The investigation of the antidiabetic Dominican traditional medicinal plants *Costus spicatus* Sw. and *Momordica charantia* L.

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Abstract

The investigation of the antidiabetic Dominican traditional medicinal plants *Costus spicatus* and *Momordica charantia*

by

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Diabetes is a serious disease affecting many people throughout the world, and is expected to increase in the coming decades. Traditional medicine is used in many places around the globe, including the Dominican Republic, for the treatment of diabetes along with modern medicines. Fieldwork conducted in the Dominican community by the Institute of Economic Botany identified *Costus* species and *Momordica charantia* L. as being widely used for diabetes treatment, thus pointing to these plants for further investigation.

In an in vivo study, *Costus spicatus* Sw. tea or water were fed ad libitum to a C57BLKS/J mice (KS) *db/db* mouse model of obesity and type 2 diabetes mellitus (T2DM). The *C. spicatus* tea did not improve glucose or insulin tolerance, or moderate hyperglycemia or insulin sensitivity.

To analyze the hypoglycemic effect of *Momordica charantia* fruit, initial studies were conducted in vitro. Both an ethanol extract and saponin-rich fraction of fruit, along with the five isolated triterpene saponin compounds 3*β*,7*β*,25-trihydroxycucurbita-5,23(*E*)-dien-19-al, momordicine I, momordicine II, 3-hydroxycucurbita-5,24-dien-19-al-7,23-di-*O*-*β*-glucopyranoside, and

kuguaglycoside G, were tested to assess their potential stimulation of insulin secretion. The saponin-rich fraction, along with mormordicin II and kuguaglycoside G, were active in the assay, suggesting saponins as the active hypoglycemic compounds in *M. charantia*.

To further characterize the antidiabetic activity of *Momordica charantia*, a saponin-rich fraction and ethanol extract of the fruit was gavaged daily to C57BL/6 mice fed a high-fat diet. Both treatments lowered fasting glucose and improved glucose tolerance after three weeks. Also, the ethanol-extract treated group had significantly less β-cell mass at the end of the study, pointing to improved β-cell function. The results of this study again suggest saponins in *M. charantia* as the therapeutic constituents.

In conclusion, the studies described aimed to investigate the efficacy of traditional medicine in a rigorous scientific setting and found that although *Costus spicatus* was not active, *Momordica charantia* displayed significant antidiabetic activity. Information about safety and efficacy of herbal medicine will continue to be important as these traditional treatments increase in use around the world for health conditions, including diabetes.

Foreword and Acknowledgments

People pursue PhD degrees for many reasons; mine were simple. I wanted to have the perfect job. This perfect job would be somehow related to medicinal plants, include adventurous world travel, involve rigorous scientific research, and somehow contribute to the well-being of humanity and the planet. I wanted to complete a project that would grace me with the most diverse skills and varied experiences as possible. On all these points, I succeeded and achieved all I set out to do at the City University of New York. However, I was able to live my dream by surrounding myself with brilliant advisors and mentors, clever colleagues, and many other knowledgeable and helpful people. I have so many to thank:

I owe so much to my advisor, Dr. Edward J. Kennelly, who did whatever he could to help me succeed, and only rarely told me “no,” usually for my own good. Dr. Kennelly facilitates excellent teams and resources, and defines his lab and work through collaboration with cutting edge medical research scientists and settings. He works tirelessly to create a rigorous and dynamic environment, and offers so much to those who join his lab. I believe his lab is unique among phytochemistry for these reasons. I owe my success as a graduate student in everything from grants to publications largely through his efforts. I also wish to thank many members of Dr. Kennelly’s lab both past and present, including Drs. Jun Ma, Kurt Reynertson, Bei Jiang, Hui Yang, Keyvan Dastmalchi, Mario

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On a more personal note, I owe many thanks for the love and support of my incomparable family who nurtured my curiosity growing up, and provided countless opportunities to develop my interest in science. My parents, Moe and Steve Keller, have set such a wonderful example for many of us, and I have deep gratitude for their lives well lived, and their endless contributions. My brother Tim always accommodated my complaining, and never failed with a kind word of encouragement and support. In addition, my aunts April and Renee cheered me

up through their humor and caring conversation. Many other members of my extended family welcomed me for visits and holidays. I am grateful to my friends, too numerous to name, who treated me to heavy metal shows, hand-written letters and gifts, art exhibits, performances, drinks, and other urban adventures to remind me of the greater world.

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Even when times were tough, I tried to remember that doing work you love is a privilege, and the efforts and sacrifices of those who came before paved the way to my career in science.

Lastly, I was generously awarded funding from the National Institutes of Health's National Center for Complementary and Alternative Medicine (NIH/NCCAM), and the majority of this research was supported by the NIH/NCCAM fellowship F31-AT004548 "Antidiabetic Constituents from the Dominican Medicinal Plant *Momordica charantia*." I am grateful to NCCAM for allowing me and many others the opportunity to research medicinal plants. I am also thankful for Dr. Flor Henderson's CUNY Community College Collaborative Research Grant, which supported our fieldwork in the Dominican Republic.

Portions of this work have been published in the *Journal of Ethnopharmacology*, and *Planta Medica*. The contents of this study are solely the responsibility of the author and do not necessarily represent the official views of NIH/NCCAM.

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Chapter 1

Introduction

Diabetes

Diabetes is a prevalent and widespread disease in human health. As of 2007, in the United States over 23.6 million people 20 years or older have diabetes, representing 7.8% of the population (*1*). If uncontrolled, diabetes can lead to up to four times the risk of developing cardiovascular disease and stroke, conditions which were responsible for 65% of diabetes-related deaths in 2005 (*1, 2*). In addition to these, diabetes is the leading cause of new cases of blindness for adults over 20 years old, and is associated with a group of health complications such as high blood pressure, obesity, and high blood glucose concentrations known as metabolic syndrome (*1, 3*). In addition, diabetes is thought to correlate with the increase of certain cancers due to the alteration of normal hormonal activity (*3*).

The total diabetes-related cost in the United States for 2007 was $174 billion, including direct medical costs as well as costs corresponding to loss of work, premature death, and disability (*1*). In 2006, diabetes was the seventh leading cause of death in the United States, with the risk of death being twice as high among diabetics than among non-diabetics from the same age groups (*1*). Worldwide, 171 million people had diabetes in 2000, and twice as many people are predicted to have the disease by 2030 (*4*). Globally, high body-mass index, a measure of obesity, accounts for 16% of the general disease burden, and

between 2% - 7% of the developed world's health care cost is from obesity, a diabetes precursor (*5*). Also, death rates related to diabetes are projected to increase worldwide by 50% in the next 10 years with a more than 80% increase occurring in relatively wealthier nations (*6*).

Diabetes is disproportionate across various ethnic populations. For example, in 2008, 11% of the United States Hispanic adult population was diagnosed with diabetes as opposed to 7.1% of the non-Hispanic white population (*7*). The diabetes death rate for Hispanics in 2006 was 29 per 100,000 as opposed to 20.4 per 100,000 in the non-Hispanic white population (*7*). As Hispanics are the fastest growing minority group in the Unites States (*8*), this disparate incidence points to a serious and growing health problem among Hispanic Americans and the United States in general. According to a 2007 study, 12% of adults living in the Bronx have diabetes (*9*).

The main forms of diabetes are Type I diabetes mellitus (T1DM) and Type II diabetes mellitus (T2DM). T1DM is defined as an autoimmune disease in which the autoimmune inflammation destroys the β-cells located in the pancreas (*10*). The β-cells are responsible for the production of the insulin hormone, which regulates glucose uptake into insulin-sensitive tissue, such as skeletal muscle, liver, and adipose tissue (*1, 10*). Usually diagnosed in childhood or young adulthood, T1DM is controlled by regular insulin injections (*10*), and accounts for 5% to 10% of all diabetes diagnoses (*1*). In contrast, T2DM accounts for 90% to 95% of all diagnoses and is characterized by increased resistance to insulin (*11*). T2DM is also characterized by β-cell dysfunction leading to decreased insulin

secretion (*11*). T2DM is commonly associated with older age, obesity, lack of exercise, genetic history and ethnicity, and physical parameters such as impaired glucose tolerance, and decline of glucose control (*1, 11, 12*). Although commonly diagnosed in later adulthood, the prevalence of diabetes in young people and children is increasing, especially among minority ethnicities (*1*).

In addition to various lifestyle changes such as a healthy diet and regular exercise, there are also pharmaceutical therapies for T2DM. The classes of drugs currently used for T2DM are summarized below (**Table 1.1**).

Table 1.1. Drugs used for T2DM treatment in the United States.

Class	**Type and Brand Name**	**Mechanism of Action**
Sulfonylureas	glipizide, Glucotrol®	Stimulates β-cells to increase insulin production (*13*).
Biguanides	metformin, Glucophage®	Decreases glucose produced by liver; makes certain tissues more sensitive to insulin (*13*).
Thiazolidinediones	pioglitazone, ACTOS®	Decreases glucose produced by liver; increases insulin's effectiveness (*13*).

Of particular interest is metformin, belonging to the biguanide class of plant-based compounds and containing these compounds' common structure (**Figure 1.1**). The biguanides have a variety of bioactivity, including antibacterial, antiviral, and antimalarial. A precursor to metformin, galegine, was originally isolated from *Galega officinalis* L. (Fabaceae), or goat's rue (*14*). A common plant both in the United Kingdom and the United States, it has served as a source of various antidiabetic compounds (*15*). Although widely used in Europe

for more than 50 years, metformin only gained the approval of the United States Food and Drug Administration in late 1994 (*15*). However, since this time it has been widely prescribed to treat T2DM, both in conjunction with lifestyle changes and other drugs (*15*).

Metformin's main mechanism of action is lowering the glucose output from the liver (*15*). Although the exact mechanism is not known, metformin has been shown to enhance insulin's repression of gluconeogenesis by glucagon. In addition, it improves glucose transport, formation of glycogen and skeletal muscle glucose uptake (*15*). Metformin also slows the rate of glucose absorption in the intestines (*15*). All together, these mechanisms of action cause a lowering of blood glucose concentrations, thus defining an especially useful therapy for T2DM.

Figure 1.1. Chemical structure of the plant-derived drug, metformin.

Since these biguanides are derived from plants, it is reasonable to hypothesize that other naturally occurring compounds exist that could be used for the treatment of diabetes. Plant-based remedies used in traditional medicine provide an initial direction to identify novel antidiabetic compounds. In fact, many compounds with hypoglycemic activity have been reported from plant origins.

Compounds such as the plant hormone indole-3-acetic acid has been found to have hypoglycemic activity, as well as nicotinic acid, anthranilic acid, and the plant growth inhibitor, salicylic acid (*16*). Plant alkaloid trigonelline, as well as many alkaloids isolated from *Catharanthus roseus* G. Don (Apocynaceae), catharanthine, leurosine, lochnerine, tetrahydroalstonine, vindoline, and vindolinine, have been reported as having hypoglycemic activity (*16*). In addition, sulfur-containing plant compounds such as allyl propyl disulfide and diallyl disulfide oxide have been reported to be antidiabetic in both normal and alloxan-induced animals (*16*). Coumarins, glycans, several vitamins, amino acids, and inorganic ions of plant origins are also antidiabetic (*16*). As seen from these examples, a strong precedent has been set for investigating plants for antidiabetic compounds.

There are many mechanisms of action that may explain hypoglycemic effects in plant treatments. In addition to the repression of glucose output, the slowing of digestive absorption of glucose, and the increased sensitivity to insulin associated with metformin (*15*), insulin production may also be increased, as seen with the sulfonylurea class of drugs (*13*). A plant may also contain a compound or compounds that act as an insulin mimetic, imitating insulin's activity (*16*).

In addition to the commonly prescribed courses of treatment for T2DM (**Table 1.1**), it has been observed that the use of complementary and alternative medicine (CAM), as defined by practices not associated with traditional medical schools or hospitals in the United States, is increasing in general, and among

people with chronic health conditions (*17, 18*). According to the National Institutes of Health (NIH) National Center for Complementary and Alternative Medicine (NCCAM), these practices may include meditation or prayer, herbs and dietary remedies, chiropractics, energy therapies such as Reiki or therapeutic touch, or whole systems such as Ayurveda or traditional Chinese medicine (*19*). It has been shown that people with diabetes were more likely to use CAM than those without a chronic disease (*17*). People with diabetes were also observed to be more likely to report using CAM for a specific health problem, discuss it with their doctor, and be referred to a specific CAM specialist (*17*). In light of this reported use of CAM to treat diabetes, a prevalent disease in the Hispanic population, it might be assumed that traditional medicine may be a source of new treatments or therapies. In a survey on the use of herbal treatments for diabetes, the authors report that evidence is lacking for the efficacy or complete safety of the herbs used (*20*). Research investigating these modalities is crucial to the future of CAM use for diabetes.

Dominican Population and Traditional Medicine

A recent study on Dominican herbal practitioners in New York City showed that the practitioners, having migrated to the United States from the Dominican Republic, reported acquiring their healing skills from apprenticeships or family oral history (*21*). This points to the prevalence of alternative medicine in the community, as the practitioners also reported that a majority of their patients are Hispanic (*21*). The bulk of the treatments recommended by the healers were

herbal teas, followed by baths with flowers or herbs, and are influenced by the regional availability of the specific plants (*21*). Interestingly, conventional medicine was often used in conjunction with traditional medicine, as cited by certain healers in the study (*21*). In reference to their own health, for example, the practitioners mention incorporation of a conventional treatment or diagnosis with herbal treatments or regimens (*21*).

Dominican Ethnomedicine Project

The Institute of Economic Botany (IEB), based at The New York Botanical Garden (NYBG) and directed by Dr. Michael Balick, has an ongoing project involving traditional medicine of the Dominican population, both in New York City and the Dominican Republic, directed by Dr. Ina Vandebroek (*22*). This work assesses how plants are used in traditional medicine in both populations of Dominicans, and involves interviews, sourcing specific plants, and plant collections and identifications. The IEB composed a standard questionnaire in English and Spanish that posed detailed questions about the use of plants to treat a variety of illnesses. The results of the questionnaire from New York City respondents were then compared to those in the Dominican Republic.

In conjunction with the IEB, I accompanied a team led by Dr. Flor Henderson to the Dominican Republic in January of 2006. We conducted interviews using the questionnaire at many locations within the country. In community health centers in Santiago (**Figure 1.2, 1**), we spoke with various directors and health professionals. From the interviews and questions asked of

people using the clinics, it was clear that herbal medicine was used in conjunction with more mainstream treatment options. Santiago also had extensive open produce markets, almost always including stalls and merchants selling medicinal plants. The many merchants also acted as herbal practitioners, providing advice for different ailments. In Santo Domingo (**Figure 1.2, 3**), the capital of the Dominican Republic, we examined specific plants in the Dr. Rafael M. Moscoso National Botanic Garden's herbarium.

In small farming communities in the mountains not far from Pico Duarte, the highest point on the island of Hispaniola (*23*) (**Figure 1.2, 2**), we observed how local people farmed. Also, many medicinally useful plants grew wild in this environment and it served to show how plants could be harvested and used locally in addition to food crops.

Lastly, we met colleagues at the Punta Cana Ecological Foundation on the eastern coast (**Figure 1.2, 4**), a facility dedicated to the study, preservation, and sustainability of the Dominican environmental resources. The facility houses a laboratory and dormitory facilities for visiting students, in addition to extensive gardens, a greenhouse and horticulture facility, and a land preserve. The Foundation greatly contributes to the management and ongoing effects to protect the land and resources of the Dominican Republic.

Figure 1.2. Map of the Dominican Republic, Santiago (1), Pico Duarte (2), Santo Domingo (3), and Punta Cana (4). Source: en.18dao.net.

During the spring of 2006, Dr. Ina Vandebroek continued the fieldwork of extensive interviews and plant collections. After her return to the IEB, she compiled a database of most frequently mentioned plant species for use in treating diabetes, among other specific conditions and ailments. The results of this data showed that *Costus* species were second most-mentioned as an herbal remedy for diabetes, and *Momordica charantia* L. (Cucurbitaceae) ranked as fourth most- mentioned (*24*). Based on these results, a study was begun on the potential antidiabetic activity of *Costus* species and *M. charantia*. We found that

material available at our source for Dominican traditional medicinal plants, Botanica Reyes, was a mix of *Costus* species, primarily consisting of *Costus spicatus* Sw. (Costaceae). As this is the plant material purchased and used by Dominicans in New York City, we focused on this species for our further investigations.

Costus spicatus

Taxonomic treatment: The pantropical genus *Costus* is placed in the family Costaceae, in the order Zingiberales, and can be found in swamps, cloud forests, and along riverbanks (*25*). The members of the Costaceae are herbs of up to 8 m tall with horizontal rhizomes and simple, alternate leaves (*25*). The colorful flowers, white, yellow, orange, or red, appear as spikes and are terminal on a shoot, or are single in a leaf axis, and zygomorphic and bisexual, with bracts ranging in color from green to red (*25*). The fruit are dehiscent capsules and bees and hummingbirds pollinate these plants, attracted by nectar. The seeds, both water and bird-dispersed, are dark colored, and numerous (*25*).

General economic uses: *Costus* species are often used as ornamentals due to their bright colored flowers, and as diuretics, treatments for kidney and other infections, and as food (*25*). In Mexico, infusions of the aerial parts are used to treat diabetes (*26*). In Brazil, the leaf and rhizome of *C. spicatus* are used for anemia and kidney infections, as well as for fever and diabetes (*27, 28*).

Chemistry: Not many phytochemical studies have been carried out on *Costus spicatus*, but furostanol glycosides have been isolated from the rhizomes

(**Figure 1.3**), flavonoids from the leaves, and polysaccharides from the stems (*29-31*).

Hypoglycemic activity: To our knowledge, this study detailed in the second chapter is the first investigation of potential antidiabetic activity of *C. spicatus* in vivo.

Figure 1.3. The chemical structure of (3β,22α,25*R*)- 26-(β-D-glucopyranosyloxy)-22-methoxyfurost-5-en-3-yl O-D-apio-β-D-furanosyl-(1--2)-O-[6-deoxy-α-L-mannopyranosyl-(1--4)]-β-D-glucopyranoside, a furostanol glycoside isolated from *C. spicatus*.

Momordica charantia

Taxonomic treatment: *Momordica charantia* is a member of the family Cucurbitaceae, in the order Violales, characterized by annuals and occasionally perennials that can occur as climbing, woody, or trailing on the ground (*32*). The plants of this family also have simple or sometimes compound, palmate, and alternate leaves with distinct coiled tendrils at the base of the petiole (*32*). Members of the Cucurbitaceae can be either monoecious or dioecious with imperfect, yellow or white, and actinomorphic flowers (*32*). The fruits of this family are berries with a hard pericarp and contain many seeds (*32*). The Cucurbitaceae also contain bitter tasting saponin compounds, triterpenoid glycosides, known as cucurbitacins (*32*). The family Cucurbitaceae is both pantropical and subtropical, and has members living in cooler and temperate regions (*32*). The major genera include *Momordica*, containing 45 spp. and *Cayaponia* with 60 spp (*32*). Economically, the Cucurbitaceae family includes many important food crops, such as *Cucumis*, or cantaloupe, cucumber, and honeydew among others, and *Cucurbita*, representing the gourds such as pumpkins and squashes (*32*).

General economic uses: *Momordica charantia* is widely used for food and medicine throughout the world. Some of the various medicinal uses include treating topical wounds and ulcers in Turkish traditional medicine, and intestinal parasites (*33*). In India, *M. charantia* is used for many purposes and conditions ranging from contraceptives, antimalarial treatment, and eczema to jaundice, kidney stones, and leprosy (*33*). Although the uses around the world for *M.*

charantia are varied, it is the most widely used plant-based treatment for diabetes (*16*). Its antidiabetic uses are seen as far as the Middle East, Africa, Indo-China, the Caribbean, and Central America (*16*).

Cultivar variation: It is thought that *Momordica charantia* was first domesticated in India and China, and numerous cultivars continue to be used in parts of Asia and India (*34, 35*). Cultivars commonly and commercially used for food are characterized by elongated, smooth or spiky, fruit of a bright or pale green color when immature (*34, 35*). The wild relative of the cultivar, growing throughout the Dominican Republic, is characterized by smaller, round fruits that turn bright orange or yellow when mature.

As the previous literature on the hypoglycemic effects of *M. charantia* describe using the cultivated varieties for experimentation, the studies herein used a variety cultivated in China.

Human studies of hypoglycemic activity: There have been a handful of experiments with the fruit of *Momordica charantia* that verify its hypoglycemic activity in patients with T2DM. Particularly notable are studies that observed a significant lowering of blood glucose levels when measuring how efficiently glucose was cleared from the blood during a glucose tolerance test (GTT) (*36-39*). In one study, eight patients were given powdered domestic *M. charantia* fruit for seven days, with a significant increase in glucose tolerance observed via a GTT at the end of the study (*39*). In a second study, fruit juice of domestic *M. charantia* was administered to 18 patients 30 minutes prior to a GTT, and 73% displayed improved glucose tolerance (*38*). As a GTT can be indicative of

improved glucose-induced insulin secretion by the pancreatic β-cells, these results suggest improved β-cell function as a likely mechanism of hypoglycemic effect.

Certain studies have had mixed results. In a double-blind study assessing the effect of *Momordica charantia* on HbA1c levels, glycosylated hemoglobin concentrations that correlate to average blood glucose concentrations, the researchers reported a decrease in HbA1c of patients treated with the plant extract; however, the study suggests that the decrease was too minimal (0.24%) for sufficient statistical power in a group of 20 individuals, and is thus inconclusive (*40*). A recent study reported no effect on the glucose tolerance, energy expenditure, or carbohydrate and lipid oxidation rates of overweight men treated with freeze-dried *M. charantia* (*41*). Another study reported no effect on sialic acid levels of T2DM patients fed *M. charantia* juice (*42*).

Animal studies of hypoglycemic activity: Despite ambiguous reports on human physiology, there are a multitude of studies involving animal models of both T1DM and T2DM that substantiate the hypoglycemic activity of the domestic *Momordica charantia* fruit.

There are many notable studies involving T1DM animal models. Oral administration of the fruit extract of *Momordica charantia* resulted in significantly lowered blood glucose (*43-45*). In one 45-day study, T1DM rats fed 100 mg/kg and 120 mg/kg dosages of the dried methanol extract of domestic *M. charantia* showed significantly lowered glucose concentrations when measured on the 15th day of treatment (*44*). In a second study, T1DM rats given 10 ml/kg of fruit juice

over 10 weeks had significantly lower blood glucose levels when compared with T1DM control animals (*43*).

Additionally, a large number of studies have observed a significant lowering of blood glucose levels upon oral administration of various preparations of the fruit. For example, the freeze-dried methanol and ethanol extracts of the fruit, administered to rats at 125 mg/ml and 200 mg/kg respectively, resulted in a lowering of blood glucose levels (*46, 47*). The same results were reported in T1DM mice when fed the freeze-dried and fresh water extract at dosages of 200 mg per animal and 4 ml/kg, respectively (*48, 49*), and when feeding the dried water and acetone extracts of the fruit to T1DM rats at dosages of 20 mg/kg and 7.5 g/kg, respectively (*50, 51*). When simply administering a 1.5 g/kg dose of the dried fruit to T1DM rabbits, and adding 10% of it to the total food mix of T1DM rats, lowered blood glucose concentrations resulted (*52, 53*). In another study, administering the fruit juice at a dose of 20 ml/kg to T1DM rats, the authors also observed lowered blood glucose concentrations (*54*).

Other reports have continued to confirm the hypoglycemic effect of *Momordica charantia* in vivo. For example, T1DM rats fed 300 mg/kg of an ethanol extract displayed lower blood glucose concentrations (*55*), and T1DM rats fed 10mg/kg daily of a water extract of *M. charantia* for 30 days displayed the same effect (*56*). As the above-mentioned studies illustrate, *M. charantia* fruit has proven to be hypoglycemic at various preparations and dosages.

Furthermore, several studies have noted the improvement of glucose tolerance in T1DM animal models fed *Momordica charantia* fruit after conducting

a GTT (*46, 55, 57-59*). Improved glucose tolerance was also observed in a study that conducted GTTs in T2DM models fed *M. charantia* fruit (*60*). Again, these results suggest an increase of glucose-induced insulin secretion.

Current investigations have begun to explore the specific biological or molecular mechanism behind this plant's antidiabetic activity. In one such study, animals fed 150 and 300 mg/kg of an ethanol extract of *Momordica charantia* displayed a reduction in HbA1c levels (*55*). Various preparations of *M. charantia* have been shown to improve metabolic syndrome by inhibiting adipocyte hypertrophy, and limiting fat accumulation in diet-induced obese rats, as well as the inhibition of α-amylase and protein typrosine phosphatase 1B, two mechanisms thought to be related to improvement in blood glucose concentrations (*61, 62*).

In other publications, *Momordica charantia* preparations moderated kidney heparan sulfate concentrations in T1DM rats and improved downstream insulin signaling, indicating that the plant could improve diabetes-related kidney complications (*63, 64*). The plant was also found to increase insulin receptor phosphorylation in rats fed a high-fat diet (*65*), and to moderate complex carbohydrates in T1DM (*66*). Various extracts of *M. charantia* were shown to up-regulate mechanisms associated with glucose transport, including GLUT4, PPARγ, and P13K (*67*), and *M. charantia* extract increased the expression of PPARγ in adipose tissue, and GLUT4 in skeletal muscle, in addition to lowering glucose and insulin levels in high-fructose diet-fed rats (*68*). Also, *M. charantia* reduced liver damage due to diabetes in T1DM rats (*69*).

A standardized extract of *Momordica charantia* containing 20 mg/kg of possible antidiabetic compound D-*chiro*-inositol, reduced blood glucose in TIDM rats (*70*), and semi-purified peptides from *M. charantia*, in addition to significantly lowering fasting blood glucose, also reduced glycogen levels, as well as serum cholesterol triglycerides and low-density lipoprotein concentrations (*71*).

Pancreatic β-cells as a target of antidiabetic activity: Although only a handful of studies address specific targets of activity, ones that examine the effect of the fruit of the domestic *Momordica charantia*'s on β-cells in T1DM models substantiate these cells as a target in the mechanism of hypoglycemic activity of *M. charantia* (**Table 1.2**). In a recent study, a significant difference was observed at the end of a 10-week study in the level of plasma insulin of T1DM rat models treated with *M. charantia* fruit juice (1.14 ng/ml), as compared with the diabetic control (0.56 ng/ml) (*43*). This was also inversely correlated with a difference in blood glucose levels of the treated group (300.25 mg/dl) as opposed to the diabetic control group (428.5 mg/dl) (*43*). The data from this study point to an increase in insulin production, resulting in the difference in blood glucose levels.

In a separate study, T1DM rats treated with *Momordica charantia* fruit juice over a 10-week period showed a significant difference in β-cells of the treated group (16.61 cells/islet) when compared to the untreated T1DM control group (10.79 cells/islet) (**Table 1.2**) (*72*), showing an effect on β-cell growth. Additionally, a study involving a T1DM moderately diabetic animal model (characterized in the study by a blood glucose level lower than 400 mg/dl),

observed a continuation of lowered blood sugar levels 2 weeks after the termination of a 30-day study (*51*). The authors conclude that this may be due to β-cell activity and regeneration, as the moderate T1DM animals seem to retain some β-cell function (*51*).

Also, T1DM animals fed 150 and 300 mg/kg of an ethanol extract of the plant had less damage to their β-cells than a control group (*55*). An alcohol extract of *Momordica charantia* improved β-cell granulation and islet size in T1DM rats (**Table 1.2**) (*73*), and an acetone extract increased β-cell recovery in T1DM rats (**Table 1.2**) (*74*). In HIT-T15 β-cells, a water extract of *M. charantia* increased cell proliferation (**Table 1.2**) (*75*). A study examining the combined effect of the drug compound rosiglitazone and *M. charantia* found an increase in pancreatic islets as compared to a control in T1DM rats (**Table 1.2**) (*76*).

Taken together, these studies suggest that the domestic *Momordica charantia* fruit extract contains bioactive compounds that can act specifically to improve β-cell insulin secretion, resulting in improved glucose homeostasis. This may be achieved by an increase in β-cell mass, an improvement in β-cell function, or an increase in β-cell proliferation. However, as these studies were only conducted with extracts, there is a need for more comprehensive studies to fully ascertain the specific compounds behind this plant's mechanism of hypoglycemic action via the β-cells.

Table 1.2: Literature reporting effects of *M. charantia* on pancreatic β-cells.

Extract/ Fraction	Administration/ Dosage	Animal Model/ Number	Metabolic Parameters	Study Length	Results	Source
methanol extract of MC, standardized .51% charantin	Oral, 500 mg/kg body weight	Sprague-Dawley rats/5/group/streptozitocin	Measured the synergy between *M. charantia* and rosiglitizone on pancreatic islets, glucose tolerance, and blood glucose.	28 days	Increase in islets, improvement in glucose tolerance, decreased blood glucose	(*76*)
alcohol extract	Oral, 25, 50, 75 mg/100 g body weight	Wister rats/alloxan/18/group	Measured islet regeneration, body weight, and blood sugar.	45 days	Islet regeneration and blood glucose concentrations decreased	(*73*)
acetone extract	Oral, 25, 50, and 75 mg/100g body weight, once daily	normal albino rats/alloxan, 18/group	Measured islet regeneration, glycogen deposition in liver.	45 days	Islet regeneration and improved glycogen localization.	(*74*)
2 water extracts, MW>3000, MW<3000	2%, 0.2%, 0.02% water extract, 2% LW and HW extracts, 24 incubation	Cells- hamster HIT-T15 pancreatic β-cells, alloxan damaged	Measured insulin secretion, β-cell proliferation, SOD activity.	24 hours	Increase insulin secretion, some β-cell proliferation	(*75*)
Fresh juice	Oral, 10 ml/kg	Wister rats, male/streptozitocin, 4-5/group	Post-mortem pancreatic histology analyzed.	10 weeks	Increase in β-cells	(*72*)

In vitro studies of hypoglycemic activity: In addition to human and animal models, there are a few examples of in vitro experiments that test specific mechanisms of action particular to *Momordica charantia*. For example, juice of the *M. charantia* fruits inhibited proteins involved with previously reported low-density lipoprotein activity in human hepatoma and HepG2 cells (*77*), and increased glucose and amino acid uptake by treated rat muscle cells (*78*). A water extract of *M. charantia* moderated glucose uptake when combined with insulin in 3T3-L1 cells (*79*). In HepG2 cells, an extract of the plant increased mRNA expression and promoter activity of PPARδ, a receptor important to obesity and metabolic syndrome (*80*).

Chemistry: In general, *Momordica charantia* contains several classes of compounds with biological activity. For example, glycosides, saponins, triterpenes, alkaloids, proteins, and steroids have all been reported to be present in the plant (*81*). Prevalent compounds include cucurbitane triterpenoids and triterpene saponins (*82, 83*). One compound thought to be largely responsible for the antidiabetic activity of *M. charantia* is known as p-insulin, a peptide shown to have insulin-like effects when subcutaneously administered to animal models and both T1DM and T2DM patients (*84*).

Also considered largely responsible for the hypoglycemic effect of *Momordica charantia*, is a mixture of two steroid glycosides, β-sitosterol-D-glucoside (**Figure 1.2**) and 5, 2 5-stigmastadien-3-β-ol-D-glucoside (**Figure 1.2**), known as charantin (*16*). Although widely cited as a component of *M. charantia* fruit's antidiabetic activity, there are few actual studies on the hypoglycemic

activity of these compounds. Those few studies involving the antidiabetic activity of charantin show inconsistent results. One study noted hypoglycemic activity in normal animals, but not necessarily verifiable activity in alloxan-treated mice or depancreatized cats (*16*), and no drop in blood glucose levels was seen in a recent, limited study involving only the glucose levels of alloxan-induced diabetic mice (*82*).

β-sitosterol-D-glucoside 5, 2 5-stigmastadien-3-β-ol-D-glucoside

Figure 1.4. The chemical structures of the two compounds known collectively as charantin.

As charantin is repeatedly cited as being a compound causing the hypoglycemic activity seen in the fruit of *Momordica charantia*, it would follow that experiments testing that activity would verify these previous reports. As there are very few actual studies, and some contradiction about the results, there is little evidence to substantiate the assertion that charantin is responsible for the hypoglycemic activity of *M. charantia*.

Saponins

Saponins are triterpene and steroidal glycoside compounds, naturally distributed throughout the plant kingdom (*85*). These compounds consist of a terpenoid basal skeleton known as an aglycone, paired with a sugar moiety (*85*). This combination of compounds with varying solubility gives the total saponin a soap-like property of foaming in agitated water-based solutions (*85*). The wide variety of saponins are classified based on their skeleton; the type of saponins found in the Cucurbitaceae are known as the cucurbitane type, and typically are glycosylated at carbon 7 and 23, and sometimes contain an aldehyde group attached at carbon 9 (**Figure 1.3**) (*85, 86*). Some of the most common saponins found in *M. charantia* are known as momordicosides. These compounds are labeled by letter beginning with A, and have been isolated from fruit, seeds, and leaves (**Figure 1.4**) (*81*).

Figure 1.5. The chemical structure of 3β,7β,25-trihydroxycucurbita-5,23(*E*)-dien-19-al, a cucurbitane-type saponin.

Saponins are well known bioactive phytochemicals, with reports of medicinal properties ranging from antimicrobial, and cytotoxic to anti-inflammatory and immunostimulatory (*87, 88*). Also, recent studies have begun to show that saponins may be responsible, at least in part, for the antidiabetic activity seen in *Momordica charantia* (**Table 1.3**). For example, the isolated cucurbitane triterpenoids 5β,19-epoxy-3β,25-dihydroxycucurbita-6,23(*E*)-diene, and 3β ,7β ,25-trihydroxycucurbita-5,23(*E*)-dien-19-al lowered blood sugar in diabetic mice (*82*) while a saponin-rich fraction isolated from *M. charantia* was also found to lower blood sugar and small intestine disaccharidase activity (**Table 1.3**) (*89*). Momordicosides Q, R, S, and T increased GLUT4 translocation via the AMPK pathway in vitro, and momordicoside T improved glucose tolerance in mice fed a high-fat diet (**Table 1.3**) (*90*). Saponins isolated from *M. charantia* stems improved glucose uptake, insulin signaling, and overall insulin resistance in a mouse hepatic cell line (*91*). In a series of recent studies, butanol fractions of *M. charantia* fruit methanol extracts, most likely containing saponins, were shown to increase the expression of GLUT4, PPARγ, and PPARα, in addition to lowering blood glucose and leptin concentrations in rats fed a high-fat diet (**Table 1.3**) (*68, 92*).

In summary, there are very high health, societal, and financial costs for diabetes. As diagnoses for T2DM are expected to grow in the coming decades, finding new treatments and investigating existing traditional modalities are important ways forward. Ethnobotanical investigations of Dominican traditional medicine show that *Costus* species and *Momordica charantia* are highly-used

medicinal plants for diabetes treatment, thus, researching their potential activity could yield promising new directions in managing this disease. The aim of this thesis is to investigate traditional herbal medicine and contribute not only toward their knowledge, but to a better understanding of the potential of treating diabetes through plant remedies.

Extract/ Fraction	Administration/ Dosage	Animal Model/ Number	Metabolic Parameters	Study Length	Results	Source
Methanol extract, standardized to 0.51% charantin	Oral, 500 mg/kg body weight	Sprague-Dawley rats/5/group/streptozitocin	Measured the synergy between *M. charantia* and rosiglitizone on pancreatic islets, glucose tolerance, and blood glucose.	28 days	Increase in islets, improvement in glucose tolerance, decreased blood glucose.	(*76*)
Butanol fraction, multiple partitions	Oral, 1.0 g/kg body weight and 0.2, 1.0 g/kg body weight/day	Male Sprague-Dawley rats/high and low fat diets	Measured adipocytokine, plasma markers, skeletal muscle membrane protein, GLUT4, leptin, PPARγ, GAPDH, glucose, insulin, and body weight.	2 weeks	Increased PPARγ and GLUT 4 activity, decreased glucose and insulin, and decreased leptin.	(*68*)
Saponin fraction	Oral, 100, 200, 500 mg/kg/day	Female Kunming mice/alloxan/ 12-group	Measured glucose, insulin, body weight, hepatic glycogen, and sugar tolerance.	20 days, 8 days-sugar tolerance	Glucose decrease, insulin increase, hepatic glycogen increased, sugar tolerance improved.	(*93*)
Butanol fraction, multiple partitions	Oral, 0.5, 1.0 g/kg body weight and 0.2, 1.0 g/kg body weight/day	C57BL/6J mice/high-fat diet/	Measured blood glucose, various tissues, mRNA, insulin, body weight, food intake, and various PPARs.	4 weeks	Improved blood glucose and insulin concentrations, and insulin sensitivity, increased PPARγ expression.	(*92*)
Purified saponins	Subcutaneous or intraperitoneal injection of pure compound, 10 mg/kg and 100mg/kg	C57BL/6 mice, standard or high-fat diet/ number not mentioned	Measured energy expenditure fat oxidation blood glucose, GTT, GLUT4, and AMPK phosphorylation.	1-2 days, either 24 hr or 2 hr experiments	Increased GLUT4 translocation via AMPK phosphorylation, and improved glucose tolerance.	(*90*)
Butanol/ saponin rich fraction	Oral, dissolved in water- 50, 100 mg/kg body weight, only once	6-wk-old Wister rats, 6/group	Blood glucose and disaccharidase activity.	1-2 days	Decrease in blood glucose, and disaccharidase activity.	(*89*)

Table 1.3. Selected literature from 2008-2010 reporting in vivo bioactivity of *M. charantia* saponins.

Figure 1.6. Common saponins from *M. charantia*.

Chapter 2

Costus spicatus tea failed to improve diabetic progression in C57BLKS/J *db/db* mice, a model of type 2 diabetes mellitus (*24*)*

Introduction

According to the Center for Disease Control and Prevention (CDCP) in 2005, Hispanic adults had diabetes prevalence rates 1.7 times that of non-Hispanic whites (*7*). A recent study has shown a genetic predisposition to insulin resistance and T2DM in the Dominican Republic (*94*). In a recent meta-analysis, Hispanics had higher hemaglobin $A1_C$ values than non-Hispanics, indicating that glycemic control is poorer among the Hispanic population (*95*). As Hispanics are the largest (12.5%) and fastest-growing minority group in the United States (*8*), it is important to focus on their specific healthcare needs.

Currently available standard anti-diabetic medications have all been well-studied for their efficacies in lowering blood glucose and ultimately lowering rates of secondary complications of this disease (*96*). Nevertheless, several studies have documented the common use of traditional medicines for diabetes, such as herbal treatments, in the Hispanic immigrant population in the United States (*21, 97-99*).

*This chapter has been excerpted from "*Costus spicatus* tea failed to improve diabetic progression in C57BLKS/J *db/db* mice, a model of type 2 diabetes mellitus," published in the *Journal of Ethnopharmacology* **2009**, 121, 248-254.

Specifically, within the Dominican population, traditional medicine is often and consistently used as a source of health care (*22, 100*). According to a study done on Dominican patients in an emergency room, 24% of those interviewed were using alternative medicine to treat their emergency room complaint (*99*). Also, Dominican herbal practitioners and their patients in New York City reported using conventional medicine in conjunction with traditional medicine (*21*).

In the Dominican Republic, the herbal treatment called *insulina* is used as a treatment for hyperglycemia (*101*). No research exists on its ethnobotanical importance among the Dominican community in New York City to treat diabetes. The Dominican community is the fastest growing Latino immigrant population in New York City, with a population of between 369,200-555,000 in 2000 (*22*). Given the potentially wide application of *insulina* for the treatment of diabetes among the Dominican population, it is critical to define its efficacy. Therefore, the purpose of this study was to find out whether *insulina* is also known and used by Dominicans living in New York City to treat diabetes and to determine if any hypoglycemic effects can be brought about by consumption of *insulina* tea in a well-characterized mouse model of obesity-induced diabetes fed a standard chow diet. We studied the effects of 10 weeks of *insulina* tea consumption on weight gain, plasma glucose and serum insulin concentrations, and insulin sensitivity in male C57BLKSJ *db/db* mice consuming solely *insulina* tea as compared to control mice consuming water.

Methods and Materials

Ethnobotany. As part of a larger, in-depth ethnobotanical survey that included 84 questions and addressed a variety of topics (including past and current use of medicinal plants, treatment modalities, provenance of herbal remedies, preference for using medicinal plants or pharmaceuticals, demographic information, acculturation, common health conditions and folk illnesses, harmful or toxic plants, and transmission of plant knowledge), we conducted individual interviews with 175 Dominican participants (166 laypeople who self-medicate with medicinal plants and 9 plant specialists or traditional healers) about the nature of plant remedies used for 30 common health conditions, including diabetes. The City University of New York granted IRB approval for this survey (IRB #04-06-0599; PI: Dr. Michael Balick) and surveyors obtained oral informed consent from participants prior to interviewing. Convenience and snowball sampling were used to recruit participants of both sexes. We interviewed subjects in the waiting room of the Associates in Internal Medicine Clinic (Columbia University), in a community-based organization (*Alianza Dominicana* Inc.), at their homes, and a few interviews were also conducted at the Institute of Economic Botany of The New York Botanical Garden. Inclusion criteria were: been born in the Dominican Republic, currently living in New York City, 18 years or older, and some knowledge of medicinal plants. During the interview, which lasted between 1 and 2 hours, each participant was asked whether they knew of any plants used to treat diabetes

and whether they had ever been diagnosed with this disease. After affirmation, further questions were asked about the Dominican name(s) of the plant, plant part(s) used, and mode of administration. Dominican plant names recorded from the interviews were ranked according to their frequency of mention by participants. Those participants who reported information about *insulina* were subsequently contacted during a follow-up phone survey at a later date, and asked additional questions about the exact amount of plant material needed, the amount of water, possible coadjuvants, the preparation time, and the dosage and duration of administration of this remedy. We were able to obtain in-depth information from 11 participants (55% of those who had initially mentioned *insulina* as a remedy for diabetes).

Ethnopharmacology. *Materials:* Animal experimentation utilized a glucometer (Bayer), glucometer strips (Bayer), a mouse insulin ELISA kit (ALPCO), glucose (Sigma) and insulin solutions (Humulin R, Eli Lilly). Tea was filtered with 0.45 μm nylon filter syringes (Phenomenex), and thin-layer chromatography used silica plates (Merck), vanillin (Sigma), sulfuric acid (Sigma), ethanol (J.T. Baker), and G.R. grade chloroform and methanol (J.T. Baker).

Plant material: Dried plant material from *insulina*, (voucher *AK004*), was purchased at a *botánica*, a common source of traditional Dominican herbs, as well as other items of religious or healing value, in the Washington Heights neighborhood of Manhattan, New York. The botanical identity of this plant material was determined to be *Costus spicatus* Sw. (Costaceae) by Drs. Ina Vandebroek and Tom Zanoni at the New York Botanical Garden.

A tea of *Costus spicatus* leaves was prepared according to a consensus of dosage and administration reported by Dominicans who self-medicate with this medicinal plant, and specifications given by the *botánica* staff. This preparation consisted of soaking for 10 minutes and boiling for 5 minutes an average of 17.31 g of the dried leaves in 1.890 L of distilled, deionized water. The resulting water extract was strained and stored at 4°C for no longer than 48 hours. An aliquot of 5-10 ml of tea was re-filtered through a 0.45 μm nylon syringe filter and dried. Thin-layer chromatography was used to assess the tea's phytochemical profile. After resuspending the dried tea in 60% methanol, 20 μl was added to normal phase silica plates and developed with a solvent system of chloroform and methanol (7:3). Plates were then sprayed with a 1% vanillin solution in sulfuric acid and methanol (1:9), and observed under ultra-violet light.

Four weeks into the study, the tea was concentrated two-fold, and nine weeks into the study, the tea was concentrated four-fold. The average weight/weight yields were 6.92% for the initial tea concentration, and 7.16%, and 5.73% for the two and four-fold tea concentrations, respectively.

Methods. *Animal studies*: All animal studies were approved by the Institutional Animal Care and Use Committee at Columbia University Medical Center (#AC-AAAA7756). The C57BLKS/J mice (KS), when made genetically obese via a complete knock-out of the leptin receptor via the *db* mutation, develop severe insulin resistance and progressive insulinopenia (diminishing

levels of circulating insulin) resulting in severe hyperglycemia and ultimate premature death due to insulin deficiency (*102*). In the context of the KS genetic background, hyperglycemia worsens with age secondary to severe obesity-induced insulin resistance and progressive insulinopenia (*103*). This particular inbred strain was chosen so that any potential effects of *insulina* tea on either insulin resistance or preservation of beta cell insulin secretion could be detected.

A power analysis was conducted to determine the adequate number of animals to use for our studies. Previously published results characterizing the long term treatment (6 weeks) of hyperglycemic mice using a standard oral hypoglycemic agent for the treatment of T2DM, the sulfonylurea, glipizide, showed a ~40% reduction in mean plasma glucose concentrations. For our studies, we used a more conservative goal of achieving a 15% reduction in plasma glucose concentrations in the treatment group in determining adequate sample size. Therefore, assuming mean plasma glucose concentrations of 585 mg/dl with a standard deviation (SD) of 75 mg/dl (mean and SD of control obese animals at ~4 months of age in this study), a sample size of n=4 for both treated and untreated groups would be adequate to see a statistically significant difference with treatment. We used a sample size of n=6 for both groups, which should have been adequate to test for moderate effects of the *insulina* tea.

Insulina tea or distilled water was administered *ad lib* to 6-week-old male obese KS *db/db* mice. The Experimental (Tea) and Control (Water) groups consisted of six animals each. The Control Group received only water and the Experimental Group received only tea throughout the study. At the start of the

treatment protocol, Experimental and Control Group mice were 5 weeks of age and were metabolically matched at baseline for weight, fed, and fasting glucose concentrations, and fed and fasting serum insulin concentrations using blood collected via tail vein (**Table 2.2**). In addition, both groups were matched for relative insulin resistance as estimated by HOMA-IR values, a model used to assess insulin resistance using fasting insulin levels (*104*) (**Table 2.2**).

After baseline measurements of weight, glucose, serum insulin, and insulin tolerance were determined, the mice in the Experimental Group were given *ad lib* access solely to *Costus spicatus* tea, which was placed in their water bottles. Similarly, mice in the Control Group were allowed *ad lib* access to water. Throughout the study period, both Experimental and Control animals gained weight at similar rates achieving maximum body weights of ~40 g by 15 weeks of age (**Figure 2.1A**).

All animals were analyzed 1 week pre-treatment, 2, 4, 6, 8, 10 weeks and 1-2 weeks post-treatment. Prior to the start of the 10 week protocol, fasting serum insulin and plasma glucose concentrations were determined on all animals, which were then subjected to intraperitoneal glucose tolerance testing (IP-GTT). Fed plasma glucose and serum insulin concentrations were determined pre-treatment and at 2-week intervals. At the conclusion of the study, IP-GTT and IP-ITT were performed.

Intraperitoneal Glucose Tolerance Testing (IP-GTT): Animals were fasted overnight for 12-14 hours. Fasting serum insulin and plasma glucose concentrations were determined prior to intraperitoneal injection of 1 mg

glucose/gm body weight with sterile 10% glucose. Glucose concentrations were determined at 15, 30, 60, 90, and 120 minutes post-injection. Serum insulin concentrations were determined at 15 minutes post-injection.

Intraperitoneal Insulin Tolerance Testing (IP-ITT): To determine whether *insulina* tea consumption altered insulin resistance levels between Experimental and Control Groups, we performed IP-ITTs at the conclusion of the 10-week study protocol. Due to the severity of the insulin resistance within these mice, an insulin dose of 8 units/g body weight was administered to achieve adequate glucose lowering. All animals were fasted for 4 hours prior to determination of baseline plasma glucose and serum insulin concentrations. Glucose concentrations were determined at 15, 30, 60, 90, and 120 minutes post-injection.

Statistics: Student's t-test (two-tailed) with a $p < 0.05$ was used to determine statistical significance.

Results

Insulina was the second most frequently reported plant to treat diabetes in our survey among 175 Dominicans in New York City. One hundred and twenty-nine participants in the survey (74% of subjects) reported knowing about or using at least one remedy for diabetes. One in four participants (25 % of subjects) declared that they had been diagnosed with diabetes, a percentage that is double the New York City average. In total, 90 plants were reported for diabetes

and 33 plants were mentioned by at least three participants. A tea of *insulina* was the second most frequently reported plant remedy for either Type 1 (T1DM) or T2DM. The highest ranking plant species in the survey for the treatment of diabetes was *sábila*, *Aloe vera* (L.) Burm. F., (Aloaceae), followed by *insulina (Costus* spp., Costaceae), *naranja agria (*bitter orange, *Citrus aurantium* L., Rutaceae), *cundeamor* (*Momordica charantia* L., Cucurbitaceae), *pepino (*cucumber, *Cucumis sativus* L., Cucurbitaceae), and noni (*Morinda citrifolia* L., Rubiaceae). The number of people who reported these remedies was 23, 20, 13, 12, 12, and 10, respectively.

Detailed data on the preparation, amount used, and administration regime of *insulina* according to 11 Dominican participants who used this plant in New York City to treat their diabetes is shown in **Table 2.1**. The table shows that the amount of plant material and water used, as well as the preparation time, are highly variable. For example, variations in the amount of boiling time for the tea range from 1 to 10 minutes, with some preparations simply calling for a general decoction. Also variable is the dosage, varying from administration once or twice per day, to anytime thirst occurs (**Table 2.1**).

Also, the traditional preparation of *insulina* tea in the Dominican Republic most likely involves the use of fresh plant material, as plants used in traditional medicine are typically locally grown and available.

Participant Code	Sex	Amount of Plant	Amount of Water	Coadjuvants	Preparation Time	Dosage	Duration of Administration
C-2005-005	M	3-4 leaves (for beginning diabetes); 7-8 leaves (one pound per week)	3 liter	cun de amor (*Momordica charantia*)	boil until 1 liter is left	twice a day	until sugar level is normalized, from then on take only once a day
C-2005-008	M	2-3 leaves	2 cups	-	4 minutes (do not boil too long because the effect will be lost)	twice a day	until blood sugar is under control
C-2006-006	F	4-5 leaves	16 oz.	-	boil until half the amount of water is left	twice a day	-
G-2005-025	F	1 leaf	1 cup (more or less)	-	5 minutes	once a day	Forever
G-2005-050	F	2-3 leaves	1 regular coffee cup	-	boil for 1 minute, turn off fire, cover cup and soak for another 2-3 minutes	once-three times a day	15 days - 1 month
G-2005-054	F	10 leaves	2-3 cups	-	boil until the amount for 1 cup is left	every time when thirsty	depends on sugar level
G-2005-060	F	1 leaf	1 cup	-	boil water, add leaf, cover cup (infusion, do not boil anymore when leaf is in the water)	twice a day	take to control diabetes, when it works keep taking it
G-2005-096	F	2 leaves	2 cups	Cinnamon	10 minutes	twice a day	6 months (she now takes pills)
G-2005-110	F	4 leaves	6 cups	-	boil until the amount of 4 cups is left	once a day	3 months
G-2005-117	F	10 leaves	2 liter	-	boil until 1 liter is left	when thirsty (put in fridge and drink)	
G-2005-129	F	5-6 leaves	8 oz	-	10 minutes	once-twice a day	15 years

Table 2.1: Variations of *Insulina* Usage. Preparation, dosage, and administration of *insulina* were found to vary among traditional Dominican medicine users and practitioners interviewed.

Continuous consumption of increasingly concentrated *insulina* tea did not improve diabetes progression in obese C57BLKS/J (KS) animals. Although a significant difference was observed between the Experimental Group's HOMA-IR values at baseline and 10 weeks (187 ± 40 vs. 132 ± 40, $p<0.05$, **Table 2.2**), no significant differences existed between parallel values of the Control Group, or between the values of the Experimental Group and Control Group.

Obese animals consuming regular chow and water (Control Group) for 10 weeks progressed from a baseline mean fasting glucose of 171 ± 90 mg/dl to fasting glucose concentrations of 339 ± 140 mg/dl ($p<0.05$) (**Table 2.2**). This significant worsening of their hyperglycemia was reflected in their baseline and 10-week fed glucose concentrations as well. In a similar group of animals consuming *insulina* tea for 10 weeks, a statistically identical picture of glucose homeostasis developed.

On addition, both Experimental and Control Groups had comparable concentrations of serum insulin at baseline and after 10 weeks (**Table 2.2**). Therefore, *insulina* tea did not appear to improve beta cell function.

In order to control for various variables that may impact glucose homeostasis, we followed progression of weight gain, and food and liquid intake for both animal groups. Both groups gained similar amounts of weight during the study protocol (**Figure 2.1A**). Interestingly, there was a consistent trend of slightly decreased food consumption in the Experimental Group as compared to the Control Group, which became statistically significant in week 5 (8.60 $\pm$ 0.8 vs. 9.77 $\pm$ 0.7 mg/day/animal, respectively), week 6 (8.89 $\pm$ 0.6 vs. 9.62 $\pm$ 0.5

mg/day/animal, respectively), week 9 (7.39 ± 0.5 vs. 9.35 ± 0.2 mg/day/animal, respectively), and week 10 (7.48 ± 0.3 vs. 8.69 ± 0.5 mg/day/animal, respectively), ($p<0.05$, **Figure 2.1 B**).

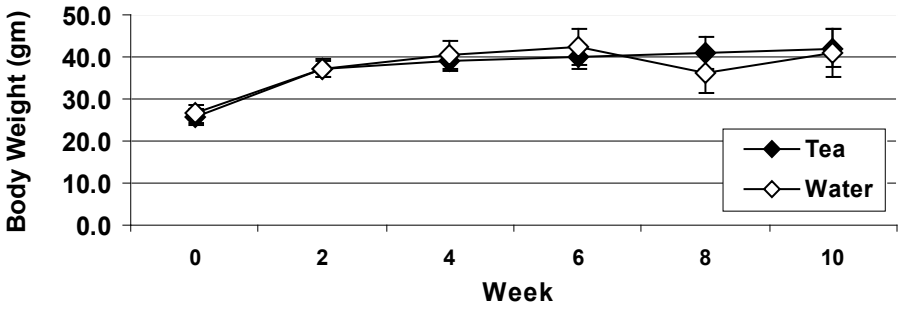
	Baseline		10 Weeks	
Metabolic Parameter	Tea	Water	Tea	Water
Body weight (g)	25.85 ± 1.94	26.53 ± 2.17	42.10 ± 4.64	41.10 ± 5.63
Fasting Glucose (mg/dl)	168 ± 55	171 ± 87	353 ±126*	339 ± 143*
Fasting Serum Insulin (ng/ml)	14.4232 ± 6.1799	13.9132 ± 7.3697	4.7743 ± 1.5597*	4.8770 ± 3.0978*
Fed Glucose (mg/dl)	241 ± 103	302 ± 147	585 ± 36	559 ± 77
Fed Serum Insulin (ng/ml)	9.9020 ± 4.7268	9.9928 ± 4.4549	5.0332 ± 2.3356	5.0073 ± 1.7152
HOMA-IR	187 ± 36	175 ± 96	132 ± 36*	140 ± 173

Table 2.2: Metabolic data at baseline and 10 weeks. The animals' weight and fed and fasting glucose and serum insulin levels were measured at baseline and at the 10-week conclusion of the study, plus or minus standard deviation. In addition, the HOMA-IR model was used to assess insulin resistance. Significant differences were observed between both group's fasting glucose levels at baseline and 10 weeks, the fasting insulin levels at baseline and 10 weeks, and the HOMA-IR values of the Experimental Group at baseline and 10 weeks (* $p < 0.05$, Student's t-test, two-tailed).

There was a corresponding trend towards decreased total liquid consumption in the Experimental Group versus the Control Group with significant differences at week 6 (26.4 $\pm$ 3 vs. 23.6 $\pm$ 1 ml/day/animal) and week 10 (24.3 $\pm$ 2 vs. 31.9 $\pm$ 7 ml/day/animal, respectively), ($p<0.05$, **Figure 2.1 C**). This decreased consumption of food and liquid in the Experimental group may reflect decreasing palatability of the more concentrated tea mixture, which was reflected as a relative decrease in appetite. Nevertheless, these differences in food and liquid consumption did not significantly alter weight gain between the two groups.

We also followed weekly glucose and serum insulin concentrations to determine whether a similar pattern of the progression of hyperglycemia and hypoinsulinemia developed between the two groups. From baseline to 10 weeks of the study protocol, worsening levels of hyperglycemia progressed in parallel between Experimental and Control groups. Mean glucose concentrations of the Experimental and Control Groups ranged from 241 $\pm$ 100 vs. 302 $\pm$ 150 mg/dl, respectively, at the start of the protocol, and progressively increased to mean glucose concentrations of 585 $\pm$ 40 vs. 559 $\pm$ 80 mg/dl, respectively (**Table 2.2**). There was a significant difference between fasting glucose levels when measured at baseline and 10 weeks, for both the Experimental Group ($p=0.008$), and the Control Group ($p=0.04$).

Consistent with their hyperglycemia, serum insulin concentrations were relatively elevated at 5 weeks of age in both Experimental (9.9020 $\pm$ 4.727 ng/ml) and Control (9.9928 $\pm$ 4.455 ng/ml) Groups, and progressively declined



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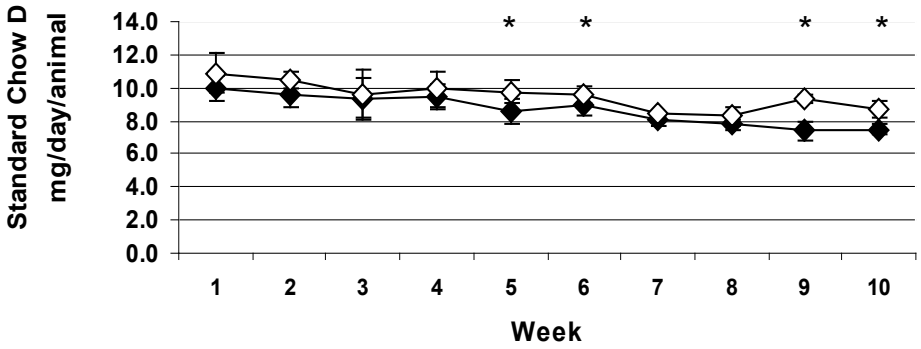

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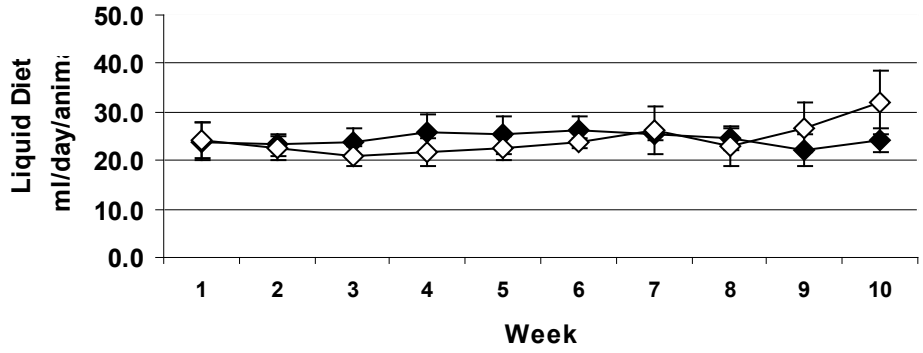


C:

Figure 2.1 A-C: Biweekly weights (A), and weekly food (B) and water or *insulina* tea (C) consumption. Error bars are expressed as ± standard deviation.

throughout the 10-week study protocol (**Table 2.2**). This progressive decrease in serum insulin concentrations in the face of worsening hyperglycemia reflects the decline in total beta cell mass typically seen in obese KS animals (*103*). Serum insulin concentrations were not significantly different between the two groups at 0, 2, 4, 6, 8, and 10 weeks. Additionally, both Experimental and Control Groups' fasting serum insulin levels were significantly decreased at 10 weeks, as compared to baseline ($p=0.04$ and $p=0.03$, respectively, **Table 2.2**).

Insulina tea failed to improve insulin sensitivity in obese KS mice. No differences in glucose concentrations were found at any time point after insulin administration up to 120 minutes. In addition, total area over the curve (AOC) analysis, which integrates total glucose dispersal over the 2-hour study period, shows no significant difference between the two study groups. These data show that *Costus spicatus* tea treatment did not improve insulin sensitivity in these obese mice.

Although glucose tolerance tests were performed on all Experimental and Control mice at the conclusion of the 10-week protocol, extremely elevated fasting glucose concentrations with relatively low serum insulin concentrations at baseline suggested ongoing maximal insulin secretion by the remaining functional beta cells. This was confirmed by post-challenge glucose concentrations which were beyond the range of the glucometer device, and therefore immeasurable. Nevertheless, average fasting glucose and serum insulin concentrations were not different between Experimental and Control

groups, suggesting that no measurable beneficial effect on insulin secretion or beta cell function was derived from *Costus spicatus* tea consumption.

Our results showed no improvement in glucose homeostasis in the Experimental Group as compared to the Control Group. At weekly intervals, there were no differences in average glucose or serum insulin concentrations. At the conclusion of 10 weeks of drinking *Costus spicatus* tea, the Experimental Group was as hyperglycemic as the Control Group, with similarly decreased serum insulin concentrations suggesting matched degrees of beta cell failure and loss. In addition, the Experimental Group showed no measurable improvement in insulin resistance as shown by insulin tolerance testing and area over the curve (AOC) analysis.

Discussion

In the obese C57BLKS/J *db/db* mouse model, *Costus spicatus* tea was not effective in improving hyperglycemia or alleviating the severe insulin resistance that develops with obesity.

One challenge in this study was the determination of what should be the optimal *Costus spicatus* tea concentration. Therefore, we chose to start with a baseline dose, guided by local users and vendors of the plant, and concentrated the dose by two-fold and four-fold during the course of the study. This was an attempt to maximize our ability to detect any beneficial effects of the tea within the animal model. Nevertheless, despite utilizing a four-fold concentrated tea

during the last 2 weeks of the study protocol, no improvements in glucose or insulin concentrations were seen.

Another potential explanation as to why our studies showed no hypoglycemic activity of the tea may be due to the severity of the insulin resistance associated with this particular animal model. Secondary to the hyperphagia and metabolic effects of disrupting leptin receptor signaling, C57BLKS/J *db/db* mice develop a rapid, and severe obesity-induced insulin resistance (*103*). If the anti-hyperglycemic activity of the *C. spicatus* tea is low, likely no effect would be observed in the severely diabetic animals used in this study.

Although we failed to find other published studies about the hypoglycemic activity of *C. spicatus*, other species of the genus *Costus* have been shown to demonstrate hypoglycemic activity. An extract of *C. speciosus* Sm. lowered blood glucose concentrations of streptozotocin-induced hyperglycemic rats (*105*). Additionally, a methanol extract of *C. pictus* D. Don ex Lindl. lowered blood glucose concentrations and increased plasma insulin concentrations in alloxan-induced diabetic rats (*106*). A methanol extract of dried *C. afer* Ker Gawl. also reduced blood glucose concentrations in streptozotocin-induced hyperglycemic rats and stimulated glucose transport in adipocyte cells, suggesting an ability to improve glucose uptake *in vivo* (*107*).

To our knowledge, this is the first animal study of the utility of *Costus spicatus* tea, known by Dominicans in New York City as *insulina*, and commonly used for the treatment of diabetes. Our study in a mouse model of obesity-

induced hyperglycemia provides no data to suggest that *Costus spicatus* tea can benefit the treatment of obesity-induced diabetes, the most common form of T2DM. Based on these results, tea of the leaves of *Costus spicatus* should not be recommended as an alternative to standard oral hypoglycemics. Investigations of this sort are critical in light of the many known and unknown herb-drug interactions for common ailments (*108*). Given that Dominican patients have been found to use herbal medicine in conjunction with or instead of conventional treatments (*21, 97*), it is crucial that the botanical identity and efficacy of the herbal treatments be further investigated. Examining potential herb-drug interactions for a widespread and serious disease such as diabetes should be a research priority, given the high prevalence of the disease and herb use in ethnic populations.

Also, based on the prevalence of use of herbal treatments, it is important to understand cultural differences that may impact or influence diabetic patients' perspectives on health care. For example, Hispanic diabetic patients told investigators that they believed that herbal remedies were effective in treating diabetes, and that they wanted their conventional health practitioners to know more about herbal treatments (*109*).

Due to its current, common use in some communities for treating diabetes, it is important to further study *insulina* and its potential utility in the management of hyperglycemia. The effects of tea made from different plant parts, including rhizomes, of *C. spicatus* and other *Costus* species in the present animal model of obesity-induced diabetes and other diabetes models (T1DM) also deserves

further study. Thus, additional study is necessary in order to definitively assess the antidiabetic activity of *insulina*.

Chapter 3

Saponins from the traditional medicinal plant *Momordica charantia* stimulate insulin secretion in vitro

Introduction

Type 2 diabetes (T2DM), also known as non-insulin dependent diabetes mellitus (NIDDM), is responsible for 90% to 95% of diagnosed diabetes (*1*). The progression of T2DM can be characterized both by insulin resistance, and loss of normal β-cell activity, such as hyperplasia of the pancreas and gradual decrease of insulin secretion (*110*). As impaired β-cell function is central to the progression of T2DM, novel therapies that regulate insulin secretion and prevent β-cell damage and subsequent impaired function may be integral to the future of treatment (*110*).

In addition to widely used medications, people throughout the world increasingly rely on complementary and alternative medicine, including plant-based traditional medicines, as a form of health care (*111*). In as study conducted in 2007, 4 out of 10 adults interviewed used alternative and complementary medicine within the past year, 17.7% of this being natural products (*112*). A study done in the United States in 2002 reported that 19% of subjects interviewed had used natural products in the form of herbal remedies, functional foods, and supplements in the prior 12 month period (*112*). With this

increase in the use of herbal medicine, it is of paramount importance that these herbs are investigated.

Of traditional medicines used for diabetes treatment, *Momordica charantia* is reported to be the most widely used (*16*). The hypoglycemic activity of various preparations of *M. charantia* is well substantiated with many recent in vivo models of diet-induced obesity and type 2 diabetes (*60, 64, 92, 113*). Also, recent studies described in the introductory chapter have begun to show that isolated triterpene glycosides, known as saponins, lower blood glucose and improve glucose uptake and tolerance (*82, 89, 90*).

Although the insulin secretion activity of *Momordica charantia* has begun to be explored in vitro (*75*), the exact mechanisms of the plant's activity have yet to be fully elucidated. As this plant is used so widely throughout the world in traditional medicine for diabetes, knowledge of its bioactivity, including potential toxicity, and possible mechanisms of action are critical for informed public health decisions. It is also important to assess the potential efficacy of pure compounds, both acting together in a concentrated fraction of plant extract, and independently.

We hypothesized that the hypoglycemic activity of *Momordica charantia* is due, at least in part, to the stimulation of insulin secretion in pancreatic β-cells by cucurbitane triterpenoids. To test this hypothesis, we investigated the insulin secretion activity of *M. charantia* ethanol extract and a LC-ToF-MS characterized saponin-rich fraction; additionally, the five triterpene saponin compounds 3*β*,7*β*,25-trihydroxycucurbita-5,23(*E*)-dien-19-al (1), momordicine I (2), momordicine II (3), 3-hydroxycucurbita-5,24-dien-19-al-7,23-di-*O*-*β*-

glucopyranoside (4), and kuguaglycoside G (5) were also tested to ascertain the potential mechanism of the glucose-lowering activity of *M. charantia* in MIN6 β-cells, a mouse insulinoma β-cell line (**Figure 3.1**). Of the clonal cell lines appropriate for testing potential insulin secretion, the glucose-stimulated insulin secretion previously observed with MIN6 cells was close to that of normal islets (*114*). Because the MIN6 cells closely resemble normal physiologic β-cell function, we chose this cell line to test our hypothesis in order to more precisely extrapolate a true pancreatic islet insulin secretion response. Extracts and compounds were tested in a static incubation assay, and insulin secretion and cell viability measured.

Materials and Methods

Plant material

Dried fruits, including seeds (1.94 kg), of *Momordica charantia* were identified by Naturex, Inc., and extracted in 75% ethanol, resulting in 578 g total solid extracted material. Voucher material was deposited in Naturex's herbarium (South Hackensack, New Jersey). This resulting ethanol-extracted material was tested as the ethanol extract. Aliquots of the ethanol extract (24 g) were partitioned sequentially with methylene chloride and water-saturated butanol repeatedly. The average yield of the dried butanol extract was 5.67% w/w of starting material. All dried butanol extracts were combined, and subsequently used for experiments.

Compound isolation

Compounds 1-5 were isolated from *Momordica charantia* as described previously (*115*).

β-cell assay

MIN6 β-cells were grown at 37 ºC with 5% CO_2 in DMEM media (Invitrogen, California) with 15% fetal bovine serum (Invitrogen, California), 1% penicillin/streptomycin (Invitrogen, California), 1:200 gentamicin (Invitrogen, California), and 1% basel medium eagle (Invitrogen, California). Cells were trypsinized off the plate, split, and replated into 6-well plates and grown until confluent. The ethanol extract, saponin-rich fraction, and isolated compounds were re-suspended in 100% DMSO to make a stock solution of 125 mg/mL. The assay was performed similarly to a previously described method (*116*). Cells were incubated with Kreb's Ringer buffer (KRB) for 60 min on the plate. Cells were then rinsed with fresh KRB twice, and an aliquot of the second wash was saved as a baseline insulin measurement. Cells were then incubated for 60 min with KRB (negative control), 50 μM glipizide with 27 mM glucose in KRB (positive control), or 0.1% DMSO in KRB (vehicle control). The ethanol extract and saponin-rich fraction were tested at 25, 75, and 125 μg/ml concentrations, while compounds 1-5 were tested at 5, 10, and 25 μg/ml. Treatment solutions were collected at 60 min, and insulin concentrations were determined by ELISA (ALPCO, New Hampshire).

Cell viability

Immediately after incubation with treatment solutions, cells were trypsinized and resuspended in PBS buffer (Invitrogen, California). Cells (10 µl) were added to an equal amount of Trypan Blue (Invitrogen, California) and counted on a hemocytometer. Cells excluding Trypan Blue were assumed viable. Both live and dead cells were counted and are presented as an average of four counts for each treatment.

DNA extraction

For a total cell count, DNA was extracted by incubating cells overnight with 1% SDS/10 mM EDTA/10 mM Tris lysis buffer and 1% proteinase K. Phenol-chloroform 1:1 was added, and cells were centrifuged. Sodium acetate-isopropanol 1:3 was added to the supernatant, and material was incubated at -80 ºC for 60 min. Precipitated DNA was then washed twice with 70% ethanol, and air-dried. DNA was reconstituted with 10:1 Tris-EDTA buffer (pH 8.0), and read with a spectrophotometer at 260 nm. The conversion factor of 5.8 µg of DNA = 1.0×10^6 cells was used.

LC-ToF-MS analysis

Samples were analyzed by LC-MS using a Waters LCT Premiere XE Time of Flight (ToF) mass spectrometer (Waters MS Technologies, Manchester, UK). Ionization was achieved using a multi-mode ESCI source in electrospray (ESI)

mode at the following conditions: +ESI capillary 3000 V, -ESI capillary 2800 V, both + and – ESI: cone: 20 V, aperture 1: 0 V, ion guide 1: 0 V, multichannel plate (MCP): 2600 V. Nitrogen was used for both cone and desolvation gases, with a cone gas flow of 20 L h-1, and desolvation gas flow of 600 L h-1 at 400 °C. The source temperature was 120 °C. Leucine-enkephaline was used as a reference mass and infused by a secondary reference probe. The reference mass was scanned once every five scans for each positive and negative data collection. Both positive and negative ESI data were collected using a scan time of 0.2 s, with an interscan time of 0.01 s, and a polarity switch time of 0.3 s. MS data was collected in centroid mode using MassLynx V4.1 Scn 727.

LC separation was conducted using a Waters Alliance 2695 HPLC coupled to a Waters 2998 PDA. Separation was achieved on a 150 x 2.0 mm 2.6 mm Kinetex C-18 column (Phenomenex, California) held at a constant temperature of 45 °C, using the following solvent gradient system: (A) 0.1% formic acid in water, (B) 0.1% formic acid in acetonitrile at a flow of 0.2 ml/min: 0-15 min B:10-70%, 15-20 min B:70-100%, 20-42 min B:100%.

Statistics

The JMP (SAS) software, version 8.0, was used to determine statistical significance between measured values. Significant differences were determined using least square means contrast within a one-way ANOVA.

Results

We tested a saponin-rich fraction of the total ethanol extract from *Momordica charantia* to investigate the mechanism of action behind the plant's hypoglycemic activity. This fraction stimulated insulin secretion above the DMSO vehicle at the 125 µg/ml concentration (p=0.02, **Figure 3.2**). The activity of the saponin-rich fraction at 125 µg/ml was 4.9-fold greater than the DMSO vehicle.

To ascertain that the potentially active compounds present in the saponin-rich fraction of *Momordica charantia* were saponins, the fraction was characterized using LC-ToF-MS. These compounds are characterized by their dammarane-type triterpene skeleton, found in cucurbitanes (*88*). The cucurbitane saponins found in *M. charantia* are glycosylated at carbon 7 and 23, and sometimes contain an aldehyde group attached at carbon 9 (**Figure 3.1**) (*86*). Using the extracted ion chromatogram which corresponds to a common saponin basal skeleton with m/z 437.3290, we were able to characterize most of the ionizable components in the extract as saponins (**Figure 3.3**).

Five pure compounds isolated from *M. charantia* fruit were tested in the β-cell insulin secretion assay. Although in a previous study, glycoside compound momordicoside U was found to be moderately active in the β-cell assay at both 10 and 25 µg/ml concentrations (*115*), its aglycone, compound 1 (**Figure 3.1**), did not stimulate insulin secretion.

Figure 3.1. Compounds 3β,7β,25-trihydroxycucurbita-5,23(*E*)-dien-19-al (1), momordicine I (2), momordicine II (3), 3-hydroxycucurbita-5,24-dien-19-al-7,23-di-O-β-glucopyranoside (4), and kuguaglycoside G (5)

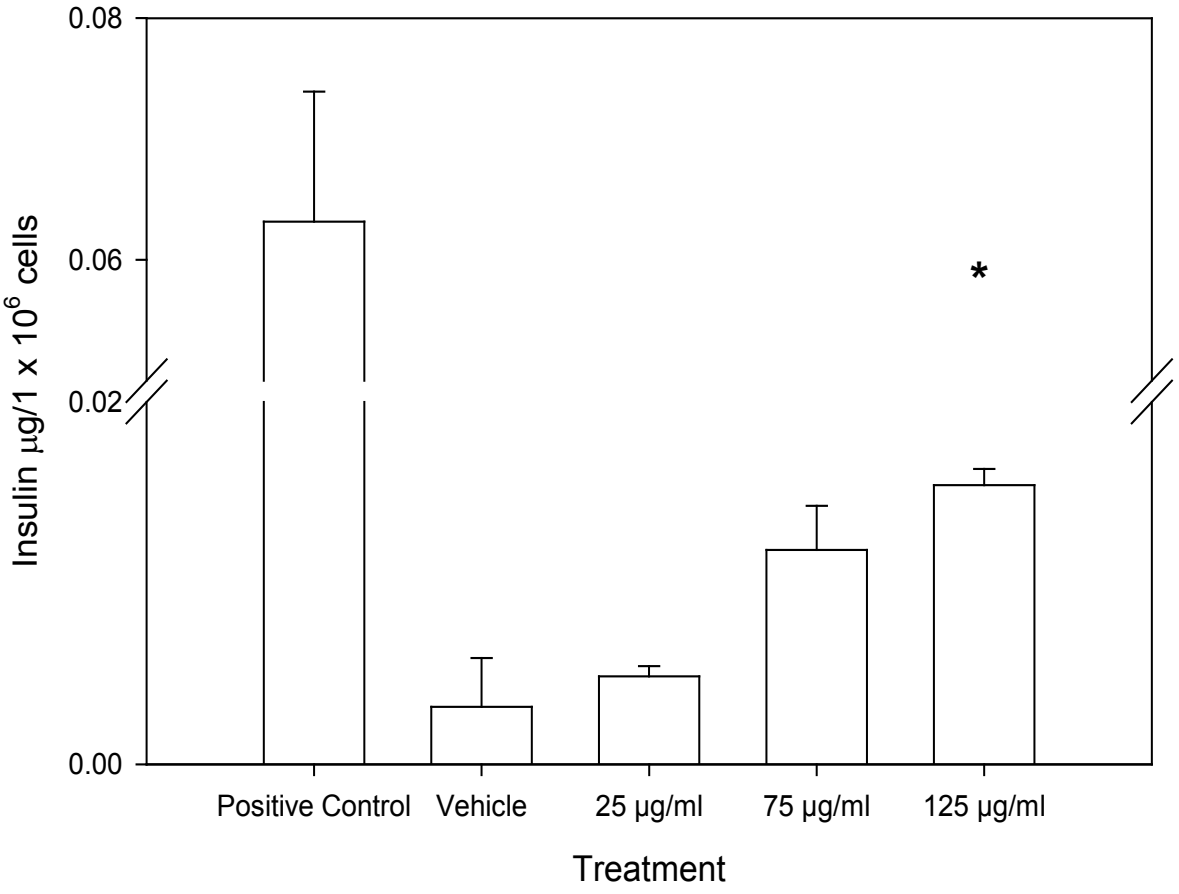


Figure 3.2: Insulin secretion activity of the saponin-rich fraction, after 60 min incubation with MIN6 β-cells. The (*) denotes significantly different activity as compared to DMSO vehicle, n=3. Error bars represent standard error.

Compound 3 (**Figure 3.4**) significantly stimulated insulin secretion at 0.1 µg/1 x 10^6 cells for both concentrations 10 and 25 µg/ml, 7.3 and 7.1 times more than the vehicle (**Figure 3.4 A**, p=0.006, 0.007, respectively). However, its aglycone, compound 2, was not active.

Compound 5 (**Figure 3.4**) stimulated insulin secretion at 0.1 µg/1 x 10^6 cells at both 10 and 25 µg/ml concentrations, 8.1 and 7.8 times the vehicle (**Figure 3.4 B**, p=0.002), although its aldehyde, compound 4 (**Figure 4.1**), showed no activity.

To ensure that the insulin secretion was due to treatment-induced insulin secretion and not cell death, cells were stained with Trypan Blue and counted for viability. The viability of cells exposed to compound 2 at 25 µg/ml was significantly less as compared to the vehicle (**Table 3.1 C**, p=0.002). As there was no significantly lower viability of any of the cells incubated with the ethanol extract, saponin-rich fraction, or pure compounds tested when compared to the DMSO control (**Table 3.1**), insulin detected was due to the treatments' ability to stimulate insulin secretion in the cells, as opposed to cell death.

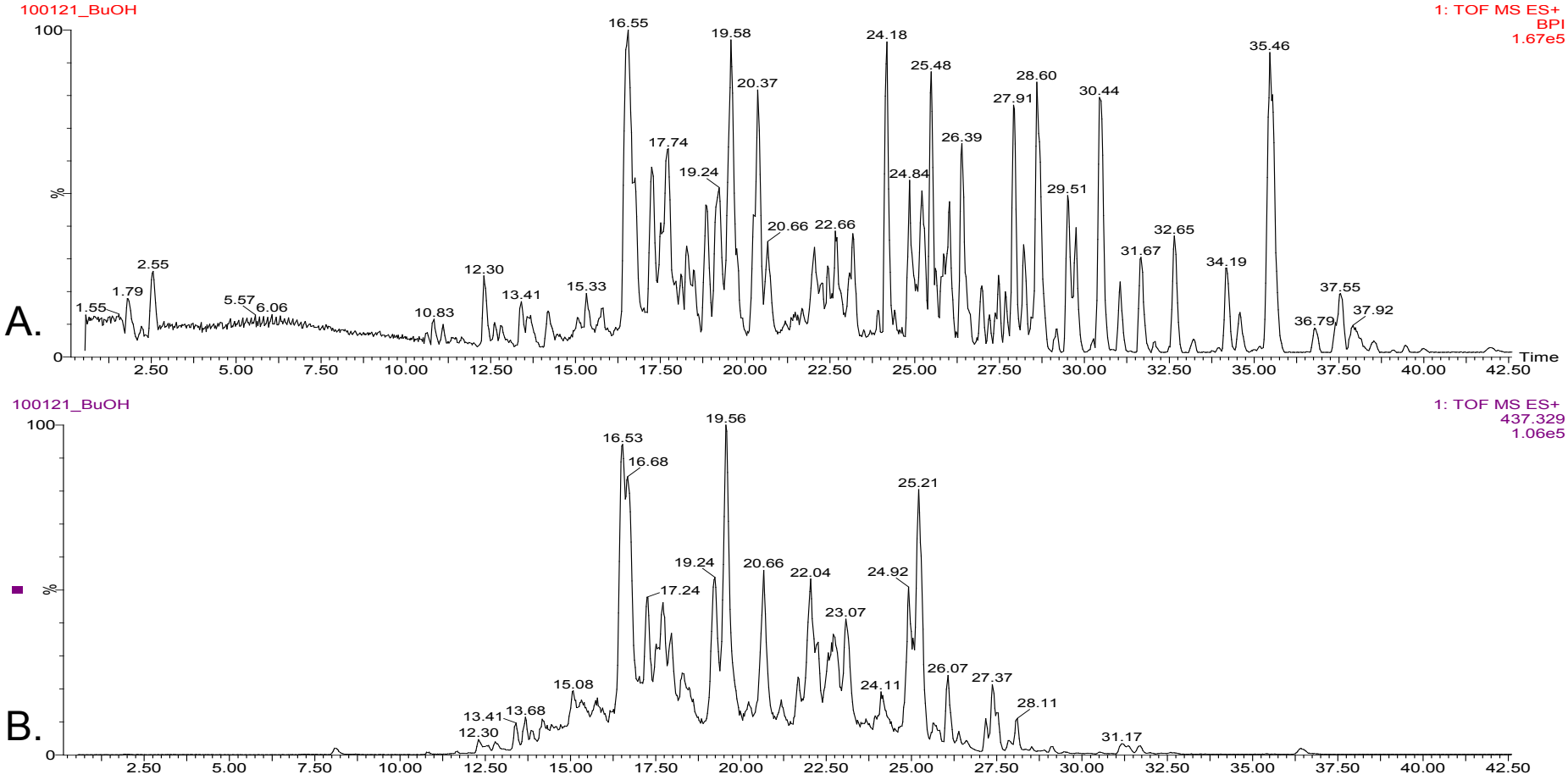
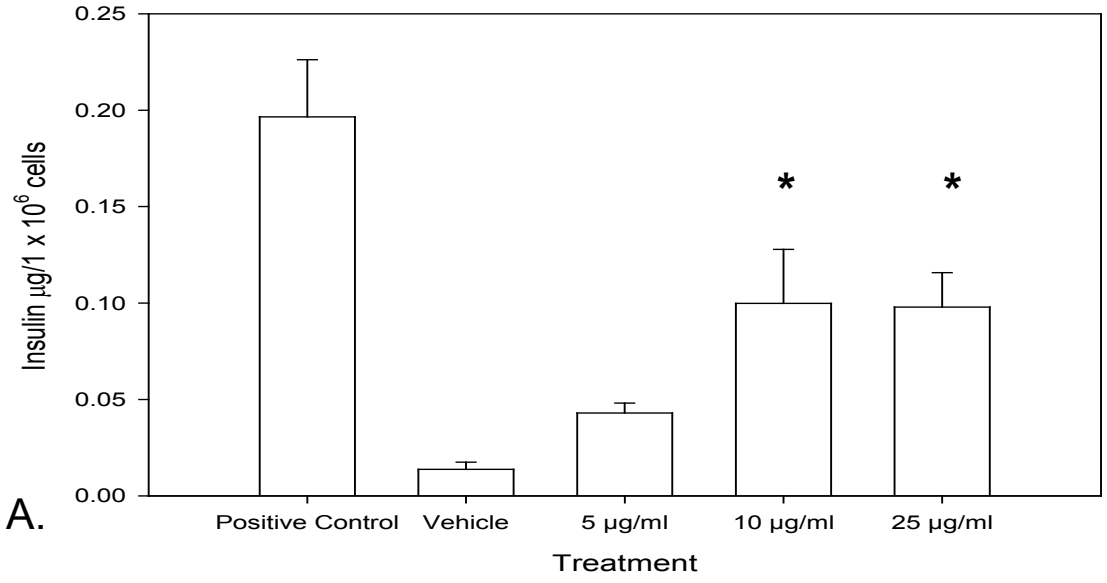


Figure 3.3: Base peak intensity chromatogram (A), derived from a total ion chromatogram, of a saponin-rich fraction of *M. charantia*. Extracted ion chromatogram (B) for a common cucurbitane skeleton, *m/z* = 437.3290.



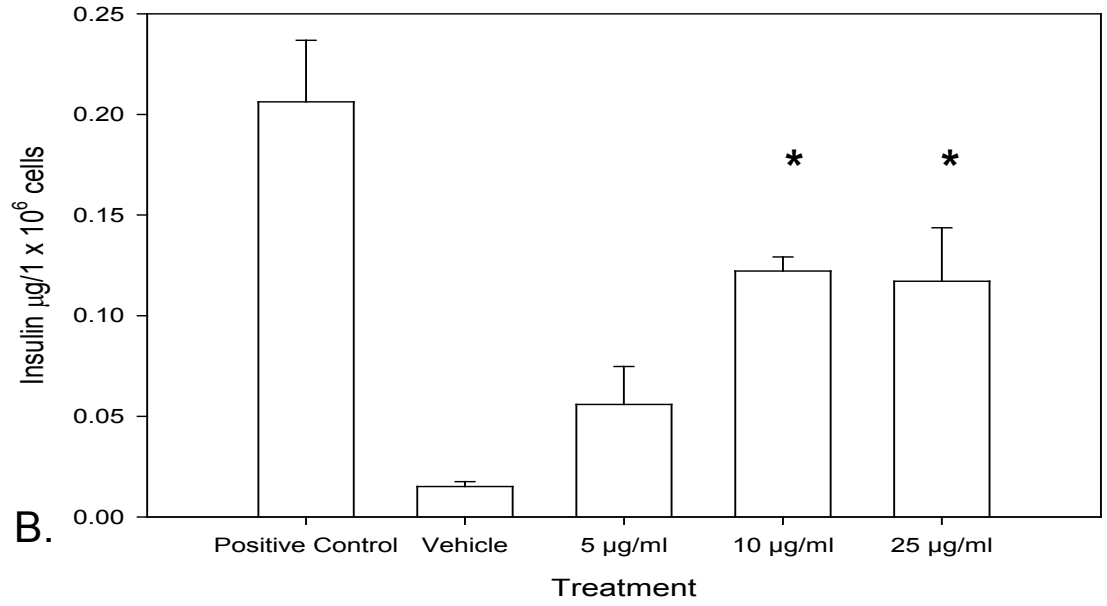


Figure 3.4 A and B: Insulin secretion activity of compounds 3 and 5, after 60 min incubation with MIN6 β-cells. Positive control is 27 mM glucose together with 50 µM glipizide, and vehicle is DMSO. The (*) denotes significantly different activity as compared to vehicle, n=3. Error bars represent standard error.

Table 3.1 A-F: Cell viability: β-Cell viability was measured by staining cells with Trypan Blue immediately after static incubation, and is an average of counting four quadrants on a hemocytometer. Viability was assessed as percentage (%) live cells and is ± standard error, n=3. Error bars represent standard error.

A:

Treatment	Live Cells (%)
Ethanol extract 25 µg/ml	77 ± 5
Ethanol extract 75 µg/ml	76 ± 4
Ethanol extract 125 µg/ml	73 ± 5
Saponin-rich fraction 25 µg/ml	94 ± 5
Saponin-rich fraction 75 µg/ml	93 ± 2
Saponin-rich fraction 125 µg/ml	93 ± 3

B:

Treatment	Live Cells (%)
Compound 1 (0.005 mg/mL)	97 ± 1
Compound 1 (0.010 mg/mL)	97 ± 1
Compound 1 (0.025 mg/mL)	93 ± 3

C:

Treatment	Live Cells (%)
Compound 2 (0.005 mg/mL)	96 ± 1
Compound 2 (0.010 mg/mL)	95 ± 1
Compound 2 (0.025 mg/mL)	89 ± 3*

D:

Treatment	Live Cells (%)
Compound 3 (0.005 mg/mL)	98 ± 0
Compound 3 (0.010 mg/mL)	98 ± 0
Compound 3 (0.025 mg/mL)	99 ± 0

E:

Treatment	Live Cells (%)
Compound 4 (0.005 mg/mL)	98 ± 0
Compound 4 (0.010 mg/mL)	99 ± 0
Compound 4 (0.025 mg/mL)	98 ± 0

F:

Treatment	Live Cells (%)
Compound 5 (0.005 mg/mL)	97 ± 0
Compound 5 (0.010 mg/mL)	95 ± 1
Compound 5 (0.025 mg/mL)	96 ± 1

Discussion

Saponins are well known bioactive phytochemicals, and have been investigated for a multitude of activities, including antimicrobial, cytotoxic, anti-inflammatory, and immunostimulatory (*87, 88*). Although saponins are known to have hypoglycemic activity (*82, 89, 90*), the cellular and molecular mechanisms of action are only beginning to be explored, and may be varied.

The saponin-rich fraction from *Momordica charantia*, along with compounds 3 and 5, stimulated insulin secretion in MIN6 pancreatic β-cells in a concentration-dependent manner. The saponin-rich fraction was also found to contain saponins as detected by LC-ToF-MS, suggesting that these compounds may be responsible for part or all of the hypoglycemic activity of *M. charantia*. These results are consistent with previous reports of hypoglycemic activity of saponins (*82, 87, 89, 90*).

The ranges in bioactivity of compounds 1-5 may be due to their differences in structure. The previously tested compound, momordicoside U (*115*) along with the active compound 3, are monodesmoside cucurbitanes as opposed to their aglycones, inactive compounds 2 and 4, respectively (**Figure 3.1**). Although compound 4 is a bidesmoside and is inactive, it also contains an aldehyde group whereas the bidesmoside compound 5 does not, and is the only compound tested to contain both of these moieties simultaneously (**Figure 3.1**). Toxicity was observed only when testing the 125 µg/ml concentration of

compound 2, and may help explain its lack of bioactivity in this insulin secretion assay, in addition to the structural differences mentioned above.

These detailed structural differences, especially glycosylation, most likely account for the bioactivity and lack thereof observed. It has been previously reported that both the type of sugar and other moieties along with the specific triterpene skeleton factor greatly in a saponin's bioactivity (*117*). For example, when investigating lupine-type saponins for cytotoxic activity, it was reported that the different sugar moieties reportedly explained the variation in activity of both monodesmosides and bidesmosides (*117*). The authors also mention that the basal skeleton of saponins can impact the mechanism of action of bioactivity, in addition to general activity (*117*). In other studies, saponin functional groups, such as sugars, also influenced the compounds' effects on cell membranes (*87*). Two saponins without an acyl group and differing in only one glucose group showed significantly different activity stimulating insulin absorption, despite their other closely related bioactivity (*87*). In another study, the presence or absence of sugar moities affected several saponins' anticancer activity (*118*).

As compounds 1-5 all shared the same basal skeleton, we observed that the sugar moities, and possibly the presence of an aldehyde group, may have been responsible for their bioactivity or lack thereof, in agreement with the studies described above.

Also, the pattern of insulin secretion in response to the cells' incubation with the saponin-rich fraction is different than the response seen with compound 3 and 5 (**Figure 3.4**). The response from the saponin-rich fraction is

concentration-dependent from 25 to 125 μg/ml, whereas the response to compounds 3 and 5 levels off between 10 and 25 μg/ml (**Figure 3.2, 3.4**). As certain bioactive saponins have previously been reported as being agonists, this phenomena may suggest that compounds 3 and 5 are binding to specific receptors, resulting in the observed bioactivity (*119*).

Momordica charantia is one of the most popular medicinal plants used worldwide for diabetes treatment (*16*), and is also a widely used food (*120*). With the prevalence of this plant used both for diabetes treatment and food, it is crucial that health practitioners and researchers know and investigate the possible mechanisms of action of its hypoglycemic activity. The more that is known about the efficacy and mechanism of action of *M. charantia*, the better its use in health care settings throughout the world. All of this points to the potential to provide more informed care, and to avoid any potential ill effects such as herb-drug interactions, among other risks associated with herbal medicine.

Although studies suggest the consistent, but limited bioavailability of saponins (*121, 122*), we are currently investigating the saponin-rich fraction in an animal model of T2DM. In summary, our data supports our hypothesis that *Momordica charantia* may work to lower blood glucose via promoting insulin secretion, and we theorize that this mechanism may contribute substantially to the plant's overall hypoglycemic effect.

Chapter 4

A characterized saponin-rich fraction of *Momordica charantia* shows antidiabetic activity in C57BLK/6 mice fed a high-fat diet

Introduction

As discussed in the previous chapters, type 2 diabetes (T2DM), the cause of 90% to 95% of diagnosed diabetes (*1*), is typified by the increase of insulin resistance, loss of normal β-cell activity, and subsequent decrease of insulin secretion (*110*). As these metabolic parameters are central to T2DM, research that targets modalities capable of normalizing insulin secretion and preventing β-cell damage and their decrease of function may be integral to the future of treatment (*110*).

Since previously-mentioned studies have narrowed in on the *Momordica charantia* saponins as active antidiabetic compounds, and the in vitro experiments described in Chapter 3 demonstrated the insulin secretion activity of saponins, we continued to explore the possible mechanisms of action behind this activity with an in vivo experiment. We maintained our hypothesis that the hypoglycemic activity reported in *M. charantia* fruit is due to the saponins' ability to stimulate insulin secretion. In addition, we explored whether these saponins can impact the extent of compensatory β-cell mass seen in obese, insulin resistant animals.

To test these hypotheses, we gavaged two groups of high-fat diet-induced hyperglycemic C57BLK/6 mice with either a crude ethanol extract or a characterized saponin-rich fraction, both from the *Momordica charantia* fruit. A third group was gavaged with vehicle as a high-fat diet-fed control (HF control). As an additional negative control, a fourth group fed a low-fat diet was gavaged vehicle (LF control). We chose this diet induced mouse model as it mimics the human lifestyle causes of T2DM. The B6 mice are susceptible to obesity, and subsequently T2DM when fed a high fat diet (*123*). In testing *M. charantia* for specific antidiabetic activity, approximating the human use of the plant in the mouse model as closely as possible allows the results to be extrapolated more specifically for potential human use. We compared weights, food, and water consumption, and fasting insulin and glucose concentrations throughout the course of the study. Both intraperitoneal glucose and insulin tolerance tests were performed, and immunohistochemical staining and subsequent analysis of the pancreatic tissue were completed at the end of the study.

Methods and materials

Plant material: Dried fruits, including seeds (1.94 kg), of *Momordica charantia* were identified by Naturex, Inc., and extracted in 75% ethanol, resulting in 578 g total solid extracted material. Part of this extract was deposited in Naturex's extract repository and herbarium (South Hackensack, New Jersey). This ethanol-extracted material was gavaged to mice as the crude extract.

Aliquots of the ethanol extract (24 g) were partitioned sequentially with methylene chloride and water-saturated butanol repeatedly. The average yield of the dried butanol extract was 5.67% w/w of starting material. All dried butanol extracts were combined, and subsequently gavaged as the saponin-rich fraction.

Animal studies: All animal studies were approved by the Institutional Animal Care and Use Committee at Columbia University Medical Center (protocol AAAB4156). Age-matched 16-week old C57BL/6 mice (Jackson Labs, Bar Harbor) arrived prefed with either 60 kcal% fat high fat diet (12492, Research Diets, New Brunswick) or 10 kcal% fat low fat control diet (12450, Research Diets, New Brunswick) for 12 weeks. Each treatment group contained a sample size of 8/9 animals, and had access to food and water ad libitum. Weights and food and water consumption were measured weekly throughout the study. Baseline and endpoint fasting glucose and insulin concentrations were also assessed via tail vein blood samples.

Treatments: The ethanol extract and saponin-rich fraction were resuspended in 10% Tween-20 solution (vehicle), and gavaged at a dosage of 0.5 mg/g body weight daily for four weeks, with dosages being reassessed weekly as the animals were weighed. The high and low fat control groups were gavaged daily with vehicle only.

Intraperitoneal glucose tolerance test (IP-GTT): Animals were fasted for 6 hours and injected with 1 mg/g body weight of 25% sterile glucose solution. Plasma glucose concentrations were measured at 0, 15, 30, 60, 90, and 120 min post-injection using a CONTOUR™ Meter (Bayer, New York). Fasting glucoses

were also measured as the 0 min timepoint. In addition, area under the curve (AUC) was calculated and assessed as a secondary measurement of glucose tolerance.

Intraperitoneal insulin tolerance test (IP-ITT): After a 4 hour fast, high fat diet-fed animal were injected with 0.5 U/kg body weight of insulin, and low fat diet-fed animals were injected with 0.25 U/kg body weight insulin to prevent hypoglycemic shock. Plasma glucose concentrations were measured at 0, 20, 40, 60, 90, and 120 min post-injection, with fasting insulin concentrations measured as the 0 min time point using ELISA insulin plates (ALPCO, Salem).

Immunohistochemical staining: At the conclusion of the study, animals were euthanized and their pancreata removed and the formalin-fixed paraffin-embedded tissue subsequently stained for β-cell mass using 1:500 insulin primary (Cell Signalling Technology, Inc., Danvers) and 1:200 rabbit secondary (Vector Labs, Burlingame) antibodies. Final staining was achieved using the diaminobenzidine tetrahydrochloride (DAB) Peroxidase Substrate Kit (Vector Labs, Burlingame). The β-cell mass was analyzed using Microsoft Digital ImagePro™ software, and calculated as % total β-cell mass of total area of the tissue. All slides contained an extra section, used as a negative control.

Statistics: Significant differences were detected using one or two-way ANOVAs and Tukey HSD comparisons therein, or student *t*-tests, as appropriate.

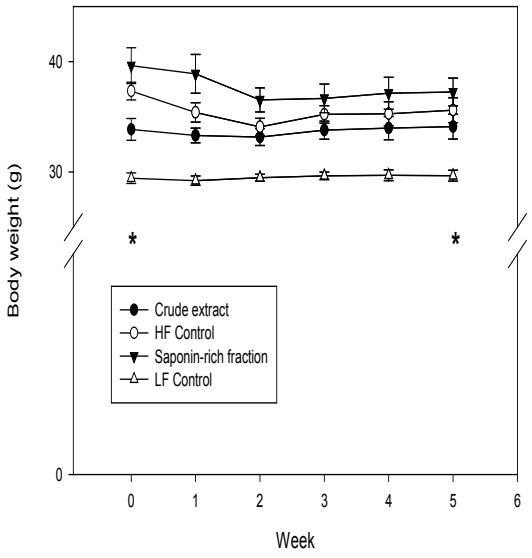
LC-ToF-MS analysis: The LC-ToF-MS analysis was conducted as described in Chapter 3.

Results

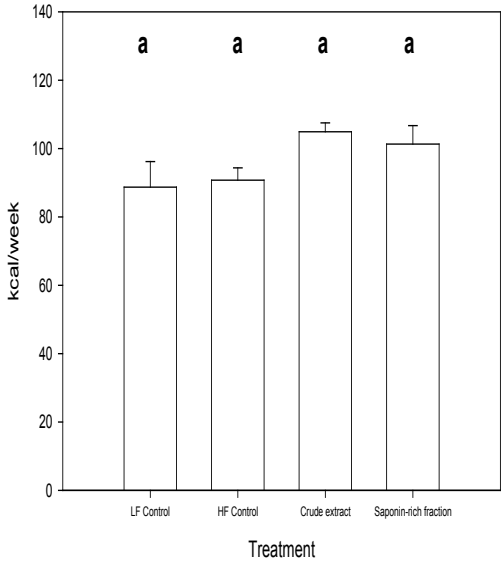
To test the hypothesis that *Momordica charantia* saponins stimulate insulin secretion and support β-cell mass growth, we measured glucose homeostasis via fasting glucose concentrations, GTT and ITT, and β-cell mass, in obese hyperglycemic animals that were gavaged a saponin-rich fraction from the plant, alongside the crude extract.

Metabolic parameters: The LF control group consistently weighed less than the other treatment groups. At baseline, these animals weighed less than the HF control, crude extract, and saponin-rich fraction groups (29.4 ± 1.4 g versus 37.3 ± 2.4 g, 33.9 ± 3.0 g, and 39.6 ± 4.6 g, respectively, $p \leq 0.02$ as compared with the LF control group, **Figure 4.1A**). At the study's endpoint, the LF control group also weighed significantly less than the HF control, crude extract, and saponin-rich fraction groups (29.7 ± 1.4 g versus 35.6 ± 3.2 g, 34.1 ± 3.2 g, and 37.3 ± 3.6 g, respectively, $p \leq 0.02$ as compared to the LF control group, **Figure 4.1A**). None of the groups showed any significant change in body weight during the treatment period (**Figure 4.1A**). No significant differences in kcal consumption were observed between any of the groups (**Figure 4.1B**). However, the LF control group and saponin-rich fraction group animals drank significantly more water than the HF control group (63.4 ± 13.8 ml and 68.3 ± 6.6 ml versus 42.0 ± 3.7 ml, respectively, $p \leq 0.03$ a s compared to the HF control group, **Figure 4.1C**).

A.



B.



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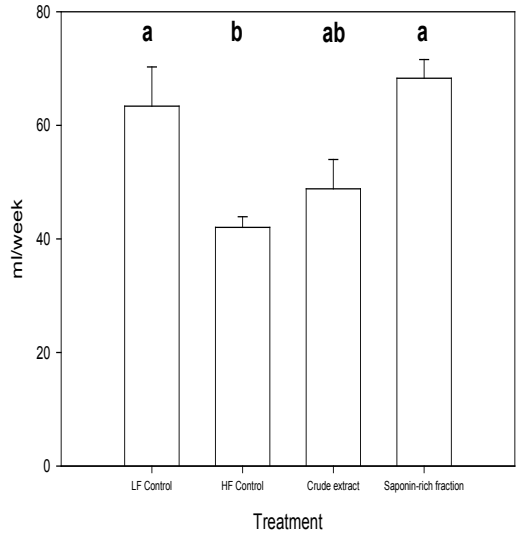
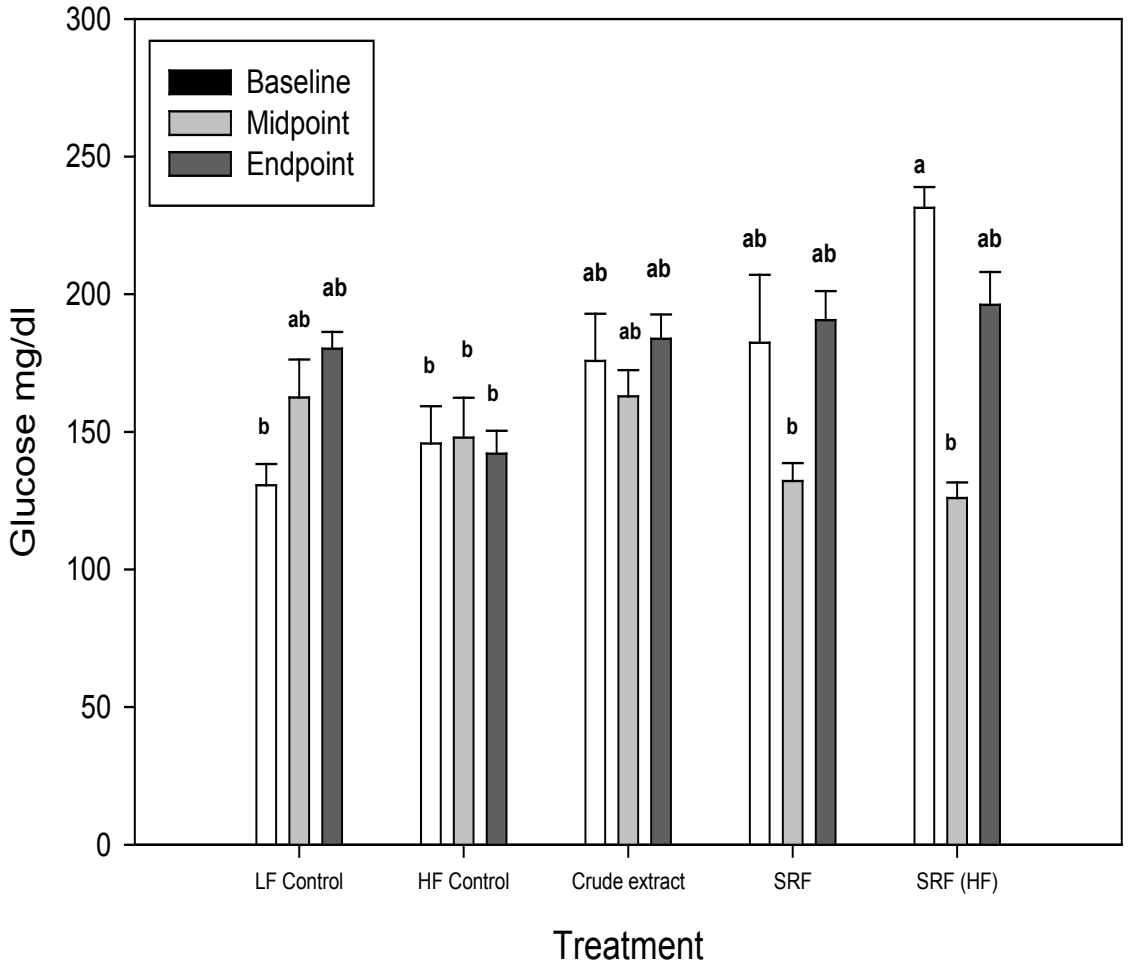


Figure 4.1 A-C. Weights were measured weekly during the treatment (A), and weekly consumption of food (kcal) (B), and water (ml) (C) were measured throughout the study. Weights are expressed as average per group per week, food and water consumption are represented as the average of total consumption per group during the study. The (*) represents significance as compared to the LF control group at weeks 0 and 5, $p \leq 0.02$, and data not connected by the same letter are significantly different, ≤0.03, Tukey HSD within a one-way ANOVA. Error bars represent ± SEM.

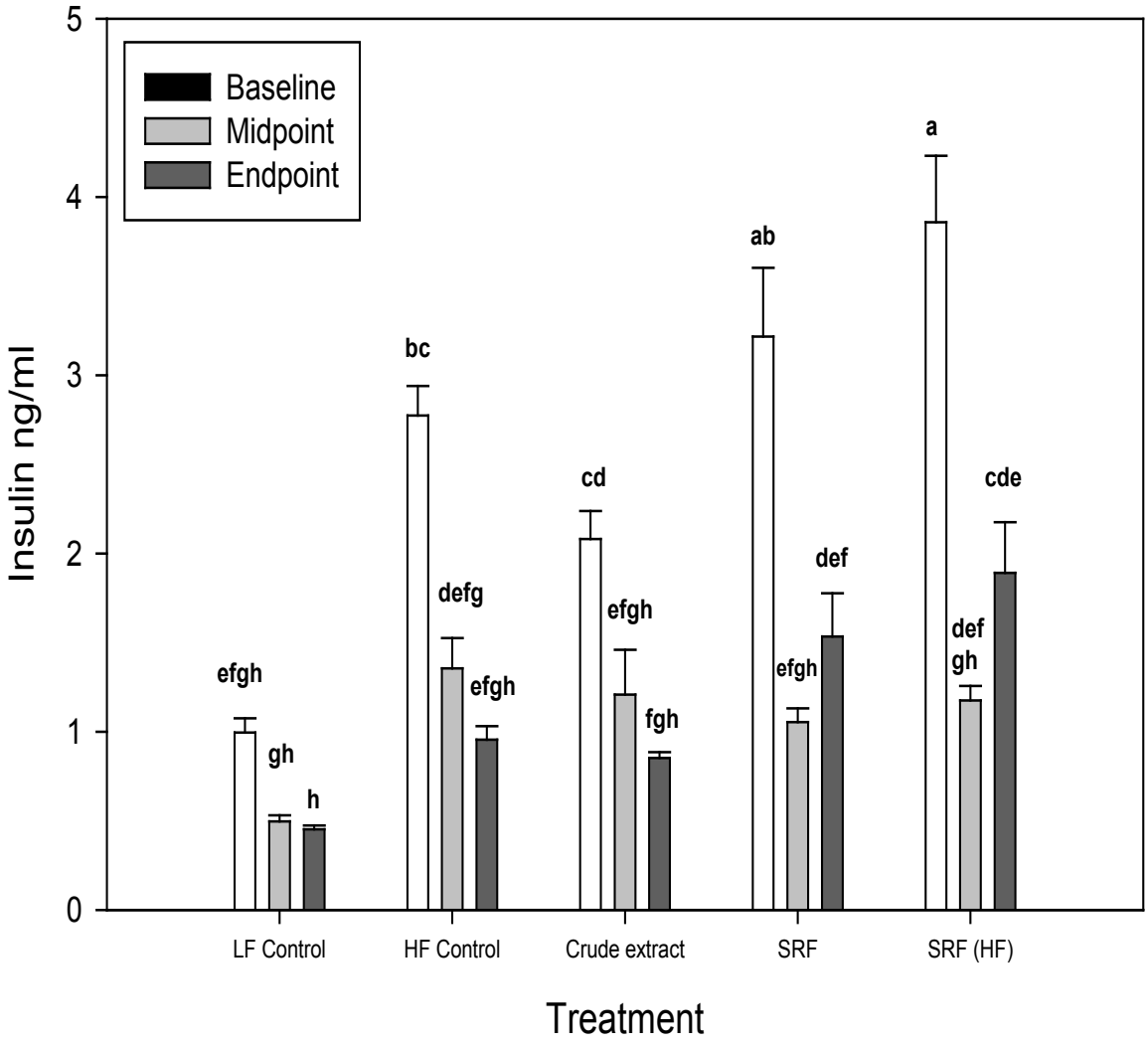
Fasting glucose concentrations: The fasting glucose concentrations of the heaviest animals of the saponin-rich fraction group (n=5, 37.6 g to 42.2 g) taken at the time of the IP-ITT, were significantly less than the baseline concentrations after 2 weeks of treatment (231 ± 21 mg/dl to 126 ± 16 mg/dl, p=0.0016, **Figure 4.2A**). However, by the end of the study, the glucose concentrations of the saponin-rich fraction group (n=8) and the heaviest animals were both greater than the midpoint concentrations, although not significant (**Figure 4.2A**). The lowered glucose concentrations at midpoint suggest a strong hypoglycemic effect of saponins, especially in the most hyperglycemic animals. However, the higher endpoint concentrations may indicate that these effects are fleeting.

Fasting insulin concentrations: The fasting insulin concentrations of all groups dropped during the course of the study as compared to their baseline concentrations ($p \leq 0.04$, **Figure 4.2B**); however, the concurrent fasting glucose concentrations of the saponin-rich fraction group also dropped from baseline to midpoint, significantly in the heaviest animals of this group (**Figure 4.2A**). This concurrent drop in glucose concentrations suggests an improvement of insulin sensitivity in the saponin-rich fraction treated animals. In contrast, the corresponding glucose concentrations of the HF control and crude extract groups did not change significantly, perhaps indicating a continued decrease in insulin sensitivity.



A

Figure 4.2. Fasting glucose (A), and insulin concentrations (B) taken at IP-ITT. Measurements were made at time 0 (baseline), after 2 weeks of treatment (midpoint), and after 4 weeks of treatment (endpoint). The heaviest animals in the saponin-rich fraction (SRF) group are presented as SRF HF (n=5). Data not connected by the same letter are significantly different, $p \leq 0.008$, Tukey HSD within a two-way ANOVA. Error bars represent ± SEM.



B

Figure 4.2. Fasting glucose (A), and insulin concentrations (B) taken at IP-ITT. Measurements were made at time 0 (baseline), after 2 weeks of treatment (midpoint), and after 4 weeks of treatment (endpoint). The heaviest animals in the saponin-rich fraction (SRF) group are presented as SRF HF (n=5). Data not connected by the same letter are significantly different, p≤0.04, Tukey HSD within a two-way ANOVA. Error bars represent ± SEM.

Although not significant, in contrast to other treatment groups, the endpoint insulin concentrations of the saponin-rich fraction group increased from midpoint in both the total group and the heaviest animals. Also, the concurrent fasting glucoses were significantly reduced at midpoint, and then increased at endpoint (**Figure 4.2A**), suggesting that, although the treatments may have a hypoglycemic effect, hyperglycemia persisted in these animals toward the end of the study, and decreased midpoint glucose concentrations were not due to increased insulin secretion. Also, although not significant, the midpoint fasting insulin concentrations of the saponin-rich fraction group were 19% less than that of the HF control group; similarly, the corresponding glucose concentrations were 11% less than the HF control group (**Figure 4.2 A and B**). This may indicate a slight positive effect of the saponin-rich fraction on insulin sensitivity and normalization.

IP-GTT: The HF control group's glucose concentrations were significantly higher during the endpoint IP-GTT at 120 min, 225 ± 85 mg/dl as opposed to 156 ± 41 mg/dl at baseline (p=0.04, **Figure 4.3B**). The area under the curve (AUC) of the HF control group was slightly, but not significantly, higher at endpoint than baseline (**Table 4.1**). Taken together, these data suggest that insulin secretion mildly worsened in the HF group. No significant differences were observed between the IP-GTTs or AUC of the LF control group (**Figure 4.3A, Table 4.1**). The crude extract-treated group showed significantly lower glucose concentrations at 0, 15, and 30 min at the end of the study as compared to baseline (108 ± 18 mg/dl, 291 ± 55 mg/dl, and 314 ± 40 mg/dl versus 171 ± 33

mg/dl, 401 ± 50 mg/dl, and 394 ± 64 mg/dl, respectively, p≤0.006, **Figure 4.3C**). In addition the crude-extract group's AUC at the end of the study was significantly lower than at baseline (p=0.01, **Table 4.1**). These data suggest an improvement of glucose-induced insulin secretion. In addition, there was a definite trend towards improved glucose concentrations in the saponin-rich fraction treated animals. More strikingly, the heaviest animals in the group had significantly lower glucose concentrations throughout the IP-GTT at the end of the study as opposed to the baseline (116 ± 27 mg/dl, 341 ± 83 mg/dl, 260 ± 64 mg/dl, 229 ± 37 mg/dl, and 191 ± 38 mg/dl and 212 ± 60 mg/dl, 469 ± 42 mg/dl, 455 ± 49 mg/dl, 375 ± 49 mg/dl, and 319 ± 68 mg/dl, respectively, p<0.02, n=5, **Figure 4.4B**). This group's AUC was also significantly lower at the end of the study (p=0.003, **Table 4.1**). Therefore, both the crude extract and the saponin-rich fraction appeared to improve glucose tolerance in these high-fat diet induced hyperglycemic animals.

In contrast to the fasting glucose concentrations taken during the IP-ITT (**Figure 4.2A**), fasting glucose measurements taken at the time of the IP-GTT showed that the crude extract-treated groups' glucose concentrations were significantly lower at the endpoint of the study, as compared to the baseline concentrations, 171 ± 33 mg/dl and 108 ± 18 mg/dl, respectively (p=0.0001, **Figure 4.3C**). Also, the animals fed the saponin-rich fraction showed a decline in their fasting glucose concentrations over the treatment period, 165 ± 71 mg/dl to 120 ± 22 mg/dl. Although this reduction in fasting glucose concentrations approached significance (p=0.06, **Figure 4.4A**), the concentrations of the

heaviest animals fed the saponin-rich fraction showed a significant reduction in fasting glucose concentrations at the end of the study (212 ± 60 mg/dl to 116 ± 27 mg/dl, p=0.01, n=5, **Figure 4.4B**), showing further evidence that both *M. charantia* preparations improved glucose tolerance. The HF and LF control groups' fasting glucose concentrations were unaffected (**Figure 4.3A and B**).

IP-ITT: During the IP-ITT at endpoint, the crude extract-treated group showed significantly lower percent of 0 min glucose concentrations at 120 min at endpoint than at baseline, 48% ± 10 and 66% ± 20, respectively (p=0.02, *t*-test, **Figure 4.5C**). The LF control group's percent glucose concentrations were significantly lower at 120 min during the endpoint IP-ITT, 56% ± 13 as compared to 91% ± 27 at baseline (p≤0.005, *t*-test, **Figure 4.5A**). No significant differences were observed between the baseline and endpoint IP-ITT measurements of the saponin-rich fraction group (**Figure 4.5D**). In light of these results, none of the treatments appear to have a dramatic effect on insulin resistance.

Immunohistochemistry: Relative pancreatic β-cell mass, measured as percent insulin-positive area of total pancreatic tissue, was significantly decreased in the crude extract group as compared to the HF and LF controls, and saponin-rich fraction groups, 0.44 ± 0.21% and 0.64 ± 0.29%, 0.66 ± 0.27%, 0.77 ± 0.31% (p≤0.04 as compared with the ethanol extract group, **Figure 4.6**). This may suggest greater efficiency of insulin secretion. No other significant differences were observed between the groups.

LC-ToF-MS: To ensure the integrity and saponin content of the saponin-rich fraction of *Momordica charantia*, the fraction was analyzed by LC-ToF-MS

(**Figure 4.7**). A common basal compound of most saponins has a *m/z* of 437.34, so this *m/z* was used to characterize the fraction. The compounds containing this fragment comprised a large part of the fraction, and remained consistent in the fraction both before and after the study (**Figure 4.7**).

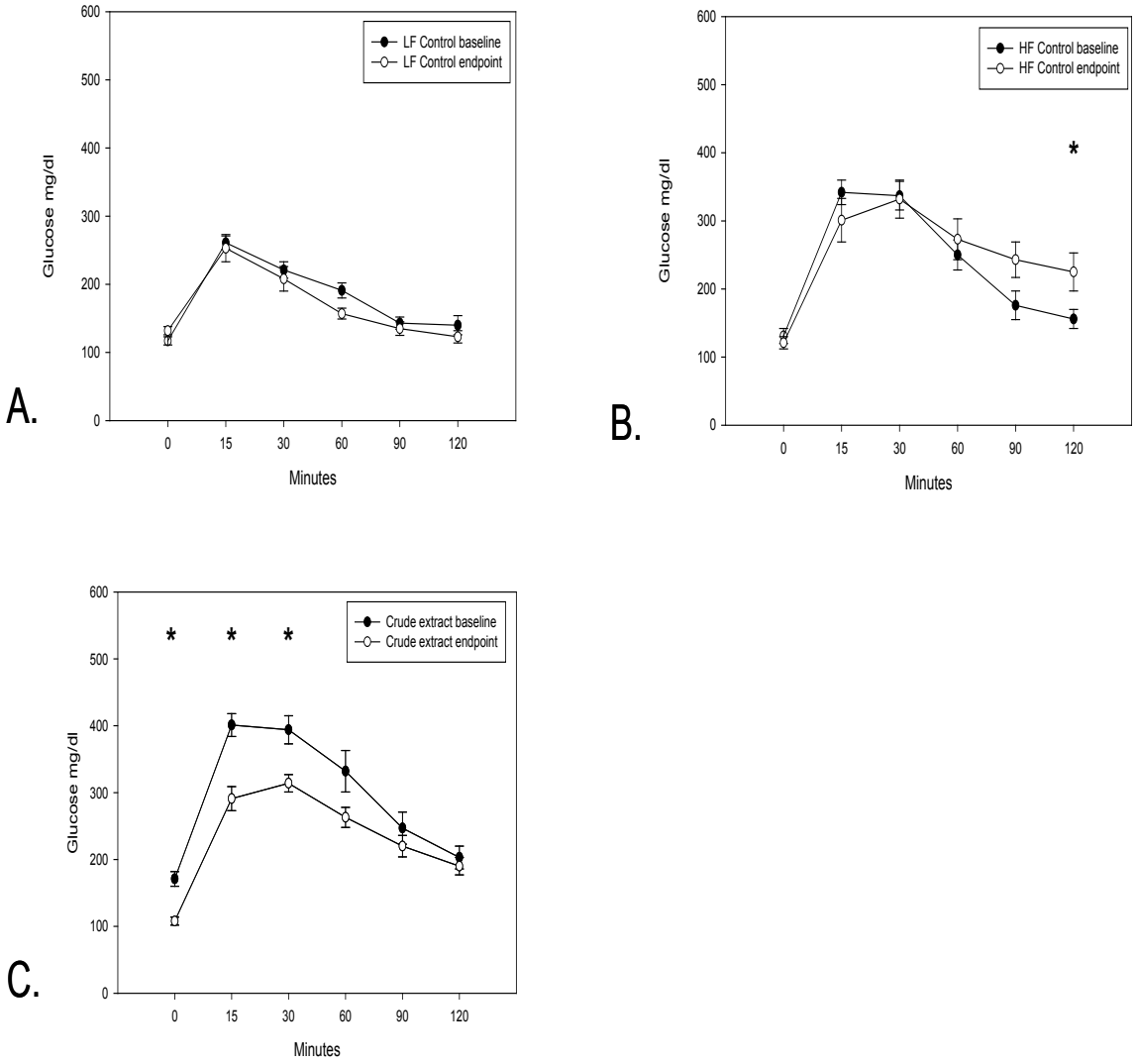


Figure 4.3: Intraperitoneal glucose tolerance test, LF control (A), HF control (B), crude extract (C) groups: Glucose was injected at 0 min, and glucose measurements taken at 15, 30, 60, 90, and 120 min. Error bars represent ± SEM, and (*) denotes significant differences, $p \leq 0.05$, student *t*-test.

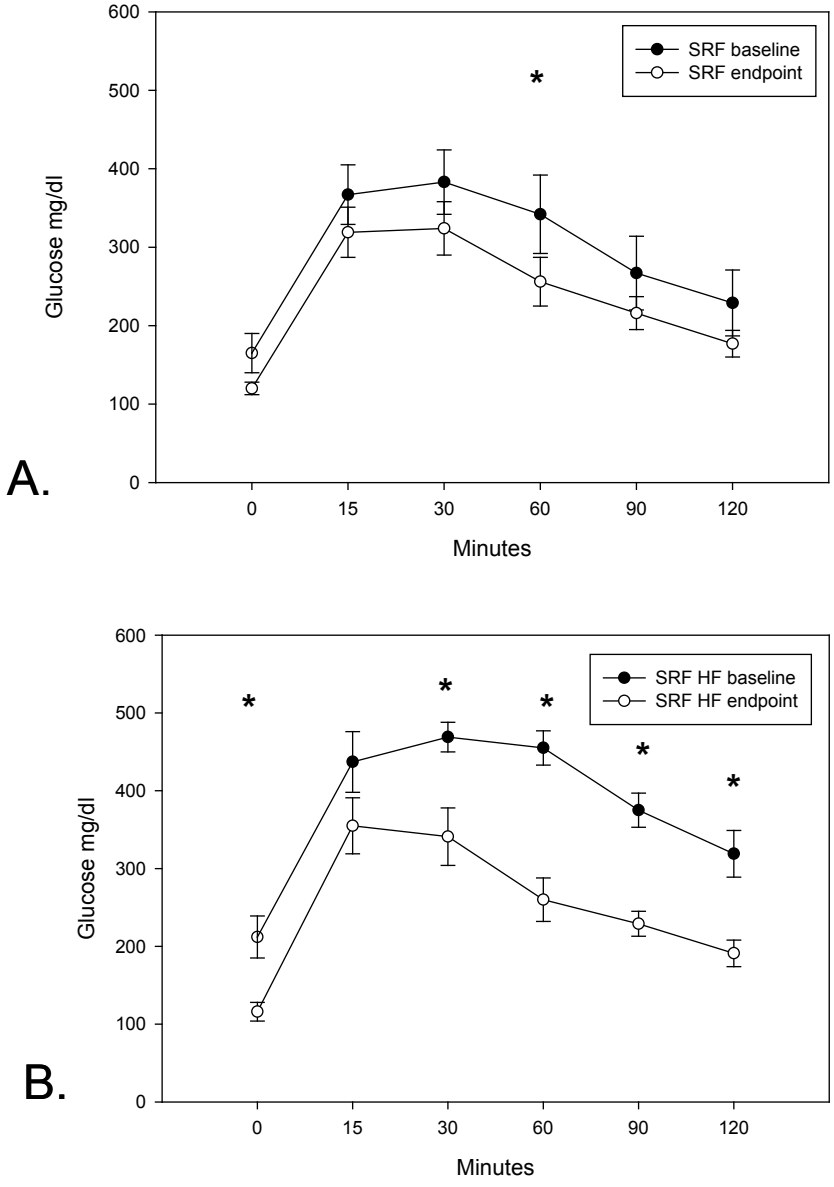


Figure 4.4: Intraperitoneal glucose tolerance test, saponin-rich group (SRF, A), both all animals, n=8, and heaviest animals (SRF HF, B), n=5. Glucose was injected at 0 min, and glucose measurements taken at 15, 30, 60, 90, and 120 min. Error bars represent ± SEM, and (*) denotes significant differences, $p \leq 0.05$, student *t*-test.

Treatment	Baseline (mg/dl*min)	Endpoint (mg/dl*min)
LF control	14,118 ± 565	13,215 ± 777
HF control	18,732 ± 1,276	19,808 ± 1,774
Crude extract	23,410 ± 1,505	18,563 ± 779*
Saponin-rich fraction	24,894 ± 2,666	18,949 ± 1,780
Saponin-rich fraction (HF)	30,012 ± 1,542	20,094 ± 1,756*

Table 4.1: Area under the curve (AUC) analysis of IP-GTT of all groups' averages ± SEM, baseline and endpoint: The (*) denotes significant differences between baseline and endpoint AUC of each group ≤0.01, student *t*-test.

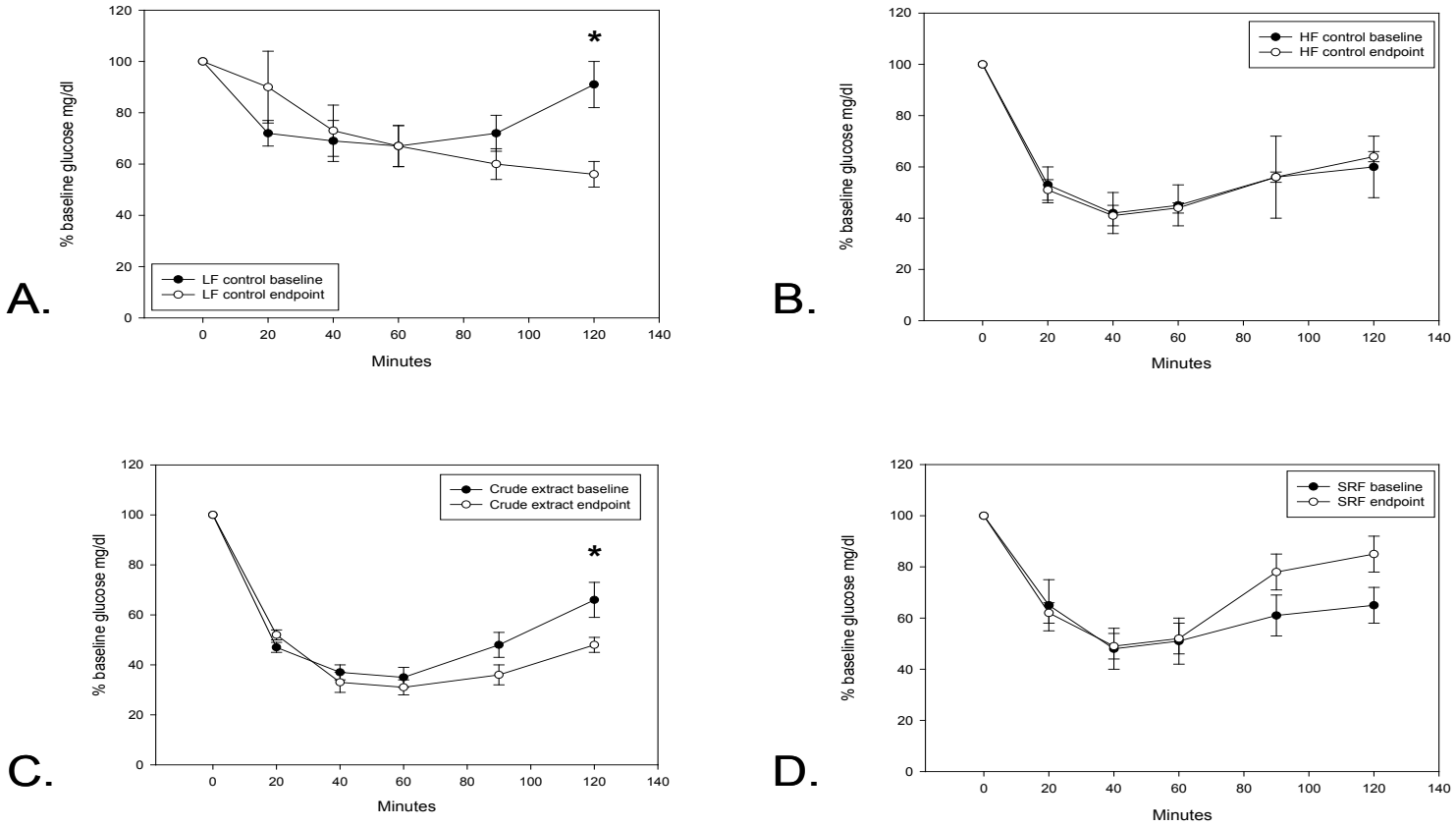


Figure 4.5: Intraperitoneal insulin tolerance test, expressed as percent of baseline measurement for LF control (A), HF control (B), crude extract (C), and saponin-rich fraction (D) treated groups. Insulin was injected at 0 min, and glucose measurements taken at 0, 20, 40, 60, 90, and 120 min. Error bars represent ± SEM, and (*) denotes significant differences, p≤0.05, student *t*-test.

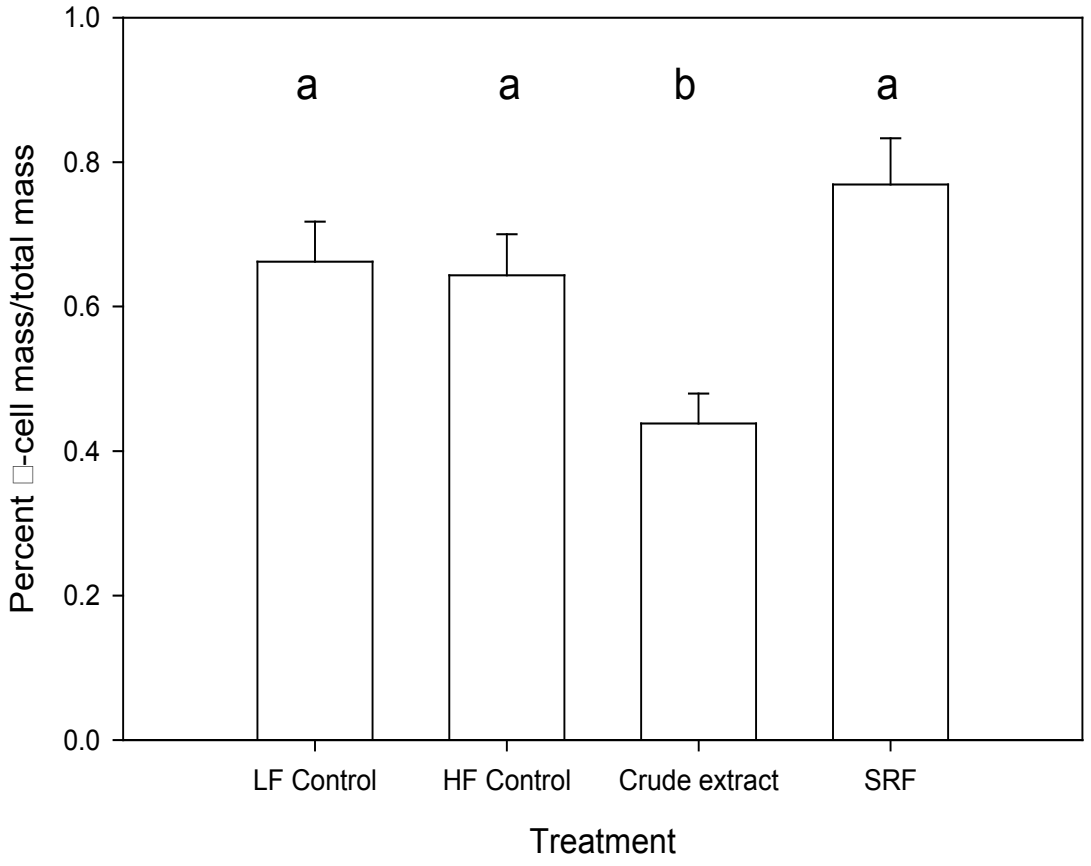


Figure 4.6: Immunohistochemical analysis of all groups: Mass of pancreatic β-cells expressed as percent β-cell per total tissue mass. Data not connected by the same letter are significantly different, ≤0.04, Tukey HSD within a one-way ANOVA. Error bars represent ± SEM.

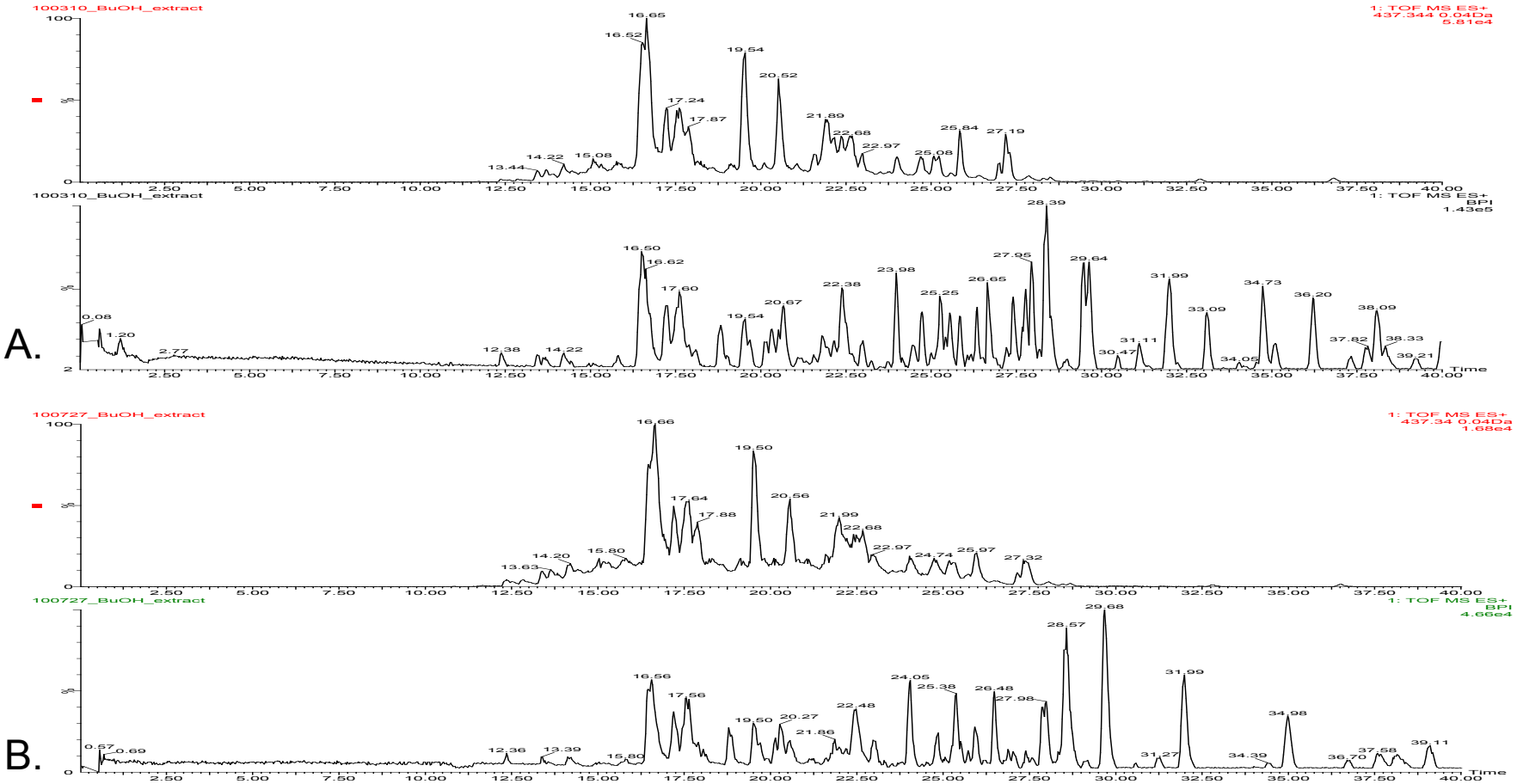


Figure 4.7. Base peak intensity chromatograms of a saponin-rich fraction of *M. charantia* before (A) and after (B) the study. Upper chromatograms show extracted ion chromatogram for a common cucurbitane skeleton, *m/z* = 437.3290.

Discussion

The main findings of this study were that the crude extract and the saponin-rich fraction were able to both significantly lower fasting glucose concentrations and significantly improve glucose tolerance in obese hyperglycemic mice. The most obese animals in the saponin-rich fraction group had the quickest and most dramatic drop in fasting glucose concentrations and the greatest improvement in glucose tolerance testing. This activity agrees with the in vivo activity reported in the *Momordica charantia* literature for purified saponins (*90*), or fractions thought to contain saponins (*89, 92, 93*). However, to our knowledge this is the first report of robust hypoglycemic activity of a characterized saponin-rich fraction over a multi-week study period.

The crude extract improved glucose tolerance, a surrogate test for glucose-induced insulin release, with apparently less total β-cell mass than in the saponin-rich fraction-treated animals. Although *Momordica charantia* crude extract has been found to improve glucose tolerance in a T2DM animal model (*60*), the concurrent β-cell function and morphology has not been previously assessed for high-fat diet-induced T2DM animals. As the HF control animals did not have a significant improvement in the IP-GTT over the treatment period, the improved IP-GTT appears to be a true effect of the crude extract and saponin-rich fraction treatment. Therefore, the most profound effect from these treatments is consistent with improvement in glucose-induced insulin secretion. These results are in agreement with the acute insulin secretion activity of the saponin-rich fraction and isolated saponins described in Chapter 3.

Although the exact etiology is unclear, the fasting insulin concentrations of the LF and HF control groups trended to decrease over the study period. The crude extract and saponin-rich fraction treated groups appeared to have the same general trend. However, only the saponin-rich fraction treated group's concurrent glucose concentrations dropped at midpoint as well, likely indicating a temporary improvement in insulin sensitivity. Despite this decrease of the fasting insulin concentrations at midpoint of the saponin-rich fraction group and the HF control group, it remains unclear if the changes in insulin concentrations in these treatment groups were necessarily secondary to the treatments. In addition, the baseline insulin concentrations between the groups were somewhat variable, and this may have masked more subtle effects of the plant treatments.

During the IP-ITT, the group fed *Momordica charantia* crude extract had significantly less endpoint glucose concentrations at 120 min than at baseline. Although a small effect, this may reflect some improvement of insulin sensitivity, especially when taken along with the improved glucose tolerance observed in this group. The LF control group also showed significantly decreased endpoint glucose concentrations at 120 min, suggesting the same result. As no significant differences were observed in the saponin-rich fraction treated group, we conclude from these results that *M. charantia* saponins failed to improve insulin tolerance. Although saponin-containing fractions of the *M. charantia* crude extract have been shown to increase insulin sensitivity via increasing PPARy expression (*92*), we did not observe any improvement of insulin tolerance. Despite this observation, these studies cannot be compared since the material in the previous

study was not characterized using LC-ToF-MS; however, we cannot rule out any possible effect on PPARy expression or additional molecular markers.

We observed significant differences between the weights of the LF control group and those of the other treatment groups at the beginning and end of the study. This indicates that the animals fed the high-fat diet were appropriate for diet-induced obesity and subsequent diabetes as they reached a weight level significantly higher than those animals fed the low-fat diet. Also, with the exception of the HF control group, all the high-fat diet-fed groups had significantly higher fasting glucose levels than the LF control group.

In addition, the fact that no significant differences were observed between the individual endpoint and baseline weights of any of the groups suggests that neither the vehicle, plant material, or experimental procedures caused undue stress or toxicity-induced weight loss; this is consistent with other studies. The saponin rich-fraction animals consumed significantly more water than the HF control group, perhaps indicating a possible improvement in metabolism, as also observed with the LF control group's water consumption. In short, no indication of toxicity of any of the treatments was reflected in the weight, or food and water consumption measurements described herein, and the increase in the saponin-rich fraction group's water consumption suggests a beneficial effect of saponins on metabolism.

Given the improvement in glucose tolerance with the crude extract treatment, the decreased β-cell mass seen in this group may reflect an effect of the extract in improving β-cell insulin secretion per mass of β-cell. There may

also be some improvement in insulin sensitivity as slightly suggested by IP-ITT testing. In previous studies, the crude extract of *Momordica charantia* has increased the β-cell mass and proliferation in T1DM animal models (*72-76*). However, according to the etiology of β-cells in T2DM, a decrease in β-cell mass and proliferation can be interpreted as an improvement in function. As the crude extract has been shown to affect β-cell morphology and function in various in vivo models, it follows that the crude extract also improves β-cell function in the animals in this study. In addition, the saponin-rich fraction did not have a significantly different β-cell mass as compared to the HF control, and showed no improvement in the IP-ITT, suggesting no improvement in insulin sensitivity. Therefore, the saponin-rich fraction had no effect on compensatory β-cell mass. This is the first time a characterized saponin-rich fraction of *M. charantia* has been analyzed for improvement of insulin tolerance over a multi-week study period.

The stability and composition of the saponin-rich fraction was analyzed by LC-ToF-MS and found to be consistent at the beginning and end of the study. This helps to show that the bioactivity observed in the animals treated with the saponin-rich fraction is most likely due to saponins, and that these compounds were consistently present in the fraction during the treatment. However, other compounds in addition to saponins are likely to be present in the butanol fraction.

In summary, this study suggests that the crude extract of *Momordica charantia* improves fasting glucose, glucose-induced insulin secretion, and to a lesser extent, insulin resistance. This diversity of antidiabetic activity is most

likely due to the wide variety of compounds in the crude extract, including saponins, and their potential synergy. Although the saponin-rich fraction showed a more immediate and dramatic hyperglycemic effect, and also improved glucose-induced insulin secretion, any compounds responsible for the effects on insulin resistance or β-cells are likely contained in the crude extract. As the effect on fasting glucose concentrations was temporary, and fasting insulin baselines were varied, we cannot conclusively assess the bioactivity observed. However, the robust improvement in the animals' glucose tolerance strongly suggests that *M. charantia* saponins, independently of the crude extract, improve hyperglycemia and may improve insulin secretion. Fractions described as containing saponins have been found to stimulate the activity of the molecular mechanisms behind insulin sensitivity and glucose transport (*68, 92*); isolated saponins improved short-term glucose tolerance, and increased fatty acid oxidation (*90*). It is likely that the bioactivity of the saponin-rich fraction is due to these mechanisms.

Despite the previously mentioned strong hypoglycemic activity, no improvement on insulin tolerance, fasting insulin concentrations or pancreatic β-cell mass was observed in the saponin-rich fraction group, again indicating that the mechanism behind the improvement in glucose tolerance and fasting glucose concentrations may likely be at the molecular level of glucose uptake or β-cell activity, as preliminarily investigated with saponins isolated from *M. charantia* fruit and stems (*90, 91*).

Although our data partially supports our hypothesis that the hypoglycemic activity reported in *Momordica charantia* fruit is due to the saponins' ability to stimulate insulin secretion, the *M. charantia* crude extract showed the broadest bioactivity in our various measurements. This suggests that consuming the fruit in entirety may be more appropriate to treating diabetes in the long term, as opposed to consuming concentrated compounds such as saponins with more short-term, specific activity. This study helps to further establish antidiabetic activity of both the crude extract and saponins of *M. charantia*, and this plant could be useful in moderating and alleviating metabolic disorders associated with obesity-induced diabetes.

Chapter 5

Conclusions

Diabetes is a growing, worldwide epidemic. Many people throughout the world rely on traditional medicine to treat diabetes, among other health ailments. According to current literature, use of traditional medicine is on the rise among those diagnosed with a chronic disease, such as diabetes (*111*). Ethnobotanical field work documented the use of *Costus* species and *Momordica charantia* as treatments for diabetes. As *M. charantia* is also a popular plant for diabetes sufferers worldwide, it follows that investigating these plants' potential antidiabetic activity, or mechanisms of reported activity, is necessary. This dissertation study aimed to substantiate or further characterize any antidiabetic activity in the commonly used plants in Dominican traditional medicine, *C. spicatus*, and *M. charantia*.

The tea of *Costus spicatus* failed to improve fasting glucose or insulin concentrations, glucose tolerance or insulin sensitivity. In short, the results of this investigation show no antidiabetic activity in *C. spicatus* tea in vivo. There could be many reasons for this lack of activity, including the species used for the study; a mix of closely related species, or even contamination with unrelated plant material, can occur with botanicals available commercially. Also, the animal model used was severely diabetic, and perhaps masked any moderate effects on metabolic parameters. In any case, it is important to report any results of bioactivity of plants used by the general public.

For *Momordica charantia*, our study showed that both the ethanol and saponin-rich fraction lowered fasting blood glucose levels and improved glucose tolerance (*46, 47, 55, 57, 58, 90, 124*). In addition, we noted that the saponin-rich fraction increased insulin secretion over a 4-week time period, and had the most dramatic effect on those animals with the heaviest weights. As we did not observe any increase in the pancreatic β-cell mass of the saponin-rich fraction treated animals, the stimulation of insulin secretion and corresponding improvement in glucose tolerance points to mechanisms at the molecular, as opposed to the cellular level. In addition, the above-mentioned work verifies that saponins are responsible for all, or part, of the hypoglycemic activity observed when testing a *M. charantia* saponin-rich fraction in vitro and in vivo. This confirms and contributes to the recent direction other studies are taking in addressing the antidiabetic activity of *M. charantia* (*68, 89-92, 124*).

Future directions for the research of *Costus spicatus* could include the broadening of bioactivity screening, such as in vitro testing, and repeating an in vivo experiment with a model suited to detecting mild or moderate bioactivity. *Momordica charantia* has hypoglycemic activity as reported in other studies and verified herein. Based on this evidence, it seems appropriate to explore the potential of this plant in clinical trials and more human-based investigations. Further studies elucidating the mechanism of action behind this plant's activity would also be prudent. In general, studies like those described herein should be undertaken for the most-used herbal treatments in traditional medicine worldwide.

Many medicinal plants used around the world have not been investigated for either efficacy or safety. In the United States, the contrast between drug and herb regulation is quite pronounced. According to the 1994 Dietary Supplement Health and Education Act (DSHEA), medicinal herbs are classified as dietary supplements (*125*). The Food and Drug Administration (FDA) places the responsibility of safety on the manufacturer and does not require that products made in the United States be registered with the FDA (*125*). The DSHEA also says nothing about verifying the efficacy of medicinal herbs (*125*). In contrast to dietary supplements, the FDA requires all new drugs to be proven safe and effective before their release on the market (*126*). The general public uses herbal medicine as a form of health care, and as the FDA does not require efficacy of herbs prior to their marketing, scientific focus on these modalities is important to support this form of available health care. In addition, heavily used herbs such as *M. charantia* should also be subjected to toxicity research as well.

Access to modern medicine is not always feasible. For example, the results of a recent survey show that 23.1% of people in the United States reported skipping a dose or not getting a prescription filled due to the cost of the medication in the past year, as opposed to 5.4% in the United Kingdom (*127*). With the current and increasing unequal access to health care around the world (*128*), coupled with increasingly expensive pharmaceuticals (*129*), it follows that modern medicine is often priced out of reach of the general public. As local, traditional medicines such as herbs might be the best health option for people, these plants should be scientifically supported as a health option. In specific

ways, the work described in this dissertation contributed to the knowledge about two medicinal plants used in diabetes treatment.

Knowledge about plants used in traditional medicine is increasingly needed in the modern medical sector. In ethnically diverse larger cities such as New York City, health practitioners may have contact with patients from many countries and health traditions. In addition to information about medicinal plants generally informing medical practitioners about what their patients may be using, this information can be crucial to avoiding serious herb-drug interactions, as well as other health concerns. If a practitioner knows how a plant may act on a person's health, this can help inform treatment and drug decision.

The inspiration of this thesis project was to contribute to the scientific support of traditional medicine, by investigating plants used for diabetes with Western medical research techniques. It is this author's hope that the information and publications generated herein can be accessed by health professionals treating diabetics that use either *Costus spicatus* or *Momordica charantia*. Accessible research of this type can contribute in some way to a more sustainable future of health care, a future where many forms of health care are available and affordable to all.

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