UNIVERSITI PUTRA MALAYSIA

ANTIDIABETIC EFFECT OF POLYPEPTIDE-K AND ESSENTIAL OIL FROM Momordica charantia

BY
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ABSTRACT

Background: *Momordica charantia* known as bitter gourd is one of the useful herbs in traditional medicine especially in antidiabetic properties. Antidiabetic effects of bitter gourd extracts have been demonstrated in cell culture, animal, and human studies which can stimulate pancreatic insulin secretion and peripheral glucose metabolism, including stimulation of glucose uptake and inhibit gluconeogenesis. In this study, we investigated the effect of essential oil and polypeptide-k from extraction of *Momordica charantia* on α-glucosidase and α-amylase activity. **Hypothesis**: Polypeptide-k and essential oil has antidiabetic properties by inhibit α-glycosidase and α-amylase activity. **Objectives**: To assess the effect of polypeptide-k and essential oil for antidiabetic properties by assayed the α-glycosidase and α-amylase activity. **Material and method**: The essential oil and polypeptide-k were assayed for inhibition of α-amylase and α-glycosidase activity by using Alpha-Amylase Assayed Kit and Alpha-Glucosidase Assayed Kit respectively. Initial dosage of two samples is 2.0 mg/ml and undergoes serial dilution with ¾ dilution factor to reduce the concentration. 0.5ul from each sample and product of serial dilution will added to 0.5ul of alpha-amylase enzyme and alpha-glucosidase enzyme. Then, the mixtures were tested by using the kit and absorbance readings were recorded at 595nm for α-glycosidase assay and 405 nm for α-amylase assay. **Result:** From this study, polypeptide-k and essential oil from extraction of *Momordica charantia* has antidiabetic properties by showed significant α-glycosidase and α-amylase inhibitory activities. Different dosage has different inhibitory activity and the highest inhibitory activities are from the highest dosage of samples by showed the most effective and significant (p<0.0005) result of inhibition activity followed by lower concentration. Polypeptide-k has strong and effective inhibitory effect to α-glycosidase and α-amylase activity than essential oil by showing a high rate of inhibition activity by the equivalent of dosage use.

KESAN ANTIDIABETIS OLEH MINYAK ESENSIAL DAN POLYPEPTIDA-K DARI *Momordica Charantia*

KHAIRUL FAIZI BIN ZAMZURI

ABSTRAK

Latar Belakang: *Momordica charantia* atau lebih dikenali sebagai buah peria adalah salah satu herba yang sangat berguna dalam perubatan tradisional terutamanya dalam merawat penyakit diabetis. Kesan antidiabetis oleh ekstrak peria telah dibuktikan dalam kultur sel, haiwan, dan kajian manusia yang dapat merangsang perembesan insulin daripada pankreas dan meningkatkan kadar metabolisma glukosa periferi, termasuklah mengurangkan pengambilan glukosa dan menghalang pencernaan glukosa. Dalam kajian ini, kita mengkaji kesan minyak esensial dan polipeptida-k dari ekstrak *Momordica charantia* terhadap aktiviti α-glukosidase dan α-amilase. **Hipotesis:** Polypeptide-k dan minyak esensial memiliki sifat antidiabetis dengan menghalang aktiviti enzim α-glikosidase dan α-amilase. **Tujuan**: Untuk menilai kesan polipeptida-k dan minyak esensial sebagai agen antidiabetes dengan menguji aktiviti enzim α-glikosidase dan α-amilase. **Bahan dan Kaedah**: Minyak esensial dan polipeptida-k akan diuji kesannya terhadap aktiviti enzim α-amilase dan enzim α-glikosidase masing-masing dengan menggunakan Kit Alpha-Amilase dan Kit Alpha-Glukosidase. Kepekatan awal untuk kedua-dua sampel ini adalah 2.0 mg/ml dan pencairan bersiri dilakukan dengan faktor pencairannya adalah 3/4 yang bertujuan untuk mengurangkan kepekatan sampel secara berkala. 0.5 µl dari setiap sampel dan dari setiap hasil siri pencairan akan bertindak balas dengan 0.5 µl enzim α -amilase dan enzim α -glukosidase. Kemudian, campuran tersebut diuji dengan menggunakan kit dan bacaan absorbansi tercatat sebanyak 595nm untuk ujian α-glukosidase dan 405 nm untuk ujian α -amilase. **Keputusan**: Dari kajian ini, polipeptida-k dan minyak esensial dari ekstrak *Momordica charantia* memiliki sifat antidiabetis dengan menunjukkan perencatan yang signifikan kepada aktiviti enzim α-glukosidase dan enzim α-amilase. Kepekatan yang berbeza mempunyai aktiviti perencatan yang berbeza dan aktiviti perencatan tertinggi adalah dari kepekatan sampel yang tertinggi dengan menunjukkan kadar perencatan yang paling berkesan dan signifikan ($p < 0.05$) diikuti dengan kepekatan sampel yang rendah. Polipeptida-k mempunyai kesan perencatan yang kuat dan berkesan untuk aktiviti α-glikosidase dan aktiviti α-amilase berbanding minyak esensial dengan menunjukkan kadar perencatan yang tinggi bagi setiap kepekatan yang sama.

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APPROVAL

It is hereby certified that I have read this project paper entitled Antidiabetic Effect of Essential oil and Polypeptide-K from *Momordica Charantia* by Khairul Faizi Bin Zamzuri, and in my opinion it is satisfactory in term of scope, quality and presentation as a fulfillment of the requirements for the course SBP 3999.

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DECLARATION

I hereby declare that the thesis is based on my original work except for the quotations and citations, which have been duly acknowledged.

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Matric No: 140595

Date: 29th April 2011

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LIST OF ABBREVIATIONS

%	Percent
ºC	degree Celsius
±	Plus minus (varied)
DM	Diabetes Mellitus
ANOVA	Analysis of Variance
PPK	Polypeptide-k
HCl	Hydrochloric Acid
EtOH	Ethanol
g	gram
mg	milligram
ml	milliliter
S.E.M	Standard Error Mean
M. charantia	*Momordica charantia*
UPM	Universiti Putra Malaysia
α-amylase	alpha-amylase
α-glucosidase	alpha-glucosidase
Lada	latent autoimmune diabetes in adults

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Overview

Diabetes Mellitus (DM) is a chronic disease that is obtained through a genetic or acquired deficiency in the secretion of insulin and lack of organs response to insulin is secreted. This problem will cause damage to body systems, including damage to blood vessels and nerves. (Matsui *et al.*, 2007). According King, Aubert and Herman, 1998, DM at present is one of the chronic diseases that are very expensive and burdensome, and an increase in the proportion of outbreaks around the world. Diabetes affect nearly 5% of world population (WHO, 2002) and management of this disease without any side effects is a challenge for the world medical system. (Chakraborty *et al.*, 2002; Kameswararao *et al.*, 2003).

One of the approaches to restore and treat this disease is by lowering the post-prandial hyperglycemia. This is done by slowing the absorption of glucose by inhibits the carbohydrate-hydrolysing enzymes, such as α -glucosidase and α-amylase in the

digestive tract. Inhibition of this enzyme activity will slow down and prolong the digestion of carbohydrates. This will decrease the absorption of glucose and resulted in blunting the postprandial plasma glucose rise (Rhabasa *et al.*, 2004).Many natural resources were tested to determine its effect on suppression of glucose production from carbohydrates in the gut or glucose absorption from the intestine (Fernando *et al.*, 1991, Welsh *et al.*, 1989).

At present, many developing countries like Nepal have been using traditional medicine and herbal medicine is particular sometimes the only source for affordable healthcare (Bhattarai *et al.*, 1993; Manandhar *et al.*, 1995; Shrestha *et al.*, 1993). For developed countries, the use of herbal medicine by the sufferers of chronic disease is recommended because they are concerned about side effects of modern drug that contains many chemicals that can affect their health and has shown the effectiveness treatment. Using Medicines natural origin appears to offer more gentle means of managing disease and Poor (Hamdan *et al.*, 2004; Klepser *et al.*, 1999: WHO, 2002). Traditional medicines and herbs have been used extensively throughout the world because of their effectiveness, fewer side effects and relatively low cost. For this purpose, the study has begun to see effect of traditional Medicines from various cultures, as scientists search for clues to discover new therapeutic drugs for diabetes (Li *et al.,* 2004). Since time immemorial, Indian and Chinese Traditional Medicines have used plant and herbal extracts as anti-diabetic agents (Chen *et al*., 2001; Grover,*et al*., 2002). Therefore, investigations on agents from traditional medicinal plants have become more important and the scientists

are competing to find the new effective and safe therapeutic agent for the treatment of diabetes.

1.2 Hypothesis of the study

i. *Momordica charantia* possess a significant hypoglycemic effect and have potential benefit to treat diabetic disease.

ii. Essential oil and polypeptide-k from extraction product of *Momordica charantia* have inhibitor effect to α-amylase and α-glucosidase activity.

1.3 Objectives

i. To assess the effect of polypeptide-k and essential oil from *Momordica charantia* to α-amylase and α-glucosidase activity.

CHAPTER 2

LITERATURE REVIEW

2.1 Diabetes Mellitus

Diabetes mellitus is a chronic metabolic disorder caused by one for lack of insulin secretion, decreased insulin, or both. (Gardner *et al.,* 2007). Effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. Diabetes mellitus can be identified by a number of typical symptoms such as thirst, polyuria, blurred vision, and weight loss. In the most severe conditions, ketoacidosis or non-ketotic state hyperosmolar will arise and lead to fainting, coma, and if there is no effective treatment, it can lead to death. Usually the symptoms are not as severe or may not have showed any symptoms and consequences of hyperglycemia sufficient to cause pathological changes and changes in body function in a long time before the diagnosis is made. Long term effects of diabetes mellitus include progressive development of specific complications of retinopathy with potential blindness; nephropathy can lead to kidney failure, and neuropathy with risk of foot ulcer, amputation, Charcot joints, and features of autonomic dysfunction, including sexual dysfunction. People with

diabetes are high risk of cardiovascular disease, peripheral vascular disease and cerebrovascular. Several pathogenetic processes involved in the occurrence of diabetes. These include processes that destroy the pancreatic beta cells that would lead to a lack of insulin is produced, in addition, diabetes is also produced when resistance to insulin action. Abnormal metabolism of carbohydrates, fats and proteins, and defects occur when the lack of insulin activity on target tissues.

2.2 Classification of Diabetes

2.2.1 Type 1 Diabetes Mellitus

Diabetes mellitus type 1 can be classified as a loss of insulin producing beta cells in the island of Langerhans in the pancreas that produced insulin deficiency would result in the body. This type of diabetes can be interpreted as an immune mediated or idiopathic. Most of the properties of type 1diabetes are mediated immunity, in which the beta cells in the pancreas removed because of offensive T-cell mediated autoimmune. (Rother *et al.,* 2007.) Right now, there is no known preventive measure against type 1 diabetes, which causes approximately 10% of cases of diabetes mellitus in North America and Europe. Most people who suffer from this type of diabetes is in good health and did not experience any weight loss at first. In the early stages as well, and the effectiveness of insulin sensitivity is normal. 1diabetes type can affect children or adults, but are traditionally referred to as "diabetic children" because it is the majority of cases of This type of

diabetes, previously known as insulin dependent diabetes terms, type 1 diabetes, or diabetes, children's early, resulting from autoimmune mediated destruction of pancreatic beta cells. According to Zimmet *et al.,*1994, the rate of destruction is quite different for all people, quickly, in some individuals and slows in some of other individuals. Rapidly progressive destruction rates are generally observed in children, but can also occur in adults (Humphrey *et al.,* 1998.). Is slowly progressive form generally occurs in adults and is sometimes referred to as latent autoimmune diabetes in adults (Lada). Some patients, especially children and adolescents, may have ketoacidosis as the first manifestation of the disease diabetes. (Japan and its Pittsburgh Diabetes Research Group, 1985). But for most patients, moderate fasting hyperglycemia that would quickly turn into severe hyperglycemia and / or ketoacidosis caused by an infection or other stress. Some of the others, especially adults, may retain residual beta-cell function, simply to avoid ketoacidosis, for many years (Zimmet *et al.,* 1995). Individuals with diabetes type 1 are often dependent on insulin for life and the risk continues to experience ketoacidosis (Willis *et al.,* 1996.). Disease at this stage, there is little or no insulin secretion as spoken by low or no detectable levels of plasma C-peptide (Nielsen *et al.,* 1988.).

Markers of immune destruction, including islet sell autoantibodies , and / or autoantibody to insulin, and autoantibodies to glutamic acid decarboxylase (GAD) are present in 85-90% of individuals who suffer from diabetes mellitus type 1 diabetes when fasting hyperglycemia is initially detected (Verge *et al.,* 1996). Peak incidence of type1 diabetes this occurs in childhood and adolescence, but initially may have occurred at all

ages, from infancy to the ninth decade of life. (Molbak *et al.,* 1994.). In this case, there is a possibility of genetic involvement in the autoimmune destruction of beta cells, and it is also closely linked to environmental factors, but still not authentic. Although patients are usually not obese when they are suffering from this type of obesity is not inconsistent with the diagnosis. These patients may also have other autoimmune disorders such as Graves's disease, Hashimoto tyroiditis, and Addison's disease (Betterle *et al.,* 1983.).

2.2.2 Type 2 Diabetes Mellitus

Diabetes mellitus type 2 is shown as a defense of insulin that can be said as well as the relative reduction in insulin secretion. Response by the body tissue defects of insulin activity is believed to involve the insulin receptor itself. However, the specific defect is unknown. Diabetes mellitus due to a known defect are classified separately. Diabetes type 2 is the most common type. In the early stages of type 2 diabetes, the most dominant disorder that can be seen is a decrease in sensitivity to insulin. At this stage hyperglycemia can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce glucose production by the liver.

Diabetes mellitus types of diabetes known as non-insulin-dependent, or adult onset diabetes. This term is used for individuals who are insulin deficient relative (not absolute). Patients with diabetes type are often resistant to insulin action (DeFronzo e *et al.,* 1997:

Lillioja *et al.*, 1993.). At the outset, and throughout his life, these people do not need insulin treatment to survive. This type of diabetes usually cannot be diagnosed for years because the hyperglycemia it is usually not severe enough to cause obvious symptoms of diabetes (Mooy *et al.*, 1995; Harris *et al.,* 1993.), but such these patients run the risk of macrovascular and microvascular complications. (JM *et al.,* 1995).

Most of the patients with this type of diabetes is an obese, and obesity itself causes or aggravating insulin defense (Campbell *et al.,* 1993; Bogardus *et al.*, 1985). Most of them are not suffer from obesity and a traditional weight may have increased the percentage of body fat distribution, particularly to the pelvic area (Kissebah *et al.*, 1982.). Diabetic ketoacidosis is rare in this type, and if seen, it usually occurs when there is a correlation with stress and other illnesses such as infections (Banerji *et al.,* 1994; Umpierrez *et al.,* 1995). The patient has the disease of this type may have an normal or increased insulin, blood glucose levels higher in patients with diabetes is expected to arise despite the high insulin but have a functioning beta cells, normal (Polonsky *et al.,* 1996). As a result, insulin secretion is not normal and it not sufficient to provide compensation to the defense of insulin. On the other hand, some individuals have normal insulin activity, but have problems in terms of the secretion of insulin. Insulin sensitivity can be improved by weight reduction, increased physical activity, and pharmacological treatment of hyperglycemia but is not returned to normal (Simonson *et al.,* 1984; Wing *et al.,* 1994). Increased risk of type 2 diabetes occurs with age, obesity, and lack of physical activity (Zimmet *et al.,* 1992; Harris *et al.,* 1995). This often occurs in women and in individuals

suffering from hypertension or dyslexia. However, the frequency varies in different racial / ethnic sub-groups (Zimmet *et al.,* 1992, Harris *et al.,* 1995: T Valle *et al.,* 1997: John Wiley *et al.,* 1997). It is often associated with a strong family, the possibility of genetic trends (Valle *et al.,* 1997: John Wiley *et al.,* 1997: Knowler WC *et al.,* 1993). However, the genetics for this type of diabetes are quite complex and are not clear.

2.2.3 Gestational Diabetes Mellitus

Gestational diabetes mellitus (GDM) resembles type 2 diabetes in several aspects, which involves a combination of the terms of the relative lack of insulin secretion and responsiveness. It happened at around 2% -5% of all pregnancies and may improve or disappear after delivery. Gestational diabetes is fully treatable but requires careful monitoring in terms of medicine during pregnancy. About 20% -50% of women, who had lung cancer, may develop type 2 diabetes later in life. Even though it may be temporary, untreated gestational diabetes can have adverse effect on the health of the fetus or mother. Risks may occur in infants, including macrosomia (high birth weight), congenital heart anomalies and central nervous system and skeletal muscle abnormalities. Increased fetal insulin in the body can prevent fetal surfactant production and cause respiratory syndrome. Hyperbilirubinaemia may be generated from the destruction of red blood cells. In severe cases, prenatal death may occur, and most commonly is the result of abnormal placental perfusion due to vascular impairment. Induction to birth may be indicated by decreased

placental function. Caesarean section may be performed if any signs of stress in the fetus or increase the risk of injury associated with macrosomia, as distortion shoulder. A study in 2008, settled in the U.S. found that the number of American women entering pregnancy with preexisting diabetes increases. Even the level of diabetes in pregnant women has more than doubled in the last 6 years (Lawrence *et al.,* 2008.). This is a very member of the rising problem of diabetes and increased risk of complications during pregnancy. It also increases the potential that children of mothers who have diabetes will also become diabetic in the future.

2.3 Bitter gourd *(Momordica charantia)*

2.3.1 Overview of *Momordica charantia*

The bitter gourd (*Momordica charantia*) belong to the family Cucurbitaceae is found throughout the tropics, probably has been used by human and their immediate ancestors species for at least a million years. *Momordica charantia* (bitter gourd) is a plant native to the semi-tropical climate of China, India, Asia and Africa. This plant traditionally used medicinal herbs as, anti-HIV, anti-ulcer, anti-inflammatory, anti-microbial, anti-diabetic, and anti-tumor (Taylor *et al.,* 2002; Grover *et al.,* 2004) and is one of the most promising alternative medicines for the disease. Momordica means, “to bite” referring to the jagged edges of the leaf, which appear as if bitten. All parts of the plant, including the fruit, taste bitter. The fruit is oblong and resembles a small cucumber. The young fruit is emerald green that turns to orange-yellow when ripe. At maturity the fruit splits into three

irregular valves that curl backwards and release numerous brown or white seeds encased in scarlet arils (Grover *et al.*, 2004). *Momordica charantia* has been used traditionally as medicine in developing countries like Brazil, China, Colombia, Cuba, Ghana, Haiti, India Mexico, Malaya, New Zealand, Nicaragua, Panama and Peru. Some of its common uses in most countries are for diabetes, as a carminative and in treatment of colics (Yesilada *et al.,* 1999; Satyawati *et al.,* 1987).

Figure 2.1: Fruit of *Momordica charantia* plant.

Figure 2.2: Flower of *Momordica charantia* plant.

Figure 2.3: Rind of *Momordica charantia* plant.

Figure 2.4: Seeds from a ripe fruit of *Momordica charantia.*

2.3.2 Ethnobotanical uses of *Momordica charantia*

Country	Uses
Brazil	Abortifacient, anthelmintic, aphrodisiac, burn, catarrh, colic, dermatosis, diabetes, diarrhea, eczema, emetic, emmenagogue, emollient, fever, febrifuge, hemorrhoids, hepatitis, hypoglycemic, inflammation (liver), leprosy, leucorrhoea, leukemia, malaria, menstrual colic, pain, pruritus, purgative, rheumatism, scabies, skin, tumor, vaginitis, vermifuge, wound,
China	Aphrodisiac, cancer (breast), diabetes, food, glucosuria, halitosis, hematuria, polyuria, refrigerant
Colombia	Bite (snake), malaria
Cuba	Anemia, colitis, emmenagogue, fever, hepatosis, hypoglycemic, kidney (stone), sterility (female), vermifuge
Ghana	Aphrodisiac, dysentery, fever, gonorrhea
Haiti	Anemia, appetite stimulant, dermatosis, eye, fever, insecticide, laxative, liver, sk in, rage, rhinitis
India	Abortifacient, anthelmintic, bite(snake), contraceptive, diabetes mellitus, dysmenorrhea, eczema, emmenagogue, fat loss, fever (malarial), galactagogue, gout, hydrophobia, hyperglycemia, jaundice, kidney (stone), laxative, leprosy,

	leucorrhea, liver, piles, pneumonia, psoriasis, purgative, rheumatism, scabies, skin, tonic, vegetable
Mexico	Aphrodisiac, burn, diabetes, dysentery, purgative, scabies, sore, vermifuge
Malaya	Abdomen, asthma, burn, dermatosis, diarrhea, headache, scald, sprue, stomachache, vermifuge,
Panama	Cold, emmenagogue, diabetes, fever, gallbladder, hypertension, insecticide, malaria, pruritus
Peru	Colic, contusions, diabetes, diarrhea, emetic, emmenagogue, febrifuge, hepatitis, inflammation, lung, malaria, measles, purgative, skin (sores), suppurative, vermifuge, wound
Trinidad	Diabetes, dysentery, fever, hypertension, malaria, rheumatism, vermifuge

Table 2.1: Worldwide Ethnobotanical uses of *Momordica charantia* (Adapted from Leslie Taylor, 2002)

2.3.3 Phytochemistry of *Momordica charantia*

Momordica charantia contains biologically active compound that include glycosides, saponins, alkaloids, fixed oils, triterpenes, proteins and steroids (Raman and Lau, 1996). Several phytochemicals such as momorcharins, momordenol, momordicilin, momordicins, momordicinin, momordin, momordolol, charantin, charine, cryptoxanthin, cucurbitins, cucurbitacins, cucurbitanes, cycloartenols, diosgenin, elaeostearic acids, erythrodiol, galacturonic acids, gentisic acid, goyaglycosides, goyasaponins and multiflorenol have been isolated (Husain *et al*., 1994; Xie*et al*., 1998;Yuan *et al*., 1999; Parkash*et al*., 2002).The hypoglycemic/ antihyperglycemic chemicals of *Momordica charantia* are a mixture of steroidal saponins known as charantins, insulin-like peptides and alkaloids (Raman and Lau, 1996) and these chemicals are concentrated in fruits of *Momordica charantia* (Ali *et al*., 1993).

There are two types of hypoglycemic substances in *Momordica charantia* with different time dependent effects. The first one with fast antihyperglycemic activity of around 1 h present in the aqueous and the residue after alkaline chloroform extraction

of aqueous extract and another with a slow hypoglycemic activity in acidic wash of the chloroform extract remaining after alkaline water wash (Day et al., 1990). *Momordica charantia* also contain HIV inhibitory proteins like MRK29 (MW: 28.6 kDa), MAP30 (MW: 30,000 kDa) and lectin (Putnam and Tainer, 2000). Protein (MAP30) has potential for the treatment of HIV and a host of other infections. It would be better if MAP30 are used in combination with current antiretroviral drugs. The presence of trypsin inhibitors (Miura and Funatsu, 1995), elastase inhibitors (Hamato*et al*., 1995), guanylatecyclase inhibitors (Vesely*et al*., 1977; Takemoto*et al*., 1980) and alpha-glucosidase inhibitor like D-(+)-trehalose in *Momordica charantia* are well reported (Matsuur *et al*., 2002).

CHAPTER 3

MATERIAL AND METHOD

3. 1 Material and sources

Material	Source
Plants–*Momordica charantia* (essential oil and polypeptide-k)	Were purchases from Magna Mission SDN. BHD
Assay Kits – - QuantiChrom™ α-Amylase Assay Kit - QuantiChrom™ α-Glucosidase Assay Kit	Purchase from BioAssay Systems Co. (Hayward,U.S.A).
Enzymes – - α-Glucosidase Enzyme(EC 3.2.1.20) - α-Amylase Enzyme (EC 3.2.1.10)	Purchase from Sigma Chemical Co. (St. Louis, MO)

Table 3.1: Material and sources

3.1.1 Preparation of essential oil and polypeptide-k

Essential oil and PPK was obtained from the dried seeds of *Momordica charantia*. Essential oil was obtained by hydrodistillation of the dried seed using the Clevenger-type apparatus. Air dried seeds of the *Momordica charantia* were ground and subjected to hydrosillation for 3 hours by use of a Clevenger-type apparatus. The essential oil was isolated and dried over anhydrous Sodium Sulphate to remove trace of moisture (Cabral, 2005).

Accordingly to European Patent Specification, method for the extraction of PPK from the seeds of *Momordica charantia* comprising the steps of;

(i) Providing dry seeds from the fruit of *Momordica charantia*;

⇩

(ii) Pulverizing the seeds to fine powder;

⇩

(iii) Treating the fine powder obtained in step (ii) with acetone hexane solvent mixed in the ratio 1:2

⇩

(iv)Dissolving the residual mass in 20% acetone;

⇩

(v) Adjusting the pH to 9. 5 with an organic buffer;

⇩

(vi)Buffering the supernatant to pH 3 by adding sulfuric acid, to obtain a flocculant precipitate;

⇩

(vii) Collecting the precipitate; and

⇩

(viii) Crystallising the polypeptide

3.2 Chemical and drug

- Dimethyl sulfoxide (*DMSO*)
- Ethanol
- Tween 20
- Distilled Water

3.3 Experimental design

3.3.1 Dissolving of essential oil and Polypeptide-K (PPK)

Essential oil is in the form of liquid oil whereas PPK are in the powder form. Both of these samples should dissolve to facilitate the reaction of PPK and essential oil with assays. Essential oil was dissolved in ethanol, tween 20 and distilled water in ratio 0.5:0.5:9. PPK were dissolved in 20% DMSO and distilled water with ratio 1: 9. Ensure that the sample products resulting from the dissolving is concentrated 2mg/ml. (A. Braca *et al*., 2008)

3.3.2 Serial dilution

After dissolving of samples was done, the serial dilution performed on both of the samples. Serial dilutions are performed by using 1.78-fold (100.25-fold) dilution factor or called as quarter-logarithmic dilution or quarter-log dilution. The initial concentration for both of the samples is 2 mg/ml. From this serial dilution, we can get 8 samples with different concentrations which is (2.000, 1.500, 1.125, 0.844, 0.633, 0.475, 0.356, 0.267) mg/ml.(K. R. Aneja., 2005)

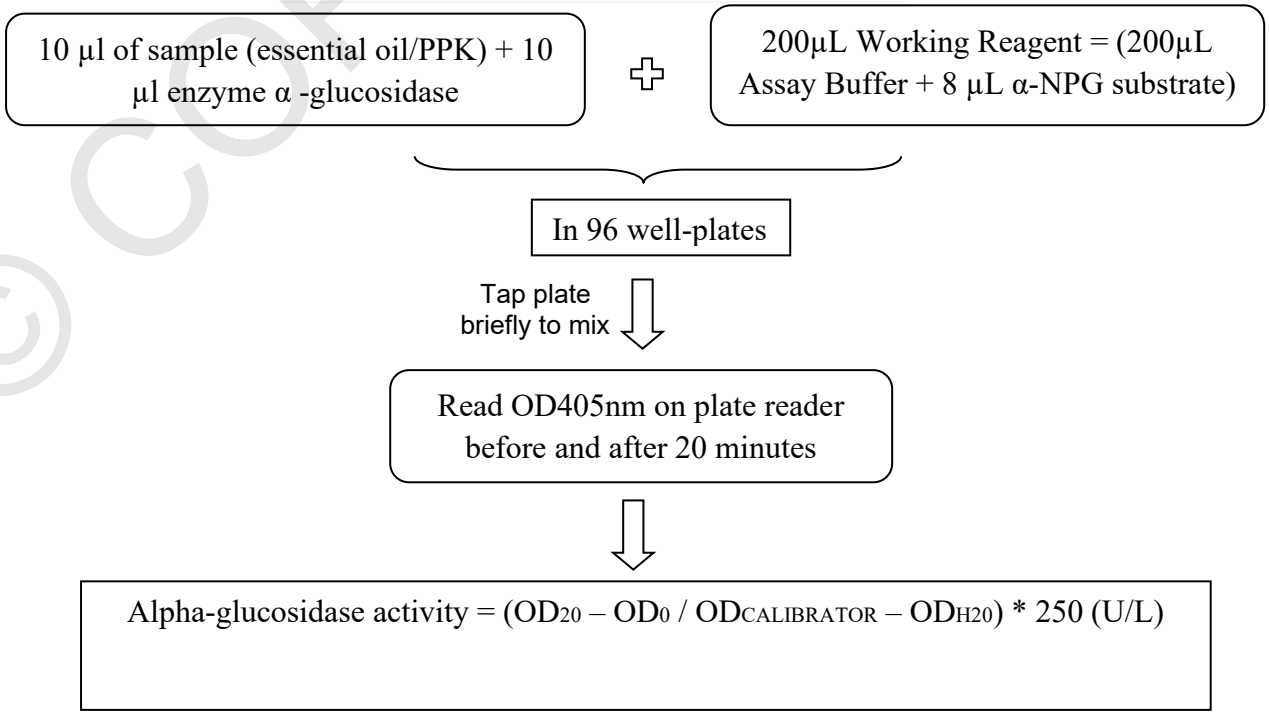
3.3.3 Assayed the samples

3.3.3.1 Determination of α-glucosidase inhibitory activity by essential oil and polypeptide-k (PPK)

The bioassay method was adopted and modified from Bioassay System (Yamamoto *et al,* 2004). This assay is based on a kinetic reaction. The QuantiChrom™ α-Glucosidase Assay Kit contents is

- Assay Buffer :24ml (Ph 7.0)
- α-NPG Substrate : 1 ml
- Calibrator : 10 ml (equivalent to 250 U/L)

The steps of method are shown below.



OD_{20} and OD_0 are OD_{405nm} values of sample at 20 and 0 min, respectively. $OD_{CALIBRATOR}$ and OD_{H20} are OD405nm values of calibrator and H_2O at 20 min.

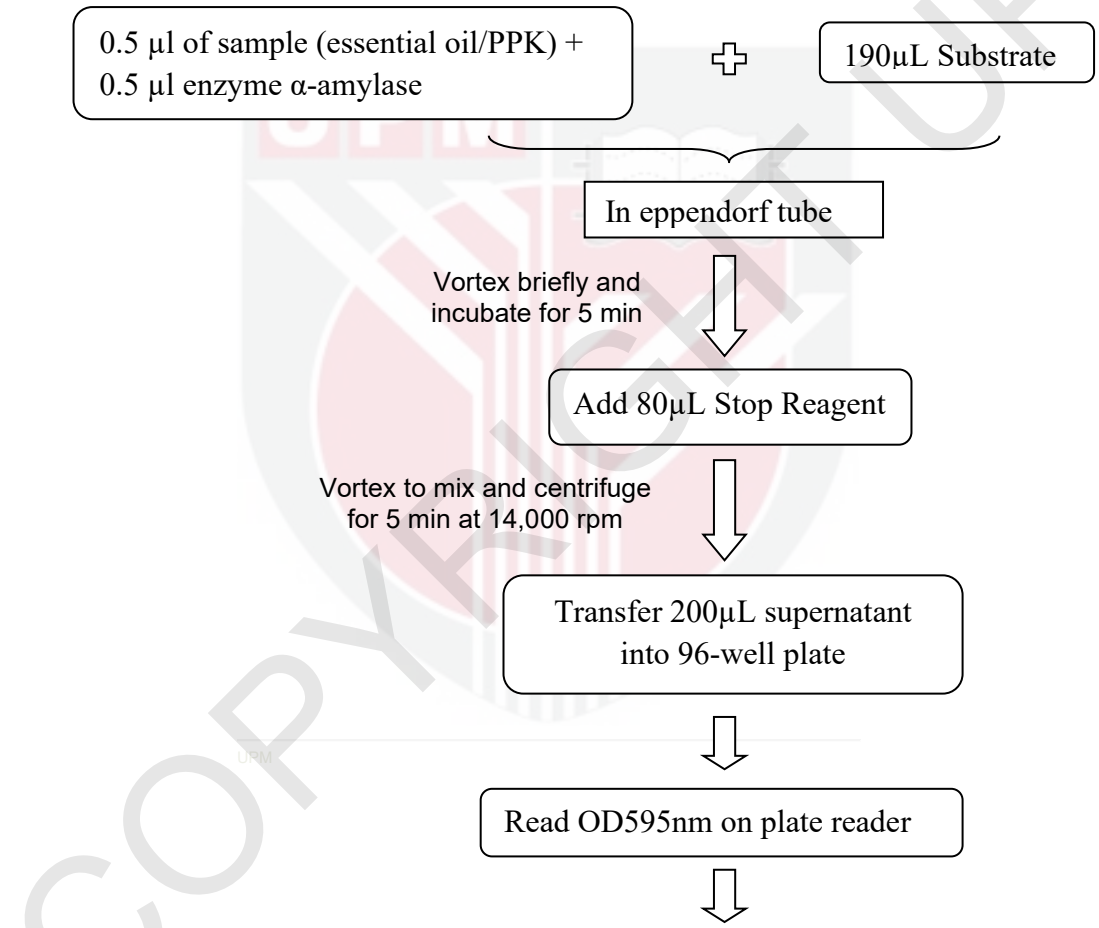
3.3.3.2 Determination of α-amylase inhibitory activity by essential oil and polypeptide-k (PPK).

The bioassay method was adopted and modified from Bioassay System (Rindeknecht H et.al., 1967) The QuantiChrom™ α-Amylase Assay Kit contents is:

- Substrate (Ph 7.0) : 2 ml
- Stop reagent : 10 ml

- Calibrator : 2 ml (equivalent to 550 U/L)

The steps of method are shown below.



$$\text{Alpha-amylase activity} = (OD_{SAMPLE} - OD_{BLANK} / OD_{CALIBRATOR} - OD_{H20}) * 25(U/L)$$

Where OD_{SAMPLE} and OD_{BLANK} are the OD 585nm values of the sample and blank respectively. OD_{CAL} and OD_{H2O} are the OD 595 nm values of the calibrator and water.

3.3.5 Spectrometric analysis

Spectrometric analysis is performed by using UVM 340 Monochromator Based Microplate Reader. For α -amylase assay, we read OD595nm on plate reader once for each experiment whereas for α-glucosidase assay, we read OD405nm before and after 20 min for each experiment. Then, the activity of enzyme is calculated by using equation shown at procedure steps.

3.4 Statistical analysis

Data was expressed as mean ± S.E.M. the results of ulcer scoring and scoring data of histopathology study was analyzed with non parametric statistical analysis by Kruskal-Wallis test, followed by Man-Whitney U test for comparison within the group. For the ulcer index data was analyzed with one way ANOVA, followed by Dunnets multiple comparison test post-hoc comparison of group means. For all tests, effect of probability of $p<0.05$ were considered significant.

CHAPTER 4

RESULT

4.1 Enzyme activity

4.1.1 α-Glucosidase activity by polypeptide-k (PPK)

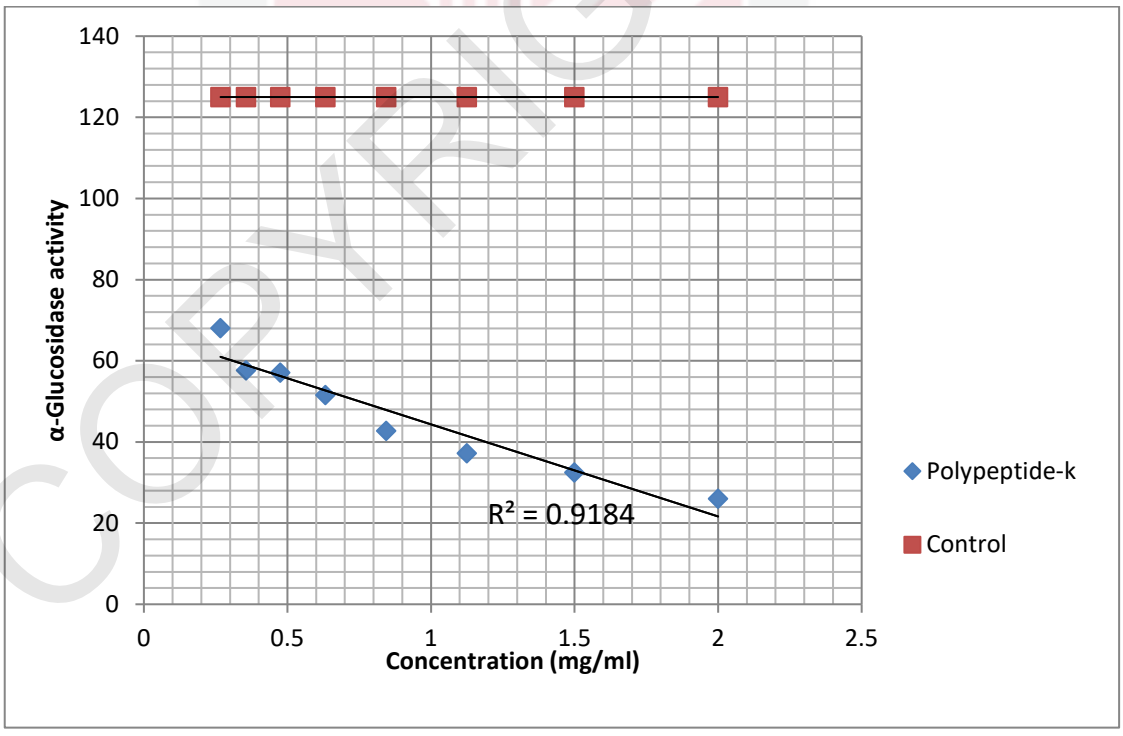


Figure 4.1: Dose-dependent inhibition of α-glucosidase activity by Polypeptide-k.

Table 4.1: Effect of polypeptide-k on α-glucosidase activity.

Concentration (mg/ml)	α -Glucosidase activity (mean±SEM)	α –Glucosidase inhibition (%)
0.267	70.6628±5.0856	45.55
0.356	51.4710±9.9259	53.89
0.475	55.4295±2.9121	54.33
0.633	48.5088±3.6516	58.73
0.844	42.7553±4.6282	65.79
1.125	37.2303±4.8153	70.21
1.5	32.4798±2.0861	74.01
2	26.0133±2.5803	79.18
Total	45.568±3.1322	

All values are mean±SEM, n = 8

As shown in figure 4.1, the graph shows activity of α-glucosidase enzyme versus concentration (mg/ml) of polypeptide-k. From that graph, we can see that polypeptide-k from *Momordica charantia* showed a dose dependent and significant inhibition to activity of α-glucosidase enzyme activity. The higher concentration of polypeptide-k shows the most effective and strong inhibition to enzyme activity. The highest concentration as 2mg/ml showed the lowest activity of enzyme which has 26.0133±2.5803 enzyme activity and highest percent of inhibition (79.18%) whereas the lowest concentration or 0.267mg/ml showed the highest enzyme activity or 70.6628±5.0856 enzyme activity and the lowest percent of inhibition which is 45.55%

4.1.2 α Glucosidase activity by essential oil

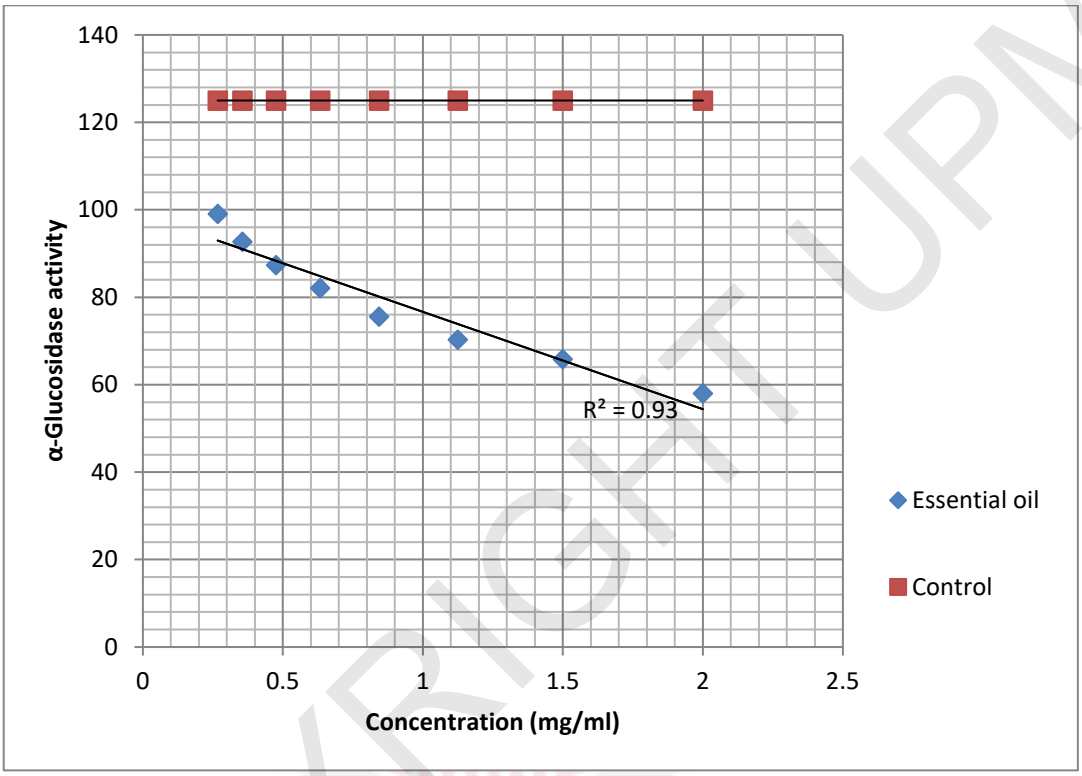


Figure 4.2: Dose-dependent inhibition of α-glucosidase by Essential oil

Table 4.2: Effect of essential oil on α-glucosidase activity.

Concentration (mg/ml)	α -Glucosidase activity (mean±SEM)	α -Glucosidase inhibition (%)
0.267	97.0091±1.0726	20.71
0.356	90.7401±1.1301	25.84
0.475	85.6295±3.0594	30.09
0.633	80.4882±3.4838	34.30
0.844	74.0152±2.1990	39.55
1.125	68.8970±2.5743	43.75
1.5	64.5213±2.0793	47.31
2	56.8900±2.9721	53.55
Total	77.2738±2.7652	

All values are mean±SEM, n = 8

As shown in figure 4.2, the graph shows activity of α-glucosidase enzyme versus concentration (mg/ml) of essential oil. From that graph, we can see that the essential oil from *Momordica charantia* showed a dose dependent and significant inhibition to activity of α-glucosidase enzyme. The higher concentration of essential oil shows the most effective and strong inhibition to enzyme activity. The highest concentration as 2mg/ml showed the lowest activity of enzyme which has 56.8900±2.9721 enzyme activity and highest percent of inhibition (53.55%) whereas the lowest concentration or 0.267mg/ml showed the highest enzyme activity or 97.009±1.0726 enzyme activity and lowest percent of inhibition (20.71%).

4.1.3 α -Amylase activity by polypeptide-k (PPK)

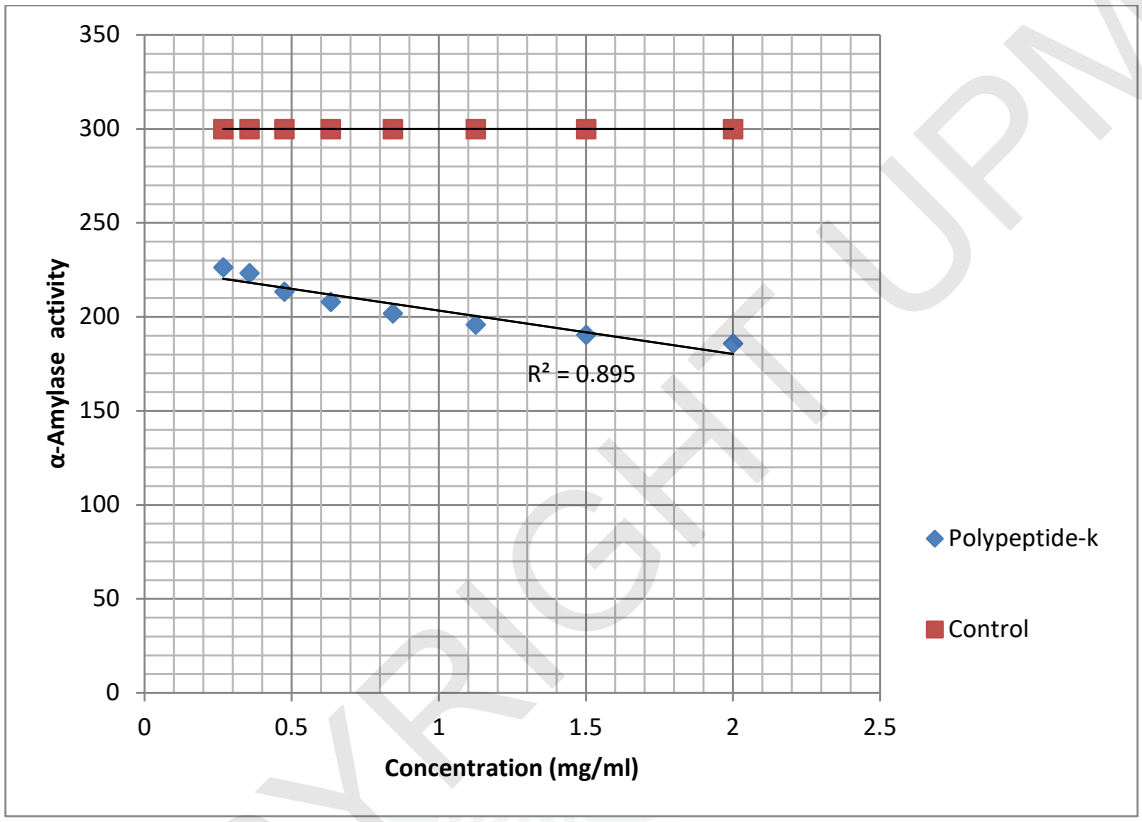


Figure 4.3: Dose-dependent inhibition of α-amylase by polypeptide-k

Table 4.3: Effect of polypeptide-k on alpha-amylase activity**.**

Concentration (mg/ml)	α-Amylase activity (mean±SEM)	α-Amylase inhibition (%)
0.267	226.3502±9.3639	18.20
0.356	223.3028±9.3302	21.54
0.475	213.3901±8.4586	22.42
0.633	208.0655±9.1613	25.43
0.844	201.9705±9.0938	28.09
1.125	195.8756±9.0263	31.10
1.5	190.5341±8.2053	32.88
2	185.9291±5.1072	35.58
Total	205.6772±4.2033	

All values are mean±SEM, n = 8

As shown in figure 4.3, the graph shows activity of α-amylase enzyme versus concentration (mg/ml) of polypeptide-k. From that graph, we can see that the polypeptide-k from *Momordica charantia* showed a dose dependent and significant inhibition to activity of α-amylase enzyme. The higher concentration of polypeptide-k shows the most effective and strong inhibition to enzyme activity. The highest concentration as 2mg/ml showed the lowest activity of enzyme which has 205.677±4.2033 of enzyme activity and has highest percent of inhibition (35.58%) whereas the lowest concentration or 0.267mg/ml showed the highest enzyme activity or 226.350±9.3639 of enzyme activity and lowest percent of inhibition(18.20%).

4.1.4 α -Amylase activity by essential oil

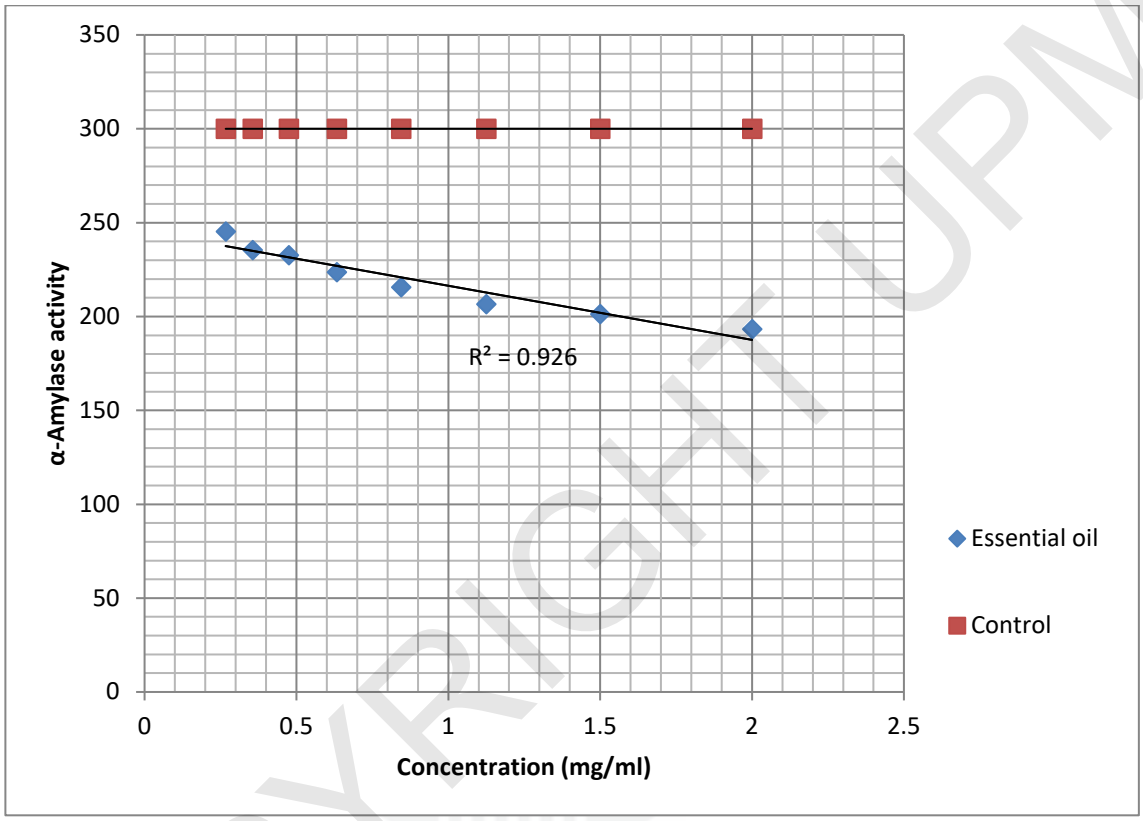


Figure 4.4: Dose-dependent inhibition of α-amylase activity by essential oil

Table 4.4: Effect of essential oil on α-amylase activity.

Concentration (mg/ml)	α -Amylase activity (mean±SEM)	α -Amylase inhibition (%)
0.267	245.3911±9.8317	24.54
0.356	235.3548±12.4998	25.56
0.475	232.7123±12.9344	28.86
0.633	223.6822±12.3970	30.64
0.844	215.7165±12.3385	32.67
1.125	206.6906±13.2289	34.70
1.5	201.3527±11.8301	36.48
2	193.2560±7.6110	38.02
Total	219.2696±4.9496	

All values are mean±SEM, n = 8

As shown in figure 4.4, the graph shows activity of α-amylase enzyme versus concentration (mg/ml) of essential oil. From that graph, we can see that the essential oil from *Momordica charantia* showed a dose dependent and significant inhibition to activity of α-amylase enzyme. The higher concentration of essential oil shows the most effective and strong inhibition to enzyme activity. The highest concentration as 2mg/ml showed the lowest activity of enzyme which has 193.256±7.6110 of enzyme activity and has highest percent of inhibition (38.02%) whereas the lowest concentration or 0.267mg/ml showed the highest enzyme activity or 245.391±9.8317 of enzyme activity and lowest percent of inhibition(24.54%).

4.2 Comparisons

4.2.1 α- Glucosidase activity by essential oil and polypeptide-k (PPK).

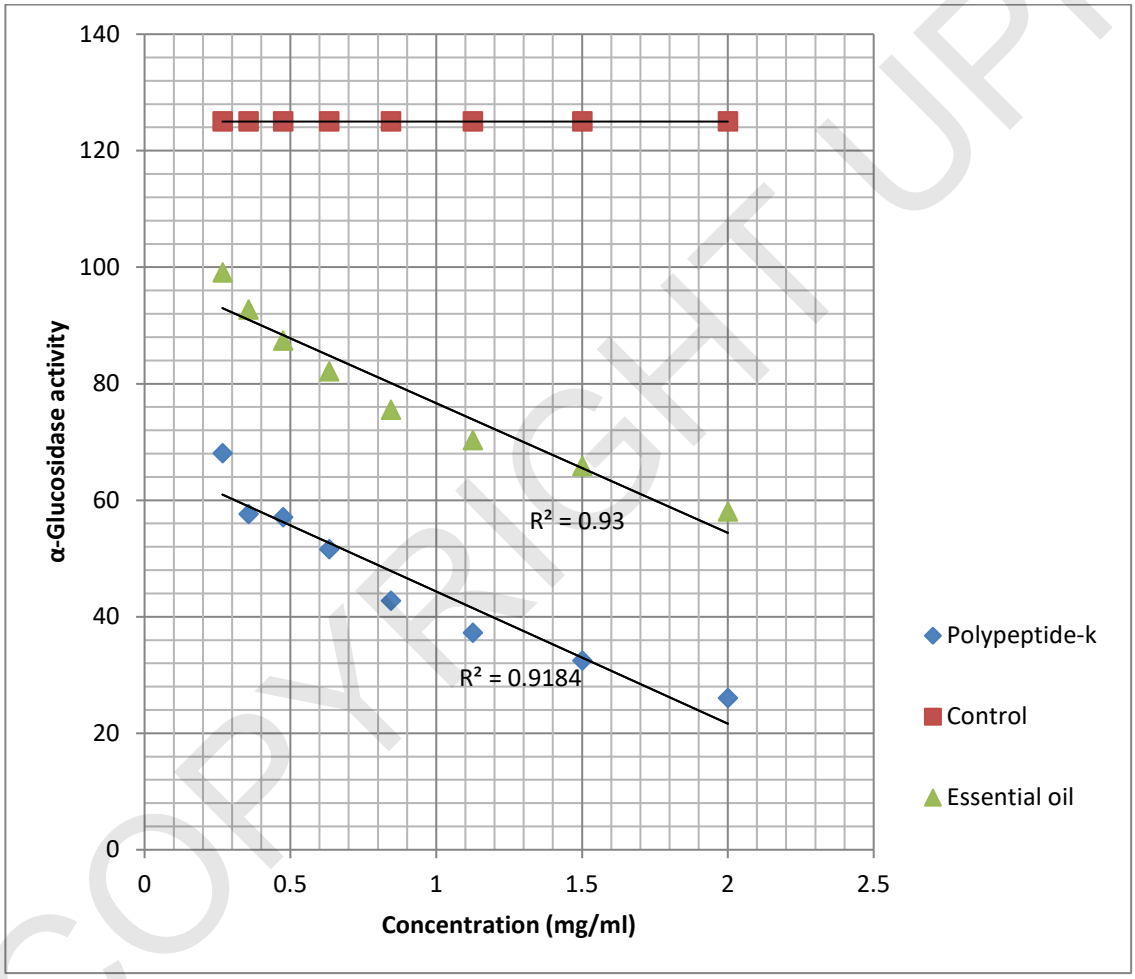


Figure 4.5: Dose-dependent inhibition of α-glucosidase activity by polypeptide-k and essential oil.

Table 4.5: Effect of essential oil and polypeptide-k on α-glucosidase activity.

Concentration	α-Glucosidase activity by Polypeptide-k (mean±SEM)	α-Glucosidase activity by essential oil (mean±SEM)
0.267	70.6628±5.0856	97.0091±1.0726
0.356	51.4710±9.9259	90.7401±1.1301
0.475	55.4295±2.9121	85.6295±3.0594
0.633	48.5088±3.6516	80.4882±3.4838
0.844	42.7553±4.6282	74.0152±2.1990
1.125	37.2303±4.8153	68.8970±2.5743
1.500	32.4798±2.0861	64.5213±2.0793
2.000	26.0133±2.5803	56.8900±2.9721
Total	45.5689±3.1322	77.2738±2.7652

All values are mean±SEM, n = 8

From the figure 4.5, that shows graph of α-glucosidase enzyme activity against the concentration of samples, essential oil and the polypeptide-k. This graph is to make a comparison between the action of polypeptide-k and essential oil to activity of α-glucosidase enzyme. As we can see, both of the samples show inhibition action to enzyme activity. The two samples with the equivalent concentration showed the same rate of inhibition to enzyme activity. However, polypeptide-k showed a relatively high rate of inhibition and effective than essential oil to inhibit this enzyme. As we can see from table 4.5, the total activity α-glucosidase activity treated by Polypeptide-k which has 45.5689±3.1322 and its lower than total α-glucosidase activity treated by essential oil which has 77.2738±2.7652. From the graph, we can conclude that, concentration of samples inversely proportional to the activity of α-glucosidase enzyme.

4.2.2 α-Amylase activity by polypeptide-k and essential oil

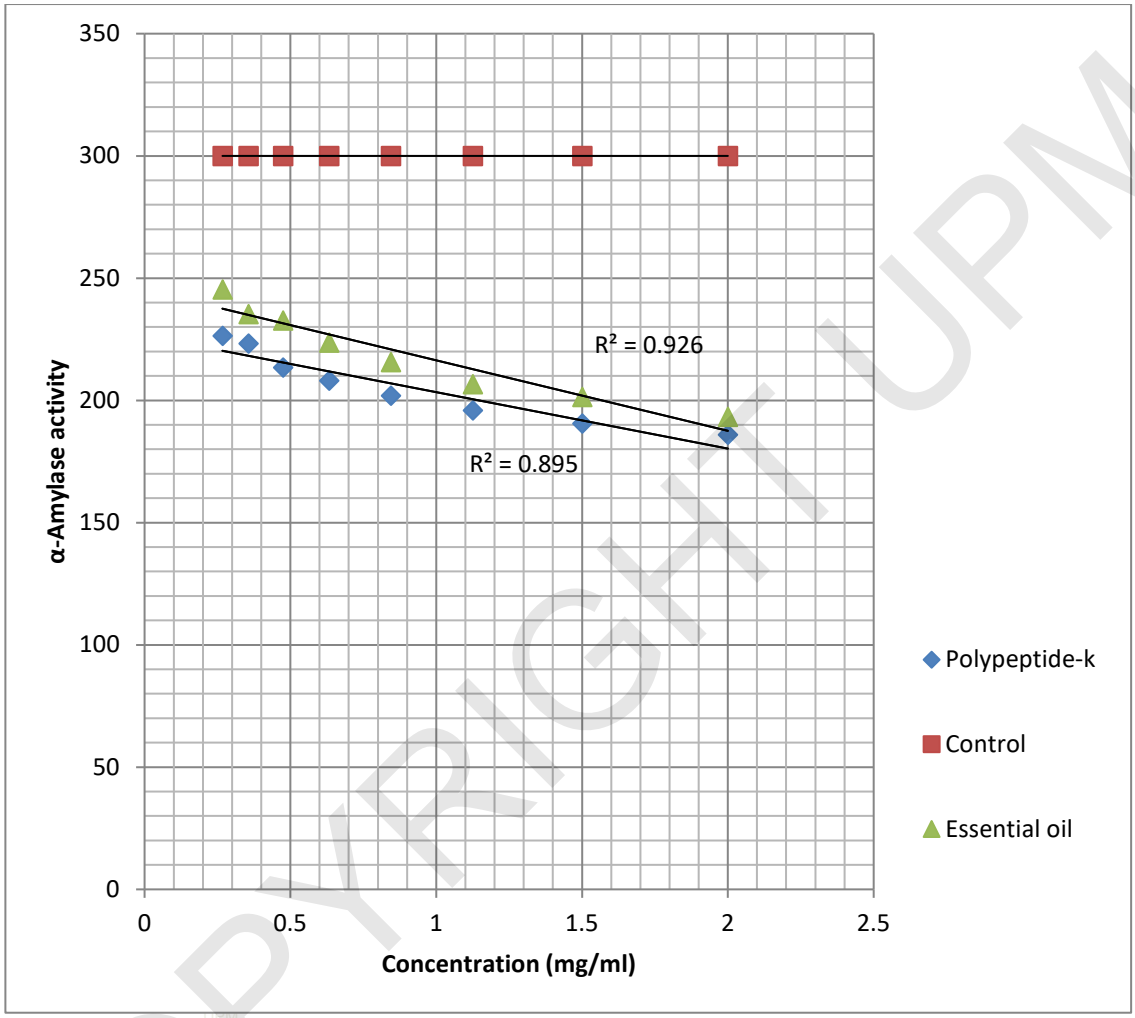


Figure 4.6: Dose-dependent inhibition of α-amylase activity by polypeptide-k and essential oil.

Table 4.6: Effect of essential oil and polypeptide-k on α-amylase activity.

Concentration	α-Amylase activity by polypeptide-k (mean±SEM)	α-Glucosidase activity by essential oil (mean±SEM)
0.267	226.3502±9.3639	245.3911±9.8317
0.356	223.3028±9.3302	235.3548±12.4998
0.475	213.3901±8.4586	232.7123±12.9344
0.633	208.0655±9.1613	223.6827±12.3970
0.844	201.9705±9.0938	215.7165±12.3385
1.125	195.8756±9.0263	206.6906±13.2289
1.500	190.5341±8.2053	201.3527±11.8301
2.000	185.9291±5.1072	193.2560±7.6110
Total	205.6772±4.2033	219.2696±4.9496

All values are mean±SEM, n = 8

From the figure 4.6, that shows graph of α-amylase enzyme activity against the concentration of samples, essential oil and the polypeptide-k. This graph is to make a comparison between the action of polypeptide-k and essential oil to activity of α-amylase enzyme. As we can see, both of the samples show inhibition action to enzyme activity. The two samples with the equivalent concentration showed the same rate of inhibition to enzyme activity. However, polypeptide-k showed a slightly high in rate of inhibition than essential oil to inhibit this enzyme. As we can see from table 4.6, the total activity α-amylase activity treated by polypeptide-k which has 205.6772±4.2033 and its lower than total α-glucosidase activity treated by essential oil which has 219.2696±4.9496. From the graph, we can conclude that, concentration of samples inversely proportional to the activity of α-glucosidase enzyme.

CHAPTER 5

DISCUSSION

Diabetes mellitus (DM) is a chronic disease caused by genetic inheritance or by reason of lack of insulin secreted and the lack of response by the organ to secrete insulin. Lack of insulin secretion would lead to higher rates of glucose in the blood, and also will cause damage to the body system, including blood vessels and nerves. (Matsui *et al.,* 2007). DM is currently one of the chronic diseases and has taken millions of money in the deal. it is very burdensome and is more infectious disease outbreaks around the world (King *et al.,* 1998). According to (World Health Organization) WHO, in 2002, diabetes disease affects about 5% of the world's population. For the management of diabetes without any side effects, it is still a challenge for the medical system (Chakraborty *et al.*, 2002; Kameswararao *et al.,* 2003).

The purpose of treatment for patients with diabetes is to maintain the rate of glucose in the body back to near normal glycemic control, either when fasting or after the meal. Lot of natural resources has been investigated in relation to the inhibition of glucose production from carbohydrates in the gut or the intestinal absorption of glucose (Matsui

et al., 2007.). α-Amylase and α-glucosidase is the enzymes that hydrolyze carbohydrates into complex forms. α-Amylase catalyses the hydrolysis of

α--1-4-glucosidic linkages of starch, glycogen and other attractions Oligosaccharides and α-glucosidase further breakdown the disaccharides into simpler form, readily available for the intestinal absorption. Inhibition activity of this enzyme in the human digestive tract, considered as an effective way to control diabetes by reducing the absorption of glucose from starch broken down by enzymes (Hara & Honda, 1990). Therefore, for α-amylase inhibitor and α-glucosidase is effective and safe has long sought.

In this study we have investigated the anti-diabetic potential of the *Momordica charantia* (Cucurbitaceae), is one such plant that has been frequently used as traditional medicine (Giron *et al*., 1991; Lans and Brown, 1998). *Momordica charantia* has been traditionally used as medicinal herb in treating HIV, inflammation, antileukemic, microbial infection, diabetes mellitus, and for tumor (Taylor, 2002; Grover and Yadav, 2004). MC is most widely studied with regard to its antidiabetic effect and all parts of the plant (fruit pulp, seed, leaves and whole plant) have shown hypoglycemic activity in normal animals (Bailey *et al.,* 1985; Day *et al.,* 1990; Shibib *et al.,* 1993; Ali *et al.,* 1993; Cakici *et al.,* 1994; Sarkar *et al.,* 1996 and Jayasooriya *et al.,* 2000); and antihyperglycemic activity in alloxan (Akhtar, 1982; Karunanayake *et al.,* 1984; Singh *et al.,* 1989; Pari *et al.,* 2001; Rathi *et al.,* 2002a and Kar *et al.,* 2003) or streptozotocin-induced (Kedar and Chakrabarti, 1982; Bailey *et al.,* 1985; Day *et al.,* 1990; Karunanayake *et al.,* 1990;Higashino *et al.,* 1992; Shibib *et al.,* 1993; Sarkar *et al.,* 1996;

Ahmed *et al.,* 1998; Ahmed *et al.,* 2001; Sitasawad *et al.,* 2000; Grover *et al.,* 2002 and Rathi *et al.,* 2002a) as well as genetic models of diabetes (Miura *et al.,* 2001).

In this study, we are used the essential oil and the polypeptide-k derived from the extraction product of *Momordica charantia.* Essential oils are in liquid oil form while the polypeptide-k is in the powder form. For use both of the sample in the assay kit, we were dissolving the samples into a liquid form that is suitable to react with enzymes and chemicals in the assay kit. Dissolving results for each sample was concentrated 2 mg /ml. In this study, we attempted to investigate the effect of sample concentration on the activity of alpha-amylase enzyme and alpha-glucosidase. So, a serial dilution (3 / 4 dilution factor) is practiced to reduce the concentration regularly. Sample concentration resulting from the serial dilution was (2.000, 1.500, 1.125, 0.844, 0.633, 0.475, 0.356, and 0.267) mg/ml.

From the experiment result that has been obtained, essential oil and polypeptide-k has a significant inhibition effect on activity of α -amylase enzyme and α -glucosidase enzyme but different effectiveness and strengthens to inhibit enzyme activity. From 8 dose from the two samples were tested, we can see that the all dose sample has inhibition effect to activity of α -amylase enzyme and α -glucosidase enzyme. From observation, the highest dose of the sample showed the lowest enzyme activity among all samples tested. Therefore, the sample concentration is inversely proportional to the enzyme activity for the both graph.

From the graph that shows α -glucosidase activity, we can see that polypeptide-k and essential oil inhibit the activity of α -glucosidase. Both of the samples that equivalent in the concentration showed similar inhibition rates but differ in the quality and strengthens of inhibition. From the graph, polypeptide-k showed the higher inhibition rates than essential oil. From the graph that show α -amylase activity versus concentration, polypeptide-k and essential oil also show inhibition to the activity to this enzyme but the rate of inhibition for both of the samples are not significantly difference. However, polypeptide-k still shows high inhibition rates than essential oil.

In my study, there are 2 factors that contribute to the effectiveness and strengthen of polypeptide-k to inhibit enzyme activity especially α -glucosidase enzyme. According to Khanna *et al.,* polypeptide-k comprising 160 amino acid residues and from this, it contain 18 standards amino acid. Standard amino acid is twenty-two amino acids are naturally incorporated into polypeptides and are called proteoigenic or natural amino acids. In polypeptide-k, this 18 standard amino acid incorporated to form proteins – that have hypoglycemic effect and works similar manner as human insulin. (Khanna *et al.,* 2004). Compare to the composition of essential oil, according to the A. Braca *et al.,* twenty-five compounds were identified in the seed oil of M. charantia amounting to 90.9% of the total oil. The constituents are represented by sesquiterpenes (10.1%), phenylpropanoids (11.0%), and monoterpenes (7.6%), trans-nerolidol being the major constituent (61.6%). But from these 4 constituents, only 2 of them are shown

hypoglycemic effect which is sesquiterpenes (10.1%) and monoterpenes (7.6%). (W.L Li *et al*., 2004). So, we can see that only 17.7% of the total 90.9% oil is useful for hypoglycemic effect.

Second factor that make polypeptide-k is better inhibitor than essential oil is because of their structural. Based on Thomas *et al.,* from his book (Structure, Function, and Genetics) reported that structural of polypeptide-k is slightly same with structural of human insulin. It show 34% homology to human insulin if we merged this 2 structural.

However, in this study, inhibition of enzyme activity of the individual compounds tested in vitro, the results of this study must be relevant to the human body. Inhibition the activity of enzymes α-glucosidase and α-amylase from the isolated compound was also reported to have been using a model in vivo and in vitro. (Barca *et al.,* 2003; Hara & Honda, 1990;.He *et al.*, 2006; Matsui *et al.,* 2007, Wan *et al.,* 2004). In addition to inhibition of enzyme activity α-amylase and α-glucosidase, isolated compound was also reported several other biological effects including anti-bacterial, anti-oxidative, etc., anti-cancer (He *et al.,* 2006.). Evidence supporting this has increased the importance of herbal medicine *Momordica charantia*, and it shows that this herb is not only beneficial for diabetes, but may also be useful for others health problem.

CHAPTER 6

CONCLUSION

As a conclusion, the enzyme α-glucosidase and α-amylase is enzymes that digest carbohydrates into complex shapes. Inhibitor of the enzyme α-glucosidase and α-amylase activity would interfere with these two-enzyme in the brush border of small intestine, and it may delay the release of D-glucose from oligosaccharides and disaccharides, thus delaying the absorption of glucose and decrease postprandial glucose levels. In this study, essential oil and polypeptide-k of *Momordica charantia* show significantly inhibit activity of α-glucosidase and α-amylase activity thus can significantly reduce the post-prandial increase of blood glucose and therefore can be an important strategy in the management of blood glucose level in type 2 diabetic and borderline patients. From the results that have been obtained, the polypeptide-k showed the effective rate of inhibition against both of the enzymes when compared with the essential oil

For the future, it is suggested that another experiment will be performed by using the extract from other parts of *Momordica charantia* such as, leather, flowers and leaves. In addition, other herbs can also be used to study the effects of diabetes. Moreover, further study should be conducted to determine and later confirm the mechanism of action involved in this particular antidiabetic activity. In addition, the experiment should be performed in - vitro as better to study the real pictures of antidiabetic effect on living things. We also can study about the mechanisms that occur inside our body. As we know, the environment between in-vitro and in-vivo is very different.

REFFERENCES

Ahrén B, Corrigan CB. Intermittent need for insulin in a subgroup of diabetic patients in Tanzania. *Diabet Med* 1984; **2:** 262-64.

Assan R, Perronne C, Assan D, Chotard L, Mayaud C, Matheron S *et al*. Pentamidine-induced derangements of glucose homeostasis. *Diabetes Care* 1995; **18:** 47-55.

Banerji MA, Chaiken RI, Huey H, Tuomi T, Norin AJ, MacKay IR *et al*. GAD antibody negative NIDDM in adult black subjects with diabetic ketoacidosis and increased frequency of human leukocyte antigen DR3 and DR4: flatbush diabetes. *Diabetes* 1994; **43:** 741-45.

Barrett TG, Bundey SE, Macleod AF. Neurodegeneration and diabetes: UK nationwide study of Wolfram (DIDMOAD) syndrome. *Lancet* 1995; **346:** 1458-63.

Betterle C, Zanette F, Pedini B, Presotto F, Rapp LB, Monsciotti CM *et al*. Clinical and subclinical organ-specific autoimmune manifestations in type 1 (insulin-dependent) diabetic patients and their first-degree relatives. *Diabetologia* 1983; **26:** 431-36.

Bodansky HJ, Grant PJ, Dean BM, McNally J, Bottazzo GF, Hambling MH *et al*. Islet-cell antibodies and insulin autoantibodies in association with common viral infections. *Lancet* 1986; **ii:** 1351-53.

Bogardus C, Lillioja S, Mott DM, Hollenbeck C, Reaven G. Relationship between degree of obesity and in vivo insulin action in man. *Am J Physiol* 1985; **248:** E286-E291.

Byrne MM, Sturis J, Menzel S, Yamagata K, Fajans SS, Dronsfield MJ *et al*. Altered insulin secretory response to glucose in diabetic and nondiabetic subjects with mutations in the diabetes susceptibility gene MODY 3 on chromosome 20. *Diabetes* 1996; **45:** 1503-10.

Campbell PJ, Carlson MG. Impact of obesity on insulin action in NIDDM. *Diabetes* 1993; **42:** 405-10.

Clement K, Pueyo ME, Vaxillaire M, Rakotoambinina B, Thuillier F, Passa P *et al*. Assessment of insulin sensitivity in glucokinase-deficient subjects. *Diabetologia* 1996; **39:** 82-90.

Conn JW. Hypertension, the potassium ion and impaired carbohydrate tolerance. *N Engl J Med* 1965; **273:** 1135-43.

deCourten M, Bennett PH, Tuomilehto J, Zimmet P. Epidemiology of NIDDM in Non-Europids. In: Alberti KGMM, Zimmet P, DeFronzo RA, eds. *International Textbook of Diabetes Mellitus*. 2nd edn.Chichester: John Wiley, 1997: pp 143-70.

DeFronzo RA, Bonadonna RC, Ferrannini E. Pathogenesis of NIDDM. In: Alberti KGMM, Zimmet P, DeFronzo RA, eds. *International Textbook of Diabetes Mellitus*. 2nd edn.Chichester: John Wiley, 1997: pp 635-712.

Eriksson KF, Lindegärde F. Prevention of Type 2 (non-insulin-dependent) diabetes mellitus by diet and physical exercise. *Diabetologia* 1991; **34:** 891-98.

Esposti MD, Ngo A, Myers MA. Inhibition of mitochondrial complex I may account for IDDM induced by intoxication with rodenticide Vacor. *Diabetes* 1996; **45:** 1531-34.

European Arterial Risk Policy Group on behalf of the International Diabetes Federation (European Region).A strategy for arterial risk assessment and management in Type 2 (non-insulin-dependent) diabetes. *Diabet Med* 1997; **14:** 611-21.

Fabris P, Betterle C, Floreani A, Greggio NA, de Lazzari F, Naccarato R *et al*. Development of type 1 diabetes mellitus during interferon alfa therapy for chronic HCV hepatitis (Letter). *Lancet* 1992;**340:** 548.

Flier JS. Lilly Lecture: syndromes of insulin resistance: from patient to gene and back again. *Diabetes* 1992; **41:** 1207-19.

Forrest JA, Menser MA, Burgess JA. High frequency of diabetes mellitus in young patients with congenital rubella. *Lancet* 1971; **ii:** 332-34.

Froguel P, Vaxillaire M, Sun F, Velho G, Zouali H, Butel MO *et al*. Close linkage of glucokinase locus on chromosome 7p to early-onset non-insulin-dependent diabetes. *Nature* 1992; **356:** 162-64.

Gallanosa AG, Spyker DA, Curnow RT. Diabetes mellitus associated with autonomic and peripheral neuropathy after Vacor poisoning: a review. *ClinToxicol* 1981; **18:** 441-49.

Gruppuso PA, Gorden P, Kahn CR, Cornblath M, Zeller WP, Schwartz R. Familial hyperproinsulinemia due to a proposed defect in conversion of proinsulin to insulin. *N Engl J Med* 1984; **311:** 629-34.

Gullo L, Pezzilli R, Morselli-Labate AM, and the Italian Pancreatic Cancer Study Group.Diabetes and the risk of pancreatic cancer. *N Engl J Med* 1994; **331:** 81-84.

Haffner SM, Valdez RA, Hazuda HP, Mitchell BD, Morales PA, Stern MP.Prospective analysis of the insulin resistance syndrome (Syndrome X). *Diabetes* 1992; **41:** 715-22.

Haneda M, Polonsky KS, Bergenstal RM, Jaspan JB, Shoelson SE, Blix PM *et al*. Familial hyperinsulinemia due to a structurally abnormal insulin. Definition of an emerging new clinical syndrome. *N Engl J Med* 1984; **310:** 1288-94.

Harris MI, Cowie CC, Stern MP, Boyko ES, Reiber GE, Bennett PH, eds. *Diabetes in America*.2nd edn. Washington DC: US Government Printing Office, 1995 (NIH publ. No. 95-1468).

Harris MI. Undiagnosed NIDDM; clinical and public health issues. *Diabetes Care* 1993; **16:** 642-52.

Hirata Y, Ishizu H, Ouchi N *et al*. Insulin autoimmunity in a case of spontaneous hypoglycaemia. *J JpnDiabetSoc* 1970; **13:** 312-20.

Hother-Nielsen O, Faber O, Sørensen NS, Beck-Nielsen H. Classification of newly diagnosed diabetic patients as insulin-requiring or non-insulin-requiring based on clinical and biochemical variables.*Diabetes Care* 1988; **11:** 531-37.

Humphrey ARG, McCarty DJ, Mackay IR, Rowley MJ, Dwyer T, Zimmet P. Autoantibodies to glutamic acid decarboxylase and phenotypic features associated with early insulin treatment in individuals with adult-onset diabetes mellitus. *Diabetic Med* 1998; **15:** 113-19.

Japan and Pittsburgh Childhood Diabetes Research Groups. Coma at onset of young insulin-dependent diabetes in Japan: the result of a nationwide survey. *Diabetes* 1985; **34:** 1241-46.

Johns DR. Mitochondrial DNA and disease. *N Engl J Med* 1995; **333:** 638-44.

Kahn CR, Baird KL, Flier JS, Jarrett DB.Effects of autoantibodies to the insulin receptor on isolated adipocytes. *J Clin Invest* 1977; **60:** 1094-106.

Kahn CR, Flier JS, Bar RS, Archer JA, Gorden P, Martin MM *et al*.The syndromes of insulin resistance and acanthosisnigricans. *N Engl J Med* 1976; **294:** 739-45.

Kaplan NM. The deadly quartet: upper body adiposity, glucose intolerance, hypertriglyceridaemia and hypertension. *Arch Intern Med* 1989; **149:** 1514-20.

Karjalainen J, Knip M, Hyoty H, Linikki P, Ilonen J, Kaar M-L *et al*. Relationship between serum insulin antibodies, islet cell antibodies and Coxsackie-B4 and mumps virus-specific antibodies at the clinical manifestation of type 1 (insulin-dependent) diabetes. *Diabetologia* 1988; **31:** 146-52.

King ML, Bidwell D, Shaikh A, Voller A, Banatvala JE. Coxsackie-B-virus-specific IgM responses in children with insulin-dependent (juvenile-onset; type 1) diabetes mellitus. *Lancet* 1983; **i:** 1397-99.

Kissebah AH, Vydelingum N, Murray R, Evans DF, Hartz AJ, Kalkhoff RK *et al*. Relationship of body fat distribution to metabolic complications of obesity. *J ClinEndocrinolMetab* 1982; **54:** 254-60.

Knowler WC, Nelson RG, Saad M, Bennett PH, Pettitt DJ. Determinants of diabetes mellitus in the Pima Indians. *Diabetes Care* 1993; **16:** 216-27.

Krejs GJ, Orci L, Conlon JM, Ravazzola M, Davis GR, Raskin P *et al*. Somatostatinoma syndrome. *N Engl J Med* 1979; **301:** 285-92.

Larsen S, Hilsted J, Tronier B, Worning H. Metabolic control and B cell function in patients with insulin-dependent diabetes mellitus secondary to chronic pancreatitis. *Metabolism* 1987; **36:** 964-67.

Lillioja S, Mott DM, Spraul M, Ferraro R, Foley JE, Ravussin E *et al*. Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes. Prospective Study of Pima Indians. *N Engl J Med* 1993; **329:** 1988-92.

MacFarlane IA. Endocrine diseases and diabetes mellitus. In: Pickup JC, Williams G, eds. *Textbook of Diabetes*. 2nd edn. Oxford: Blackwell, 1997: pp 64.1-64.20.

McLarty DG, Athaide I, Bottazzo GF, Swai ABM, Alberti KGMM. Islet cell antibodies are not specifically associated with insulin-dependent diabetes in rural Tanzanian Africans. *Diabetes Res ClinPract* 1990; **9:** 219-24.

Mølbak AG, Christau B, Marner B, Borch-Johnsen K, Nerup J. Incidence of insulin-dependent diabetes mellitus in age groups over 30 years in Denmark. *Diabet Med* 1994; **11:** 650-55.

Mooy JM, Grootenhuis PA, de Vries H, Valkenburg HA, Bouter LM, Kostense PJ *et al*. Prevalence and determinants of glucose intolerance in a Dutch population. The Hoorn Study. *Diabetes Care*1995; **18:** 1270-73.

Moran A, Pyzdrowski KL, Weinreb J, Kahn BB, Smith SA, Adams KS *et al*. Insulin sensitivity in cystic fibrosis. *Diabetes* 1994; **43:** 1020-26.

Mykkänen L, Kuusisto J, Pyörälä K, Laakso M. Cardiovascular disease risk factors as predictors of Type 2 (non-insulin-dependent) diabetes mellitus in elderly subjects. *Diabetologia* 1993; **36:** 553-59.

O'Byrne S, Feely J. Effects of drugs on glucose tolerance in non-insulin-dependent diabetes (parts I and II). *Drugs* 1990; **40:** 203-19.

Pak CY, Eun H, McArthur RG, Yoon J. Association of cytomegalovirus infection with autoimmune type 1 diabetes. *Lancet* 1988; **ii:** 1-4.

Pandit MK, Burke J, Gustafson AB, Minocha A, Peiris AN. Drug-induced disorders of glucose tolerance. *Ann Intern Med* 1993; **118:** 529-40.

Permert J, Larsson J, Westermark GT, Herrington MK, Christmanson L, Pour PM *et al*. Islet amyloid polypeptide in patients with pancreatic cancer and diabetes. *N Engl J Med* 1994; **330:** 313-18.

Phelps G, Chapman I, Hall P, Braund W, Mackinnon M. Prevalence of genetic haemochromatosis among diabetic patients. *Lancet* 1989; **ii:** 233-34.

Polonsky KS, Sturis J, Bell GI. Non-insulin-dependent diabetes mellitus: a genetically programmed failure of the beta cell to compensate for insulin resistance. *N Engl J Med* 1996; **334:** 777-84.

Robbins DC, Shoelson SE, Rubenstein AH, Tager HS. Familial hyperproinsulinemia: two cohorts secreting indistinguishable type II intermediates of proinsulin conversion. *J Clin Invest* 1984; **73:** 714-19.

Sanz N, Karam JH, Horita S, Bell GI. Prevalence of insulin-gene mutations in non-insulin-dependent diabetes mellitus. *N Engl J Med* 1986; **314:** 1322-23.

Simonson DC, Ferrannini E, Bevilacqua S, Smith D, Barrett E, Carlson R *et al*. Mechanism of improvement in glucose metabolism after chronic glyburide therapy. *Diabetes* 1984; **33:** 838-45.

Solimena M, De Camilli P. Autoimmunity to glutamic acid decarboxylase (GAD) in Stiff-Man syndrome and insulin-dependent diabetes mellitus. *Trends Neurosci* 1991; **14:** 452-57.

Stern MP. The insulin resistance syndrome. In: Alberti KGMM, Zimmet P, DeFronzo RA, eds. *International Textbook of Diabetes Mellitus*. 2nd edn.Chichester: John Wiley, 1997: pp 255-83.

Stoffers DA, Ferrer J, Clarke WL, Habener JF. Early-onset type-II diabetes mellitus (MODY4) linked to *IPF1*. *Nature Genetics* 1997; **117:** 138-39.

Taylor SI. Lilly Lecture: molecular mechanisms of insulin resistance: lessons from patients with mutations in the insulin-receptor gene. *Diabetes* 1992; **41:** 1473-90.

Tsokos GC, Gorden P, Antonovych T, Wilson CB, Balow JE.Lupus nephritis and other autoimmune features in patients with diabetes mellitus due to autoantibody to insulin receptors. *Ann Intern Med*1985; **102:** 176-81.

Umpierrez GE, Casals MMC, Gebhardt SSP, Mixon PS, Clark WS, Phillips LS.Diabetic ketoacidosis in obese African-Americans. *Diabetes* 1995; **44:** 790-95.

Valle T, Tuomilehto J, Eriksson J. Epidemiology of NIDDM in Europids. In: Alberti KGMM, Zimmet P, DeFronzo RA, eds. *International Textbook of Diabetes Mellitus*. 2nd edn.Chichester: John Wiley, 1997: pp 125-42.

Verge CF, Gianani R, Kawasaki E, Yu L, Pietropaolo M, Jackson RA *et al*. Predicting type I diabetes in first-degree relatives using a combination of insulin, GAD, and ICA512bdc/IA-2 autoantibodies.*Diabetes* 1996; **45:** 926-33.

Vionnet N, Stoffel M, Takeda J, Yasuda K, Bell GI, Zouali H *et al*. Nonsense mutation in the glucokinase gene causes early-onset non-insulin-dependent diabetes. *Nature* 1992; **356:** 721-22.

Walker M, Turnbull DM. Mitochondrial related diabetes: a clinical perspective. *Diabet Med* 1997; **14:** 1007-09.

Willis JA, Scott RS, Brown LJ, Forbes LV, Schmidli RS, Zimmet PZ *et al*. Islet cell antibodies and antibodies against glutamic acid decarboxylase in newly diagnosed adult-onset diabetes mellitus.*Diabetes Res ClinPract* 1996; **33:** 89-97.

Wing RR, Blair EH, Bononi P, Marcus MD, Watanabe R, Bergman RN. Caloric restriction per se is a significant factor in improvements in glycemic control and insulin sensitivity during weight loss in obese NIDDM patients. *Diabetes Care* 1994; **17:** 30-36.

Yajnik CS, Shelgikar KM, Naik SS, Kanitkar SV, Orskov H, Alberti KGMM *et al*.The ketoacidosis-resistance in fibro-calculous-pancreatic-diabetes. *Diabetes Res ClinPract* 1992; **15:** 149-56.

Yamagata K, Furuta H, Oda N, Kaisaki PJ, Menzel S, Cox NJ *et al*. Mutations in the hepatocyte nuclear factor-4alpha gene in maturity-onset diabetes of the young (MODY 1). *Nature* 1996; **384:**458-60.

Yamagata K, Oda N, Kaisaki PJ, Menzel S, Furuta H, Vaxillaire M *et al*. Mutations in the hepatocyte nuclear factor-1alpha gene in maturity-onset diabetes of the young (MODY 3). *Nature* 1996; **384:**455-58.

Zimmet PZ, Tuomi T, Mackay R, Rowley MJ, Knowles W, Cohen M *et al*. Latent autoimmune diabetes mellitus in adults (LADA): the role of antibodies to glutamic acid decarboxylase in diagnosis and prediction of insulin dependency. *Diabetic Med* 1994; **11:** 299-303.

Zimmet PZ. Kelly West Lecture 1991: challenges in diabetes epidemiology: from West to the rest. *Diabetes Care* 1992; **15:** 232-52.

Zimmet PZ. The pathogenesis and prevention of diabetes in adults. *Diabetes Care* 1995; **18:** 1050-64.

Appendix C

Statistical analysis results for activity of α-glucodidase and α-amylase enzyme treated with polypeptide-k and essential oil.

One-way ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
α-glucodidase – essential oil	Between Groups	3929.667	7	561,381	30,837	,000
	Within Groups	291,273	16	18,205		
	Total	4220,940	23			
	Between Groups	4204,815	7	600,688	7,937	,000

α-glucodidase – PPK	Within Groups	1210,928	16	75,683		
	Total	5415,743	23			
α-amylase – PPK	Between Groups	3064,680	7	437,811	2,979	,075
	Within Groups	1175,603	8	146,950		
	Total	4240,283	15			
α-amylase – essential oil	Between Groups	6929,478	7	989,925	2,402	,070
	Within Groups	6594,129	16	412,133		
	Total	13523,607	23			

Duncan multiple comparisons test

Activity of α-glucosidase treated with essential oil.

Concentration (mg/ml)	N	Subset for alpha = .05						
	1	2	3	4	5	6	7	1
2	3	56,8900						
1.5	3		64,5213					
1.125	3		68,8970	68,8970				
0.844	3			74,0152	74,0152			
0.633	3				80,4882	80,4882		
0.475	3					85,6295	85,6295	

0.356	3						90,7401	90,7401
0.267	3							97,0091
Sig.		1,000	,227	,161	,082	,159	,162	,091

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,000.

Activity of α-glucosidase treated with polypeptide-k.

Concentration (mg/ml)	N	Subset for alpha = .05				
	1	2	3	4	5	1
2	3	26,0133975				
1.5	3	32,4798442	32,4798442			
1.125	3	37,2303473	37,2303473	37,2303473		
0.844	3		42,7553617	42,7553617	42,7553617	
0.633	3		48,5088832	48,5088832	48,5088832	
0.475	3			51,4710649	51,4710649	
0.356	3				55,4295045	
0.267	3					70,6628402

Sig.		,153	,053	,082	,119	1,000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,000.

Activity of α-amylase treated with polypeptide-k.

Concentration (mg/ml)	N	Subset for alpha = .05		
	1	2	3	1
2	2	185,9291662		
1.5	2	190,5341315		
1.125	2	195,8756380	195,8756380	
0.844	2	201,9705691	201,9705691	201,9705691
0.633	2	208,0655002	208,0655002	208,0655002
0.475	2	213,3901232	213,3901232	213,3901232
0.356	2		223,3028280	223,3028280
0.267	2			226,3502936

Sig.		,070	,069	,099

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 2,000.

Activity of α-amylase treated with essential oil.

Concentration (mg/ml)	N	Subset for alpha = .05		
	1	2	3	1
2	3	193,2560078		
1.5	3	201,3527786	201,3527786	
1.125	3	206,6906616	206,6906616	206,6906616
0.844	3	215,7165860	215,7165860	215,7165860
0.633	3	223,6827802	223,6827802	223,6827802
0.475	3		232,7123481	232,7123481
0.356	3		235,3548099	235,3548099

0.267	3			245,3911487
Sig.		,116	,085	,053

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,0

α- Glucosidase activity by essential oil and polypeptide-k

Duncan

				N				Subset for alpha = .05			
Concentration (mg/ml)	1	2	3	4	5	6	7	8	9	10	1
2 (PPK)	3	26,01 33975									
1.5(PPK)	3	32,47 98442	32,47 98442								
1.125(PPK)	3	37,23 03473	37,23 03473								
0.844(PPK)	3		42,75 53617	42,75 53617							
0.633(PPK)	3			51,58 65347	51,58 65347						
0.475(PPK)	3				57,08 67015	57,08 67015					
0.356(PPK)	3				57,62 63679	57,62 63679					
2(E.OIL)	3				58,05 31581	58,05 31581					
1.5(E.OIL)	3					65,85 50979	65,85 50979				
0.267 (PPK)	3					68,05 86735	68,05 86735				
1.5 (E.OIL)	3					70,30 83039	70,30 83039	70,30 83039			

1.125(E.OIL)	3						75,55 06379	75,55 06379	75,55 06379		
0.844(E.OIL)	3							82,11 66775	82,11 66775	82,11 66775	
0.633(E.OIL)	3								87,38 20380	87,38 20380	87,38 20380
0.475(E.OIL)	3									92,69 42389	92,69 42389
0.356(E.OIL)	3										99,10 27640
Sig.		,081	,109	,144	,327	,055	,143	,066	,066	,099	,068

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,000.

α-Amylase activity by polypeptide-k and essential oil

Duncan

		N	Subset for alpha = .05			
Concentration (mg/ml)	1	2	3	4	5	1
2 (PPK)	2	185,9291662				

1.5(PPK)	2	190,5341315	190,5341315			
2(E.OIL)	3	193,2560078	193,2560078	193,2560078		
1.125(PPK)	2	195,8756380	195,8756380	195,8756380	195,8756380	
1.5(E.OIL)	3	201,3527786	201,3527786	201,3527786	201,3527786	
0.844(PPK)	2	201,9705691	201,9705691	201,9705691	201,9705691	
1.125(E.OIL)	3	206,6906616	206,6906616	206,6906616	206,6906616	206,6906616
0.633(PPK)	2	208,0655002	208,0655002	208,0655002	208,0655002	208,0655002
0.475(PPK)	2	213,3901232	213,3901232	213,3901232	213,3901232	213,3901232
0.844(E.OIL)	3	215,7165860	215,7165860	215,7165860	215,7165860	215,7165860
0.356(PPK)	2	223,3028280	223,3028280	223,3028280	223,3028280	223,3028280
0.633(E.OIL)	3	223,6827802	223,6827802	223,6827802	223,6827802	223,6827802
0.267 (PPK)	2		226,3502936	226,3502936	226,3502936	226,3502936
0.475(E.OIL)	3			232,7123481	232,7123481	232,7123481
0.356(E.OIL)	3				235,3548099	235,3548099
0.267(E.OIL)	3					245,3911487

Sig.		,062	,075	,051	,051	,054

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 2,400.
b The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.