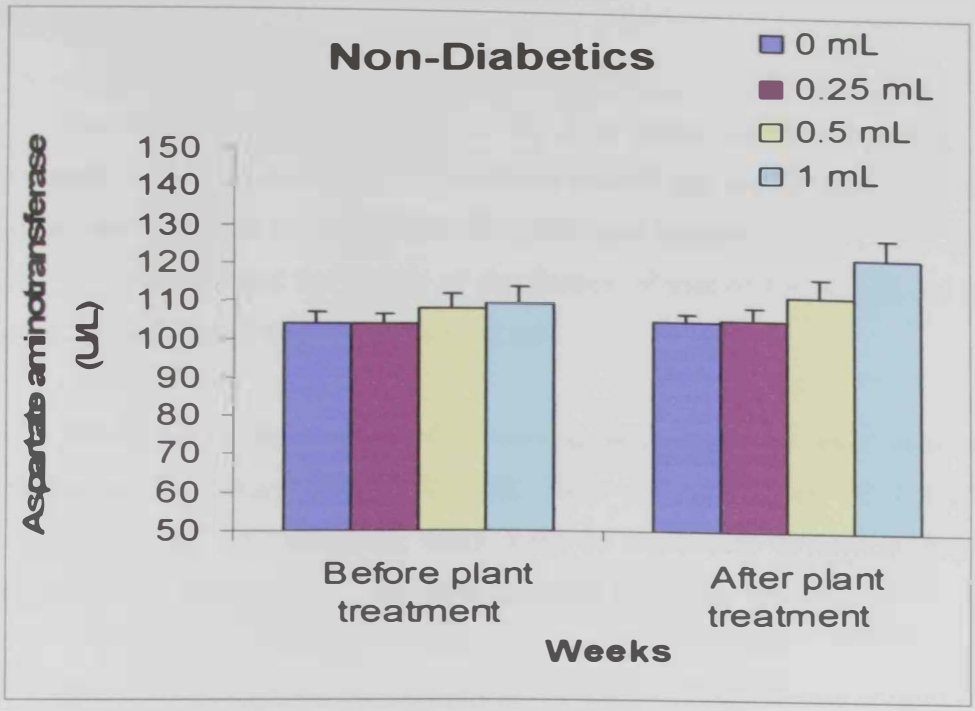


Figure 35: Effect of aqueous extract of *Citrullus colocynthis* on plasma aspartate aminotransferase in fasted non-diabetic rats (Data are mean ± SD, n=9). The plant extracts cause a small, but not significant changes in the plasma aspartate aminotransferase level compared to control.

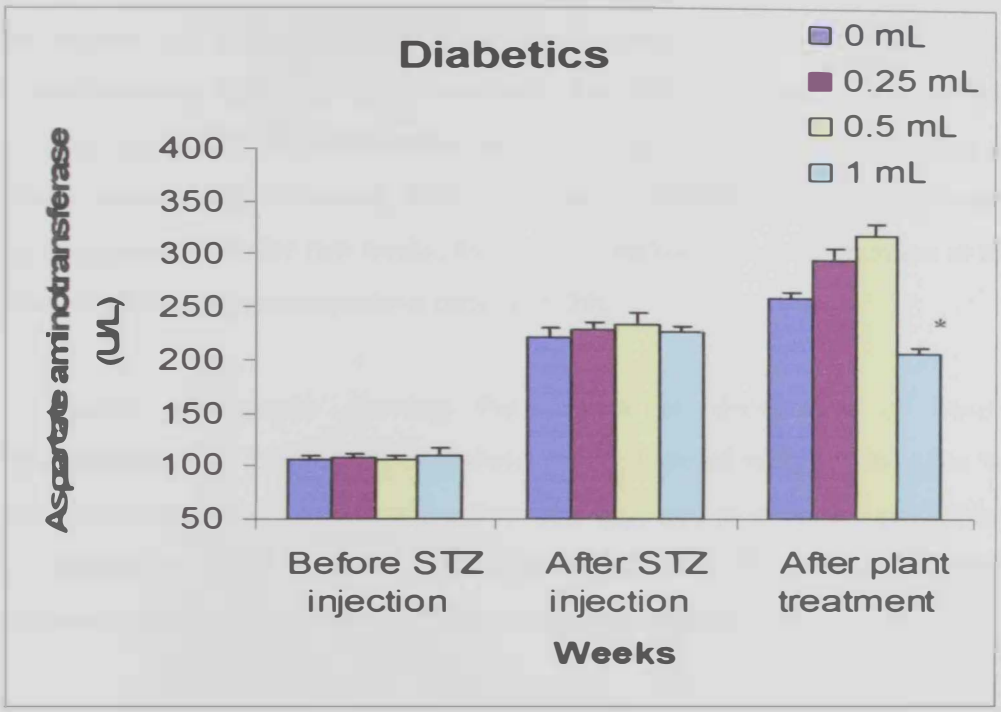


Figure 36: Effect of aqueous extract of *Citrullus colocynthis* on plasma aspartate aminotransferase in fasted diabetic rats (Data are mean ± SD, n=9). 1ml of the plant extract induced significant (* = $p < 0.0001$) decrease in plasma aspartate aminotransferase level compared to control.

III. Histology results

In order to determine the effect of the plant extract on the morphology of pancreatic islet cells, we stained the islets of normal and treated rats. The plant extract did not cause any significant morphological changes to pancreatic islets (Fig. 37). The size and the pattern of distribution of islet of treated rats did not appear to be different from that of normal rats

The size and the number of pancreatic islets appear to have increased (qualitative assessment) after treatment with different doses of *Citrullus colocynthis* (Fig. 38). However, there were no significant differences in the morphological appearances of the acinar cells and pancreatic tubules.

Figure 39 shows light micrographs of the pattern of distribution of insulin in the pancreas of normal rats. Insulin immunoreactive cells were numerous and found in both the central and peripheral parts of the islet of Langerhans. Morphometric analysis of pancreatic islet cells suggests that there is increase in the number of insulin-positive cells. The increase in the number of insulin immunoreactive cells was dose-correlated. The administration of a low dose (0.25ml) of the plant did not cause any significant increase in the number of insulin-positive cells. However, when moderate and high doses (0.5ml, 1ml) were given to normal rats for two weeks, there was a marked ($p < 0.02$) increase in the number of insulin immunopositive cells (Fig. 39).

Light micrographs showing the pattern of distribution of insulin immunoreactive of insulin cells in diabetic rats is depicted in figure 40. After the onset of diabetes, insulin immunoreactive cells appeared to be fewer (quantitative assessment) in diabetic pancreas without treatment. But in the treated rats insulin immunoreactive cells increase correlate on the dose of plant.

In normal rats, glucagon (GLU) immunoreactive cells were observed mainly in the periphery of the islet of Langerhans where they form a mantle around the cells of the inner core of the islet (Fig 43). In experimental diabetes, GLU-positive cells were located in both the central and peripheral parts of the islet of Langerhans (Fig. 44). In addition, the number of glucagon-immunoreactive cells appeared to have increased significantly after the onset of diabetes.

The oral administration of a low dose (0.25 ml) of the aqueous extract of the plant extract appear to have caused a significant increase in the number of glucagon-positive cells in the islet of Langerhans of normal rats (Fig. 46). In contrast, higher doses (0.5 ml, 1 ml) of the plant extract did not cause any significant changes in the percentage distribution of glucagon-immunoreactive cells in the islets of normal rats (Fig. 46).

Morphometric analysis suggests that higher doses of the aqueous extract of *Citrullus colocynthis* induced large and significant ($p < 0.03$) increases in the percentage distribution of glucagon-immunopositive cells in the pancreatic islets of diabetic compared to control rats (Fig. 47).

Figure 37. Light micrographs of pancreatic islets (I) of normal rats. (Hematoxylin and eosin stain).

A. Normal control.

B. Normal rats treated with *C. colocynthis* (1ml/kg of aqueous extract).

C. Normal rats treated with *C. colocynthis* (2ml/kg of aqueous extract).

D. Normal rats treated with *C. colocynthis* (4ml/kg of aqueous extract).

Note that pancreatic islets of Langerhans are located between acinar tissues (thick arrow). There is no morphological difference between the pancreas of normal rats and those treated with different doses of the plant extract. N = peripheral nerve, thin arrow = pancreatic duct. Magnification: X 400.

Figure 38. Light micrographs of the pancreas of diabetic rats, showing pancreatic islets (I), duct (thin arrow), blood vessel (bv). Haematoxylin and Eosin stain.

A. Diabetic control.

B. Diabetic rats treated with *C. colocynthis* (1ml/kg of aqueous extract).

C. Diabetic rats treated with *C. colocynthis* (2ml/kg of aqueous extract).

D. Diabetic rats treated with *C. colocynthis* (4ml/kg of aqueous extract).

The islet of Langerhans in the diabetic group appeared to be smaller than that of normal rat. However, the pancreatic islets of diabetic rat treated with different doses of the plant extract appeared to be bigger compared to control. Magnification X400

Figure 39. Micrographs of insulin-immunoreactive cells (arrow) in pancreatic islets of non-diabetic rats.

A. Normal control.

B. Normal rats treated with *C. colocynthis* (1ml/kg of aqueous extract).

C. Normal rats treated with *C. colocynthis* (2ml/kg of aqueous extract).

D. Normal rats treated with *C. colocynthis* (4ml/kg of aqueous extract).

Note that there is no apparent difference in the number and pattern of distribution of insulin positive cells in the treated groups compared to control. bv = blood vessel. Magnification X400

Figure 40. Micrographs of insulin-immunoreactive cells (arrow) in pancreatic islets of diabetic rats.

A. Diabetic control

B. Diabetic rats treated with *C. colocynthis* (1ml/kg of aqueous extract).

C. Diabetic rats treated with *C. colocynthis* (2ml/kg of aqueous extract).

D. Diabetic rats treated with *C. colocynthis* (4ml/kg of aqueous extract).

Note that there is apparent increase in the number of insulin positive cells with increase in the dosage of the plant extract. Magnification X400

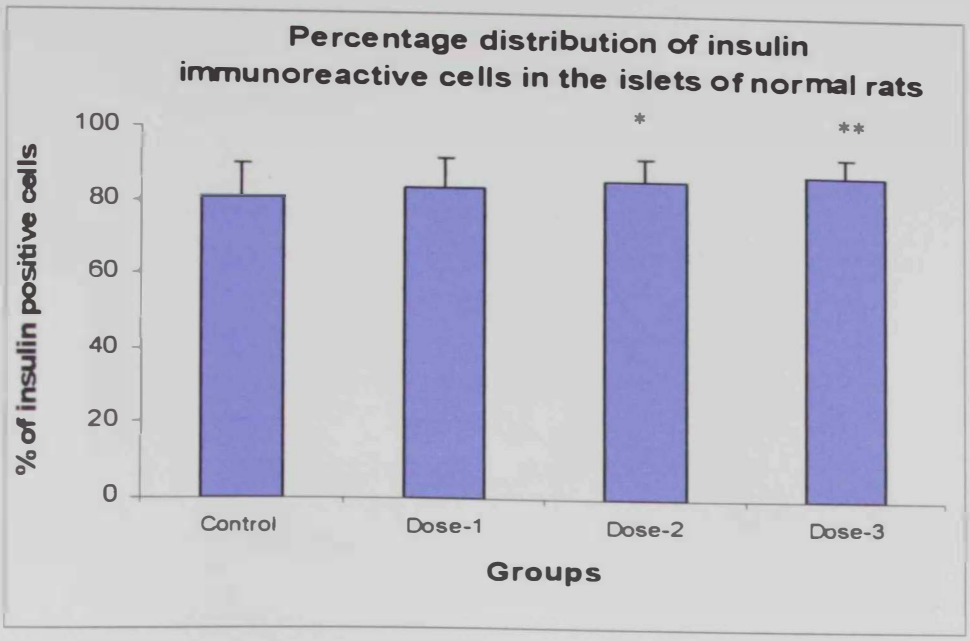


Figure 41: Effect of aqueous extract of *Citrullus colocynthis* on percentage distribution of insulin immunoreactive cells in pancreatic islet cells in fasted normal non-diabetic rats (Data are mean ± SD, n=18-30). (Low dose=0.25ml, moderate dose=0.5ml, high dose=1 ml) 0.5ml, 1ml of the plant extract induced significant (* = p <0.02, ** = p <0.002) increase in the percentage of insulin immunoreactive cells compared to control.

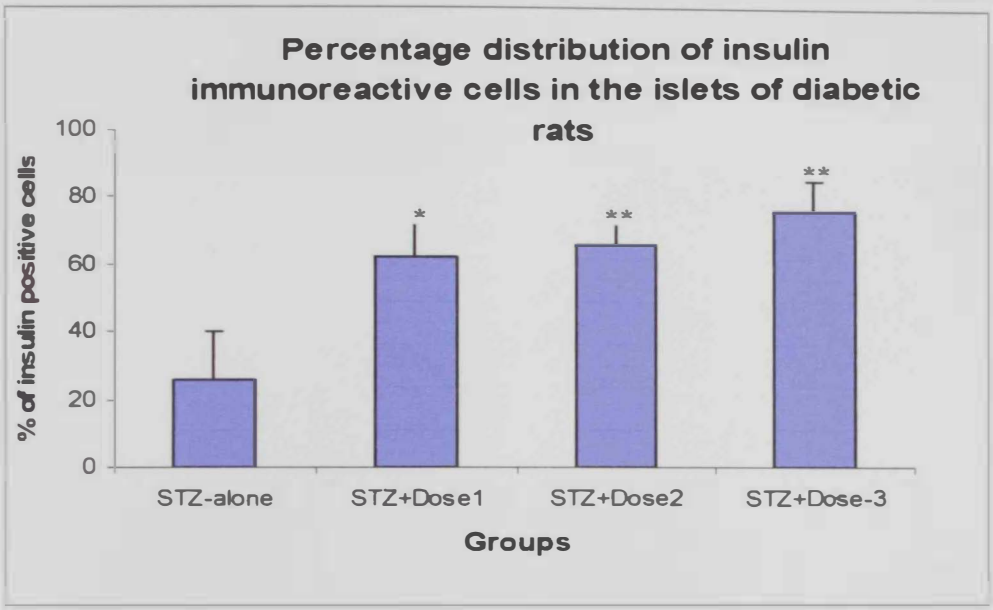


Figure 42: Effect of aqueous *Citrullus colocynthis* extract on percentage of insulin immunoreactive cells of fasted diabetic groups (Data are mean ± SD, n= 12-23). (Low dose=0.25ml, moderate dose=0.5ml, high dose=1 ml) 0.25ml, 0.5ml, 1ml of the plant extract induced significant (* = p <0.00005, ** = p <0.00000) increase in percentage of insulin immunoreactive cells compared to control.

Figure 43. Micrographs of glucagon-immunoreactive cells (arrow) in pancreatic islets of non-diabetic rats.

A. Normal control.

B. Normal rats treated with *C. colocynthis* (1ml/kg of aqueous extract).

C. Normal rats treated with *C. colocynthis* (2ml/kg of aqueous extract).

D. Normal rats treated with *C. colocynthis* (4ml/kg of aqueous extract).

Note that the pattern of distribution of glucagon-positive cells in the treated group is similar to that of normal control. Magnification: X400

Figure 44. Micrographs of glucagon-immunoreactive cells (arrow) in pancreatic islets of diabetic rats.

A. Diabetic control.

B. Diabetic rats treated with *C. colocynthis* (1ml/kg of aqueous extract).

C. Diabetic rats treated with *C. colocynthis* (2ml/kg of aqueous extract).

D. Diabetic rats treated with *C. colocynthis* (4ml/kg of aqueous extract).

The number and pattern of distribution of glucagon-positive cells in the pancreatic islets of rats treated with *C. colocynthis* extract appear to be fewer compared to that of untreated diabetic rats. Magnification: X400.

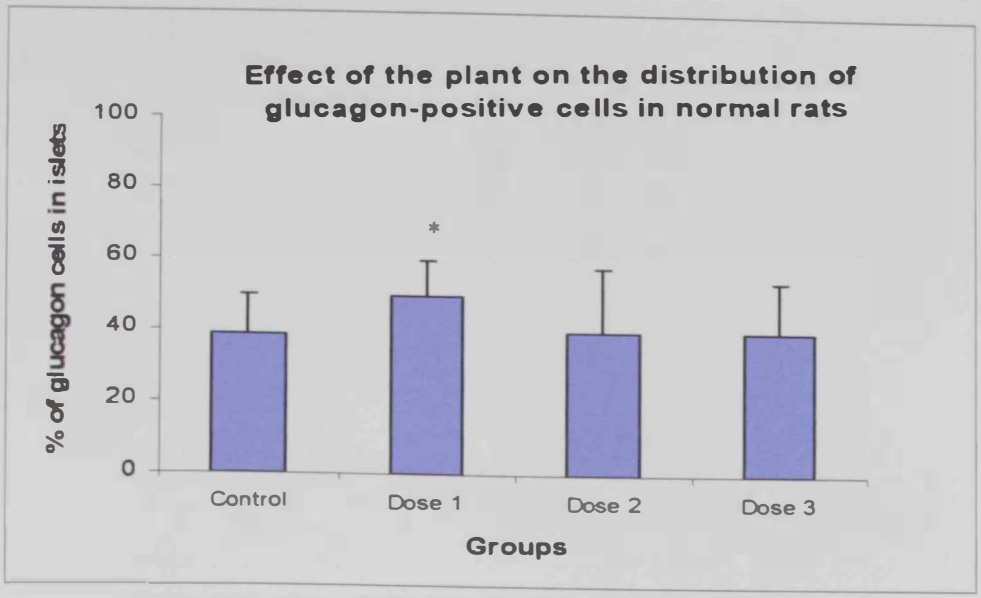


Figure 46: Effect of aqueous extract of *Citrullus colocynthis* on the percentage distribution of glucagons immunoreactive cells of fasted normal non-diabetic groups (Data are mean ± SD, n=18-30). (Low dose=0.25ml, moderate dose=0.5ml, high dose=1 ml). 0.25ml of the plant extract induced significant (* = $p < 0.001$) increase in the percentage of glucagon immunoreactive cells compared to control.

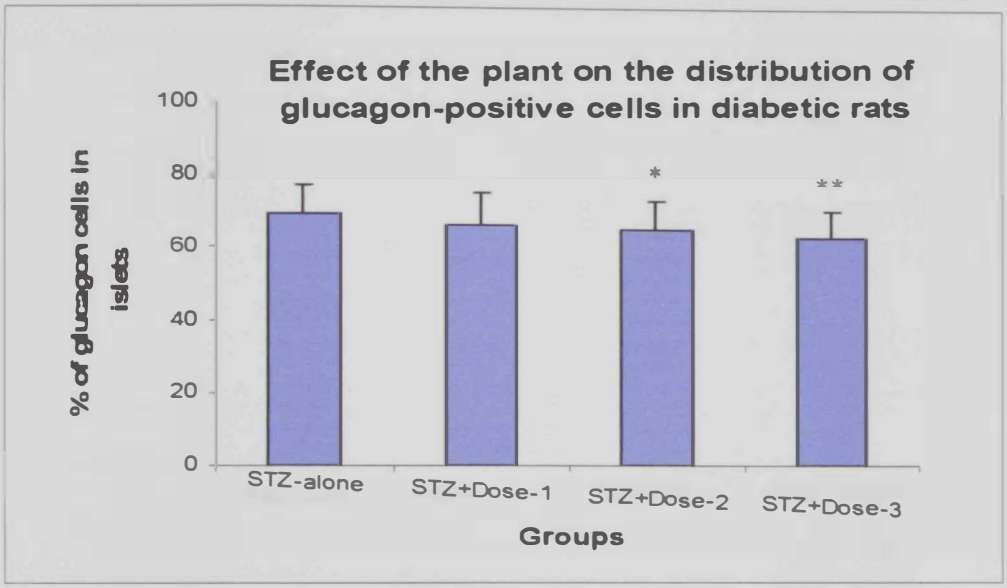


Figure 47: Effect of aqueous extract of *Citrullus colocynthis* on the percentage distribution of glucagon immunoreactive cells of fasted diabetic groups (Data are mean ± SD, n=20-30). (Low dose=0.25ml, moderate dose=0.5ml, high dose=1 ml). 0.5ml, 1ml of the plant extract induced significant (* = $p < 0.03$, ** = $p < 0.002$) increase in the percentage of glucagon immunoreactive cells compared to control.

Diabetes is possibly the world's fastest growing metabolic disease. While the knowledge of the heterogeneity of this disorder increases, also the need for more appropriate therapies increases (Baily and Flatt, 1986). Traditional medicinal plants are used through out the world for a wide range of diabetic presentations. The study of such medicines might offer a natural key to unlock a diabetologist's pharmacy for the future (Abdel-Barry et al, 1997).

I-*In Vitro* Experiment

Many local herbs, including *Zygophyllum simplex, Tephrosia apollinea, Teucrium mascatense, Rhazya stricta, Ziziphus spina-cristi, Moringa peregrina,* which were considered to have anti-hyperglycaemic effect (El-Ghonemy, 1993, Ghazanfar, 1994) failed to stimulate insulin secretion from pancreatic tissue fragments. The reason for the inability of these plant extracts to evoke insulin release from the pancreas of normal rat is unknown. It is possible that the hyperglycaemic effect of these plants may be initiated via a different mechanism such as inhibition of absorption of nutrients, improvement of the accessibility of glucose target cells and insulin sensitivity. However, more studies are needed in this area to elucidate the mechanism by which these plants initiate their beneficial effects in the treatment of diabetes mellitus. Of all the plant extracts screened for their insulinotropic effects, only Citrullus colocynthis had a significant effect on increasing insulin secretion.

The result of this study showed that different doses of the extract of *Citrullus colocynthis* can evoke large and significant increases in insulin release from the pancreas of normal rats. This was observed at higher concentrations of the plant extract. This result corroborates with that of previous studies (Nmila et al, 2000) on the effect of *Citrullus colocynthis* on insulin secretion. Different extracts obtained from *Citrullus colocynthis* seeds were shown to stimulate insulin secretion *in vitro* in isolated perfused rat pancreas, in the presence of a slightly stimulating glucose concentration (Nmila et al, 2000). This effect is through a direct action of the extract on endocrine pancreatic β-cells (Nmila et al, 2000). There are reports indicating that *Citrullus colocynthis* seeds contain one or several insulinotropic substances (Nmila et

al., 2000). Because of the beneficial effects of these insulinotropic substances in the seeds of *Citrullus colocynthis*, it is widely used as a traditional medicine in Morocco and in the Arabia Peninsula for the treatment of diabetes mellitus. In fact, this insulin stimulating effect could improve glucose-induced insulin secretion, thereby counteracting the deficiency of pancreatic cell which occurs in some types of non-insulin dependent diabetic cases (Cerasi and Luft, 1967; Kahn and Porte, 1988). This herb could be a cheaper alternative to expensive drugs used in the treatment of diabetes mellitus.

II-*In Vivo* Experiment

STZ selectively destroys the pancreatic islet cells that secrete insulin, which causes less active pancreatic cells and produces diabetes mellitus (Peungvicha et al, 1998). Oral administration of aqueous extract of *Citrullus colocynthis* seeds for two weeks produced a statistically significant dose-correlate rising activity in plasma insulin level for both non-diabetic and diabetic rats. The phytochemical screening of *Citrullus colocynthis* revealed the presence of tertiary or quaternary alkaloids, glycosides, saponin in addition to other compound such as amino acids, proteins and tribines (Afifi et al, 1973, Darwish et al, 1974). The effect of the extract of *Citrullus colocynthis* seeds in increasing plasma insulin may be due to a mixture of amino acids such as leucine or iso-leucine, which are generally known to stimulate insulin secretion when given either alone or in combination with each other (Fajans et al., 1972; Sener et al, 1981, 1982).

The results of this study showed that, the aqueous extract of *C. colocynthis* has a significant glucose-lowering effect in diabetic as well as in non-diabetic rats. This effect could, possibly, be due to increased peripheral glucose utilization. Inhibition of the proximal tubular reabsorption mechanism for glucose in the kidney, if any, can also contribute towards blood lowering effect (Sharma et al, 1983). Also, the oral hypoglycaemic effect of the aqueous extract of the *C. colocynthis* could be due to the presence of saponin components, which possess a strong hypoglycaemic effect (Abdel-Hassan et al, 2000). In addition, the effect of *C. colocynthis* may also be exerted indirectly through insulin.

At the end of this experiment the body weight of normal and diabetic rats did not improve significantly after oral administration of the aqueous extract of *C. colocynthis.*. There were no literature data on the effect of *C. colocynthis* on the weight of diabetes rats. However, studies performed on diabetic rabbits showed that *C. colocynthis* decreased the weight of diabetic rats (Abdel-Hassan et al., 2000).

II-1. Biochemical analysis

The effect of aqueous *C. colocynthis* extract on potassium in fasted non-diabetic groups was not significant. On other hand, in diabetic groups the potassium concentration was increased after treatment. It is well known that diabetes mellitus causes an influx of potassium from cells into the extracellular space. In view of this, serum potassium is usually normal to slightly elevated in diabetes mellitus (Greenspan and Strewler, 1997). The oral administration of the aqueous extracts of the *C. colocynthis* failed to cause significant changes in the plasma level of potassium compared to control. This may be due to the short duration of the experiment or the fact that biochemical and physiological parameters of diabetes mellitus were not completely normalized. In a similar trend, the plasma level of sodium of normal and diabetic rats did not change significantly after the oral administration of the aqueous extract of *C. colocynthis*.

Liver enzymes used as markers for hepatotoxicity include the following ALT, AST, GGT, LDH and ALP. Serum ALT is thought to be a more specific indicator for liver damage. The plasma level of ALT increased significantly after the onset of experimental diabetes. In contrast, the plasma level of ALT decreased significantly after the administration of the plant extract. All of these results raised and confirm a number of interesting issues. Firstly, that STZ has a hepatotoxic effect by increasing the plasma level of ALT and secondly, the aqueous extract of the *C. colocynthis* can ameliorate the toxic effect of STZ in the liver. Moreover, this study suggests that *C. colocynthis* is not toxic at least when given in the higher doses in this study.

GGT is an enzyme believed to be involved in the transport of amino acids and peptides into cells as well as glutathione. Its elevation may be found in liver disease or drug abuse. In diabetic rats there was a significant increase in plasma GGT level. This elevation is an indicator of hepatotoxicity caused by STZ. There were no significant changes in the level of GGT even after treating diabetic rats with low and moderate doses of the aqueous extract of the plant.

Increase in creatinine level was variable used not only to indicate impairment of kidney function (Braunlich et al, 1997; Hwang et al, 1997), but it helps to detect treatment related toxic effects of compounds on the kidney in rats (Travols et al, 1996).

There was no significant change in the concentrations of many other biochemical parameters (creatinine, calcium, sodium, phosphorus) investigated. It is not known why there were no changes in these biochemical parameters after treatment with the plant extract. It is possible that the concentration of the extract may be too weak to have a significant effect or the tissue or organ damage may be too severe to show any detectable improvement.

II-2. Morphological investigations

There were changes in the pattern of distribution of insulin and glucagon positive cells in pancreas of non-diabetic and diabetic treated animals compared to that of normal and diabetic control. The pattern of distribution of endocrine cells in normal pancreatic islets followed a specific pattern in which insulin-producing cells were located in the central and peripheral parts of the islets. Glucagon-positive cells on the other hand were observed exclusively in the peripheral region of the islet of Langerhans. Our observation on the pattern of distribution of these hormone-secreting cells is similar to that reported previously (Adeghate and Donáth, 1991)

After the onset of diabetes, there was a gross derangement in the pattern of distribution of these pancreatic peptides. The density of insulin positive cells became more sparse, leaving pancreatic islets with only a few surviving beta cells. This observation shows that STZ does not destroy all of the beta cells in pancreatic islet in

it totally. This result corroborates those reported by Adeghate (1999b). A total destruction of the beta cells in pancreatic islet will make life completely unviable and survival difficult for the animals. Glucagon-containing cells occupied areas in both the central and peripheral portions of the islet of Langerhans, a feature not seen in normal pancreas. Moreover, morphometric investigation suggests that the number of glucagon-positive cells increased significantly when compared to normal, non-diabetic rats. The increase in the number of glucagon-secreting cells may contribute to the hyperglucagonaemia observed in diabetes. Hyperglucagonaemia is a constant feature of diabetes and has been implicated in the pathogenesis of complications associated with diabetes mellitus. This observation is in agreement with previous reports (Adeghate, 1999b).

Oral administration of the aqueous extract of *C. colocynthis* appeared to have induced a significant increase in the number of insulin-immunoreactive cells in the islet of Langerhans of diabetic rats. The mechanism by which the aqueous extracts of *C. colocynthis* causes increase in the number of insulin-positive cells in unknown. It is possible that the plant extract may help in the regeneration of pancreatic beta cells damaged by STZ. It may also be involved in the production of new beta cells from progenitor cells such as those in pancreatic duct. Pancreatic beta cells have been to be present in the ducts of pancreas (Adeghate and Donáth, 1990). In addition, *C. colocynthis* may also contain anti-oxidants that may neutralize the destructive and tissue-damaging effects of cytokines released by STZ during the induction of experimental diabetes. It is known that the administration of STZ will induce a cascade of events during which many cytokines will be liberated to destroy pancreatic beta cells in the islets (Adeghate and Parvez, 2000). All of these indicate that that *C. colocynthis* has a regenerative effect on pancreatic beta cells. The ability of a plant extract to increase the number of insulin-positive cells has been reported for *Momordica charantia*, (Ahmed et al., 1998) a plant in the same family as *C. colocynthis*.

The results of this study suggest that the aqueous extracts of *C. colocynthis* reduced the number of glucagon-positive cells significantly when compared to control. The mechanism by which aqueous extracts of *C. colocynthis* reduces the number of glucagon-positive cells is unknown, but this could be an indirect effect of

the relative increase in the number of insulin-positive cells. The plant extract can thus reduce the severe hyperglucagonaemia by reducing the number of glucagon-immunoreactive cells in pancreatic islets of diabetic rats.

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ARABIC SUMMARY

الملخص العـــربي

يعتبر مرض السكري من أكثر الأمراض الهرمونية شيوعا في العالم، فهو يصيب 6 % من سكان العالم. و يشمل علاج هذا المرض ثلاث مراحل أساسية، أولها ممارسة الرياضة التي يصاحبها نظام غذائي متوازن. ثانيا العلاج بالأنسولين وثالثا العلاج باستخدام العقاقير الخافضة للسكر. و الكثير من هذه العقاقير ليست فعالة في خفض مستوى الجلوكوز في هؤلاء المرضى، كما انها باهضة الثمن بالأضافة الى الأعراض الجانبية التي تسببها هذه العقاقير. و نتيجة لذلك مازالت الأبحاث في هذا المجال مستمرة للبحث عن عقاقير لعلاج السكري. و قبل اكتشاف الأنسولين سنة 1922، كان الاعتماد كبيرا على الطب الشعبي لعلاج هذا المرض. و الكثير من النباتات الطبية الشعبية شائعة الاستخدام، و لكن القليل منها التي خضعت للأبحاث العلمية أو الطبية. والكثير من الأبحاث توصلت الى تأثير هذه النباتات الخافض للسكر.

و علاج السكري باستخدام النباتات الطبية منتشرة بشكل كبير في منطقة الشرق الأوسط. و في دولة الامارات الكثير من النباتات الطبية تستخدم في علاج السكر مثل الحرمل، الظفرة، الهرم، الشوع، السدر، الجعدة و الحنظل. و بالرغم من الانتشار الواسع لاستخدام النباتات الطبية في علاج السكري، الا أنها لاتوجد فحوصات مخبرية تؤكد فعالية هذه النباتات في علاج السكر.

و الهدف من هذا البحث هو دراسة فعالية هذه النباتات في علاج السكر. و قد تم تحضين المستخلص المائي لأجزاء من هذه النباتات مع قطع من البنكرياس لايجاد مدى فعالية هذه النباتات في تحفيز افراز الانسولين. و من جميع هذه النباتات المستخدمة يعتبر المستخلص المائي لبذور الحنظل هو الوحيد الذي أعطى تأثير معنوي في افراز الانسولين. و تم اختبار المستخلص المائي لبذور الحنظل في فئران طبيعية و فئران مصابة بمرض السكري عن طريق تناولها بالفم.

و قد توصل هذا البحث الى أن تناول المستخلص المائي لبذور الحنظل عن طريق الفم كان له تأثير معنوي في خفض معدل السكر في الفئران الطبيعية و المصابة بالسكر، في شكل معتمد على الجرعات. بالاضافة لذلك فان المستخلص المائي عمل على زيادة مستوى الانسولين في الدم، وزيادة عدد الخلايا المفرزة للانسولين، و خفض النسبة المئوية لتوزيع الخلايا المفرزة لهرمون الجلوكاجون. بالرغم من ذلك الا أن المستخلص لم يعد هذه المقاييس الى قيمتها الطبيعية كما هي في المجموعة الضابطة.

جامعة الإمارات العربية المتحدة
عمادة الدراسات العليا

تأثير بعض النباتات الطبية النامية بدولة الامارات العربية المتحدة على أيض الجلوكوز في الجرذان

رسالة مقدمة من الطالبة
فاطمة خادم سعيد محمد الغيثي
بكالوريوس علوم الحياة - كلية العلوم - جامعة الإمارات العربية المتحدة (١٩٩٧)

استكمالاً لمتطلبات الحصول على درجة الماجستير في العلوم
﴿علوم البيئة﴾

٢٠٠٢-٢٠٠٣