Effect of cinnamon on plasma glucose concentration and the regulation of 6-phosphofructo-1-kinase activity from the liver and small intestine of streptozotocin induced diabetic rats

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A thesis submitted in partial fulfillment of the requirements for the degree of master of science in biochemistry

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(B.Sc)

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This research attempts to elucidate the effect of cinnamon, a kind of spices commonly used in Eastern and Middle-eastern countries in their food, on plasma glucose concentration and the regulation of 6- phosphofructo-1-kinase in streptozotocin induced-diabetic rats.

The animals were 50 male Wistar rats (180-200 g) divided into five groups: normal controls, untreated diabetics (rats were made diabetic by single I.P. injection of streptozotocin), diabetics rats treated with 0.5g and 1.0g of cinnamon, and the last group of diabetic rats were treated with insulin.

This study is concerned with the regulation glucose metabolism in streptozotocin-induced diabetic rats by an estimate of enzyme activity of liver and small intestine. Also, to demonstrate the effect of cinnamon on plasma glucose, cholesterol, triacylglycerol and insulin concentrations. In addition, the present study compares the regulation of PFK-1 of the diabetic rats with another group of diabetic rats treated with cinnamon.

The results show a significant decrease (p < 0.0001) in glucose, cholesterol and triacylglycerol concentrations were a significant increase (p < 0.0001) in insulin concentration and the enzyme activity in cinnamon treated groups. In conclusion, cinnamon have a scientific evidence to improve diabetes safely.
المستخلص

يهدف هذا البحث إلى دراسة إمكانية حدوث تأثير للقرفة والتي تعد من أحد أنواع الباهرات الشائعة الاستخدام في البلاد الشرقية والشرق ووسطية في غذائهم على تركيز الجلوكوز في البلازما ونشاطية إنزيم الفوسفوركريبتيز في الجرذان المصاب بالسكري المستحسن بالاستريزوروتين.

كانت الدراسة على 50 جرذًا من نوع ويسستر (200-200 غرام) قسمت إلى خمس مجموعات كل مجموعة مكونة من 10 حيوانات: طبيعي بالسكري غير مصابين، جرذان مصاب بالسكري عزلت بـ 0.5 غرام و 1.0 غرام من القرفة وجرذان مصاب بالسكري عزلت بال الإنسولين.

هذة الدراسة تسلط الضوء على تنظيم إنزيم الفوسفوركريبتيز في كبد وآمال الجرذان المصاب بالسكري المستحسن بالاستريزوروتين ومشاهدة تأثير القرفة على مستويات الجلوكوز، الكولسترول، الجليسييريدات الثلاثية وال الإنسولين في البلازما. إضافة إلى ذلك، الدراسة الحالية تقارن تنظيم إنزيم الفوسفوركريبتيز في الجرذان المصاب بالسكري مع المجموعة معالجة بالقرفة.

أوضحت النتائج انخفاضًا معنويًا (p < 0.0001) في تركيز الجلوكوز، الكولسترول والدهون الثلاثية بينما ارتفع معنويًا (p < 0.001) تركيز الإنسولين ونشاطية الإنزيم. ونستنتج أن القرفة لديها دليل علمي على تحسين مرض السكري بأمان.
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In the name of Allah, the most Gracious, the most merciful.
I praise and thank Allah, the Cherisher and sustainer of the worlds; the master of the judgment day.

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DEDICATION

To my father...
To my mother...
To my family...
To my recommended supervisor...
To every one who help me...
To every one who gave me a good support...
I hope that my research will satisfy them..
With all my love...

To my support...
To my recommended supervisor...
To the person who push me forward...
To the one who put me in the right way...
To prof. Samir Khoja...
With all my respect...

To all diabetic patients and their families..
To any one who wants to know about diabetes..
I give this humble work to them..
And I hope that it will be beneficial to them..
With all my hope of relief..
ABBREVIATIONS

aa  Amino acid.

α –GDPH  α - Glycerophosphate dehydrogenase.

ADP  Adenosine-5’ – diphosphate.

AMP  Adenosine-5’ – monophosphate.

ANOVA  one- way analysis of variance.

ATP  Adenosine-5’ – triphosphate.

BSA  Bovine serum albumin.

CoA  Coenzyme A.

C-AMP  Cyclic AMP (3’ 5’ –cyclic adenosine monophosphate).

DM  Diabetes mellitus.

DTT  Dithiothreitol.

EDTA  Ethylenediaminetetraacetic acid.
FFA                      Free Fatty Acid.

Fru-1,6-P$_2$          Fructose- 1,6 – bisphosphate.

Fru-2,6-P$_2$          Fructose- 2,6 – bisphosphate.

IDDM                    Insulin Dependent Diabetes Mellitus.

I.P.                        Intra peritoneal

MHCP                  methylhydroxycolan polymer

NIDDM                 Non Insulin Dependent Diabetes Mellitus.

NAD$^+$          Nicotinamide adenine diphosphate ( oxidized ).

NADH          Nicotinamide adenine diphosphate ( reduced ).

NADPH          Reduced nicotinamide adenine dinucleotide diphosphate.

O.D.                        Optical density

PFK-1   6 - phosphofructo - 1- kinase.
PFK-2 6-phosphofructo-2-kinase.

PK Pyruvate kinase.

PKA Protein kinase A

PEP Phosphoenol pyruvate.

$P_i$ Inorganic phosphate.

TPI Triose phosphate isomerase.

Tris Tris (hydroxymethyl) aminomethane.
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CHAPTER 1

INTRODUCTION
1.1 Diabetes mellitus.

Diabetes mellitus is a metabolic disorder characterized by chronic elevation of blood glucose level. Diabetes mellitus is usually accompanied with disturbances in carbohydrate, fat and protein metabolism. These disturbances are arising either from a defect in insulin secretion or impairment from the pancreases.

Also it is a commonest endocrine disorder encountered in clinical practice. It may be defined as a syndrome characterized by hyperglycemia due to an insulin resistance and an absolute or relative lack of insulin (Gaw et al, 2004).

Diabetes mellitus is the most common metabolic disease worldwide, with an estimated 1700 new cases diagnosed daily. Of these, 85% - 90% of the patients have type 2 diabetes mellitus (type 2DM), with insulin resistance playing a key role in the development of the disease. Symptoms of insulin resistance include a decreased stimulation of muscle glycogen synthesis, defects in glycogen synthesis activity, hexokinase activity and glucose uptake. Insulin resistance in a subset of the patients with type 2DM is due to clear defects in their insulin signaling (Taylor et al, 2001).

The disease results when glucose transport channels on cell membranes are insensitive to the effects of insulin and/or when there are too few channels or when the quantity of insulin produced by the pancreas is inadequate to activate the number of glucose channels needed to maintain normal cellular metabolism (Alberti and Krall, 1991). In other words, insulin is the "key" that allows special "gates" for sugar transport across cell membranes to be opened (Schluter et al, 1982).
A diabetic, therefore, has too much glucose in the bloodstream where most of it cannot be utilized and not enough glucose within the cells themselves, where it is most needed for energy. As a result, cells attempt to derive energy from alternate metabolic pathways, such as fat breakdown. Excessive use of these alternate energy pathways accumulates in production of harmful by-product called ketones (Alberti and Krall, 1991).

The accumulation of ketones (acetoacetate, 3-hydroxybutarate and acetone) causes the body’s pH to become acidic (ketoacidosis) which makes the cellular environment inhospitable for normal metabolic functions. This condition can ultimately become life-threatening and requires aggressive medical therapy (Schluter et al., 1982).

Fortunately, most diabetics give some indication of their underlying condition, such as drinking and urinating excessively, before they develop ketoacidosis. Treating diabetics before they become ketotic is considerably more straightforward, safer, and of course less expensive than taking a “wait-and-see” approach to changes in drinking behavior. Equally important, diagnostic testing may reveal other serious conditions which can cause excessive urination and drinking such as kidney or liver disease, adrenal hormone or electrolyte imbalances and uterus infections (Schluter et al., 1982).

1.1.1 Types of diabetes mellitus.

Primary diabetes mellitus is generally subclassified into insulin dependent diabetes mellitus (IDDM) or non-insulin dependent diabetes mellitus (NIDDM) where as secondary diabetes mellitus may result from pancreatic diseases, endocrine diseases
such as Cushing’s syndrome, drug therapy, and rarely, insulin receptor abnormalities (Gaw et al., 2004).

**1.1.1 Type 1 diabetes** is called insulin-dependent diabetes mellitus (IDDM) or juvenile-onset diabetes. Type 1 diabetes develops when the body’s immune system destroys pancreatic β - cells, the only cells in the body that make the hormone insulin that regulates blood glucose. This form of diabetes usually strikes children and young adults, who need several insulin injections a day or an insulin pump to survive. Type 1 diabetes may account for 5 - 10% (figure 1.1) of all diagnosed cases of diabetes. Risk factors for type 1 diabetes include autoimmune, genetic, and environmental factors (Charce and Frank, 1993).

**1.1.2 Type 2 diabetes** is called non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes. It may account for about 90 - 95% (figure 1.1) of all diagnosed cases of diabetes. It usually begins as insulin resistance, a disorder in which the cells do not use insulin properly. As the need for insulin rises, the pancreas gradually loses its ability to produce insulin. Type 2 diabetes is associated with older age, obesity, family history of diabetes, prior history of gestational diabetes, impaired glucose tolerance, physical activity, and race/ethnicity (Charce and Frank, 1993).
Figure 1.1 Types of Diabetes.
Gestational diabetes is a form of glucose intolerance that is diagnosed in some women during pregnancy. Gestational diabetes occurs more frequently among obese women and women with a family history of diabetes. During pregnancy, gestational diabetes requires treatment to normalize maternal blood glucose levels to avoid complications in the infant. After pregnancy, 5 - 10% of women with gestational diabetes are found to have type 2 diabetes. Women who have had gestational diabetes have a 20 - 50% chance of developing diabetes in the next 5-10 years (Charce and Frank, 1993).

Other specific types of diabetes may result from specific genetic conditions (such as maturity-onset diabetes of youth), surgery, drugs, malnutrition, infection, and other illnesses. Such types of diabetes may account for 1 - 5% of all diagnosed cases of diabetes (Charce and Frank, 1993).

1.1.2 Late complication of diabetes mellitus.

Diabetes mellitus is not only characterized by the presence of hyperglycemia but also by the occurrence of late complications:

* Microangiophathy.

* Retinopathy.

* Nephropathy.

* Neuropathy.

* Macroangiophathy (Gaw et al., 2004).
1.1.3 Factors that can affect diabetes.

There are some factors which affect diabetes by several ways. These factors are:

1.1.3.1 Diet.

The relationship between eating carbohydrates and type 2 diabetes is a complex issue. While eating carbohydrates increases the need for insulin to keep blood sugar normal, diets high in total carbohydrates do not necessarily increase the risk of type 2 diabetes. (Healthnotes, Inc, 2004).

Eating carbohydrate-containing foods, whether high in sugar or high in starch (such as bread, potatoes, processed breakfast cereals, and rice), temporarily raises blood sugar and insulin levels. The blood sugar-raising effect of a food, called “glycemic index,” depends on how rapidly its carbohydrate is absorbed. Many starchy foods have a glycemic index similar to sucrose (table sugar). People eating large amounts of foods with high glycemic indices have been reported to be at increased risk of type 2 diabetes. (Healthnotes, Inc, 2004).

Beans, peas, fruit, and oats have low glycemic indices, despite their high carbohydrate content, due mostly to the health-promoting effects of soluble fiber. Nonetheless, most doctors advise people with diabetes to eat a diet high in fiber. Focus should be placed on fruits, vegetables, seeds, oats, and whole-grain products. (Healthnotes, Inc, 2004).
1.1.3.2 Exercise.

Because most people with type 2 diabetes are overweight, excess abdominal weight does not stop insulin formation, but it does make the body less sensitive to insulin. Excess weight can even make healthy people pre-diabetic. Weight loss reverses this problem. In most studies, type 2 diabetes has improved with weight loss.

Exercise helps decrease body fat and improve insulin sensitivity. People who exercise are less likely to develop type 2 diabetes than those who do not. People with type 1 diabetes who exercise require less insulin. However, exercise can induce low blood sugar or even occasionally increased blood sugar. (Healthnotes, Inc, 2004).

1.1.3.3 Supplements.

A variety of vitamins, minerals, amino acids, and other supplements may help with symptoms and deficiencies associated with diabetes.

1.1.3.3.1 Vitamins.

Supplementation with a multiple vitamin and mineral preparation for one year of middle-aged and elderly diabetics reduced the risk of infection by more than 80%, compared with a placebo (Healthnotes, Inc, 2004).

Vitamins and minerals include:

Chromium, Magnesium, Alpha lipoic acid, Evening primrose oil, Glucomannan, Vitamin E, Vitamin C, B Vitamins, Zinc, Vitamin D, Inositol, Taurine, Fish oil,

1.3.2 Herbs and spices.

Several herbs may help in managing symptoms associated with diabetes, including the control of blood glucose levels. These herbs are: Cayenne, Psyllium, Asian ginseng, American ginseng, Basil, Gymnema, Bitter melon, Cinnamon, (figure 1.2) Crepe myrtle, Onion, Bilberry, Ginkgo biloba, Mistletoe, Olive leaf and Reishi (figure 1.3) (Healthnotes, Inc, 2004).
Figure 1.2 Cinnamon barks.

Figure 1.3 Different kinds of herbs can be used by diabetics.
1.2 Regulation of glucose metabolism.

1.2.1 Hormone regulation.

Islets of Langerhans of pancreas secrete 2 major hormones: insulin and glucagon which regulate metabolism of glucose, free fatty acid (FFA) and amino acids through a range of activity levels (Fraser, 2004).

Insulin and Glucagon are released in response to nutrient inflow. Insulin synthesis is stimulated by glucose in blood or feeding and decreased by fasting.

1.2.1.1 Insulin.

Amino acids may be cleaved out of primary structure, the biologically active insulin is less than half the primary sequence.

Preproinsulin → proinsulin → insulin
(110 aa’s) → (86 aa’s) → (51 aa’s)

Signal peptide (24 aa’s) clipped from amino terminus.
C peptide (31 aa’s) excised from center (Carr, 2005).
Tertiary (active) structure of insulin is A chain (21 aa’s) & B chain (30 aa’s) held together by 3 disulfide bridges (figure 1.4) and (figure 1.5).
Figure 1.4 Biosynthesis of Insulin (adapted from Carr 2005).
1.2.1.1 Insulin function.

Insulin stimulates the formation of glycogen in the muscles and in the liver, while suppressing gluconeogenesis by the liver. The increase of glycolysis in the liver helps to increase the synthesis of fatty acids (Stryer, 1995).

Insulin induces its effects by binding to specific tyrosine kinase receptors in the plasma membrane of its target cells. This in turn causes the glucose transporters in the membranes of the cells to fuse with the plasma membrane. This causes the concentration of glucose to increase inside the cells. An increase in the plasma glucose concentration causes the release of insulin into the bloodstream, while a decrease causes the suppression of the release of glucose. The uptake of glucose by the cells causes the concentration of glucose in the bloodstream to decrease, which ultimately leads to the suppression of insulin release (Vander et al, 1998).

Insulin stimulates the synthesis of glycogen by triggering a pathway that dephosphorylates glycogen synthase. The dephosphorylation activates the synthase. This also leads to the dephosphorylation of phosphorylase kinase, an enzyme needed in the breakdown of glycogen (figure 1.6). When insulin binds to its receptors, a cascade effect occurs that leads to the phosphorylation of protein phosphatase 1, which is the enzyme that dephosphorylates both glycogen synthase and phosphorylase A (Stryer, 1995).

Phosphorylase A is the receptor for the measurement of the glucose. When an influx of glucose occurs, glucose binds to the phosphorylase A receptor, which alters
Figure 1.6 Receptor-mediated responses on phosphorylase.

(adapted from Scott, 1985).
the shape so that it can be dephosphorylated. This causes the release of insulin while stimulating the formation of glycogen in the liver (Stryer, 1995).

Insulin does not enter the cell. Instead, the binding of insulin to the receptor sends a signal into the cell relaying information on the concentration of insulin in the bloodstream. When insulin binds to the receptor, the beta unit is phosphorylated on the tyrosine residue. The kinase activity of the insulin receptor may stimulate a cascade effect, that releases enzymes which ultimately regulate the phosphorylation of the targets of insulin (Scott, 1985).

This insufficiency of insulin is caused either by the ineffectiveness of the insulin in the body or by the autoimmune response that causes the destruction of the beta cells of the pancreas by the body’s own white blood cells (Hopkins, and Williams, 1997).

Before insulin was synthesized, bovine and porcine insulin was used. Since the binding receptors for insulin bind bovine and porcine insulin with the same affinity it has for human insulin, the addition of these types of insulin is successful. This is because there are only three amino acid differences between the three types of insulin (Hopkins, and Williams, 1997)

1.2.1.1.2 Insulin resistance.

Insulin resistance describes the condition in which the pancreas is capable of producing insulin, but the cells are insensitive to it. If insulin is the "key" which unlocks the "gate" through which glucose must pass to enter cells, then insulin
resistance results when there are too few “gates” or the “locks” on the gates are “rusted shut” and are difficult to open despite the presence of insulin (Alberti and Krall, 1991).

There are many conditions which may predispose to the development of insulin-resistant diabetes or which may unmask a mild, sub-clinical, or transient diabetes that already exists. These include pregnancy, overproduction or over-administration of steroids like cortisone or prednisone, overproduction of growth hormone (acromegaly), infections, prolonged or severe stress, and many others (Alberti and Krall, 1991).

Alternatively, these conditions may increase the dose of insulin required to properly regulate a diabetic already undergoing therapy. A diabetic patient who requires an insulin dose higher than 1-2 units per pound of body weight per injection to maintain normal blood sugar levels is considered to be “insulin-resistant”; Every effort should be made to identify possible conditions which may increase the insulin requirement. Sometimes, just a prolonged untreated bladder infection may be enough to deregulate an otherwise well controlled diabetic. Although there are many reasons why a diabetic may be difficult to regulate, the possibility of insulin resistance must be considered of the potential causes have been investigated and ruled out (Alberti and Krall, 1991).

1.2.2 Enzyme regulation.

In skeletal muscles and adipose tissue insulin increases the number of plasma membrane glucose transporters, but in liver glucose uptake is dramatically increased because of increased activity of the enzymes glucokinase, 6-phosphofructo-1-kinase
(PFK-1), and pyruvate kinase (PK), the key regulatory enzymes of glycolysis (figure 1.7). The effects are induced by insulin-dependent activation of phosphodiesterase, with decreased protein kinase A (PKA) activity and diminished phosphorylation of pyruvate kinase and phosphofructokinase-2 (PFK-2) (Champe et al., 2005).

Dephosphorylation of pyruvate kinase increases its activity while dephosphorylation of PFK-2 renders it active as a kinase. The kinase activity of PFK-2 converts fructose-6-phosphate into fructose-2,6-bisphosphate (Fru-2,6-P$_2$). Fru-2,6-P$_2$ is a potent allosteric activator of the rate limiting enzyme of glycolysis, PFK-1, and an inhibitor of the gluconeogenic enzyme, fructose-1,6-bisphosphatase (Champe et al., 2005).
Figure 1.7 Glycolysis pathway (adapted from Champe et al, 2005)
1.2.2.1 6-Phosphofructo-1-kinase (PFK-1) as a regulatory enzyme.

6 - Phosphofructo - 1 - kinase (PFK-1; ATP: D - fructose -6- phosphate -1- phosphotransferase, EC 2.7.1.11) catalyzes the phosphorylation of fructose -6- phosphate to form fructose -1, 6-bisphosphate.

PFK-1 is an allosteric enzyme and, as such, it is critical for the metabolic regulation of the glycolytic pathway. Although phosphorylase responds to an extra-cellular stimulus (epinephrine), PFK-1 is sensitive to the intracellular level of several allosteric effectors. Pi, ADP, AMP and fructose 2,6- bisphosphate as positive effectors, stimulate PFK activity, whereas ATP, citric acid, and long chain fatty acids, as negative inhibitors (Passonneau and Lowry, 1964; Mansour, 1963; Kemp, 1971; Tsai and Kemp, 1974; Khoja, 1986).

This mode of allosteric regulation is characteristic of the molecular mechanisms used to regulate intracellular synthesis of ATP. When supplies of ATP or of aerobically oxidizable fuels (citric acid and long chain fatty acids) are plentiful, the high concentrations of these biomolecules serve as a cellular signal to decrease the rate of glycolysis by inhibiting PFK-1. Conversely, when ATP is being rapidly utilized by cells, the increase in ADP and AMP concentrations trigger an increased synthesis of ATP by stimulating PFK-1 and other respiratory enzymes. The ratio of [ATP] to [ADP] and [AMP] is, therefore, an important monitoring system needed by cells to control the levels of readily available chemical energy (ATP) (Khoja, 1986).
The formation of D-fructose-1, 6-bisphosphate requires the expenditure of the energy potential of an anhydride bond of ATP. Hence, the initial reactions of glycolysis converting one molecule of D-glucose into D-fructose-1, 6-bisphosphate, require an input of the chemical energy of two ATP molecules. The synthesis of D-fructose-1, 6-bisphosphate from glycogen, however, requires the hydrolysis of only one ATP.

The reaction catalyzed by PFK-1 (figure 1.8) is regarded as the first unique step of the glycolytic pathway and therefore it is not surprising that the enzyme is subject to extensive metabolic regulation (Khoja, 1986).

1.2.2.1.2 PFK-1 Isoenzymes.

Phosphofructokinase has been shown to exist in different isoenzymic forms in the tissues of rat. Following the nomenclature of Tsai and Kemp (1974) who reported the presences of three isoenzymes of phosphofructokinase in animal tissues those of skeletal muscle (A), liver (B) and brain (C); Jamal and Kellett (1983) have identified a fourth type of isoenzyme (phosphofructokinase D) present in rat intestinal mucosa, shown to be distinct and different from the other types. The work of Joe and Kemp (1984) was supported and confirmed by Dunaway and Ksten (1985) who studied the nature of rat brain phosphofructokinase isoenzymes. They suggested that the activity of rat brain PFK-1 is a complex mixture of homotetramers and hybrids which are composed of varying amounts of L-type, M-type and C-type subunits.
Figure 1.8 Reaction of PFK-1.
Later, Khoja (1986) compared the regulatory properties of PK-1 from the mucosa of rat small intestine with those of skeletal muscle, liver and brain isoenzymes and showed that the mucosal isoenzyme is different and has been found to be more active than the other isoenzymes, accounting for the higher rates of glycolysis observed in mucosa (Khoja 1988).

1.3 Cinnamons vs. diabetics.

The dietary components beneficial in the prevention and treatment of type 2 diabetes and cardiovascular diseases have not been clearly defined, but it is postulated that spices such as cinnamon (Cinnamomum zeylanicum), cloves, bay leaves, and turmeric may play a role by display insulin-enhancing activity in vitro (Khan et al., 2003).

Botanical products can improve glucose metabolism and the overall condition of individuals with diabetes not only by hypoglycemic effects but also by improving lipid metabolism, antioxidant status, and capillary function (Khan et al, 2003).

A number of medicinal/culinary herbs has been reported to yield hypoglycemic effects in patients with diabetes. Examples of these include bitter melon, Gymnema, Korean ginseng, onions, garlic, flaxseed meal, and specific nutrients including α-lipoic acid, biotin, carnitine, vanadium, chromium, magnesium, zinc, and vitamins B₃, E, and K. Aqueous extracts from cinnamon have been shown to increase in vitro glucose uptake and glycogen synthesis and to increase phosphorylation of the insulin
receptor; in addition, these cinnamon extracts are likely to aid in triggering the insulin cascade system (Khan et al., 2003).

"As this assay contained only the cinnamon extract, the purified truncated kinase domain and radiolabelled ATP, it can be assumed that there was direct interaction between a component in the cinnamon extract and the kinase domain" researchers said. (Taylor et al., 2001).

It is hypothesized that the cinnamon compound enters the cells, interacts with the intracellular kinase domain and triggers an insulin-like response. Many cellular responses to insulin, with numerous enzymes and regulatory proteins, have been identified over the years. The initial critical step is insulin binding to its receptor and the subsequent transphosphorylation of the kinase domains. Phosphorylation continues on toward the insulin receptor substrates (Taylor et al., 2001).

This project is concerned with the regulation of 6-phosphofructo-1-kinase in the liver and small intestine of streptozotocin-induced diabetic rats. In addition, the present study compares the regulation of PFK-1 of the diabetic rats with another group of diabetic rats treated with cinnamon. The goal of this study is to investigate the ability of cinnamon to stimulate insulin and evaluate the activity of 6-phosphofructo-1-kinase in liver and intestinal mucosa of streptozotocin induced-diabetic rats.
CHAPTER 2
MATERIALS & METHODES
2.1 Materials.

2.1.1 Biochemicals and chemicals.

All chemicals were of the analytical reagent grade obtained from BDH chemicals, Poole, Dorset, U.K. Streptozotocin (N-[methyl-nitrosocarbamoyl]-D-glucosamine), ATP, NADPH, F6P, Aldolase and G6PDH/TPI were purchased from sigma Chemical Co., Poole, Dorset, U.K., and used without further purification. Sagetal, for anaesthezing rats was obtained from May & Baker Ltd., Dagenham, U.K.

The kits used for glucose, cholesterol and triacylglycerol determinations were obtained from BioSystems reagents and instruments, Costa Brava, 30, Barcelona, Spain. The ELISA kit for the insulin measurements was bought from DRG Instruments GmbH, Germany.

Mixtard insulin (Nove Nordish A/S 2880 Bagsvaerd, Denmark) was purchased from the local pharmacy without preparation.

2.1.2 Animals.

Male Wistar rats (180-200 g), were obtained from King Fahad Medical Research Center, King Abdulaziz University, Jeddah, Saudia Arabia. The animals were housed individually in an environment in which the temperature was maintained at a constant temperature 24 ± 1 °C, with lighting for 12 hours each day. Rats were fed ad libitum on standard laboratory diet (Grain Soils and Flour Mills Organization, Jeddah, Saudia Arabia) with free access to water. Animals were divided into five groups:
1-Normal Control rats.

A total of 10 normal rats were used as a control group. Their weight and blood glucose were recorded weekly.

2-Diabetic rats.

A total of 10 rats were made diabetic by single I.P. injection of streptozotocin (65 mg/kg body weight) dissolved in 50 mM citrate buffer, pH 4.5, immediately before use. Rats showing positive hyperglycemia (> 300 mg /100ml, Accu-check Active glucose strep, Roche diagnostics GmbH, Mannheim, Germany) after the induction of diabetes. Their weight and blood glucose were recorded weekly. Animals were then sacrificed after 15 days.

3-Diabetic rats treated with 0.5g of cinnamon.

A total of 10 rats were made diabetic as above and then fed with cinnamon water extract (0.5 g of cinnamon barks incubated in 500 ml warm water for 48 hours) in drinking water for 6 weeks starting 7 days after induction of diabetes. Their weight and blood glucose were recorded weekly.

4-Diabetic rats treated with 1.0g of cinnamon.

A total of 10 rats were made diabetic as above and then fed with cinnamon water extract (1.0 g of cinnamon barks incubated in 500 ml warm water for 48 hours) in drinking water for 6 weeks starting 7 days after induction of diabetes. Their weight and blood glucose were recorded weekly.
5- Diabetic rats treated with insulin.

A total of 10 rats were made diabetic as above and the effect of diabetes were reversed with 4 intramuscular injections of insulin as described in (Khoja S. M. and Salem A. M, 1991) (20 U/kg body weight) of mixtard insulin. Insulin was injected into diabetic rats with three doses in one day (one every 8 hours). The fourth and final injection of insulin was given one hour before sacrificing the rats. Their weight and blood glucose were recorded. Animals were then sacrificed after 15 days.

2.2 Methods.

2.2.1 Collection of blood samples and separation of serum.

At the end of each period, blood samples were collected from rats by retro-orbitally from the inner acanthus of the eye under light sagetal anesthesia using capillary tubes into EDTA tubes. Blood was then separated by centrifugation at 3000 x g for 20 minutes. Plasma was divided into aliquots, and stored at -20°C until analyzed.

2.2.2 Preparation of tissue extracts.

Rats were anaesthetized with sagetal (0.1ml/100g body wt.) and fresh tissue (liver and intestinal mucosa) were removed and immediately frozen in liquid N₂ until use. Tissues were then homogenized with 3 volumes (v/w) of ice cold extraction buffer (100 mM K₂HPO₄, pH 7.5) containing 30 mM KF, 3 mM MgSO₄, 1 mM EDTA, 5mM 2- mercaptoethanol and 0.1 mM trypsin. The homogenates were centrifuged at 70,000 x g for 20 minutes at 4°C. The supernatants were used for the assays of PFK-1.
2.2.3 Plasma Glucose determination.

Determination of glucose is based on the method of Trinder (1969). Glucose was measured according to the following reaction in which glucose oxidase is highly specific for beta-D-glucose. A coloured complex that can be measured by a spectrophotometerically.

\[
\text{Glucose} + \frac{1}{2} \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{glucose oxidase}} \text{Gluconate} + \text{H}_2\text{O}
\]

2 \text{H}_2\text{O} + 4\text{-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4 \text{H}_2\text{O}

2.2.4 Plasma Total Cholesterol determination.

Free and esterified cholesterol in the sample estimated by means of coupled reactions described below, a coloured complex that can be measured by a spectrophotometer.

Cholesterol determination was based on the method of Kattermann et al., (1984).

\[
\text{Cholesterol ester} + \text{H}_2\text{O} \xrightarrow{\text{chol. esterase}} \text{Cholesterol} + \text{Fatty acid}
\]

\[
\text{Cholesterol} + \frac{1}{2} \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{chol. oxidase}} \text{Cholestenone} + \text{H}_2\text{O}
\]

2 \text{H}_2\text{O} + 4\text{-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4 \text{H}_2\text{O}
2.2.5 Plasma Triacylglycerol determination.

An enzymatic colorimetric method was applied according to the method of Wahlefeld et al., (1974). The method depends on the enzymatic hydrolysis of triacylglycerol with subsequent colorimetric determination of the liberated glycerol. Triacylglycerol in the sample estimated by means of coupled reactions described below, a coloured complex that can be measured by spectrophotometry.

\[
\text{Triacylglycerol} + \text{H}_2\text{O} \xrightarrow{\text{lipase}} \text{Glycerol} + \text{Fatty acids}
\]

\[
\text{Glycerol} + \text{ATP} \xrightarrow{\text{glycerol kinase}} \text{Glycerol-3-P} + \text{ADP}
\]

\[
2 \text{H}_2\text{O}_2 + 4\text{-Aminoantipyrine} + 4\text{-chlorohenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4 \text{H}_2\text{O}
\]

2.2.6 Plasma Insulin determination.

Roche Diagnostics GmbH Ultrasensitive Rat Insulin ELISA is a solid phase two site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. During incubation; insulin in the sample reacts with peroxidase conjugated anti-insulin antibodies and anti-insulin anti-antibodies bound to microtitration well. A simple washing step removes unbound enzyme labeled antibody. The bound conjugate is detected by reaction with 3,3',5,5'-tetramethylbenzidine. The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically.
2.2.7 The Assay of 6-Phosphofructo-1-kinase.

2.2.7.1 Enzyme assay.

PFK-1 activity under optimal conditions at pH 8.0 was assayed as described by Jamal and Kellett, (1983). The assay is based upon the coupling of fructose 1,6-bisphosphate to the oxidation of NADH as follows:
The reaction mixture contained the following reagents:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Assayed concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris- chloride, pH 8.0</td>
<td>33 mM</td>
</tr>
<tr>
<td>KCl</td>
<td>50 mM</td>
</tr>
<tr>
<td>ATP</td>
<td>2 mM</td>
</tr>
<tr>
<td>Fructose-6-phosphate</td>
<td>2 mM</td>
</tr>
<tr>
<td>MgCl$_2$</td>
<td>5 mM</td>
</tr>
<tr>
<td>DTT</td>
<td>1 mM</td>
</tr>
<tr>
<td>NADH</td>
<td>0.12 mM</td>
</tr>
<tr>
<td>Aldolase</td>
<td>1 unit</td>
</tr>
<tr>
<td>α-GPDH/TPP enzymes</td>
<td>2 units</td>
</tr>
</tbody>
</table>

Table 2.1 The reaction mixture of pH 8.0 enzyme assay.

Stock solutions of these reagents were stored for two to three weeks at 4°C. The auxiliary enzymes were dissolved in 200 mM Tris- chloride, pH 8.0. The NADH was also freshly made up in this buffer.

Each assay used 1 ml of reaction mixture, and was initiated by the addition of an aliquot of PFK-1. The rate of depletion of NADH was followed spectrophotometrically at 340 nm. One unit of enzyme activity was defined as the number of μmols of fructose 1,6-bisphosphate formed per minute. pH 8.0 is the optimal pH for PFK-1 activity, but the regulatory properties of this enzyme are only displayed at lower pH values. The regulatory properties at pH 7.0 were determined as described by Hussey et al., (1977). The mixed auxiliary enzymes were dialyzed.
overnight before use against 100 volumes of 100 mM imidazole chloride buffer, pH 7.0.

This process was completed by double changing the imidazole buffer so as to remove ammonium sulphate, since it was reported that sulphate inhibits α-GPDH (Sellinger and Miller, 1959). Ammonium sulphate was, however, added back to the reaction mixture at a concentration of 10 mM in order to stabilize the PFK-1. The NADH was also made up in 100 mM imidazole chloride buffer, pH 7.0. The reaction mixture contained the following reagents:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Assayed concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imidazole chloride, pH 7.0</td>
<td>100 mM</td>
</tr>
<tr>
<td>KCl</td>
<td>50 mM</td>
</tr>
<tr>
<td>ATP</td>
<td>Concentrations</td>
</tr>
<tr>
<td>Fructose-6-phosphate</td>
<td>as required</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>1 mM</td>
</tr>
<tr>
<td>DTT</td>
<td>10 mM</td>
</tr>
<tr>
<td>(NH₄)₂SO₄</td>
<td>10 mM</td>
</tr>
<tr>
<td>NADH</td>
<td>0.12 mM</td>
</tr>
<tr>
<td>Aldolase and auxiliary</td>
<td>activities as pH 8.0</td>
</tr>
<tr>
<td>A-GPDH/TPI</td>
<td>dialysed overnight</td>
</tr>
</tbody>
</table>

Table 2.2 The reaction mixture of pH 7.0 enzyme assay.
For pH 7.0 assay, a fresh batch of auxiliary enzymes was used for each day work. The use of a fixed excess concentration of magnesium over ATP follows the procedure of Paetkau and Lardy (1967). For each assay, the reaction mixture was incubated with PFK-1 for a short time and the assay was then initiated with fructose-6-phosphate. The activities at pH 7.0 were expressed as a percentage of the corresponding values at pH 8.0.

2.2.7.2 Activity determination.

(i) ∆ O.D./min should not be allowed to exceed 0.2, otherwise, the assay will not be linear.

(ii) The assay shows an initial lag. The steady-state rate after this lag should be measured.

(iii) The assay principle is as follows:

Fructose-6-phosphate + ATP $\xrightarrow{PFK-1}$ Fructose-1,6-bisphosphate + ADP
Fructose-1,6-bisphosphate $\xrightarrow{Aldolase}$ Dihydroxyacetone phosphate + Glyceraldehyde-3-phosphate
Dihydroxyacetone phosphate $\xrightarrow{\alpha$-Glycerophosphate dehydrogenase}$ Glycerol-3-phosphate
$\text{NAD}^+ + H^+ \rightarrow \text{NAD}^+$
As defined earlier, one unit of PFK-1 activity is the number of μmoles of fructose-1,6-bisphosphate formed per min. For each mole of fructose-1,6-bisphosphate formed, 2 moles of NADH are converted to the oxidized form NAD⁺. The extinction coefficient of NADH at 340 nm = 6.22 mM⁻¹ and since 1 mM = 1 μmol/ml.

\[
\text{Number of μmoles of NADH per ml} = \frac{\Delta \text{C.D.}}{6.22} = \frac{1.0}{6.22} = 0.16
\]

Since there are 2 moles of NADH converted per mole of fructose 1,6-bisphosphate formed,

\[
\text{Number of units in the enzyme sample added} = \frac{\Delta \text{C.D.}}{\text{min}} \times 0.08 \text{ when assay volume is 1 ml.}
\]

\[
\text{Number of units per ml of enzyme sample} = \frac{\Delta \text{C.D.}}{\text{min}} \times 0.08 \times \text{volume of enzyme used in assay ( in ml ).}
\]

2.2.8 Total Protein determination.

Proteins were measured as described by Lowery et al., (1951) with bovine serum albumin as standard as follows:

A series of test tubes were prepared including:

(1) a blank contain 1.2 ml of distilled H₂O,

(2) a set of standard tubes each contain appropriate aliquots of water and a protein standard solution (0.2 mg BSA/ml) that yield separate tubes contained 10, 20, 40, 80, 120, 160, and 200 μg of protein, all in final volumes of 1.2 ml,

(3) a set of assay tubes contained water and appropriate dilutions of the protein solution of unknown concentration, also in final volumes of 1.2 ml. 100 ml of fresh
alkaline copper reagent was separately prepared by mixing, in order, 1 ml of 1% 
CuSO$_4$, H$_2$O, 1 ml of 2% sodium tartrate and 98 ml of 2% Na$_2$CO$_3$ in 0.1 N NaOH.
6 ml of alkaline copper reagent were added to each tube and immediately mixed in.
After 10 minutes at room temperature, 0.3 ml of Folin-Ciocalteu reagent was added to 
each tube and immediately mixed in. After another 30 minutes, the absorbance of the 
standard and assay tubes was read at 600 nm against the blank (figure 2.1).

2.2.9 Statistical analysis.

Statistical analyses of the data were performed and graphs were created using 
statgraphics computer program package, SPSS ver. 14.0 for windows and Microsoft 
EXCEL ver 2003 for windows.

In this study, different methods were used to analyse the data obtained from the 
experiments using one - way analysis of variance (ANOVA). Post Hoc test 
procedure using bonferroni between groups following a significant ANOVA test when 
the value of the measured parameter were normally distributed.

Kruskallis test was used to study the difference between groups when the value of 
the measurement parameters is not normally distributed such as (glucose, cholesterol, 
triacylglycerol). Significant was assigned at $P<0.05$
Figure 2.1 BSA Standard curve.

Concentration of Bovine Serum Albumin (mg/ml)
CHAPTER 3

RESULTS
Table 3.1 shows that the food intake of streptozotocin-induced diabetic rats was significantly decreased (P< 0.001) on the first two days after the injection of streptozotocin. However, the food intake of the diabetic rats had returned back to normal by the third day. Therefore, plasma glucose concentrations were measured at 10 days after induction of diabetes since starvation is known to affect plasma glucose concentrations. Also, the activity of PFK-1 in liver and intestinal mucosa were measured at 10 days after induction since starvation is known to reduce the enzyme activity.

Table 3.2 shows the effect of cinnamon administration (6weeks) on glucose concentration (mg/dl). Administration of streptozotocin (65mg/kg) led to significant increase (p <0.0001) of blood glucose levels in untreated diabetic group companied with normal control group. After 6 weeks of daily treatment with 0.5g and 1.0g cinnamon led to significant decrease (p < 0.0001) in blood sugar levels comparing with diabetic rats started after the 15th day of the treatment.

The general characteristics of rat; weight and tissue (liver & mucosal) weights are showed in table 3.3. The untreated diabetic rats weight significantly decreased (p<0.0001) than the normal control where as the other groups ware significally increased than the untreated diabetic group. Liver and intestinal mucosal weights ware non significant change in all groups.
Table 3.1 Daily food intake of normal control and streptozotocin-induced diabetic rats.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>16.10 ± 0.38</td>
<td>15.90 ± 0.25</td>
<td>16.40 ± 0.28</td>
<td>16.30 ± 1.9</td>
<td>16.40 ± 0.25</td>
</tr>
<tr>
<td>Diabetic Untreated</td>
<td>7.80 ± 0.35*</td>
<td>8.10 ± 0.38*</td>
<td>14.20 ± 0.44</td>
<td>16.90 ± 0.51</td>
<td>16.80 ± 0.32</td>
</tr>
</tbody>
</table>

Results are presented as mean ± SEM. Significant differences between control and all other groups were made by ANOVA (*p < 0.001).
Table 3.2 Effect of cinnamon administration (6 weeks) on plasma glucose concentration (mg/dl).

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>week 0 0 day</th>
<th>week 1 0 day of treatment</th>
<th>week 3 15 days of treatment</th>
<th>week 5 30 days of treatment</th>
<th>week 7 45 days of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>105.80 ± 3.25</td>
<td>111.30 ± 4.99</td>
<td>118 ± 3.87</td>
<td>120.80 ± 3.31</td>
<td>123.80 ± 3.31</td>
</tr>
<tr>
<td>Diabetic Untreated</td>
<td>105.40 ± 3.50</td>
<td>563.80 ± 7.70**</td>
<td>408.2 ± 37.93</td>
<td>418.20 ± 37.5**</td>
<td>430.50 ± 31.80**</td>
</tr>
<tr>
<td>Diabetic + Cinnamon 0.5g</td>
<td>106.6 ± 2.96</td>
<td>547.80 ± 11.27**</td>
<td>355.2 ± 40.53 *</td>
<td>232.70 ± 28.67*</td>
<td>106.60 ± 2.96**</td>
</tr>
<tr>
<td>Diabetic + Cinnamon 1.0g</td>
<td>106.1 ± 2.98</td>
<td>509.80 ± 29.72**</td>
<td>408.2 ± 37.93</td>
<td>250.10 ± 17.41**</td>
<td>106.10 ± 2.98**</td>
</tr>
</tbody>
</table>

Results are presented as means ± SEM. Significant differences between control and all other groups by one-way ANOVA (*p < 0.001 **p < 0.0001).
Table 3.3 General characteristics of rat; weight and tissue (liver & mucosal) weights.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Animal weight g</th>
<th>Liver weight g</th>
<th>Mucosal weight g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>245.80 ± 0.98</td>
<td>8.30 ± 0.49</td>
<td>1.50 ± 0.14</td>
</tr>
<tr>
<td>Diabetic Untreated</td>
<td>102.80 ± 1.64*</td>
<td>9.70 ± 0.57</td>
<td>1.35 ± 0.13</td>
</tr>
<tr>
<td>Diabetic + Cinnamon 0.5g</td>
<td>185.10 ± 1.06*</td>
<td>8.40 ± 0.33</td>
<td>1.35 ± 0.13</td>
</tr>
<tr>
<td>Diabetic + Cinnamon 1.0g</td>
<td>164.10 ± 1.33*</td>
<td>8.30 ± 0.49</td>
<td>1.50 ± 0.14</td>
</tr>
<tr>
<td>Diabetic + Insulin</td>
<td>134.70 ± 0.70*</td>
<td>9.70 ± 0.57</td>
<td>1.55 ± 0.15</td>
</tr>
</tbody>
</table>

Results are presented as means ± SEM. Significant differences between control and all other groups by one-way ANOVA for normally distributed data and the other were used kruskallis test (*p < 0.0001).
Table 3.4 shows the rat plasma concentrations of glucose, cholesterol, triacylglycerol and insulin. The concentrations of plasma glucose, cholesterol and triacylglycerol were significantly increased \((p<0.0001)\) in the untreated diabetic group compared with the normal control group. These concentrations were significantly decreased \((p<0.0001)\) in 0.5g cinnamon treated, 1.0g cinnamon treated and insulin treated groups compared with diabetic group. On the other hand, insulin concentrations were significantly decreased \((p<0.0001)\) in untreated diabetic group compared with normal control group but increased in all other groups compared with untreated diabetic group. The results of plasma concentrations of glucose, cholesterol, triacylglycerol and insulin are summarized in (figures 3.1-3.4).

Figure 3.5 shows the fructose-6-phosphate curve of liver PFK-1 activity measured in the presence of 2.5mM ATP at pH 7.0. It appears that in diabetic group the curve has the lowest \(v_{0.5}/V\) compared with all other groups. The cinnamon treated rats show an increase in \(v_{0.5}/V\) value of the enzyme compared with diabetic rats but this value is still lower than normal rats. The enzyme showed a sigmoidal velocity curve in all groups but the sigmoidicity was lower in the untreated diabetic group. The increase in \(v_{0.5}/V\) is a reflection of an increased susceptibility to inhibition by ATP showed in (figure 3.6).
Table 3.4 The concentrations of rat plasma Glucose, Cholesterol, Triacylglycerol and Insulin.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Glucose (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>Triacylglycerol (mg/dl)</th>
<th>Insulin (μLU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>79.30 ± 0.32</td>
<td>104.90 ± 0.33</td>
<td>129.94 ± 0.39</td>
<td>69.42 ± 1.83</td>
</tr>
<tr>
<td>Diabetic Untreated</td>
<td>367.21 ± 7.15*</td>
<td>367.92 ± 0.34*</td>
<td>302.66 ± 0.49*</td>
<td>23.94 ± 2.63*</td>
</tr>
<tr>
<td>Diabetic + Cinnamon 0.5g</td>
<td>170.19 ± 1.63*</td>
<td>203.06 ± 0.46*</td>
<td>191.38 ± 5.77*</td>
<td>47.78 ± 2.41*</td>
</tr>
<tr>
<td>Diabetic + Cinnamon 1.0g</td>
<td>148.91 ± 1.46*</td>
<td>132.08 ± 0.30*</td>
<td>125.92 ± 0.29*</td>
<td>65.50 ± 4.05*</td>
</tr>
<tr>
<td>Diabetic + Insulin</td>
<td>95.34 ± 1.26*</td>
<td>89.50 ± 0.96*</td>
<td>189.27 ± 0.31*</td>
<td>84.09 ± 4.12*</td>
</tr>
</tbody>
</table>

Results are presented as means ± SEM. Significant differences between control and all other groups by one way ANOVA for normally distributed data and the other were used kruskall wallis test (*p < 0.0001).
Figure 3.1 A histogram showing the changes of glucose concentration in plasma of concerned categories.

Figure 3.2 A histogram showing the changes of cholesterol concentration in plasma of concerned categories.
Figure 3.3 A histogram showing the changes of triacylglycerol concentration in plasma of concerned categories.

Figure 3.4 A histogram showing the changes of insulin concentration in plasma of concerned categories.
Figure 3.5 Dependence of Liver PFK-1 activity on F6P concentration in the presence of 2.5 mM ATP. Assays were performed at pH 7 and the activity of pH 7 is expressed as a ratio to that at pH 8.
Figure 3.6 Dependence of Liver PFK-1 activity on ATP concentration in the presence of 0.5 mM fructose-6-phosphate, assay were performed at pH 7.0.
The specific PFK-1 activities of liver in all experimental groups are shown in table 3.5. The administration of 0.5g cinnamon, 1.0g cinnamon and insulin resulted in a significantly increased \((p<0.0001)\) in the values of \(v_{0.5}/V\) from 0.12 ± 0.002 of diabetic untreated rat liver PFK-1 to 0.18 ± 0.003 of 0.5g cinnamon treated group, 0.18 ± 0.002 of 1.0g cinnamon treated group and 0.22 ± 0.001 of insulin treated group. Similarly, the total activities of the enzyme were significantly increased \((p<0.0001)\) from 5.42 ± 0.34 of diabetic untreated rat liver PFK-1 to 6.94 ± 0.3 of 0.5g cinnamon treated group, 8.24 ± 0.43 of 1.0g cinnamon treated group and 9.75 ± 0.25 of insulin treated group.

Figure 3.7 shows the fructose-6-phosphate curve of intestinal mucosa PFK-1 activity measured in the presence of 2.5mM ATP at pH 7.0. It appears that in diabetic group the curve has lower \(v_{0.5}/V\) compared with normal control. The cinnamon treated rats show an increase in \(v_{0.5}/V\) value of the enzyme compared with diabetic rats but this value is still lower than normal rats. The enzyme showed a sigmoidal velocity curve in all groups but the sigmoidicity was lower in the untreated diabetic group. The increase in \(v_{0.5}/V\) is a reflection of an increased susceptibility to inhibition by ATP showed in (figure 3.8).

The specific PFK-1 activities of mucosal in all experimental groups are shown in table 3.6. The administration of 0.5g cinnamon, 1.0g cinnamon and insulin
### Table 3.5 Total activity of rat liver PFK and v0.5/V ratio.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Total PFK activities</th>
<th>v 0.5 / V</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unit / g</td>
<td>Unit / mg protein</td>
</tr>
<tr>
<td>Normal Control</td>
<td>3.00 ± 0.03</td>
<td>11.70 ± 0.62</td>
</tr>
<tr>
<td>Diabetic Untreated</td>
<td>1.80 ± 0.09*</td>
<td>5.42 ± 0.34*</td>
</tr>
<tr>
<td>Diabetic + Cinnamon 0.5g</td>
<td>2.40 ± 0.05*</td>
<td>6.94 ± 0.30</td>
</tr>
<tr>
<td>Diabetic + Cinnamon 1.0g</td>
<td>2.50 ± 0.05*</td>
<td>8.24 ± 0.43*</td>
</tr>
<tr>
<td>Diabetic + Insulin</td>
<td>2.79 ± 0.06*</td>
<td>9.75 ± 0.25*</td>
</tr>
</tbody>
</table>

Results are presented as means ± SEM. Significant differences between control and all other groups by one-way ANOVA (*p < 0.0001).*
Figure 3.7 Dependence of Mucosal PFK-1 activity on F6P concentration in the presence of 2.5 mM ATP assay were performed at pH 7 and the activity of pH 7 is expressed as a ratio to that at pH 8.
Figure 3.8 Dependence of Mucosal PFK-1 activity on ATP concentration in the presence of 0.5 mM fructose-6-phosphate, assay were performed at pH 7.0.
<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Total PFK activities</th>
<th>v 0.5 / V</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unit / g</td>
<td>Unit / mg protein</td>
</tr>
<tr>
<td>Normal Control</td>
<td>5.01 ± 0.04</td>
<td>21.33 ± 2.34</td>
</tr>
<tr>
<td>Diabetic Untreated</td>
<td>3.51 ± 0.04*</td>
<td>12.17 ± 1.14*</td>
</tr>
<tr>
<td>Diabetic + Cinnamon 0.5g</td>
<td>4.01 ± 0.03*</td>
<td>18.66 ± 1.49</td>
</tr>
<tr>
<td>Diabetic + Cinnamon 1.0g</td>
<td>4.11 ± 0.06*</td>
<td>15.98 ± 0.46</td>
</tr>
<tr>
<td>Diabetic + Insulin</td>
<td>4.50 ± 0.04*</td>
<td>19.74 ± 1.99*</td>
</tr>
</tbody>
</table>

Results are presented as means ± SEM. Significant differences between control and all other groups by one-way ANOVA.

(* p < 0.0001)
resulted in a significantly increased ($p<0.0001$) in the values of $v_{0.5}/V$ from $0.22 \pm 0.002$ of diabetic untreated rat mucosa to $0.25 \pm 0.005$ of 0.5g cinnamon treated group, $0.32 \pm 0.003$ of 1.0g cinnamon treated group and $0.35 \pm 0.005$ of insulin treated group. Similarly, the total activities of the enzyme were significantly increased ($p<0.0001$) from $12.17 \pm 1.14$ of diabetic untreated rat mucosa to $18.66 \pm 1.49$ of 0.5g cinnamon treated group, $15.99 \pm 0.46$ of 1.0g cinnamon treated group and $19.74 \pm 1.59$ of insulin treated group.
CHAPTER 4

DISSCTION & CONCLUSIONS
4.1 Discussion.

Cinnamon is very widely used all over the world especially in the eastern and far eastern countries. This leads to the finding of a benefit therapeutic agent for one of the most common diseases these days such as diabetes from natural sources rather than chemical ones.

Untreated diabetic group showed significant increase ($P<0.0001$) in plasma glucose concentrations by 363%. This result was reversed by significantly decrease ($P<0.0001$) by 54% and 60% in the 0.5g and 1.0g cinnamon treated rats respectively.

Similarly, untreated diabetic group showed significantly increase ($P<0.0001$) in plasma cholesterol concentrations by 250% which were reversed by the 0.5g and 1.0g cinnamon treatment and significantly decrease ($P<0.0001$) by 45% and 64% respectively.

In the case of plasma triacylglycerol, untreated diabetic group showed significantly increase ($P<0.0001$) by 133%, which were reversed by the 0.5g and 1.0g cinnamon treatment and significantly decrease ($P<0.0001$) by 37% and 58% in the 0.5g and 1.0g cinnamon treated rats respectively.

Plasma glucose concentrations were measured at 10 days after induction of diabetes since starvation is known to affect plasma glucose concentrations. Also, the activity of PFK-1 in liver and intestinal mucosa was measured at 10 days after induction since starvation is known to reduce the enzyme activity.

The total activity of liver PFK-1 at pH 8 was significantly increase ($P<0.0001$) by 33% and 39% in the 0.5g and 1.0g cinnamon treated rats respectively after a significant decrease ($P<0.0001$) by 40% in untreated diabetic rats. Also, the total activity of intestinal PFK-1 at
pH 8 was significantly increase (P<0.0001) by 14% and 36% in the 0.5g and 1.0g cinnamon treated rats respectively after a significant decrease (P<0.0001) by 30% in untreated diabetic rats.

In addition, the activity ratio v_{0.5}/V of the liver enzyme was significant increase (P<0.0001) by 50% in both cinnamon treated groups after a significant decrease (P<0.0001) by 56% in untreated diabetic rats group. Also, the activity ratio v_{0.5}/V of the intestinal enzyme was significant increase (P<0.0001) by 14% and 17% in the 0.5g and 1.0g cinnamon treated rats respectively after a significant decrease (P<0.0001) by 45% in untreated diabetic rats group.

These results proved that cinnamon could improve the glucose utilization and metabolism in the rat tissues.

It has been reported by Khan et al. (2003) that extracts of cinnamon activated insulin receptor kinase and inhibited dephosphorylation of the insulin receptor, leading to maximal phosphorylation of the insulin receptor that led to increased insulin sensitivity.

Untreated diabetic group showed significant decrease (P<0.0001) in plasma insulin level by 66% which was reversed by the 0.5g and 1.0g cinnamon treatment and significant increase (P<0.0001) by 100% and 174% respectively even with damage pancreatic cells due to streptozotocin dose was given. This may be for the structural like between the insulin and methylhydroxycolan polymer (MHCP) in cinnamon extract as described and isolated in 2004 by Anderson and others.

The cinnamon fraction directly activates the auto-phosphorylation of the insulin receptor kinase catalytic domain. However, this effect is transient with unclear reason, but the
activation is due to auto-phosphorylation on the kinase and not due to phosphorylation of the components in the insulin potentiating fraction. Therefore, since the insulin potentiating fraction inhibits a protein tyrosine phosphatase that may act in vivo to regulate the activity of the insulin receptor kinase, and activates the insulin receptor kinase autophosphorylation directly. This cinnamon fraction may act in vivo to do the same. The net result would be increased auto-phosphorylation of the receptor kinase and presumably increased insulin signaling (Impard-Radosewich et al., 1998).

It has been also reported that there were many herbs and spices which have anti-diabetic effect such as cinnamon and fenugreek. However, they appear to be generally safe. The available data suggest that several supplements may warrant further study. The best evidence for efficacy from adequately designed randomized controlled trials is available for Coccinia indica and American ginseng. Chromium has been the most widely studied supplement. Other supplements with positive preliminary results include Gymnema sylvestre, Aloe vera, vanadium, Momordica charantia, and nopal (Yeh et al., 2003).

The levels of cinnamon tested in this study, 0.1 and 0.2 g per day (0.5g incubated in 500 ml water and 1.0g incubated in 500 ml water), suggest that even small amount of cinnamon intake that may be beneficial and that intake of 0.2 g daily is likely to be beneficial in controlling blood glucose and lipid levels.

In conclusion, extracts of cinnamon activated PFK-1, increased plasma insulin concentration, and decreased plasma glucose, triacylglycerol and total cholesterol in streptozotocin-induced diabetics rats. In addition, cinnamon may be beneficial for the people to prevent and control elevated glucose and blood lipid levels.
FUTURE WORK

After this study and the other herb and spices that showed a positive effect in diabetic patient, I recommend other researchers to do more research on the activity of the most important key enzyme (PFK-1) with other herbs and/or spices.
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التعليمز

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الملخص المرجعي

مرض السكري

يُعتبر مرض السكري من أكثر الأمراض انتشارها في العصر الحديث، وهو عبارة عن خلل في خلايا الجسم الذي يؤثر على خلايا أو عضلات السكر في الدم. يتمتع الفرد بالسكري من خلال ارتفاع نسبته من السكر في الدم، ويعتمد الهدف من الجلوكوز في الجسم لتنفيذ عملية الاستفادة من الجلوكوز.

ويتم العملاء المرض بوضع عناصر من موضعين مزمنين، وهما النوع الأول لتطبيق موضع الالتباس على الأنسولين، واللوقود الثاني الذي يعتمد على الأنسولين. ويُعتبر هذان النوعان أكثر أمراض السكري انتشارًا، حيث أن نسبة الإصابة بالنوع الثاني أعلى منها النوع الأول.
السكري النوع 1

1. يسمى داء السكري المديم، على أن السكر في الدم ينتج بسبب خلل في عملية تحليل الشوكولاتة.
2. ويليامز في البحوث النموذجية عن إنتاج الأنسولين بسبب الفلل في نظرة محاكاة الأجسام.
3. هذا النوع عادةً الأطفال الذين تحت سن العشرين، المرضى الذين يعانون لمدة حسنتن أنسولين في البور. 5% - 6% من مرضى السكري يعانون من هذا النوع.

السكري النوع 2

1. يسمى داء السكري المدير بعلي الأنسولين وينتج بسبب مشاكل في الجهاز الهضم.
2. الأنسولين أو عمليات تبادل الأنسولين في الجسم ببعض البحوث نجد أن ذلك يصبح نتيجة لإنتاج الأنسولين بسبب حوالي 90% - 95% من المصابين بداء السكري عادةً يعانون من النوع الأول فور تصل العالقين، يرتبط ببعض عواملها: تقدم العمر، السمنة، تأثير العائلة المرض.

و هناك أيضا أنواع أخرى من مرض السكري مثل سكر الدم، الذي يصيب المرأة الحامل.

نتيجة لزيادة مستوى الأنسولين في الدم، غالبًا ما تزداد هذه الحالة بعد الولادة، لذا من المهم أن تصاب المرأة بعد ذلك مريضة السكري النوع الثاني.
الانسولين: طيعرٌ وظيفٌ

تفرز جزيء الإنسولين في الهرمونات المنسوجية. يلعبان الأنسولين والجلوكاجون دورًا في النظام العضلي، حيث يتمتع الأنسولين بالقدرة على تحريك العضلات، بينما يمنع الجلوكاجون من ذلك. زيادة في تصنيع الأنسولين ستساعد على glycolysis، في حين ستقلل gluconeogenesis من الإنتاج الأيضي. سيساعد الهرمونات الأخرى في زيادة تصنيع الأنسولين، مثل الأستروجين، وتقلل من تصنيع الأنسولين. في المحاصيل الزراعية، يتم استخدام الإنسولين لتعزيز نمو النباتات بواسطة تقليل مستويات الأنسولين.-negative

الرجوaldo:

هذا المكثف ليس فقط معززًا لنشاط مستوي السكري في الدم، بل أيضاً خدمت لتعزيزات متأخرة مثل الاعتداء الأيضي، والبروتيوم القصبي، وغيره.

يستعمل الأنسولين بعد تحكم سلسلة الأحماض الأسيتيك في التركيب الأولي إلى سلسلات مرتبطين بعضهم ببعضًا، مما يسمح بالبناء الصحيح الواحد بين السلسلة الأولى. حيث أن الأنسولين
السلسلة الأولى على 30 حمض أميني، الثانية على 20 حمض أميني فيما كان التركيب الأولي مخزون
على 10 حمض أميني.

يصف عمل الاكتسولين في الحلالا "الأنسان" هو "المناخ" الذي يفتح "الباب" لمهر سكر الجلوبيز. متاحة الأنسولين تنتج عندما يجوع موز "أبرة" أن "الأعمال" على هذه الأبرة
تصداً، وصعب النجع على الرغم من وجود الأنسولين.

ومن مسببات منعضة الأنسولين الإنجراط في إنتاج المنتجات مثل التهاب تسبب أن
الإنجراط في الإنتاج مع مهن السموم إصابات إجهاد مطر أو حاد. مشاكل
المأكولات الدائمة وغيرها.

الأيض الطبيعي للدواء بزونكير

يعتبر بزونكير خطرة عديدة للتفاعلات في بذور الموز، وزيدناء ومرحاس إلى
المسؤولة الحيوية، امتدت عامل (Pu,AMP,ADP) مقسمة بزونكير 30-ثاني الموت.
 كامل الإيثامي، "تُقدر نشاط 

PFK -1 ATP" بينما 

السلسلة تعمل كعامل سلبي، مشطب للفاعل.

Tsai et al. أكتسبت عدة أشكال من إنزيمات PFK-1 في أنسجة الجلد. العالمان Kemp و Dunaway عام 1992 كشفا عن ثلاثي من الإنزيمات المشابهة في الأنسجة الجلدية، مثل الفضلات çıkarها حسباً على النوع Kellett. Khoja (C) (A) (B) (C) (D) من النوع 

هذا النوع Khoja المشابه من النوع (D) الموجود في العضة المخاطي للإمساك.

الدراسة

إحتمالية أن المحتويات الغذائية مساعدة في معالجة نوع من أنواع مرض السكري وأعراض الإكيد العديد. تتطلب بعضها مساعدة لكي تتمكن من أنباض الدهون مثل الفرد التي الحليب أو أوصاف الغازات والمشتقات تلعب دوراً في تخليق الأكسائين. المنتجات البالتي: 2-4% أو أكثر الجلوكوز.

فقط هذه أيضاً قليلة الأمين. كما أنها وظيفة كافية للتغذية الليثيوم.

هدف هذا البحث إلى دراسة إحتمالية جذب تأثير الفرد، والتي تقدم من أحد أنواع الدهون.

شاركت الاستخدام في أغلب البلد الشرقية في الشرق الأوسط.

في هذا البحث كان هناك 60 جرعة من نوع ديسير (100-200 غرام) متصلة إلى حمض.

جميع الجمعيات المكونة من 10 حيوانات: طبيعي، مريضة بالسكري، غير معالجة.

(1) قد أصبحت مصابين بالسكري خلقها حصة واحدة في البطن مادة باستيرنر أو ترسيب 5 واصل.

كما أن ميزان الجسم والحموضة في 50 مل. لإغلاق محلول المرميز بـ 4.5 pH. و 0.5 غرام من التربة في جرذان مصابين.

الاستخدام

أخرج ذا مصاب بالسكري غذاء بـ 0.5 غرام من التربة في جرذان مصابين.
بالسكتري عولجت 0.1 غرام من الفرخة، مفضل مستخلص الفرخة ب량 0.5 غرام
منها في 500 مل ماء، دافئ، لمدة 60 ساعة، والجديدة المجموعة الأخرى عولجت بالأنسولين

هذا الدراسة تستنسل الفروض على تطبيق إنزيم فوسفوركريبتيز في كدب أمعاء الجرذان المصابة
بـ: السكتري المسخن، بالستروجلوسين، على ملاحظة تأثير الفرخة على مستوى الجلوكوز،
الكسطرول، الدهون الثلاثية، الأنسولين في الدم، إضافة إلى ذلك، الدراسة الحالية
تقترب من نتائج إنزيم فوسفوركريبتيز في الجرذان المصابة بالسكتري مع مجموعة المعاينة بالفرخة.

أوضحت النتائج اختلافاً معيناً (p < 0.0001) في تركيز الجلوكوز، الكسطرول،
الجليسيزيات الثلاثية في مجموعات الجرذان المخضوعين بالفريحة حيث مكثف تركيز الجلوكوز بنسبة 54.6% للالمجموعة المعالجة بـ 0.0 غرام الفرخة، 67% للمجموعة المعالجة بـ 0.5 غرام الفرخة.

كما اختلفت معيناً (p < 0.0001) تركيز الكسطرول بنسبة 64.4% للمجموعة المعالجة
بـ 0.0 غرام الفرخة، 67% للمجموعة المعالجة بـ 0.5 غرام الفرخة، 87% للمجموعة المعالجة بـ 0.0 غرام الفرخة، 87% للمجموعة المعالجة بـ 0.5 غرام الفرخة.
While there was a significant difference in the level of PKF-1 (p < 0.0001) in the control group compared to the treatment group. The difference between the two groups showed a p-value of 0.0001. The enzyme activity in the treatment group was significantly different from the control group (p < 0.0001).

We conclude that the treatment was effective in lowering the level of PKF-1. This suggests that the enzyme activity was indeed reduced in the treatment group. Additionally, it was observed that the enzyme activity was reduced by 70% in the treatment group compared to the control group. The difference in enzyme activity was statistically significant (p < 0.0001).

Furthermore, it was found that the treatment was effective in reducing the level of blood glucose in the treatment group. The decrease in blood glucose levels was significant (p < 0.0001). The difference between the two groups showed a p-value of 0.0001. The treatment was effective in reducing the level of blood glucose in the treatment group.

The treatment was effective in reducing the level of blood glucose in the treatment group. The decrease in blood glucose levels was significant (p < 0.0001). The difference between the two groups showed a p-value of 0.0001. The treatment was effective in reducing the level of blood glucose in the treatment group.
دری اهمیت روشن‌کردن

دری تکثیری

دری مصرفی

دری هولوژی

دری از دیدگاه‌ی روشن کردن و اعم‌العمل‌ها...
شكر وإعفاء

إعلان العلم...

إعلان التغيير...

فينسينزي سايدر كيرا نحنهم العلم...

الأسف الشديد، العلم الفاضل، العلماء الفهماء، العلماء الطيبين، علماء الفهماء...

تقدير كبير، التحية النافعة...

يرجى أن تفحصوا منشوراتنا...

وهكذا نسأوا الاعترافات في هذا الشكل بالقبول....
إهداء

إله رفعه الريح... 
إله تقصه بظر العصر...
إله نبوءة الصفا، وانتفاخ ...
إله عاقبة العالم...
إله ضياء الدنيا والغيب...

الخواص المدهشة للسماء في فصتنا، مع ثمور السمرة، وأدري أنا، في انتفاح...
الزينة من الطمار، والرطبة، فاضلة، التنصر مي، لنور، مي، (الباني)، نرى، وهى، نتمل، تعيئة تيلاء حراري، روابط وزارات، باسي (الباني).

وازدرى من مصلى بالدرة...
صاعب من القلق، يا ناري (المحارب)...
طأبة من مرارة اليد، نيمور، كلاجور، سمل الدين، مها (التعمير)، نور، نارو، نوروز. ...

وازدرى يمكن، وحضرارات من سلبيا، (المحرر) للحي...
(الخصب) بالهواء، (السماوية)، (القلم)، (السرد)، (النار)، (النار)، (النار)...
(نار)، (نار)، (نار)، (نار)، (نار)، (نار)، (نار)، (نار). 
hann, moon flower.
بشرع آمناً الحمد لله
تأثير الترفيع على تركيز الجلوكوز في البلازما وتنظيم نشاطه

عدد: فوسفوروكو-6-kinase من كبد الفئران المصابة بداء السكري

المستعرض بالبيرونييزين

إعداد
سهام رمسي نصوص

قدت هذه الرسالة استكشافاً لمتطلبات درجة الماجستير في العلوم

تُسيرة: الكيمياء-كلية العلوم
جامع الملك عبد العزيز-جدة
المملكة العربية السعودية

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