

DIPLOMARBEIT

SCREENING OF VARIOUS EXTRACTS OF *ARTEMISIA*
DRACUNCULUS L. FOR ANTIDIABETIC ACTIVITY IN RATS

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Chapter 1

Abstract

Previous studies have proven the antidiabetic properties of *Artemisia dracunculus* L. through various approaches. In the face of the present diabetes epidemic, looking into new approaches and developing remedies that differ from the traditional forms of treatment is pivotal. Testing extracts of *Artemisia dracunculus*, a plant that has safely been applied in traditional medicine, thus seems very promising.

In this study, a rat model was used to evaluate the effects of *A. dracunculus* extracts not only on blood glucose levels, but on dipeptidyl peptidase IV (DPPIV) levels and plasma insulin as well, employing DPPIV assay and insulin ELISA respectively. Results of basal and glucose tolerance tests were evaluated. By conducting a dose finding study, the extract concentration of 60mg/kg could be identified as being most effective in lowering blood glucose levels und therefore most suitable for further testing.

Abstract

In früheren Studien wurden die antidiabetischen Eigenschaften von *Artemisia dracunculus* L. bereits mittels verschiedener Herangehensweisen nachgewiesen. Angesichts der gegenwärtigen Diabetes-Epidemie ist es unabdingbar, nach neuen Herangehensweisen zu suchen, um Arzneimittel zu entwickeln, die von traditionellen Heilmethoden abweichen. Die Untersuchung von Extrakten von *Artemisia dracunculus* L., einer Pflanze, die in der traditionellen Medizin bereits angewendet wurde, erscheint daher vielversprechend.

In der vorliegenden Studie wurde ein Rattenmodell für die Untersuchung der Effekte von *A. dracunculus*-Extrakten nicht nur auf Blutzuckerwerte, sondern auch zur Klärung des möglichen Wirkmechanismus durchgeführt. Hierzu wurden Dipeptidylpeptidase IV (DPPIV) assays beziehungsweise Insulin ELISA angewendet. Mittels einer Dosisfindungskurve konnte die Extraktkonzentration von 60 mg/kg als diejenige identifiziert werden, die Blutzuckerwerte am effektivsten senkte und daher für weitere Tests am besten geeignet war.

Chapter 2

Objectives and Introduction

Tarragon (*Artemisia dracunculus* L.) of the family *Asteraceae*, is a perennial herb with a long history of medical and culinary use. While in traditional medicine mainly used as a remedy for gastrointestinal disorders, recent studies looked into possible antidiabetic and glucose-lowering effects of *A. dracunculus* ([Ribnicky *et al.*, 2009], [Govorko *et al.*, 2007], [Logendra *et al.*, 2006], [Wang *et al.*, 2008]). Several active compounds have already been identified: polyphenoles such as 6-demethoxycapillarisin, 2',4'-dihydroxy-4-methoxydihydrochalcone and 4,5-di-*O*-caffeoylquinic acid, flavonoids such as luteolin and apigenin, the coumarin scopoletin and the sesquiterpenoid lactone costunolide as well as cinnamates ([Govorko *et al.*, 2007], [Logendra *et al.*, 2006], [Duke, 1992], [Huang *et al.*, 1993], [Arlt *et al.*, 2004], [Liu *et al.*, 2003]). In 2001, the German 'Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin' released a statement in which the hepatocarcinogenic effects of estragole and methyleugenol were described – compounds that can also be found in *A. dracunculus* and its ethanolic extracts, while water extracts contain no detectable amounts of those components (Data courtesy of Finzelberg GmbH & Co KG).

In order to assess the effect of *A. dracunculus* extracts on blood glucose, plasma insulin levels and DPPIV activity, a standard animal model for the testing of antidiabetic activity was used. Based on literature such as Verspohls "Recommended testing in diabetes research" (2002), it was chosen to work with a rat model, with blood collection from the *vena sublingualis*.

The specific objectives of the present work consists of:

1. Conducting a dose finding study to identify the most active concentration of extract
2. To get hints on the possible mechanism of action by measuring insulin plasma levels and DPPIV enzyme activity.

The following chapter gives insight into the metabolic disease diabetes and current treatment options, draws an outline of the prevalence of diabetes in our society and gives an outlook into the future. Chapter 4 provides a detailed description of the plant *A. dracunculus*, its compounds and traditional use and presents previous studies dealing with its antidiabetic properties. Materials and methods are discussed in chapter 5. Results are presented and debated in chapters 6 and 7.

Chapter 3

Diabetes

1. Introduction

Diabetes mellitus is a metabolic disorder with elevated blood glucose levels, caused by inadequate insulin secretion, as its main clinical symptom. Based on the pathologic processes that lead to hyperglycemia and disordered metabolism, diabetes mellitus can be classified into two main groups ([Kasper, Harrison, 2005], [McPhee, Papadakis, 2008]).

1.1. Diabetes mellitus type 1

Type 1 diabetes or insulin dependent diabetes, is caused by a combination of genetic, environmental and immunological elements that eventually lead to autoimmune destruction of pancreatic beta cells and severe or absolute insulin deficiency. Over 90% of cases are immune-mediated, less than 10% are idiopathic. Although environmental factors are believed to play a major part in the development of type 1 diabetes, the causes for the autoimmune injuries are still unknown, but different hypotheses, such as infections with viruses (e.g. rubella, coxsackie B4), have been discussed. Therapy of patients with diabetes type 1 consists in lifelong supplementation of insulin ([Kasper, Harrison, 2005], [McPhee, Papadakis, 2008] [Katzung, 2007]).

1.2. Diabetes mellitus type 2

Type 2 diabetes, also known as non-insulin dependent diabetes or adult-onset diabetes, is defined as a group of syndromes characterized by the presence of hyperglycemia, which is caused by a progressive loss of beta-cell function as the central feature. ([McPhee, 2005], [The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003], [McPhee, Papadakis, 2008]).

In addition to insulin resistance, individuals with type 2 diabetes mellitus suffer from impaired insulin secretion and show a characteristic decrease in the early release of insulin, also known as first-phase insulin release, all of which results in deficient insulin action. The dominating malfunction varies, ranging from insulin resistance with relative insulin deficiency to a predominating insulin secretory defect with insulin resistance ([Kasper, Harrison, 2005], [The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003]).

The molecular or genetic defects responsible for the development of type 2 diabetes mellitus are for the most part still unclear, but it can be assumed that a combination of polygenetic origins and environmental factors, such as an unhealthy diet or a sedentary lifestyle, lead to both insulin resistance and beta cell loss ([McPhee, Papadakis, 2008], [McPhee, 2005]).

The following quote describes very well the different risk factors of developing diabetes:

„The risk of developing type 2 DM increases with age, obesity and lack of physical activity. It occurs more frequently in women with prior gestational diabetes mellitus and in individuals with hypertension or dyslipidemia, and its frequency varies in different racial/ethnic subgroups.“

(Source: The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003)

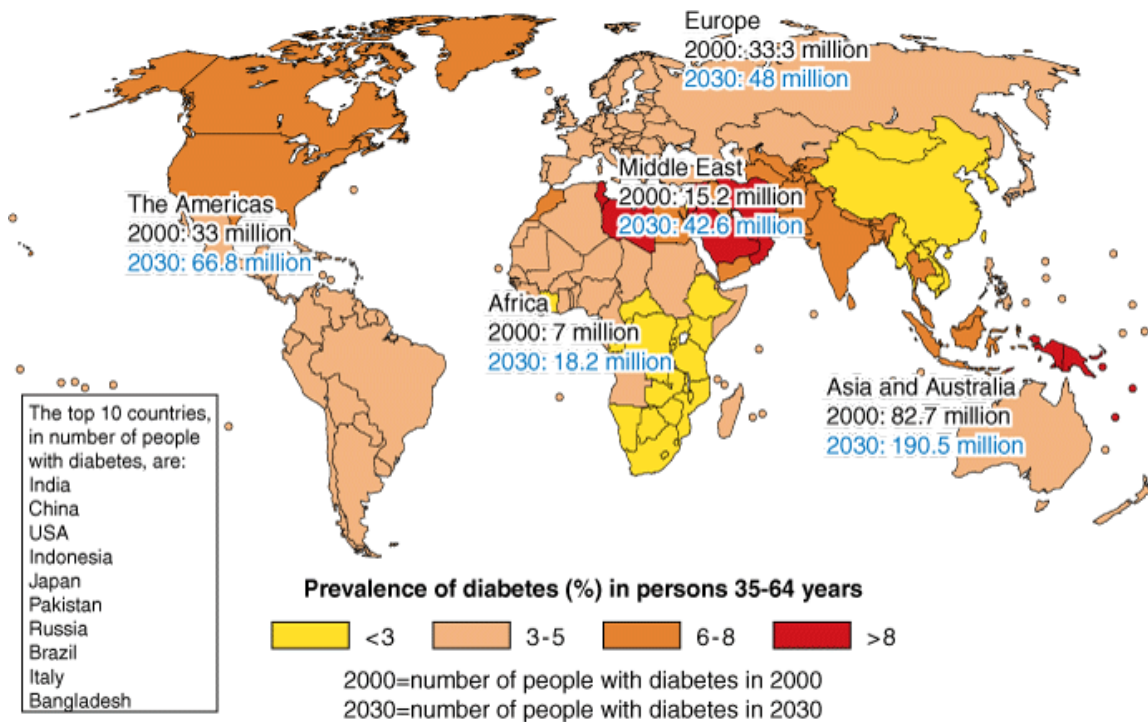
80% of patients presenting with diabetes mellitus (DM) also suffer from obesity, notably in central or visceral location, which increases insulin resistance. Insulin resistance in early and prediabetic stages is compensated by hyperplasia in beta cells ([Kasper, Harrison, 2005], [McPhee, 2005]). This correlates with the fact that factors such as increased body-mass index, heightened liver-enzyme levels, smoking, reduced insulin secretion and a corresponding family history are the main prognostic symptoms (Lyssenko *et al.*, 2008).

Before type 2 DM becomes manifest, it is preceded by abnormal glucose homeostasis, a condition known as impaired glucose tolerance (IGT) and impaired fasting glucose (IFG). This stage is also referred to as prediabetes. At this point, lifestyle modification and the prescription of oral antidiabetic agents can delay or in some cases even prevent the onset of DM, as prediabetes is considered rather a risk factor for the development of diabetes and cardiovascular disease than a clinical entity, although it is associated with the insulin resistance syndrome ([Kasper, Harrison, 2005], [The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003] [Rosenstock, 2007]). Screening for prediabetes is advisable in people older than 45 as well as in adults below the age of 45 if aforementioned risk factors are present (Crandall *et al.*, 2008).

2. The diabetes epidemic

Diabetes is one of the most common non-communicable diseases globally, with type 2 diabetes constituting about 85% to 95% of all cases in developed countries. Being the fourth to fifth leading cause of death in most developed countries and a prime cause of excess cardiovascular morbidity and mortality in Western populations, diabetes is epidemic in many developing and newly industrialized nations and can be

considered one of the main threats to human health in the 21st century. ([Zimmet *et al.*, 2001], [Allgot, Gan, *et al.*, 2003][Reimann *et al.*, 2009]).



Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: <http://www.accessmedicine.com>
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Figure 1. The prevalence of diabetes in 2000 and the projected prevalence in 2030.

The prevalence of diabetes globally is estimated to rise from 171 million in 2000 to 366 million in 2030, which is an enormous increase from 2.8% to 4.4% in 2030 (see Figure 1).

Figure 2 illustrates the 46% increase in diabetes cases worldwide from 2000 to 2010 (Zimmet et al., 2001). These figures are based on the assumption that the prevalence of obesity remains stable. As this seems highly unlikely, and taking into consideration that the number of individuals with IGT, also termed prediabetes, may be equal or even higher than that of people with diabetes, the total of diabetes cases in 2030 could be considerably higher ([Wild et al., 2004], [Goodman, Gilman 2006], [Ryan, Espeland et al., 2003]).

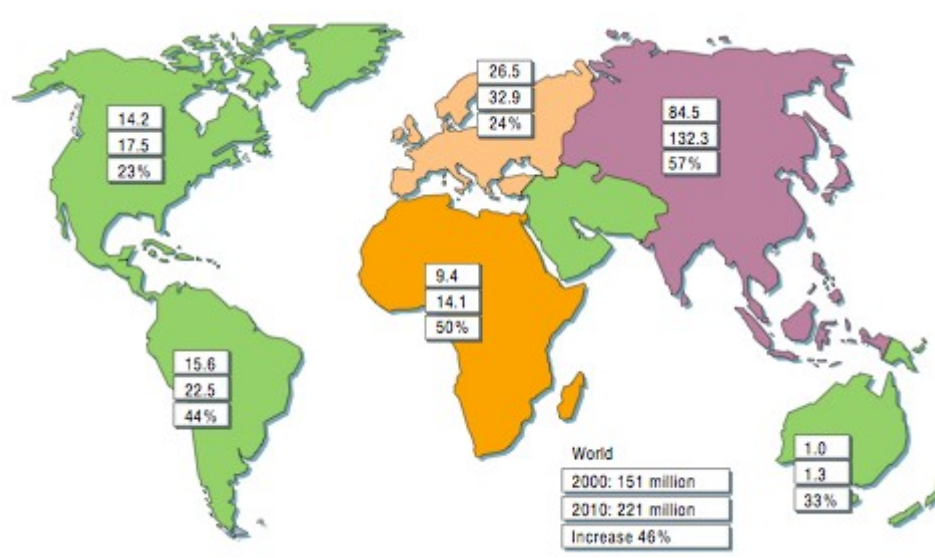


Figure 2. Numbers of people with diabetes (in millions) for 2000 and 2010 (top and middle values respectively), and the percentage increase
(Source: Zimmet *et al.*, 2001)

The human and economic costs societies all over the world will subsequently have to face will be enormous, not only due to the numerous associated illnesses, but also due to the incremental costs of diabetes, beginning at least eight years prior to the

diagnosis ([Wild *et al.*, 2004], [Logendra *et al.*, 2006], [Nichols *et al.*, 2000], [Stummvoll *et al.*, 2005]). Due to the residual insulin secretion and the fact that hyperglycemia develops gradually, patients are often asymptomatic and remain undiagnosed for a considerable time ([McPhee, 2005], [The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003]).

In this regard, early discovery of diabetes and delaying its progress is pivotal (Reimann *et al.*, 2009). The numerous complications that develop in long-term diabetes patients, such as retinopathy with potential loss of vision, nephropathy and subsequent renal failure, peripheral neuropathy and foot ulcers, autonomic neuropathy causing gastrointestinal, genitourinary and cardiovascular symptoms and sexual dysfunction all account for the rising health care costs for diabetes-related diseases ([The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003]).

Alternative strategies like nutritional supplementation with botanical agents in addition to the traditional strategy of reducing food intake and increasing physical activity could be essential ([Zuberi, 2008], [Wang *et al.* 2008]).

This is particularly important, as conventional treatments such as insulin and sulfonylureas have been proven to produce weight gain and could thus eventually exacerbate the disease (UK Prospective Diabetes Study group 1998). Plants have always been an abundant source of medicinal compounds, with numerous plants being known for their antidiabetic activity, but there is still a want of thoroughly controlled studies on efficacy and safety in the use of such products ([Wang *et al.*, 2008], [Ribnicky *et al.*, 2006]).

3. Treatment options

3.1. Hypoglycaemic drugs

When diet and physical activity programs fail to show a significant effect in lowering blood glucose levels, oral antidiabetics are required.

Depending on their mechanism of action, drugs for treating type 2 diabetes mellitus fall into several categories:

- (1) Sulfonylureas primarily stimulate insulin secretion by binding to the sulfonylurea receptor. The meglitinide analog repaglinide and the D-phenylalanine derivate nateglinide use the same mode of action.
- (2) Drugs that alter insulin action: while biguanides have their point of action in the liver, thiazolidinediones appear to be working in both skeletal muscle and adipose tissue.
- (3) α -Glucosidase inhibitors alter insulin action.
- (4) Exenatide and DPPIV inhibitors mimic incretin effect or prolong incretin action.
- (5) Other: Pramlintide suppresses glucagon and slows gastric emptying (McPhee, Papadakis, 2008).

Treatment of patients with type 2 DM must also include taking care of conditions associated with type 2 DM, such as obesity, hypertension, dyslipidemia and cardiovascular diseases, as well as the management of DM-related complications. These are numerous and highly common in patients suffering from type 2 DM, with cardiovascular complications being the leading cause of mortality (Kasper, Harrison, 2005).

3.1.1. Stimulating insulin secretion

3.1.1.1. Sulfonylureas

By binding on sulfonylurea receptors on the surface of pancreatic β -cells, efflux of potassium ions through the ATP-sensitive potassium channel is inhibited, which results in depolarisation and subsequent influx of calcium. This active promotion of insulin release is only possible if functioning pancreatic β -cells are present. Acute administration improves the early phase of insulin release, while long-term administration reduces serum glucagon levels ([Kasper, Harrison, 2005], [McPhee, Papadakis, 2008]).

Sulfonylureas can be divided into first generation (tolbutamide, tolazamide, acetohexamide, chlorprogamide) and second generation drugs (glyburide, glipizide, gliclazide, glimepiride). Second generation sulfonylureas show fewer adverse effects and drug interactions, as well as a lower incidence of hypoglycemia, but tolbutamide is probably the safest sulfonylurea to use both when liver function is normal and for elderly diabetics ([Kasper, Harrison, 2005], [McPhee, Papadakis, 2008] [Katzung, 2007]).

Glyburide (see structure in figure 3) was used as the positive control in this study to evaluate the antidiabetic potency of *Artemisia dracunculus* L. extracts .

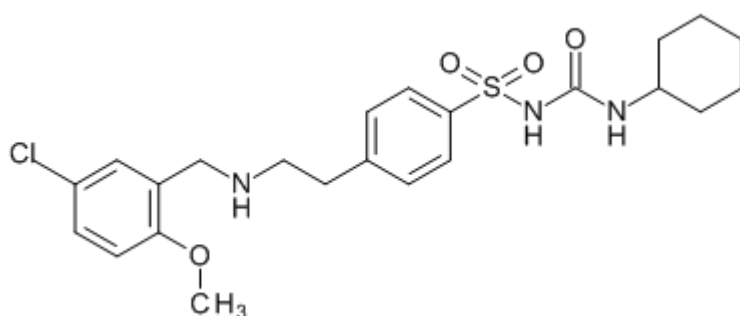


Figure 3. Structural formula of glyburide (Created with Symyx Draw 3.3).

3.1.1.2. Miglitinide and D-Phenylalanine derivative

Following the same mode of action as the sulfonylureas but being rapidly and completely metabolised, both miglitinide and nateglinide cause a brief but rapid pulse of insulin. Main side effects are, as with the other insulin secretagogues, hypoglycemia and weight gain ([Katzung, 2007], [Goodman, Gilman, 2006], [McPhee, Papadakis, 2008]).

3.1.2. Altering insulin action

3.1.2.1. Biguanides

The main biguanide in use is metformin (see figure 4 below). It is antihyperglycemic, not hypoglycemic. Its mechanism of action has not yet been fully explained, but the following effects have been proposed: a reduction of hepatic and renal gluconeogenesis, slowing of glucose absorption from the gastrointestinal tract, direct stimulation of glycolysis in tissues, thus inducing increased glucose removal from blood, and finally a reduction of plasma glucagon levels as well as moderate weight loss ([Katzung, 2007], [Goodman, Gilman, 2006], [McPhee, Papadakis, 2008]).

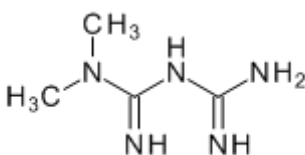


Figure 4. Structural formula of metformin (Created with Symyx Draw 3.3).

Metformin is indicated for use in combination with insulin secretagogues or thiazolidinediones in patients with type 2 DM with inadequate oral monotherapy, but can also be given alone ([Katzung, 2007], [Goodman, Gilman, 2006]).

3.1.2.2. Thiazolidinediones

Thiazolidinediones sensitize peripheral tissues to insulin. They are selective agonists for nuclear peroxisome proliferator-activated receptor γ (PPAR γ) which promotes the activation of insulin-responsive genes that regulate carbohydrate and lipid metabolism. It has also been observed that thiazolidinediones induce increased expression of glucose transporters GLUT 1 and GLUT 4, decrease free fatty acid levels and glucose production in the liver ([Katzung, 2007], [Goodman, Gilman, 2006], [McPhee, Papadakis, 2008]).

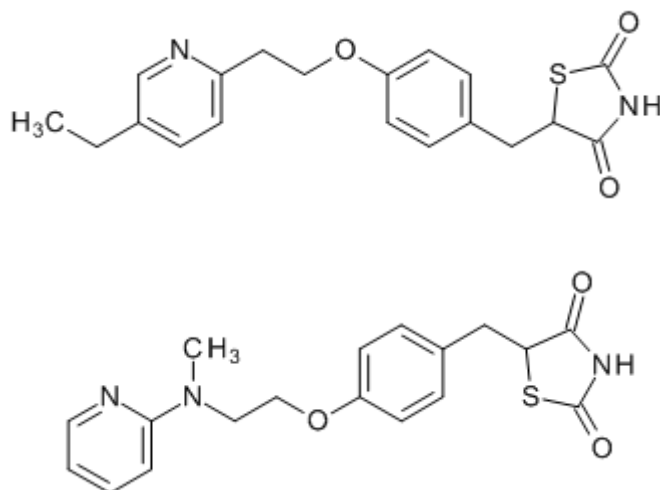


Figure 5. Structural formula of the thiazolidinediones rosiglitazone and pioglitazone (Created with Symyx Draw 3.3).

Currently available drugs of this class are pioglitazone and rosiglitazone (see figure 5 for structural formula). They can be used either as monotherapy or in combination with sulfonylureas, metformin or insulin. The combined application of a thiazolidinedione and metformin has the advantage of not causing hypoglycemia ([Katzung, 2007], [Goodman, Gilman, 2006]).

Adverse effects: thiazolidinediones have been reported to cause anemia, weight gain, edema and plasma volume extension. Additionally, liver function should be monitored (Katzung, 2007).

3.1.3. Affecting glucose absorption – α -Glucosidase inhibitors

α -Glucosidase inhibitors competitively inhibit the α -glucosidase enzymes, which delays the absorption of carbohydrates and reduces intestinal absorption of starch, dextrin and disaccharides. Both currently for clinical use available drugs – acarbose and miglitol – are potent inhibitors of glucoamylase, α -amylase and sucrase. The fundamental difference between the two drugs is their absorption. Acarbose, having structural features of a tetrasaccharide, is absorbed only to a small degree, while miglitol has a structural similarity with glucose and is absorbable.

α -Glucosidase inhibitors are typically used in combination with other oral antidiabetic agents or insulin.

Adverse effects: the most common adverse effect in both acarbose and miglitol is flatulence, followed by diarrhea and abdominal pain. All of these symptoms stem from the appearance of undigested carbohydrate in the colon ([Katzung, 2007], [Goodman, Gilman, 2006], [McPhee, Papadakis, 2008]).

3.1.4. Mimicking incretin effect or prolonging incretin action – exenatide and dipeptidyl peptidase IV inhibitors

DDPIV inhibitors are the newest class of oral antidiabetics.

The two gut hormones glucagon-dependent insulintropic polypeptide (GIP) and glucagon-like peptide (GLP-1) are also known as incretins. The secretion of GLP-1, which enhances insulin release and lowers glucose levels, is reduced in patients with type 2 DM. It also suppresses glucagon secretion and thus improves postprandial hyperglycemia. It additionally delays gastric emptying and decreases appetite ([McPhee, Papadakis, 2008], [Barnett, 2006]).

GLP-1 is rapidly inactivated by the enzyme dipeptidyl peptidase IV (DPPIV), which would make continuous infusion necessary for obtaining a clinical effect. Exenatide is a GLP-1 receptor agonist that is more resistant to DPPIV and has the same effects on glucagon suppression and gastric emptying as GLP-1 (McPhee, Papadakis, 2008).

Another approach is the application of the latest class of oral antidiabetic agents: DPPIV inhibitors such as sitagliptin (see figure 6) prolong the action of endogenously released GLP-1 and GIP and have been shown to effectively lower glucose when used alone or in combination with metformin and pioglitazone ([Goodman, Gilman, 2006], [McPhee, Papadakis, 2008], [Katzung, 2007], [Green et al., 2006], [Geelhoed-Duijvestijn, 2007]). Additionally, preclinical data suggests that DPPIV inhibitors may also preserve beta cell function and increase beta cell mass (Mu et al., 2006).

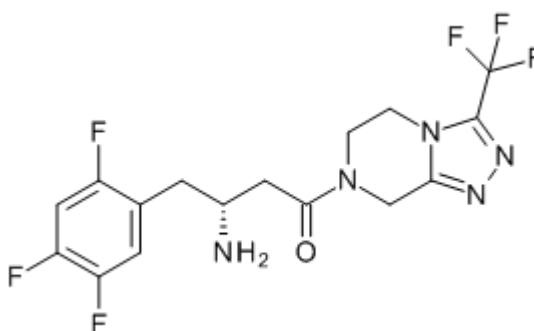


Figure 6. Structural formula of the DPP-4 inhibitor sitagliptin (Created with Symyx Draw 3.3).

3.1.5. Other – Pramlintide

Pramlintide is a synthetic analogon of amylin, a peptide produced in pancreatic beta cells. It is cosecreted with insulin and inhibits glucagon secretion, delays gastric emptying and suppresses appetite. The drug is administered subcutaneously and in addition to insulin. Major side effects include hypoglycemia and gastrointestinal symptoms such as nausea, vomiting and anorexia (Katzung, 2007).

3.2. Insulin

Due to the progressive nature of type 2 DM and the relative insulin deficiency that develops eventually, a considerable number of patients ultimately require insulin therapy (Kasper, Harrison, 2005). Insulin is injected subcutaneously and diffuses into the peripheral circulation.

Commercially available preparations of insulin can be classified according to their

duration of action into short, intermediate and long acting, and by their species of origin as human or porcine (Goodman, Gilman, 2006).

3.2.1. Unitage

For therapeutic purposes, doses and concentrations of insulin are expressed in international units (IU), ensuring that different preparations with the same biological effect contain the same number of IUs. Following the guidelines of the Expert Committee on Biological Standardization of the World Health Organisation, one IU insulin is the biological equivalent of about 45.5 µg pure crystalline insulin (1/22mg exactly). This is the amount of insulin required to reduce the concentration of blood glucose in a fasting rabbit to 45mg/dl (2.5mM).

3.2.2. Classification of insulins

As mentioned above, insulin preparations can be divided, according to their time of onset and duration of their biologic action. Rapidly/short acting insulins such as insulin lispro, insulin aspart and insulin glulisine are administered before a meal, while long acting, „peakless“ insulin formulations such as insulin glargine and insulin detemir are given to create a stable basal coverage. Neutral Protamine Hagedorn (NPH) insulin is an intermediate acting preparation. There also exist special insulin formulations that can be delivered by inhalation, are absorbed quickly and show a rapid onset of action ([Goodman, Gilman, 2006], [McPhee, Papadakis, 2008]).

Chapter 4

Artemisia dracunculus L.

1. Botany and Phytochemistry

The genus *Artemisia*, which is part of the family of composites (*Asteraceae*), comprises about 500 plants, most of which are perennials native to Europe, Russia, Mongolia, China and western and central North America. ([Jung *et al.*, 2007], [Franke, Lieberei, 1997], [Hoppe, 1975]).



Figure 7. *Artemisia dracunculus* L.

(Source: http://plants.usda.gov/gallery/standard/ardr5_001_svd.jpg)

Of the species *Artemisia dracunculus* L., commonly known as tarragon, two varieties exist. French tarragon (*Artemisia dracunculus* var. *sativa*), a popular seasoning, is known to be more aromatic and reproduces only vegetatively. Russian tarragon (*Artemisia dracunculus* var. *inodora*), has a more acrid taste and can be grown from seed. It is more sturdy and can reach a height of about 150 cm. The yellow or greenish-white flowers are small capitulae of about 2 – 4 mm in diameter that contain up to 40 florets. ([Dachler, Pelzmann, 1999], [Franke, Lieberei, 1997]).

The long and smooth leaves contain the greatest amount of volatile oil. French tarragon contains 0.15 – 3.0% essential oil, estragol (methylchavicol) being the main constituent with 68 – 80%, as well as various amounts of *cis*- and *trans*-ocimene and limonene. The oil has an aniseed-like taste. Russian tarragon yields 0.25 – 2.0% essential oil. The principal constituents are sabinene, elemicine and trans-isoelemicin, resulting in a bitter chervil-like taste of the oil [Wright, C., 2002]).

The extracts used for the present study were obtained from *Artemisia dracunculus* var. *inodora*.

2. Traditional and modern use

2.1. Background

Artemisia dracunculus is a perennial herb with a long history of medicinal and culinary use ([Swanston-Flatt *et al.*, 1991], [Wang *et al.*, 2008]). In traditional medicine, aerial parts of the plant are applied to cure various digestive complaints (Wright, C., 2002).

The drug of *A. dracunculus* is *Herba dracunculi*, the dried aerial part of the plant. The main component is the essential oil, *Oleum dracunculi* (Hoppe, 1975).

The genus *Artemisia* is not new in diabetes research: *Artemisia herba alba* has been used in Iraqi folk medicine for the treatment of diabetes mellitus, and several studies indicating its hypoglycemic activity have already been completed ([Twaij, H. A., Al-Badr, A. A., 1988], [al-Khazraji *et al.* 1993], [al-Shamaony *et al.*, 1994]).

The hypoglycemic potency of *Artemisia sphaerocephala* Krasch and the anti-diabetic effect of *Artemisia princeps* Pampanini extracts have already been proven in animal models ([Zhang *et al.*, 2006], [Jung *et al.*, 2007], [Kang *et al.*, 2008]).

Previous studies indicate that extracts of *Artemisia dracunculus* L. have antihyperglycaemic activity and could be of use in the management of diabetic states, suggesting multiple modes of action for the tested extract ([Ribnicky *et al.*, 2006], [Govorko *et al.*, 2007]). *A. dracunculus* is also being tested for its potential beneficial effect on the metabolic syndrome, an illness that is associated with insulin resistance (Cefalu *et al.*, 2008).

2.2 Mechanism of action

Artemisia dracunculus L. has been found to have several distinct methods of action valuable for the treatment of diabetes and the complications associated with it:

Ethanollic extracts of *A. dracunculus* L. were found to be active in insulin-deficient (genetically diabetic KK-A^y) mice, suggesting an insulin-like effect on peripheral tissues and enhanced GLP-1 receptor binding as probable mechanisms of action (Ribnicky *et al.*, 2006). Furthermore, it has been shown that two polyphenolic compounds isolated from *A. dracunculus* (see below) have an insulin-like effect on phosphoenolpyruvate carboxykinase (PEPCK) gene expression (Govorko *et al.*, 2007). PEPCK is an enzyme of the metabolic pathway that controls the reversible,

rate controlling step of gluconeogenesis. It is inhibited by insulin.

Likewise, three compounds of the ethanolic extract proved to inhibit aldose reductase (ALR2), an enzyme of the polyol pathway that catalyzes the conversion of blood glucose into sorbitol in the presence of NADPH (Logendra *et al.*, 2006).



Figure 8. *Artemisia dracunculus* L.

(Source: http://plants.usda.gov/gallery/standard/ardr5_1v.jpg)

In vivo tests with an alcoholic extract, using primary human skeletal muscle cultures showed enhanced insulin activity through reduction of phosphatase activities such as protein tyrosine phosphatase1-B (Wang *et al.*, 2008).

2.3. Active compounds

Some compounds of *A. dracunculus* L. that are responsible for the methods of action described above have already been identified. These compounds include polyphenoles such as 6-demethoxycapillarisin, 2',4'-dihydroxy-4-methoxydihydrochalcone and 4,5-di-*O*-caffeoylquinic acid ([Govorko et al, 2007], [Logendra *et al.*, 2006]). Additionally, other compounds of the aerial part of *A. dracunculus* have already been reported to display antidiabetic activity. This includes flavonoids, such as luteolin and apigenin, coumarins such as scopoletin, and sesquiterpenoid lactones such as costunolide, and cinnamates ([Duke, 1992], [Huang *et al.*, 1993], [Arlt *et al.*, 2004], [Liu *et al.*, 2003]).

3. Toxicity

Concerning the possible toxicity of *Artemisia dracunculus* L. extracts, inconsistent data was published lately. In a recent study, the ethanolic extract of the shoots of *A. dracunculus* showed no noteworthy signs of toxicity and appeared to be safe and nontoxic (Cefalu *et al.*, 2008). But in two statements by the German 'Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin' (BgVV), released on May 11th 2001 and November 12th 2001 respectively, it was advised to reduce the intake of products containing estragole and methyleugenol – organic compounds that can be found in plants like tarragon, basil and anis (see figures 9 and 10). This was substantiated by *in vivo* experiments with rats and mice in which those compounds proved to be hepatocarcinogenic ([BgVV, may 2001][BgVV, november 2001]).

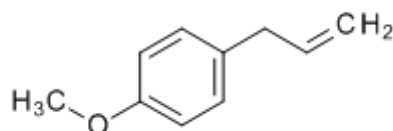


Figure 9. Chemical structure of estragole (Created with Symyx Draw 3.3).

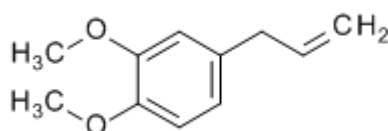


Figure 10. Chemical structure of methyleugenol (Created with Symyx Draw 3.3).

This study has however been subject to criticism for various reasons. The pivotal points of criticism concern the fact that monosubstances in concentrations as high as 1000 mg / kg bodyweight were applied, thus not accounting for the antioxidative properties of the multicomponent mixtures of plant extracts or the possibility of transferring data from the animal study to the human situation ([Iten, Saller, 2004], [Smith *et al.*, 2002]).

The „European Agency for the Evaluation of Medicinal Products“ (EMA) has since issued a reevaluation of herbal medicinal products containing estragole, in which they are deemed to not pose a significant cancer risk if consumed in appropriate quantities. Nonetheless, exposure levels of estragole to sensitive groups such as children, pregnant and breastfeeding women should be minimised (European Agency for the Evaluation of Medicinal Products, 2004).

4. Choice of extract

4.1. Extraction method

In order to reduce the amount of estragole and methyleugenol in the extract used in this study, ethanolic and water extracts were compared previously regarding the content of those critical compounds. It could be shown that while water extracts contained no detectable ('n.nw.' for 'nicht nachweisbar' in figure 11) amounts of estragole and methyleugenol, this was not true for ethanolic extracts.

Droge	<i>Artemisia dracunculus</i>	<i>Artemisia dracunculus</i>	<i>Artemisia dracunculus</i>	<i>Artemisia dracunculus</i>
Art	var. <i>sativa</i> (Franz.)	var. <i>sativa</i> (Franz.)	var. <i>inodora</i> (Russ.)	var. <i>inodora</i> (Russ.)
AZM	Wasser	Ethanol 60% (V/V)	Wasser	Ethanol 60% (V/V)
Estragol [ppm]	n.nw.	1,75	n.nw.	0,58
Methyleugenol [ppm]	n.nw.	7,69	n.nw.	7,31

Figure 11. Content of estragole and methyleugenol in water and ethanolic extracts (Courtesy of Finzelberg GmbH & Co KG, Andernach, Germany)

Extracts of French Tarragon contained higher levels of the aforementioned compounds than Russian Tarragon.

4.2. Previous studies – choice of subspecies

Studies with *Artemisia dracunculus* have already been accomplished in Dr. Veronika Butterweck's group at the University of Florida prior to the present work. The aim was to compare the antidiabetic properties of the subspecies *Artemisia dracunculus* var. *sativa* (French Tarragon) and *Artemisia dracunculus* var. *inodora* (Russian Tarragon). It could be shown that Russian Tarragon showed antidiabetic activity, with the ethanolic fraction being more active than the aqueous fraction; French Tarragon was not active (see Figure 12).

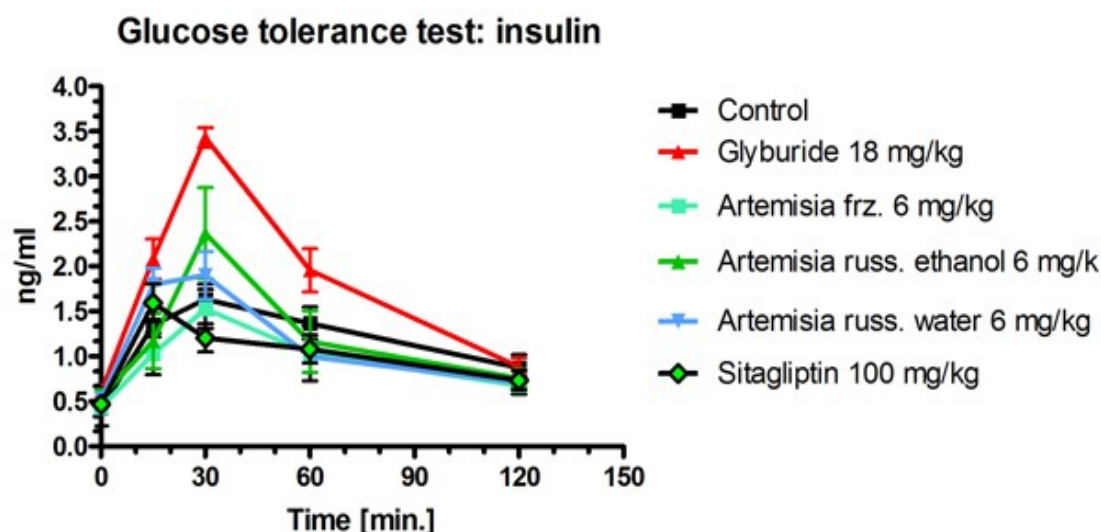


Figure 12. Comparison of Russian and French Tarragon in glucose tolerance test
(Butterweck *et al.*, unpublished)

It was therefore decided to work with the extract that had the highest antidiabetic activity and at the same time the lowest levels of estragole and methyleugenol. As a consequence, the aqueous extract of Russian tarragon was chosen for further research.

Chapter 5

Materials and Methods

1. Materials

1.1. Chemicals, drugs

The volatile anaesthetic isoflurane was purchased at Webster Veterinary, Sterling, Massachusetts, USA. Halothane and glyburide (glibenclamide) were bought from Sigma-Aldrich, St. Louis, Missouri, USA. For the second positive control with sitagliptin, Januvia™ 100 mg, 90 tablets from Merck & Co., Inc., Whitehouse Station, New Jersey, USA were purchased. The sodium hydroxide used to prepare isotonic saline for intraperitoneal injection as well as propylene glycol were obtained from Thermo Fisher Scientific, Pittsburgh, Pennsylvania, USA.

To achieve optimal results, all suspensions were homogenized, using the Power Gen 125 from Fisher Scientific (Catalogue No. 14-261-02). Blood samples were centrifugated with Eppendorf Centrifuge 5810 R.

1.2. Extracts

Different extracts of *Artemisia dracunculus* var. *inodora* were used as provided by Finzelberg GmbH & Co KG, Andernach, Germany. The extracts were weighed in plastic tubes and diluted with a 0.5% propylene glycol (PG) solution. All dilutions were homogenized with the Power Gen 125.

1. 3. Control and positive control

Animals received a 0.5% PG solution as control. For this, a stock solution of 0.5 ml PG and 99.5 ml purified water was prepared.

Two oral antidiabetics, glyburide and sitagliptin, were used as positive controls. Suspensions were prepared as follows:

Glyburide was suspended in 0.5% PG solution. Rats received 18 mg/kg glyburide, which equals a drug concentration of 3,6 mg/ml.

Sitagliptin tablets were pulverized with the aid of a mortar and subsequently suspended in 0.5% PG solution. 100 mg/kg sitagliptin were orally administered. The drug solution had a concentration of 20 mg/ml.

2. Methods

2.1 Animal Procedures

All animal procedures have been approved and monitored by the Institutional Animal Care and Use Committee, IACUC, of the University of Florida.

After each experiment, the animals were not used for other tests for a period of at least five days in order to give them time to recover from bloodloss and as a sufficient washout period.

2.1.1. Overnight Fasting

As described by E. J. Verspohl, blood glucose measurements can be carried out with either fasted or non-fasted animals. Either method has its advantages: fasted animals show a small fluctuation of blood glucose levels in a controlled setting, whereas non-

fasted rats offer a more physiological situation (Verspohl, 2002).

To determine eventual advantages of either method, both models were tested. The animals had access to water and food pellets *ad libidum*. Food deprivation was implemented for about 15 hours prior to the experiment.

2.1.2. Procedure for Oral Gavage

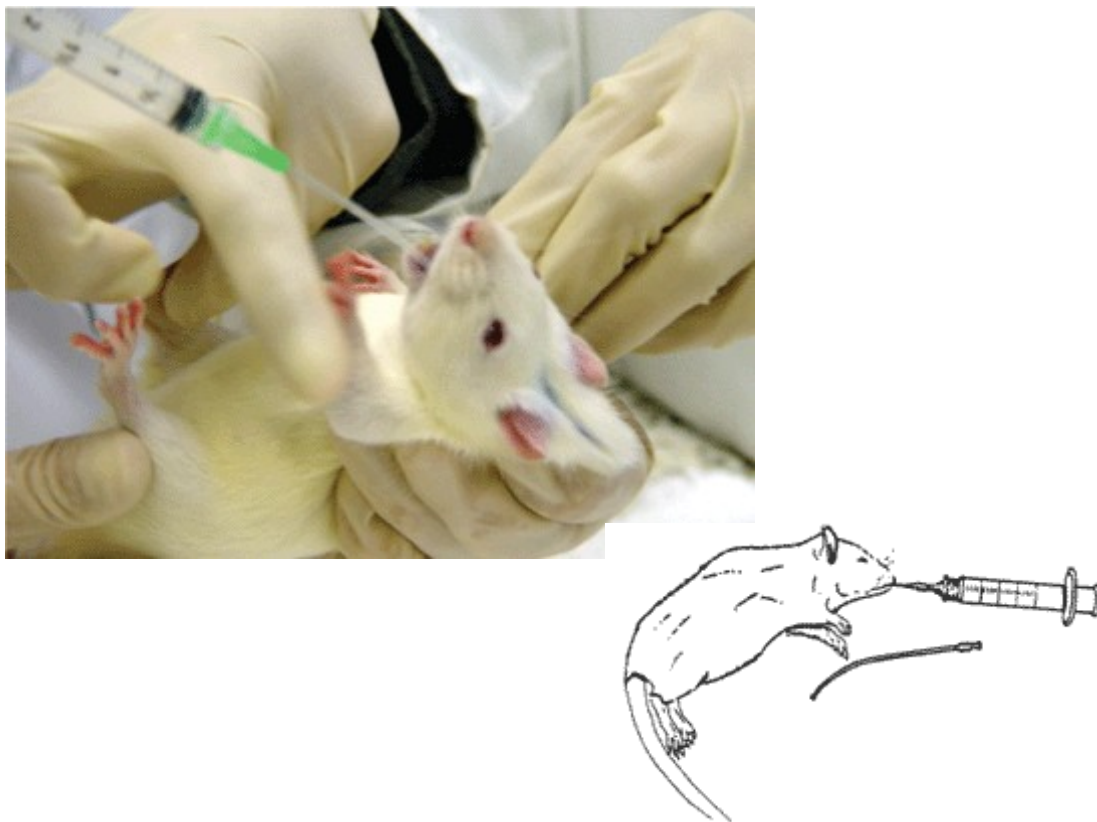


Figure 13. Oral gavage – seizing all loose skin in the neck area

(Source: www.scielo.br/img/revistas/rhc/v59n5//a06fig01.gif)

Figure 14. The slightly curved gavage reaches down to the stomach

(Source: www.urmc.rochester.edu/ucar/images/2fig7.gif)

For this, a stainless steel ball tipped gavage and a 3ml syringe were used. Following the guidelines of AALAS Learning Library for Animal Care and Use in Research and Education (www.aalaslearninglibrary.org), slightly curved needles were used.

The animal needs to be held firmly at the neck area, seizing all loose skin (see figure 13). This ensures that the procedure can be performed quickly and without harm to the animal. In this position, the mouth of the rat is already slightly opened and the gavage can be inserted. Moving the gavage forward from the side and behind the back teeth, it can now slide down the oesophagus and into the stomach (see figure 14) without pressure, on its own accord.

2.1.3. Basal glucose measurement

Male Wistar rats weighing 300 – 350 g were purchased from Harlan (Indianapolis, IN, U.S.A.). Rats were housed in cages of two at 20 ± 1 °C in a 12-h light/dark cycle. Tap water and food pellets were available *ad libidum*. Groups of six rats were randomly assigned to different treatment groups. All experiments were carried out in a quiet room between 8:00 a.m. and 1:00 p.m.

The second set of rats were white Sprague Dawley rats from Harlan, see above, purchased at around nine weeks of age and used for a period of around nine weeks.

After overnight food deprivation, control, glyburide (18mg/kg), sitagliptin (100mg/kg) and four different doses of the extract (1.5mg/kg, 6.0mg/kg, 30.0mg/kg and 60.0mg/kg) were given orally immediately after the first blood collection, equalling timepoint zero.

For the second set of rats, overnight fasting was not implemented.

Blood samples from the sublingual veins were collected into heparinized capillary tubes at timepoints 0, 30, 60, 120 and 180 min. Blood glucose was measured immediately after the puncturing of the vein, using a commercially available

glucometer.

After every blood collection, approximately 0.9 ml isotonic saline was administered i.p. (see below for further details)

2.1.4. Glucose Tolerance Test (GTT)

Herbal extracts in concentrations as described above were orally administered 30 minutes prior to the first blood collection. In order to increase plasma glucose levels, rats were challenged by glucose (2g/kg) i.p. immediately after the first blood collection (timepoint 0). Additional blood samples were collected at 15, 30, 60 and 120 min. The subsequent procedure was the same as described above for basal glucose measurement.

2.1.5. Blood collection

Blood collection from the *vena sublingualis* requires anaesthesia of the animal. For this, the rats were placed in a desiccator that had been prepared with about 3 to 4 ml of isoflurane (halothane respectively) and layers of absorbent paper. When the air in the desiccator was saturated, the rat was placed in it until breathing and heartbeat slowed down (after about two minutes).

Blood was now collected from the sublingual vein following the procedure described by Zeller *et al.* (Zeller *et al.*, 1998).

Blood was withdrawn by puncturing the *Vena sublingualis* at intervals indicated above. The vein is to be punctured by holding the needle parallel to the vein so as not to pierce through the vessel. The vein can be ruptured carefully to assure that enough blood can be collected. Both *venae* can be punctured alternately. Approximately 400µl blood were collected / animal / sampling time by holding the

rat with the head downwards and applying gentle pressure to the neck area to ease bloodflow (see Figure 13).

Blood samples were collected in special tubes with a heparin layer to avoid clotting. The tubes were kept on ice until the blood was centrifuged for serum separation. The samples were centrifuged at 4°C at 2800 rpm for 15 minutes.

Plasma was then pipetted into Eppendorff caps and stored at -20°C for subsequent measurement of insulin and DPPIV levels.



Figure 15. Blood collection.

2.1.6. Volume replacement after blood collection

In order to replace the approximately 400µl blood collected at each timepoint, about 0,9ml isotonic saline was administered i.p., using a 23 gauge $\frac{3}{4}$ needle.

2.2. Insulin ELISA

Enzyme Linked Immunosorbent Assays can be classified in six categories: indirect, direct competitive, antibody-sandwich, double antibody-sandwich, direct cellular and indirect cellular.

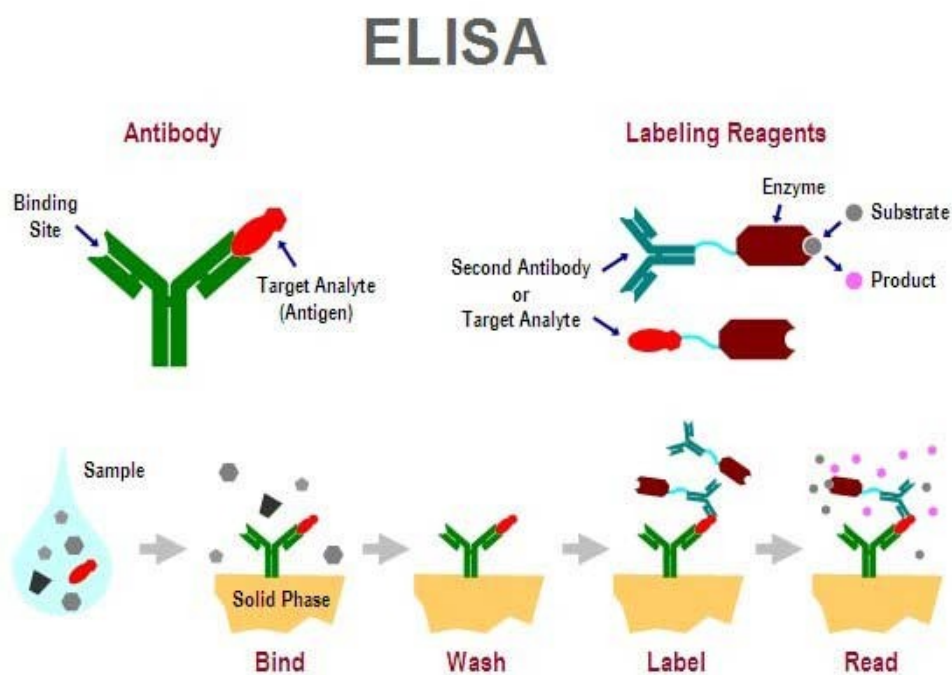


Figure 16: ELISA test-principle

(Source: <http://biosystemdevelopment.com>)

The antibody-sandwich ELISA may be the most useful assay for antigen-detection, as it generally is between 2 and 5 times more sensitive than those in which antigen is directly bound to the solid phase (Hornbeck *et al.*, 2000).

For detection of insulin, a solid phase sandwich ELISA was purchased from ALPCO Diagnostics (Insulin (Rat) Ultrasensitive EIA, Catalogue Nr.: 80-INSTRU-E01, Salem, New Hampshire, USA).

The 96 well microplate is coated with an insulin-specific (capture) antibody. After insulin standards, mammalian insulin controls and samples are added to the microplate, the plate is incubated with horseradish peroxidase enzyme (monoclonal antibody). Unbound antigen is washed out, and substrate is added, followed by another incubation period. After the stop solution has been added, the optical density is measured by a spectrophotometer at 450 nm. The degree to which substrate has been hydrolyzed corresponds proportionally to the amount of antigen in the samples ([Hornbeck *et al.*, 2000], [Alpco Diagnostics]).

2.3. DPPIV Assay

For the detection of DPPIV in rat serum, a protease assay was carried through. The following chemicals were purchased from Sigma-Aldrich, St. Louis, Missouri, USA: Trizma® base, dipeptidyl peptidase IV recombinant human, 4-nitroaniline and gly-pro-p-nitroaniline.

DPPIV is a serine protease that cleaves proteins after proline residues. The luminogenic substrate contains a Gly-Pro sequence that is cleaved by the DPPIV enzyme. The ensuing luciferase aminoluciferin is oxidized during the luciferase reaction, resulting in the production of light (see Figure 17).

The following substances were used for the assay:

- A. 0.1 M Tris-HCl, pH 8.0 at 37°C
- B. 1mM gly-pro-p-nitroaniline solution Sigma
- C. 1 mM p-nitroaniline solution (pNA)
- D. Dipeptidyl peptidase enzyme solution

In a 96-well plate, standards were pipetted: 0, 20, 40, 60, 80 and 100µl of pNA were filled up to 0.1 ml with reagent A. Enzyme samples were pipetted at 10, 20, 30, 40 and 50 µl and filled up to 0.1 ml with reagent A as well. Correspondingly, 60µl of rat plasma samples were pipetted in the remaining wells and filled up to 0.1 ml with reagent A. 0.1ml of reagent B was now added to each well, starting the reaction. After 15 minutes of incubation at 37°C, absorbance was read at 560 nm in a microplate reader.

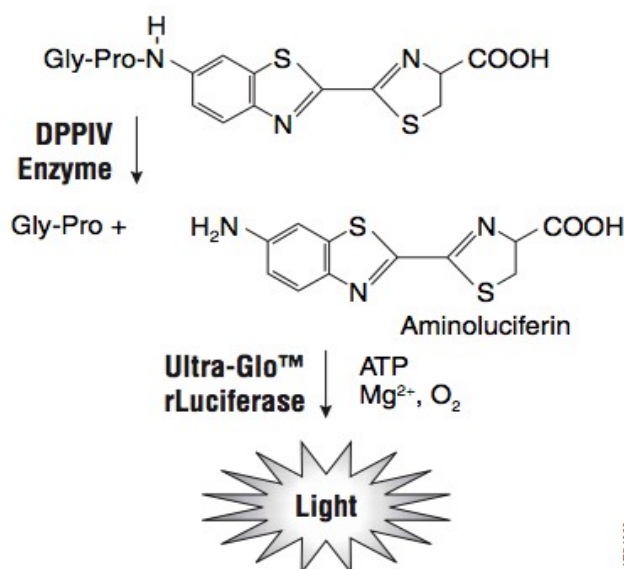


Figure 17. DPPIV immuno assay test principle

(Source: www.promega.com)

2.4. Analysis

Data are presented as mean $\pm$ SEM for each group (N = 6). Significant differences among the groups were determined by two-way analysis of variance (ANOVA). The significance of the difference between the means of test and control studies was established by Bonferroni post-hoc test. P values of less than 0.05 were considered significant. Outliers were calculated using GraphPads online Grubbs test calculator on www.graphpad.com/quickcalcs/Grubbs1.cfm

Chapter 6

Results

1. Influence of different anaesthetics on blood glucose levels

As it can be seen in figure 18, the blood glucose levels in unfasted male rats were slightly higher when halothane was used as anaesthetic than with isoflurane. Additionally, isoflurane shows a more linear increase in blood glucose levels than halothane, which accounts for the fact that depth of anaesthesia was easier to control with isoflurane. For detailed information concerning the necessity of this preliminary comparison, see chapter 7.

Isoflurane was therefore chosen for the subsequent testing of *Artemisia dracunculus* extracts.

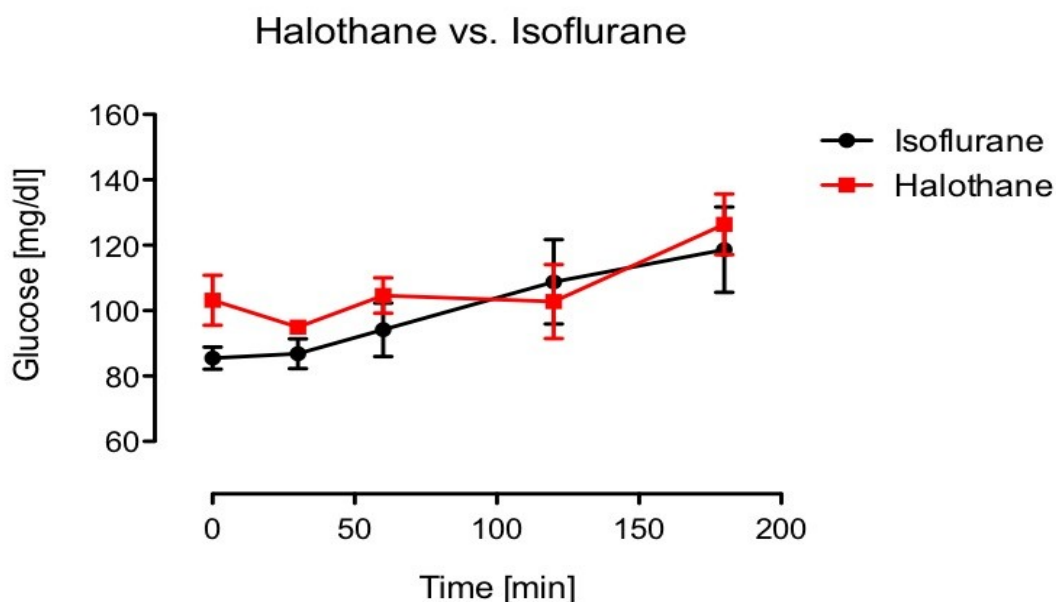


Figure 18: Blood glucose levels in unfasted rats.

2. Influence of food withdrawal on blood glucose levels

Figure 19 shows that fasting has no significant effect on blood glucose. While the rats had access to water *ad libidum*, food pellets were taken away for an overnight fasting period of about 15 hours.

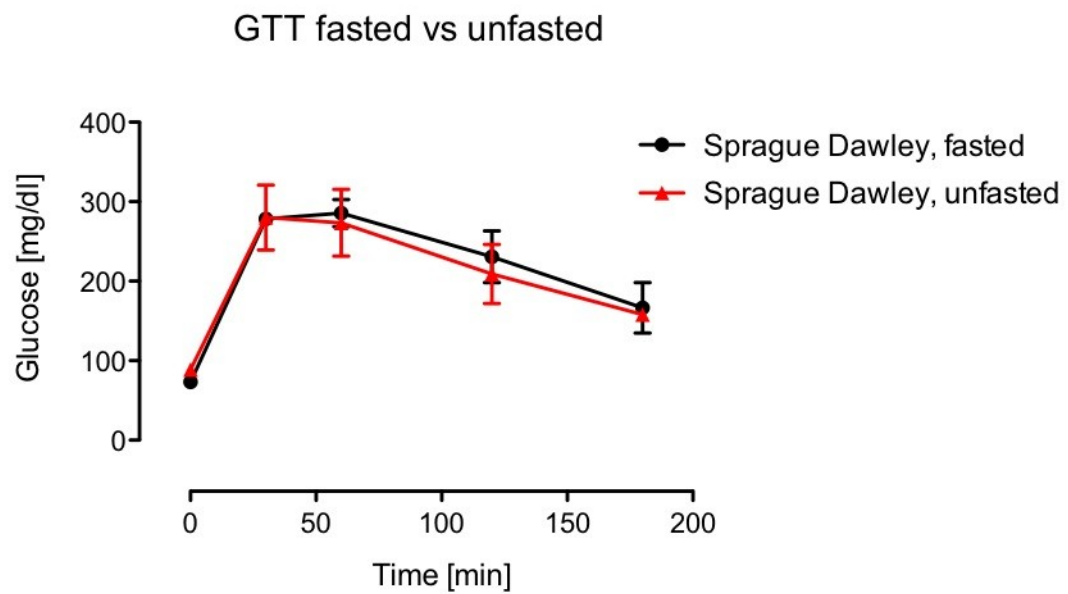


Figure 19. Fasted vs. unfasted rats.

3. Differences in blood glucose of Wistar and Sprague Dawley rats

As can be seen in figure 20, the difference between basal blood glucose levels in Wistar and Sprague Dawley rats is not significant. While the discrepancy increases slightly in the glucose tolerance test (see figure 21), it still remains insignificant.

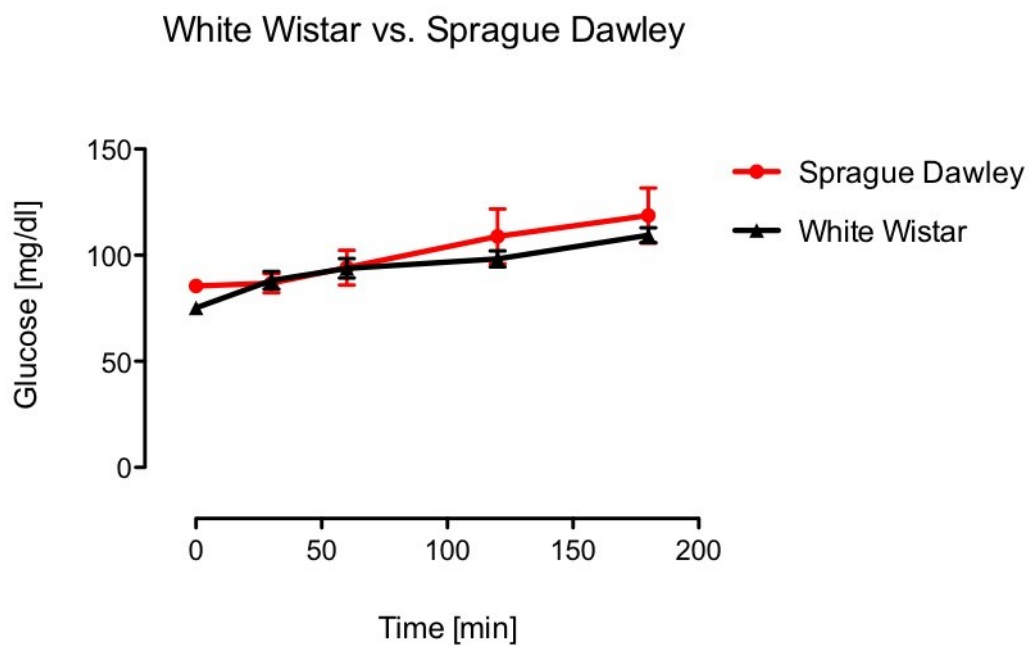


Figure 20. Wistar vs. Sprague Dawley rats, basal

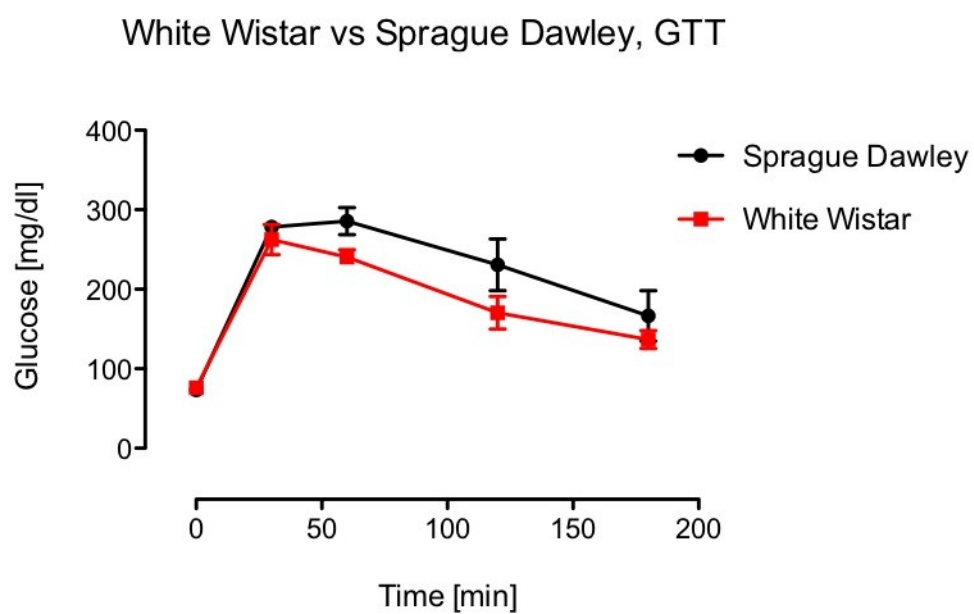


Figure 21. Wistar vs. Sprague Dawley rats, glucose tolerance test

4. Dose finding study

In the basal dose finding study (see figure 22), the positive control glyburide proved to be the only significant compound. The significant effect could be observed from the second measurement at 30 minutes to the last at 180 minutes. The second positive control sitagliptin did not affect the blood glucose levels at all.

In the glucose tolerance test, glyburide showed a significant effect at 15, 30 and 60 minutes, with the *Artemisia dracunculus* extracts of 30 and 60 mg/kg being significant at 30 minutes.

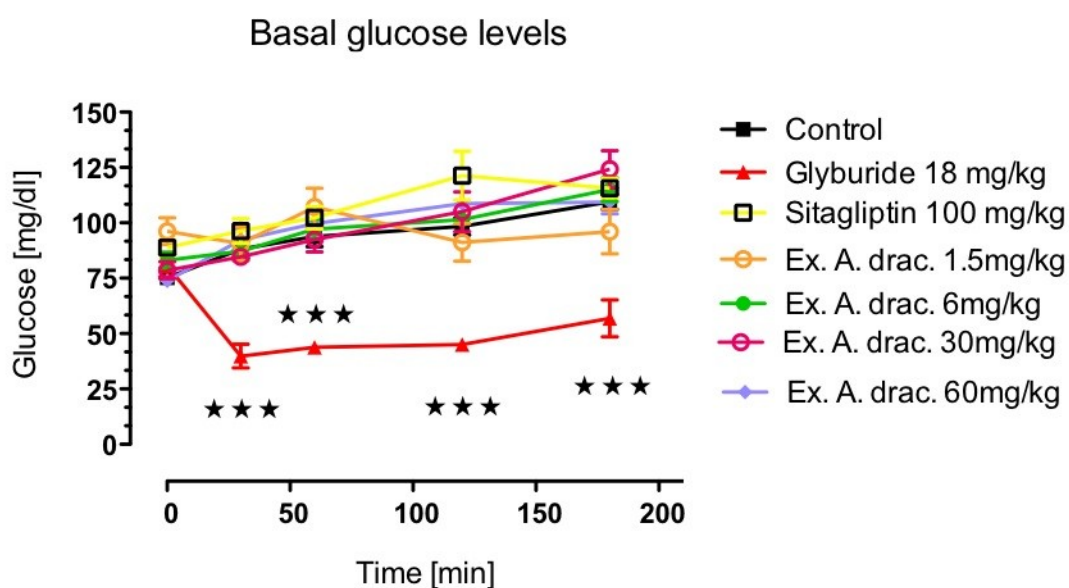


Figure 22. Dose finding study with Wistar rats. basal

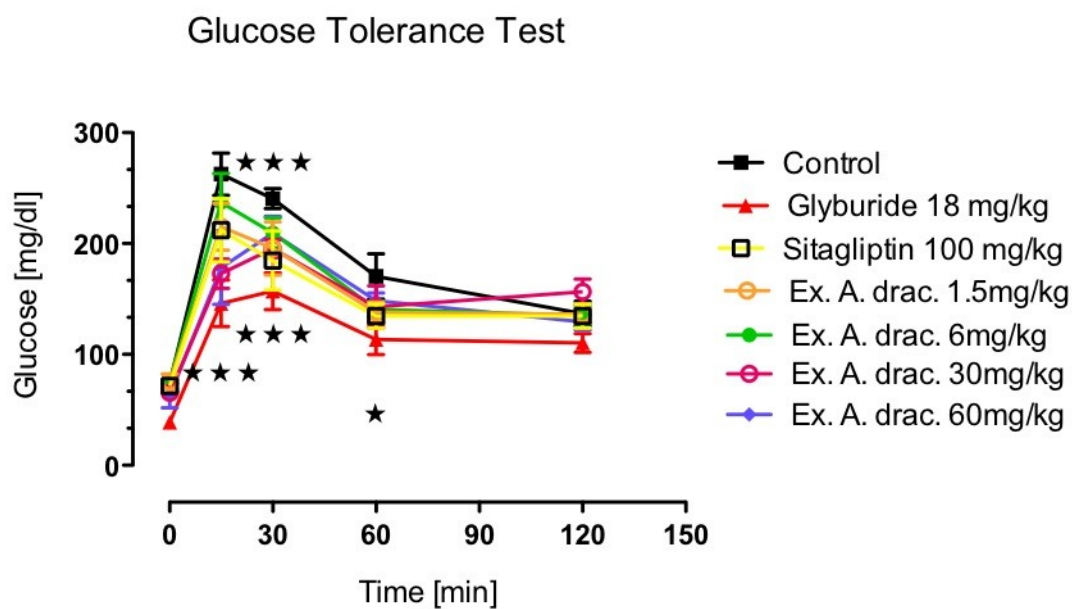


Figure 23. Dose finding study *Artemisia dracunculus* extracts with Wistar rats, glucose tolerance test

5. Plasma analysis – Insulin ELISA

As shown in figure 24, Glyburide increased insulin levels for the first 30 minutes after the treatment, while the *Artemisia dracunculus* extract exhibited some activity at 15 minutes.

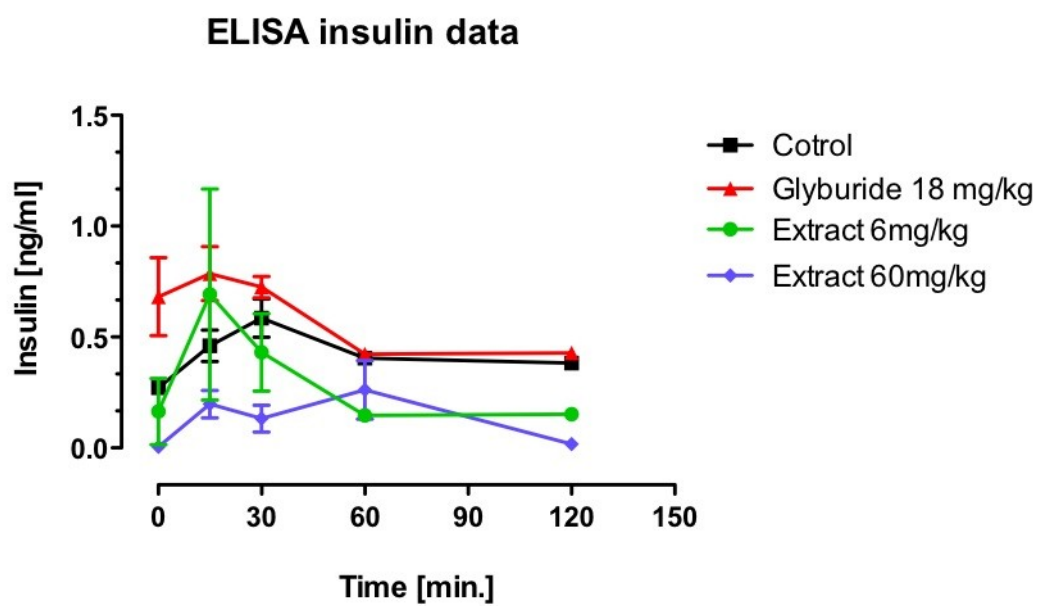


Figure 24. ELISA insulin data from glucose tolerance test

6. Plasma analysis – DPP-IV Assay

Although Sitagliptin did not achieve a significant decrease in blood glucose levels, it did show a severe decline in DPPIV values, while both extracts had no effect.

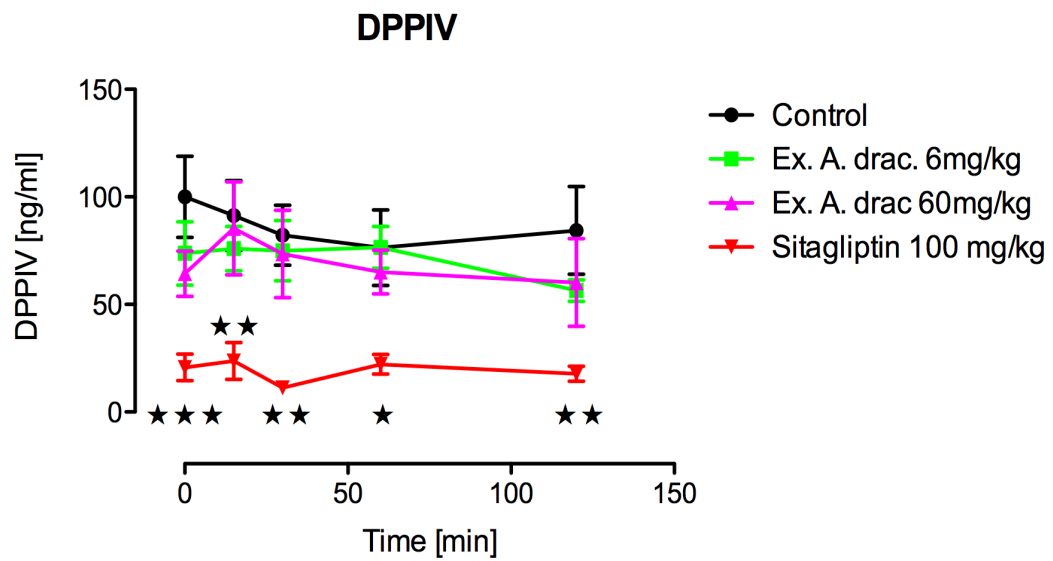


Figure 25. DPPIV data from glucose tolerance test

Chapter 7

Discussion

1. Anaesthetic

As initially planned, first experiments for this study were carried out using the anaesthetic isoflurane, but regulations changed at this point and now prohibited the use of halothane within the United States of America. Isofluran was considered a possible alternative. Due to a lack of data on the comparability of the two anaesthetics, it was deemed necessary to carry out tests to determine their impact on blood glucose levels in rats.

Although a study by Kofke *et al.* (1987) has shown that the blood glucose levels in plasma and brain were significantly elevated in the isoflurane group compared to enflurane and halothane, it was found in the present study that blood glucose levels of the isoflurane group were slightly below those of the halothane group. This could result from the fact that the rats in Kofkes study were exposed to anaesthetics for a considerably longer time, being mechanically ventilated for a period of 60 to 75 minutes, while the rats for the *Artemisia*-study were exposed to the anaesthetic five times for only about 2 minutes each, following the test setup as described for the basal glucose measurement (see above).

This outcome corresponds with a study on humans by Young *et al.* The effects of different anaesthetic techniques (isoflurane, halothane, fentanyl and sufentanil) on plasma glucose levels were compared during cerebrovascular surgery. No significant difference between the anaesthetics could be detected, but each group showed a gradual increase in plasma glucose during the procedure (Young *et al.*, 1990).

Additionally to the measurement of blood glucose levels, it could be observed that

the anaesthesia was not as deep with isoflurane as with halothane, and that consequently resuscitation was more rarely necessary. The anaesthesia was easier to control and the animals were easier to handle with isoflurane.

Another advantage of isoflurane is the fact that it is less prone to lead to hepatotoxic side effects than halothane. An additional interesting side effect in regard of the present work is the fact that isoflurane dilates peripheral blood vessels which may facilitate blood collection from the *vena sublingualis* (Mutschler *et al.*, 2001).

Summing up, isoflurane was more convenient to work with and it caused slightly less changes to glucose levels than halothane.

2. Influence of food withdrawal on blood glucose levels

Concerning the access to food animals in a diabetes study have, two possibilities with different advantages exist as described by Verspohl (2002) and has already been mentioned (see chapter 3 'Materials and Methods'). As no data could be found on the actual effect food deprivation has on blood glucose levels, this experiment had to be conducted in order to find the method best suitable for the present study, but also with regard to animal welfare and comparability of data that have already been published in various papers.

Ensuing data shows clearly that figures differ only slightly, which suggests a comparability of both approaches. In order to reduce stress and discomfort for the laboratory animals, it is advisable to provide food *at libidum*. It has to be noted that, although food was not taken away from the unfasted animals before the blood collection, it could be observed that the rats in most cases did not eat after they were brought in the animal procedure room.

3. Dose Finding Study

Artemisia dracunculus extracts (as well as sitagliptin) did not influence basal glucose levels. In the glucose tolerance test, extracts at concentrations of 30mg/kg and 60mg/kg significantly decreased blood glucose levels after 15 minutes. The dose of 60mg/kg was chosen for testing of plasma samples due to the fact that the whole curve had slightly lower glucose levels. As well, additional data confirmed the hypothesis that the antidiabetic potency of concentration 60mg/kg is higher than that of 30mg/kg.

4. Plasma analysis – Insulin ELISA and DPP-IV assay

While Glyburide showed an increased insulin secretion, the *Artemisia* extract containing 60mg/kg caused no augmentation in plasma insulin with levels being even below those of the control group. The graph for concentration 6mg/kg however showed an increase of plasma insulin at 15 minutes, followed by a sharp decline in insulin levels.

In the DPPIV assay, only Sitagliptin effectively lowered DPPIV levels, while neither *Artemisia dracunculus* extract affected plasma DPPIV concentrations. While this suggests a mechanism of action other than the inhibition of DPPIV, additional testing in this direction would be interesting.

Insulin ELISA and DPPIV assay were completed in order to compare the activity of the extracts to the positive control and to assess timepoints of activity.

5. Outlook

What sets the present work apart from previously published literature concerned with possible antidiabetic effects of *Artemisia dracunculus* L. extracts are two important points: firstly, the fact that most studies, such as Ribnicky *et al.*, 2006, Wang *et al.*, 2008, Govorko *et al.*, 2007 and various others, worked with an ethanolic extract which, though potentially more active than the aqueous extract, is also more problematic due to its potentially cancerogenic and hepatotoxic compounds methyleugenol and estragol (as discussed previously). Bearing in mind the possible use of this extract as an antidiabetic drug or functional food, it seems important to explore the possibilities of the aqueous extract more profoundly.

The second difference is the concentration of the herbal extract. The present study investigated the effect of extract concentrations ranging from 1.5 mg/kg to 60.0 mg/kg, which are more feasible in regard to possible human consumption as for example a dosage of 500 mg/kg as used by Ribnicky *et al.*, 2006 and 2009.

In this study, non-diabetic rats have been used to determine the effects of *Artemisia dracunculus* extracts on blood glucose, plasma insulin and DPPIV levels. It should also be noted that a recent diabetes study on the effects of an alcoholic extract of *A. dracunculus* L. on diabetic and non-diabetic mice found that both glucose and insulin concentrations did change significantly only in diabetic animals (Ribnicky *et al.*, 2006).

Looking at extrapancreatic effects and the mechanism of action with *in vitro* tests should yield interesting data as well.

To optimize antidiabetic potency of the extract, the effect of different extraction methods and possible additives such as the bioenhancer Labrasol could be investigated.

5.1. Functional Foods

As mentioned earlier, the incidence of diabetes is increasing worldwide, but still no treatment has been found and the current approach of oral antidiabetic agents combined with exercise and a healthy diet does not seem to be an adequate response to the diabetes epidemic. In this regard, the screening of new compounds is of eminent importance and emphasis should be laid on the evaluation of plant extracts, as this field has long been neglected by diabetes research (Verspohl, 2002).

Plant extracts as dietary supplements that can help patients control hyperglycemia could prove highly beneficial. Extracts of *Artemisia dracunculus* L. have already been examined regarding their possible use as dietary supplements (Ribnicky *et al.*, 2006).

As there is still no cure for diabetes, intervention in the prediabetic stage in order to prevent a progression of the disease seems a reasonable approach (The Diabetes Prevention Program, 1999). Several approaches could be used to achieve this: while lifestyle modifications are difficult to maintain, drug therapies may prove detrimental to the large number of individuals who may never develop type 2 diabetes mellitus (Rosenstock, 2007). Whether dietary supplements could be of use in this field, either in intensifying the effect of lifestyle modification or in addition to drug therapies has yet to be studied. But regarding the fact that the increasing diabetes prevalence is strongly linked to and preceded by a worldwide obesity epidemic ([Reimann *et al.*, 2009], [Bray, 1998], [Stumvoll *et al.*, 2005]), an individual and more targeted combination of these remedies to reduce impaired glucose tolerance and impaired fasting glucose (see chapter 1) at an early stage could prove to be successful. A long-term goal would be to develop a remedy that targets specific pathologic defects, thus restoring normoglycaemia (Stumvoll *et al.*, 2005).

But which requirements have to be met for a food to be called functional? According to Diplock *et al.*, the following working definition can be adopted:

*„A food can be regarded as 'functional' if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease.“ (Diplock *et al.*, 1999)*

Until now, evidence supporting the functionality of foods for the prevention of overweight and diabetes is, however, incomplete (Riccardi *et al.*, 2005). Studies such as the Finnish Diabetes Prevention Study (Tuomilehto *et al.*, 2001) show that moderate weight loss of about 5% can decrease the development of T2DM by half in individuals at risk for this disease. This shows the tremendous potential that lies in functional foods targeted at this indication (Hill *et al.*, 2002).

Developing functional foods also accounts for the trend in modern society: the use of complementary and alternative medicine is rising, with herbal medicine being most popular ([Tindle *et al.*, 2005], [Klein *et al.*, 2004]).

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Deutsch	Muttersprache
Englisch	Wort und Schrift
Französisch	Wort und Schrift
Latein	Gute Kenntnisse
Norwegisch	Grundkenntnisse
Italienisch	Grundkenntnisse
