Clinical Intervention Study Protocol Template

Evaluation of soy-protein isolate Russian tarragon (*Artemisia dracunculus* L.) complex as a dietary supplement to improve carbohydrate metabolism; Dosing, safety and tolerability

Submitted by:
Botanical Research Center: Pennington Biomedical Research Center, LSU System and Rutgers University

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Pennington Biomedical Research Center

FULL PROTOCOL TITLE

Evaluation of soy-protein isolate Russian tarragon (*Artemisia dracunculus* L.) complex as a dietary supplement to improve carbohydrate metabolism; Dosing, safety and tolerability

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Supported by:
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Study Intervention Provided by:

The product for the clinical studies will be obtained from the Botanical Core Lab of the Rutgers Component of the Botanical Research Center. Working in collaboration with Dr. John Finley at the Louisiana State University Food Science department, the final product will be delivered to the Pennington Biomedical Research Center where the clinical study will be performed. Specifically, *Artemisia dracunculus* herb was grown from seed by ARC Greenhouses in cooperation with Rutgers University.

Sponsor of IND (IDE):

No IND is required for this study, see attached letter (Appendix A)

Tool Revision History

Version Number: Version 4
Version Date: January 20, 2014
Summary of Revisions Made: Have revised Figure 1 to add a ten and 22 hour time point corresponding to “hour of OGTT”. Have added a new Table 2 to provide clarification of time points and blood draws of bioactives in relations to OGTT blood draws

Version Number: 4.0 Version Date: 05Oct2016
Summary of Revisions Made: Dosing of MAD phase will be done overlapping as opposed to consecutively. The change is suggested based on safety profile observed for the SAD study utilizing all doses proposed in the current proposal

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PRÉCIS

Study Title

Evaluation of soy-protein isolate Russian tarragon (*Artemisia dracunculus* L.) complex as a dietary supplement to improve carbohydrate metabolism: Dosing, safety and tolerability

Objectives

The primary objective of this study is to evaluate the effect of nutritional supplementation with a well characterized preparation of *Artemisia dracunculus* L. on safety and tolerability of the preparation. Secondary objectives will evaluate the metabolism of the preparation by assessing the appearance of bioactive compounds in the plasma.

Design and Outcomes

We will employ 3-treatment crossover designs in which each subject will receive a sequence of 3 mono treatments with washout periods between distinct treatments (extract doses). We will use ascending single oral dose evaluation in a randomized controlled trial and multiple oral dose evaluation in an additional randomized controlled trial to investigate extract safety and tolerability in healthy lean subjects and in healthy subjects who are non-diabetic but with obesity. We will assess safety in the form of biochemical assessments, collection of data on clinical adverse events and will assess plasma appearance of proposed bioactive compounds.

Interventions and Duration

A soy-protein isolate Russian tarragon (*Artemisia dracunculus* L.) complex will be used as the specific intervention. Dosing for the single ascending dose will be based on preclinical data and on referenced formulas that have been used to scale doses from mice to man or vice versa.

Sample Size and Population

For the safety and tolerability studies, healthy subjects who have passed all screening evaluations and have given informed consent will be recruited. Approximately 27 subjects (3 cohorts of 9 subjects) will participate in the single dose studies. For the multiple dose studies, we plan to enroll an additional 27 participants (3 cohorts of 9 subjects). In the single dose studies, participants will be healthy adults with BMI 18.5 – 32 kg/m2. In the multiple dose studies, participants will be stratified by body mass index (BMI) so that 1 normal weight (BMI < 25), 1 overweight (BMI 25-29) and 1 obese (BMI 30+) participant will be in each treatment sequence. Observers will be blinded to treatment sequence for each participant until the database has been locked.

1. STUDY OBJECTIVES

1.1 Primary Objectives

Phase 1 Studies: Ascending Single Oral Dose Evaluation

The **Primary objective** will be to assess the safety and tolerability of a single dose of the soy-protein isolate Russian tarragon (*Artemisia dracunculus* L.) complex in healthy lean subjects.

Although this study is not designed to evaluate efficacy, we will assess the effect of a single dose of the botanical complex on glucose tolerance to determine if there is an acute effect.

Phase 1 Studies: Multiple Oral Dose Evaluation

A **Primary objective** is to assess the safety and tolerability of multiple daily doses of the soy-protein isolate Russian tarragon (*Artemisia dracunculus* L.) complex in healthy lean subjects and in healthy subjects who are considered overweight or obese.

1.2 Secondary Objectives

A **secondary objective** is to assess the metabolism of the preparation by assessing plasma appearance of proposed bioactives after dosing.

A **secondary objective** is to assess possible mechanism of action operative *in vivo* for the soy-protein isolate Russian tarragon (*Artemisia dracunculus* L.) complex by determining effect on insulin secretion and/or effects on lipid profiles.

2. BACKGROUND AND RATIONALE

2.1 Background on Condition, Disease, or Other Primary Study Focus

It is well known that Type 2 diabetes is a progressive disorder whose pathophysiology consists of pancreatic dysfunction, hepatic glucose overproduction and insulin resistance in peripheral tissues.[1] As such, clinical strategies to achieve glycemic control endorse the use of a combination of agents from multiple pharmacologic classes and which target specific pathophysiologic defects.[1,2] In particular, the enhancement of insulin sensitivity remains as a primary clinical strategy given the role of insulin resistance in the pathophysiology of type 2 diabetes. Recent data, however, have questioned the safety of the current pharmacologic agents used to enhance insulin sensitivity.[2,3] As such, alternative strategies, e.g., nutritional supplementation with over-the-counter agents, are extensively practiced by a large number of patients and are frequently done so without the knowledge of the provider.[4,5] Based on historical human use, there has been great interest in plant extracts (botanicals) as a source for nutritional supplements intended as adjunctive therapies for human diseases. Specifically, isolated compounds identified from plant sources, i.e. phytochemicals or bioactives, have served as a source for therapeutic agents for many diseases, including malignancy, infectious diseases and diabetes.[6] Interestingly, the development of one of the most commonly used anti-hyperglycemic agents in the world today, i.e. metformin, can be traced to the traditional use of *Galega officinalis* to treat diabetes, and the subsequent search to identify active compounds with reduced toxicity.[7] However, the concern with most nutritional supplements, including those considered “natural” (e.g. botanicals) by the consumer, is the paucity of data in humans in regard to efficacy to improve metabolic abnormalities.[8,9] Thus, there remains considerable controversy regarding the use of botanical supplements for human health.

Despite the stated concerns for botanical supplements, many botanicals have shown considerable promise for human use. In particular, plants from the genus Artemisia, and

specifically *Artemisia dracunculus* L. (Russian tarragon) have a long history of medicinal and culinary use and have been reported to have efficacy as a traditional treatment for diabetes in various parts of the world.10 Specifically, the ethanolic extract of *A. dracunculus* L. significantly decreased blood glucose levels and improved insulin levels in both genetic and chemically induced murine models.[11,12] As a proposed mechanism of action, enhanced insulin receptor signaling was demonstrated in primary human skeletal muscle culture exposed to specific concentrations of the *A. dracunculus* L. extract. In addition, at least 5 bioactive compounds have been identified in *A. dracunculus* L. that have specific effects *in vitro* on mechanisms contributing to gluco-regulation.[13-16] Yet, despite the promising *in vitro* studies, clinical efficacy data for the extract in carefully controlled clinical research studies are lacking. Thus, the overall objective of this study is to conduct early human investigation and to evaluate the effect of nutritional supplementation with a well characterized extract of *Artemisia dracunculus* L. The primary objectives would be to evaluate safety, tolerability and effective dose in non-diabetic human subjects. Secondary objectives would assess metabolism of the extract and evaluate effects on proposed mechanisms such as insulin secretion, lipid levels and/or insulin resistance.

2.2 Study Rationale

Our pre-clinical studies have confirmed the potential of *Artemisia dracunculus* L. to be developed as an effective nutritional supplement to address components of metabolic syndrome. The completed studies as proposed will provide the necessary data for large scale Phase III randomized clinical intervention trials.

Experiments Proposed. Studies will assess the effect of a soy-protein isolate Russian tarragon (*Artemisia dracunculus* L.) complex (termed 5011-Nutrasorb®) as a dietary supplement to improve carbohydrate metabolism. Protocols will be designed to evaluate feasibility, dose range effects, pharmacology, safety and biological efficacy of the botanical complex. We submitted an application for review to the FDA and were informed that *"use of this product in the manner described in the information you submitted does not require an IND"*. The soy-protein isolate Russian tarragon complex will primarily be tested in this application with use of ascending single and multiple oral dose Phase 1 studies.

Source and Preparation: As outlined, PMI-5011 is an ethanolic extract of *Artemisia dracunculus* L., also known as Russian tarragon. The plant has been used as a medicinal botanical or food for centuries and continues to be cultivated in Europe. PMI-5011 has been the primary study focus of our NIH Botanical Research Center since 2005 and a study focus at Rutgers University for at least 10 additional years. Extensive safety testing has been conducted with the botanical extract including complete Ames analysis, a 14-day repeated-dose oral toxicity study in rats and an oral subchronic 90-day toxicity study in rats, none of which showed any indication of toxicity. Based on these studies, it was felt that PMI-5011 would be an attractive dietary supplement for human use in the area of improving glucose metabolism.

In the proposed clinical study, we will use an alternative preparation of *Artemisia dracunculus* L. Rather than extracting the herb with 60% ethanol and drying the extract into a sticky paste-like residue, we propose to make a hot water tea-like extract which will be mixed with soy protein

isolate, a common component of many foods. The polyphenols that have been identified as bioactive from the ethanolic extract will bind to the soy protein isolate, which can then be separated from the water and dried. This process has been used successfully to concentrate polyphenols from green tea and anthocyanins from sugary berry juices. Since the basic process involves the mixing of two foods and removal of water, it is equivalent to cooking. The resulting product remains a food, and we feel it can be Generally Regarded as Safe (GRAS). In addition to eliminating the need for organic solvent (ethanol) use and disposal, this process removes chlorophyll and other non-polar compounds which are retained when ethanol is used as an organic solvent for *Artemisia dracunculus* and which tend to make the extract insoluble and less bioavailable. Polyphenol concentrations in the soy protein mixture are comparable to those in the ethanol extract. 5011-Nutrasorb is not intended to cure, prevent or mitigate a disease. Its suggested use is to maintain normal functions of the human body. Because it is made by simple mixing and separating of two foods (Russian tarragon tea and soy protein), we believe that the resulting substance can be Generally Regarded as Safe (GRAS).

3. STUDY DESIGN

A. Phase 1 Studies: Ascending Single Oral Dose Evaluation

Objective: The **primary objective** will be to assess the safety and tolerability of a single dose of the 5011-Nutrasorb in healthy subjects. Although this study is not designed to evaluate efficacy, we will assess the effect of a single dose of the botanical complex on glucose tolerance to determine if there is an acute effect.

Subjects: Twenty seven healthy subjects (3 cohorts of 9 subjects) who have passed all screening evaluations and have given informed consent will be recruited for the Phase I studies. Healthy subjects are defined as individuals who are free of significant cardiac, pulmonary, gastrointestinal, hepatic, renal, hematological, endocrine, neurological and psychiatric disease as determined by history, physical examination and clinical laboratory tests.

Dosing: A representative dosing schedule and randomization for this study is outlined in Table 1. We will begin dosing at very low doses based on the dose response data generated from the mice studies. Based on dosing considerations (see Section 5.1), the suggested dosing range would be 3-30 g subject. Thus, our suggested doses will be 0.3, 1, 3, 10, 20 and 30 g. We will use the same extract dose for all participants irrespective of their weight. Each subject will receive three single doses (two doses of 5011-Nutrasorb and one of placebo) separated by washout periods of at least 7 days. In each treatment period a single dose of extract will be administered on day 1. Up to 9 treatment sequences (3 sequences in each of 3 cohorts) are planned for the single dose evaluations (**Table 1**).

Table 1. Ascending Oral Dose Randomization			
	Period 1	**Period 2**	**Period 3**
COHORT 1			
Sequence 1 (n=3)	Dose 1	Dose 2	Placebo
Sequence 2 (n=3)	Dose 1	Placebo	Dose 2
Sequence 3 (n=3)	Placebo	Dose 1	Dose 2
COHORT 2			
Sequence 4 (n=3)	Dose 3	Dose 4	Placebo
Sequence 5 (n=3)	Dose 3	Placebo	Dose 4
Sequence 6 (n=3)	Placebo	Dose 3	Dose 4
COHORT 3			
Sequence 7 (n=3)	Dose 5	Dose 6	Placebo
Sequence 8 (n=3)	Dose 5	Placebo	Dose 6
Sequence 9 (n=3)	Placebo	Dose 5	Dose 6

Complete blood chemistry will be obtained prior to and after dosing. After dosing, glucose tolerance studies are conducted. As part of the glucose tolerance, both glucose and insulin will be obtained at baseline, and at 30, 60, 90, 120, and 180 minutes after dosing. Plasma will also be stored and archived for later analysis of proposed bioactive compounds.

Before the next cohort is commenced, subjects in the previous cohort must have received all 3 treatments and the safety and tolerability data assessed and show good tolerability and the following information must be available in a safety summary provided by the investigator: (1) adverse events, (2) complete hematologic and biochemistry profile in blood, (3) measures of glucose and insulin response to glucose tolerance. Statistical analyses will be conducted to assess whether there is residual effect of extract after washout.

Assessments and Evaluations:

Baseline/Screening Evaluation: For the single dose study, all subjects will undergo an outpatient screening evaluation to assess their eligibility prior to entrance into the study. A complete medical history and physical examination will be performed**.** Blood samples will be taken for a complete blood chemistry, blood counts, thyroid hormone panel and urinalysis. Subjects with impaired glucose tolerance or diabetes will be excluded. During baseline, subjects will be assessed for dietary intake data and instructed on weight maintenance diets. A taste (small sip) of the study product (30g) will also be administered during screening.

Study Design of Ascending Single Dose

Objective: The **Primary objective** will be to assess the safety and tolerability of a single dose of the 5011-Nutrasorb in healthy subjects. Although this study is not designed to evaluate efficacy, we will assess the effect of a single dose of the botanical complex on glucose tolerance to determine if there is an acute effect.

Design: After all inclusion and exclusion criteria are met, subjects will be admitted to the inpatient unit of the Pennington Biomedical Research Center. After meeting inclusion and exclusion criteria, they will be randomized by the study statistician to receive a specific sequence of 3 treatments (3 doses of extract). Participants will be randomly assigned to

treatment sequences as this design balances order of treatment consumption with respect to the distribution of extraneous factors, known or unknown, that may potentially confound the association and assessment of botanical supplement use and carbohydrate metabolism. After baseline evaluation, subjects within each cohort will be randomized to one of 3 treatment sequences (order of receiving 2 doses of 5011-Nutrasorb and placebo based on the dosing plan outlined in **Table 1**. The statistician will prepare and give to the pharmacist a randomization schedule that is divided into 3 parts, one for each cohort. Nine treatment sequences will be indicated for each cohort in 3 permuted blocks of 3 treatment sequences where each treatment sequence appears exactly once in permuted block. This balances the use of treatment sequences throughout the duration of participants in the event that anticipated dose usage requires adjusting before completion of all treatment sequence assignments.

Figure 1: Single Ascending Dose Study

Day of Study	Scr	-3	1	1	1	1	1	1	1	1	1	1	2	2
Hour of OGTT			-2	-1.5	-1	-0.5	0	1	2	4	10	12	22	24
Randomization			•											
Dosing	taste		•											
Vital Signs	•	•	•					•		•		•		•
Body Weight	•													•
Complete Blood Chemistry	•													•
Physical Exam	•													•
EKG	•									•				
OGTT[a]		•					•							
Adverse Event Assessment							•	•		•				•
Fasting Lipds	•													•
Insulin/Glucose[b]	•											•		•
Bioactives:Plasma			•	•	•	•	•	•	•	•	•		•	

Figure 1: Proposed Study Design Assessing Ascending Single Dose for each period in Table 1
[a] OGTT will be conducted as described in Section 6.2 and will include assessment of insulin and glucose at -15 and 0 timepoints prior to ingestion of oral glucose load and at 30, 60, 90, 120 and 180 minutes post glucose load.
[b] Indicates insulin and glucose assessments conducted in addition to assessments as part of the OGTT

Parameters assessed as part of SAD study will consist of assessing safety with biochemical measures, and effect on insulin action (with use of HOMA-IR), and use of insulin response to glucose as assessed during oral glucose tolerance testing (OGTT) and with HOMA-□. The OGTT will be obtained at two time points, specifically at Day -3 (prior to randomization) and at Day 1. The protocol for OGTT in regard to timing of dosing of study intervention and for insulin/glucose assessments are summarized below;

1) **Day -3**: OGTT will be conducted according to the specific method as outlined in Section 6.2, entitled "Glucose Tolerance". Specifically, a 75–g oral glucose tolerance test will be used to assess for glucose tolerance and insulin secretory function according to the guidelines of the American Diabetes Association. Time points for assessment of insulin glucose will be at -15, 0, 30, 60, 90, 120, and 180 minutes.
2) **Day 1:** The subject will be given either the placebo or specified dose of the study intervention two hours prior to the OGTT. This time point on the figure is termed "-2 hours" and specified as "Hour of OGTT".

3) **Day 1:** At 1 hour and 45 minutes post study intervention dosing on Day 1 (corresponding to time point -15 minutes of the OGTT) and at two hours post study intervention dosing (corresponding to 0 time point of the OGTT), insulin and glucose levels will be obtained in the fasting state and will represent baseline labs for the OGTT. After obtaining the 0 time point baseline labs of the OGTT, 75 grams of oral glucose will be ingested. Repeat insulin and glucose levels will be obtained at 30, 60, 90, 120 and 180 minutes after glucose ingestion.
4) **Day 1:** To evaluate effect of extract dosing on insulin/glucose over time, repeat insulin and glucose level will be evaluated at 12 hours after the start of the OGTT. These levels are outlined in Figure 1
5) **Day 2:** To evaluate effect of extract dosing on insulin/glucose over time, repeat insulin and glucose levels will be evaluated at 24 hours after the start of the OGTT that was conducted on Day 1. These levels are outlined in Figure 1.

Fasting lipids will be obtained 24 hours after dosing. **Figure 1** outlines the schedule plan for metabolic testing in our single dose studies. We do not feel we need to alter any testing based on hormone/menstrual cycle issues that need to be timed, monitored, or controlled for with respect to the study outcomes.

Assessing Botanical Levels/Compliance: Levels of the bioactive components will be assessed by determining concentrations of the component in blood plasma (circulating levels) and urine (excretion) on inpatient days. Subjects will be given their randomized intervention (5011-Nutrasorb or placebo) at the -2.0 hour of OGTT (considered as 0 Hour of Bioactives) (see Table 2). Plasma will be obtained at the -2.0 hour of OGTT (i.e. 0 hours of Bioactive) then at -1.5, -1.0, -0.5, 0, 1, and 2 hour of OGTT. These time points labeled as "Hour of OGTT correspond to the following time points for bioactive assessment [Labeled as "Hour of Bioactives"] as outlined in the Table 2 which occur as follows: 0, 0.5, 1, 1.5, 2, 3, and 4 hours post ingestion (Hour of Bioactive). Blood for bioactives will also be obtained at 6, 12 and 24 hours (hours of Bioactives) after extract or placebo ingestion corresponding to 4, 10 and 22 hours post OGTT ("Hour of OGTT") (see Table 2). After initial void, urine will be collected over the 6 hour interval (corresponding to 0 through hour 6 of "hour of bioatives". These specimens will be assessed for bioactives in the Botanical Core lab.

Table 2: Correlation of Blood Draws for OGTT in relation to Bioactive Assessment Blood Draws

Day of Study	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2
Hour of OGTT	-2	-1.5	-1	-0.5	-0.25	0	0.5	1	1.5	2	3	4	10	12	22	24
OGTT Draws					X	X	X	X	X	X	X			X		X
Hour of Bioactives	0	0.5	1	1.5	1.75	2	2.5	3	3.5	4	5	6	12	14	24	26
Bioactive Draws	X	X	X	X		X		X		X		X	X		X	

B. ***Phase 1 Studies: Multiple Oral Dose Evaluation***

Objective: The **primary objective** is to assess the safety and tolerability of multiple daily doses of the 5011-Nutrasorb complex in healthy subjects that include subjects whose phenotype as assessed by BMI are in the lean, overweight and obese categories. The

secondary objective is to assess the pharmacodynamic effect of multiple daily doses of the extract on glucose tolerance. We will evaluate other proposed mechanisms of action including assessment of insulin secretion with OGTTs, plasma lipid levels and plasma appearance of proposed metabolites.

Table 3. Multiple Oral Dose Randomization

	Period 1	**Period 2**	**Period 3**
Cohort 1			
Sequence 1 (n=3)	D1	Placebo	D2
Sequence 2 (n=3)	D1	D2	Placebo
Sequence 3 (n=3)	Placebo	D1	D2
Cohort 2			
Sequence 4 (n=3)	D2	Placebo	D3
Sequence 5 (n=3)	D2	D3	Placebo
Sequence 6 (n=3)	Placebo	D2	D3
Cohort 3			
Sequence 7 (n=3)	D1	Placebo	D3
Sequence 8 (n=3)	D1	D3	Placebo
Sequence 9 (n=3)	Placebo	D1	D3

Dosing: Twenty Seven subjects (3 cohorts of 9 subjects with 3 subjects (1 normal weight, 1 overweight and 1 obese) randomly assigned to each of 3 treatment sequences) will participate in a three period crossover treatment regimen with each treatment period comprising once daily dosing for 21 days. Subjects will be randomly assigned to receive the treatments in the sequence orders shown in **Table 2**: 1) Cohort 1 will comprise 3 sequences of 3 treatments where placebo is dose 0 of 5011-Nutrasorb, D1 is a low dose (0.3 g) of 5011-Nutrasorb and D2 a medium dose (1 g) of 5011-Nutrasorb; 2) Cohort 2 will comprise 3 sequences of 3 treatments where D2 is a medium dose (1 g) of the 5011-Nutrasorb and D3 (30 g) a high dose of the 5011-Nutrasorb; and 3) Cohort 3 will comprise 3 sequences of 3 treatments where D1 is a low dose (0.3 g) of the 5011- Nutrasorb and D3 a high dose (30 g) of the 5011-Nutrasorb. Dosing for the MAD study was determined by data obtained in the SAD study as outlined above. Specifically, as outlined in Table 1, we will have data on PK studies, safety and tolerability, and biological response. In addition, based on information from the plasma appearance data and from assessment of biological response, consideration will be given to providing dosing on a BID or TID regimen. All doses used in this phase have been tested in the single-dose portion of the protocol. In the single dose protocol, there was no observed signal for toxicity, tolerability or other adverse events noted. The doses evaluated in the single dose study are spread among all 3 cohorts and no new dose is being introduced in this phase. . Blinded analysis of the glucose tolerance will be on-going during the study and dose changes will be made if necessary. Additionally, as stated, if none of these doses show adequate glucose tolerance effects then an additional cohort may be added to investigate a twice daily or three times a day dose. In each treatment period a single dose of the 5011- Nutrasorb will be administered daily

from days 1 to 21. Complete biochemical and hematologic assessments to evaluate safety will be obtained in addition to clinical monitoring for adverse events.

Assessments and Evaluations:

Baseline/Screening Evaluation: All subjects will undergo an outpatient screening evaluation to assess their eligibility prior to entrance into the study. A complete medical history and physical examination will be performed**.** Blood samples will be taken for a complete blood chemistry, blood counts, thyroid hormone panel and urinalysis. Subjects with impaired glucose tolerance or diabetes will be excluded. During baseline, subjects will be assessed for dietary intake data and instructed on weight maintenance diets.

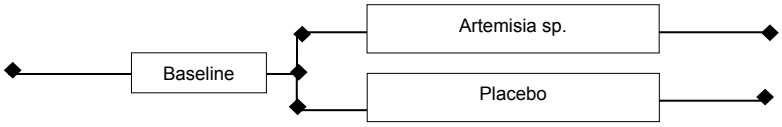
Study Design of Multiple Ascending Dose

Subjects: We will test safety, tolerability and metabolism in individuals with a wide range of BMIs representing lean, overweight and obese subjects.

Design: After meeting inclusion and exclusion criteria, subjects will be randomized by the study statistician to one of the treatment sequences within each subject's appropriate BMI stratum. Participants will be randomly assigned to treatment sequences as this design balances order of treatment consumption with respect to the distribution of extraneous factors, known or unknown, that may potentially confound the association and assessment of botanical supplement use and insulin sensitivity. After baseline evaluation, subjects will have an OGTT, insulin/glucose and effect on insulin action and response on Day -3. They will also have a baseline DXA. There will be three (3) study periods each evaluating exposure through 21 days (see **Figure 2**). At day 1, of each treatment period, subjects will be randomly assigned to treatment sequence based on the dosing scheme proposed in Table 2. Repeat insulin/glucose, safety labs, OGTT and fasting lipid profile will be assessed at 7, 14, and 21 days. Parameters assessed will consist of assessing safety with biochemical measures, bioactive metabolites and effect on insulin action (with use of HOMA-IR) and insulin response to glucose as assessed during OGTT and with use of HOMA-β. Fasting lipids will be obtained at 7, 14 and 21 days of each treatment period. **Figure 2** outlines the schedule plan for metabolic testing in our multiple dose studies. We do not feel we need to alter any testing based on hormone/menstrual cycle issues that need to be timed, monitored, or controlled for with respect to the study outcomes.

Assessments and Evaluations:

Figure 2: Multiple Dose Study



Day of Study	Scr	-3	1	3	7	10	14	17	21
Randomization			•						
Vital Signs/Body Weight	•	•	•	•	•	•	•	•	•
Complete Blood Chemistry	•				•		•		•
Physical Exam/EKG	•				•		•		•
OGTT		•			•		•		•
Plasma Metabolites					•		•		•
Insulin Secretory Assessment		•			•		•		•
Fasting Insulin/Glucose		•			•		•		•
Bioactives: Plasma/Excretion*					•		•		•
DXA		•							•
Adverse Event Assessment		•	•	•	•	•	•	•	•

Figure 2: Proposed Study Design Assessing Multiple Dose Study for each Period. There will be three study periods of assessment (see Table 2). SCR; Screening (only done in the first study period). A one day +/- visit window is allowed.

**NOTE Subjects will be given their intervention (low or high dose 5011-Nutrasorb or placebo) two hours prior to start of OGTT (0 hour) and will have plasma obtained at 0, 30 minutes, 1, 1.5, 2, 3, and 4 hours post ingestion. After initial void, urine will be collected over the 6 hour interval. These specimens will be measured in the Botanical Core lab. Note: Intervention will not be administered at Day -3 of each test period.*

Baseline/Screening Evaluation: All subjects will undergo an outpatient screening evaluation to assess their eligibility prior to entrance into the study. A complete medical history and physical examination will be performed**.** Blood samples will be taken for a complete blood chemistry, blood counts, thyroid hormone panel and urinalysis. Subjects with impaired glucose tolerance or diabetes will be excluded. During baseline, subjects will be assessed for dietary intake data and instructed on weight maintenance diets. A taste (small sip) of the study product will also be administered during screening.

Randomization: After completing initial physiological sequence, each subject will be assigned to a treatment sequence as follows: Nine subjects will be enrolled for each of the 3 cohorts to investigate 3 doses of extract as compared to placebo (Low Dose, Medium Dose and High Dose). These 9 subjects will be enrolled in 3 strata by body weight (BMI 18.5-24.9 kg/m^2, BMI 25-29.9 kg/m^2, and BMI 30-40 kg/m^2). Further, in each cohort, there are 3 possible orders (treatment sequences) in which the extract doses can be administered. In each cohort, it is desirable to have 1 participant from each BMI stratum in each treatment sequence. In each cohort, the first 3 participants enrolled in each BMI stratum will be randomly assigned to one of the 3 treatment sequences for that cohort. The statistician, Dr. Johnson, will use SAS V9.3 to prepare 3 randomization schedules (one for each BMI stratum) for the pharmacist. Each list will have 3 blocks of the 3 randomly permuted treatment sequences. The pharmacist will determine the BMI stratum for each enrollee in turn and dispense the treatments according to the next

available treatment sequence assignment on the randomization schedule for that participant's BMI stratum. Dr. Johnson and the Pennington pharmacist have a long history of collaboration in this type of trial.

The dose to be used in the multiple dose study will be based on our dose ranging Phase I study. As outlined in Section 2B, based on the data collected in the SAD study which will consist of plasma appearance of bioactives, safety and tolerability, and biological response, the dosing for the MAD will be determined. As also stated, consideration will be given to providing the extract in BID or TID dosing if data suggests that approach. The 5011-Nutrasorb used in human studies will be standardized using QA/QC concentration parameters established by the Botanical Core. The randomization will be blinded to the staff performing the physical measures, metabolic testing, performance of assays, entering or calculating data related to the study and study investigators to assure minimal potential sources of bias.

Nutrition Assessment: Subjects' usual dietary intake will be assessed by a research dietitian at screening and monitored throughout the study. The dietary intake analysis will be valuable as a method of evaluating consistency in dietary regimen over the study period. Biochemical profiles will be monitored to assess safety and body weights will be obtained at each study visit.

4. SELECTION AND ENROLLMENT OF PARTICIPANTS

4.1 Inclusion Criteria

Participants must meet all of the inclusion criteria to participate in this study.

SINGLE AND MULTIPLE DOSE COHORT

For **the single and multiple dose studies** to assess safety and tolerability, we will evaluate **healthy subjects**. Deviations from inclusion criteria for these studies are not allowed because they can potentially jeopardize the scientific integrity of the study or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

A subject will be deemed as "healthy" and eligible for inclusion in this study only if all of the following criteria apply:

- Healthy as assessed on a medical evaluation including medical history, physical examination, laboratory tests and cardiac monitoring.
- Males and females between 18 and 65 years of age inclusive at the time of signing the informed consent.
- Women of child-bearing potential must agree to use one of the approved contraception method [(i.e., barrier method, intrauterine and cervical devices, oral contraceptives, hormonal injections (Depo Provera®), condoms with spermicidal gel or foam, contraceptive patch (Ortho Evra), diaphragm, or abstinence)], prior to the start of dosing to sufficiently minimize the risk of pregnancy at that point. Female subjects must agree to use contraception until the follow-up visit.

- BMI within the range 18.5 – 32 kg/m^2 (rounding up or down as appropriate is permitted for calculation), for both males and females for the single dose studies, and a BMI of 18.5 – 40 kg/m^2 for the multiple dose study.
- Capable of giving written informed consent, which includes compliance with the requirements and restrictions listed in the consent form.

4.2 Exclusion Criteria

SINGLE AND MULTIPLE DOSE COHORT

A subject will not be eligible for inclusion in this study if any of the following criteria apply:

- Subjects with a prior history of Type 2 diabetes.
- Women who are pregnant or who are lactating.
- Women of childbearing potential who are not using an effective method of birth control(i.e., barrier method, intrauterine and cervical devices, oral contraceptives, hormonal injections (Depo Provera®), condoms with spermicidal gel or foam, contraceptive patch (Ortho Evra), diaphragm, or abstinence), are not surgically sterilized (including tubal ligation and hysterectomy), or not at least 2 years postmenopausal. All women of childbearing potential will have a pregnancy test performed prior to starting the study treatment in each phase. If a subject becomes pregnant during the study, they will be dropped from the study.
- Subjects who have type 1 diabetes.
- Subjects who are on concomitant therapy with glucocorticoids (except topical or inhalant glucocorticoids). Other medications that have an effect on glucose homeostasis (i.e. ACE inhibitors) are acceptable if they have been administered in a stable dosage during the preceding 6 months and dosage will continue unchanged during the study.
- Subjects with a history or evidence of significant gastrointestinal dysfunction, e.g. irritable bowel syndrome; inflammatory bowel disease; ulcerative colitis or Crohn's disease; regional enteritis; diverticulosis or diverticulitis; significant gastroparesis; GI stricture, partial or complete gastrectomy or small bowel resection; autonomic neuropathy consisting of dysphasia; delayed gastric emptying or diarrhea; chronic, severe constipation; peptic ulceration, colonic ulceration, or GI bleeding.
- Subjects who are taking concomitant therapy with medications known to be nephrotoxic, such as aminoglycosides, methicillin, and cyclosporin.
- Subjects who have evidence of clinically significant renal dysfunction or disease, e.g. serum creatinine >1.5 mg/dL in males and >1.4 mg/dL in females and/or BUN >50
- mg/dL, proteinuria of >1 gram/day or 4+ proteinuria on dipstick urinalysis.
- Subjects with clinically significant cardiovascular dysfunction and/or history (within the preceding 6 months) of significant cardiovascular dysfunction, e.g., congestive heart failure or serious arrhythmia, myocardial infarction, cardiac surgery; transient ischemic attacks or cerebrovascular accident during the preceding six months; diagnosis of symptomatic autonomic neuropathy with a history of orthostatic hypertension, syncope,

or hypertension with a systolic blood pressure of ≥180 mm Hg and diastolic blood pressure ≥110 mm Hg at the time of screening visit.

- Subjects with EKG findings deemed by the medical investigator as "clinically significant" will be excluded. These findings include: ST changes suggesting ischemia, myocarditis, infarction, bundle branch blocks, PVCs, ventricular arrhythmias, or ventricular paced beats from pacemaker.
- Subjects who have evidence within the preceding 6 months of hepatic disease or dysfunction, e.g. AST, ALT, alkaline phosphatase or total bilirubin twice the upper limit of normal; hepatitis; jaundice; cirrhosis.
- Subjects with clinically significant pulmonary, neurologic, hematologic, immunologic, neoplastic or metabolic disease.
- Subjects with evidence or recurrence of malignancy within the past five years, other than excised basal cell carcinoma.
- Subjects for whom surgery is anticipated during the study period.
- Subjects with a history of substance abuse or alcoholism within the past 5 years, or significant psychiatric disorder that would interfere with the subject's ability to complete the study.
- Subjects who have donated blood during the month prior to study entry or planned during the study.
- Subjects who have participated in other studies using an investigational drug during the preceding 3 months.
- Subjects who are current smokers or have smoked within the previous 6 months. No smoking will be allowed during the study.
- Subjects that have had a fluctuation in body weight >5% in the preceding 2 months.
- Subjects who are taking prescription or over the counter medication or supplements for desired weight loss.
- Subjects who have an allergy to soy

4.3 Study Enrollment Procedures

- The goal is to enroll and complete all subjects in a 9 month period. This will require enrolling 9 subjects per month.
- Potential participants will be identified through the Pennington Biomedical Research Center's participant database (over 70,000 people are in the database). The database can be queried for specific inclusion and exclusion criteria after receiving approval from the Pennington Biomedical Research Center's privacy board. Pennington Biomedical has an exceptional track record for recruiting healthy individuals into early phase trials.
- Enrollment rates will be monitored by the study coordinator, investigator and recruiting department in order to ensure goals are met.
- Alternate methods of recruitment will be implemented as needed. Examples include community events, radio advertisements, television advertisements, newspaper articles and/or advertisements.

- Screening logs will be kept and all eligibility criteria reviewed by both the study coordinator and the investigator.
- Informed consent will be obtained according to Pennington Biomedical Research Center's Standard Operating Procedures.

Randomization procedure
Each subject in the cohorts will be randomly assigned one of the relevant treatment sequences in order of enrollment. Treatment sequence assignments will be balanced so that each sequential block will have unique treatment sequences. This randomization will be done by the study statistician. As an example, for the multiple dose study, the first 3 enrolled subjects in each cohort will be randomly assigned to one of 3 distinct treatment sequences, as will enrollees 4-6 and 7-9. As a result, 3 of the 9 subjects in each cohort will be randomly assigned to each of the 3 treatment sequences.

5. STUDY INTERVENTIONS

5.1 Interventions, Administration, and Duration

The product for the clinical studies will be obtained from the Botanical Core Lab of the Rutgers Component of the Botanical Research Center. Working in collaboration with Dr. John Finley at the Louisiana State University (LSU) Food Science department, the final product will be delivered to the Pennington Biomedical Research Center where the clinical study will be performed. Specifically, Artemisia dracunculus herb was grown from seed by ARC Greenhouses. This crop of *Artemisia dracunculus* was grown in the Florida location of ARC Greenhouses. Only insecticidal soap approved for food applications was used as a pesticide. The herb was harvested and shipped to Shiloh, NJ in a cold container (4 ˚C, together with perishable fresh cut flowers) and flash frozen upon arrival. The frozen herb will be shipped frozen (-20 ˚C, together with frozen food) to LSU for processing.

The following steps for clinical product use are outlined as follows:

1. **5011-Nutrasorb preparation**
   a) Frozen *Artemisia dracunculus* is extracted in DI water 1:5 (w/v).
   b) The water is heated to near boiling for 2 hrs (slow roll) and cooled overnight. Solids are removed by filtration using MiraCloth.
   c) The Artemisia "tea" is mixed with soy protein isolate (SPI) at a ratio of 30g of SPI/1Liter of water.
   d) The pH of the mixture is adjusted to 3.5 as a slurry using HCl.
   e) The SPI is separated from the mixture by centrifugation (removing supernatant from NS) or using centrifugal filtration.
   f) The PMI-5011 Nutrasorb is lyophilized to dryness.
   g) The final product will be administered as a powder and will be mixed with water immediately before planned ingestion.

2. **Scale-up Procedure**
   a) Scale-up will be conducted in batch wise operations.
   b) Frozen *Artemisia dracunculus* tissue will be prepared in 20 kg batches. 20 kg of plant issue will be suspended in 100 kg of water in a steam jacketed kettle equipped with mechanical stirring. The suspension will be brought to 100oC for 2 hours and then transferred to another kettle, allowed to cool for several hours and filtered through multiple layers of cheesecloth followed by filtration through a double layer of miracloth. Then 3000g of soy protein will be stirred into the solution. Stirring will be continued for 30 minutes. The suspension will then be adjusted to pH 3.5 with 1M HCl and allowed to settle for one hour.
   c) The bulk of the liquid will be decanted from the surface and the protein rich material will be centrifuged and the precipitate spread in trays and freeze dried.
   d) The microbial count and heavy metal analysis of the substance used for the trial will be performed by a licensed outside laboratory (Medallion Labs - www.medlabs.com/).

Dosing Considerations

For consideration of the initial doses to be used in humans, we are aware that there are two ways that have been used to scale doses from mice to man or vice versa.[17, 18] The first one is based on body surface area and the second is the Kleiber formula of mass to the 0.75 power. For this study, we will use the Kleiber formula as we have had experience with this equation in the past (http://home.fuse.net/clymer/minor/allometry.html).

For our animal studies, successful effects were seen with PMI-5011 when used at a dose of 1%. Essentially, based on our calculations, mice were consuming approx. 4 grams of food per day. Given that the mice weigh about 40 g, this dose is approximately 1g/kg.

Based on data from the Botanical Research Center Core lab, the extract generally contains about 0.2% DMC-2 which is used as the reference standard. Therefore, based on the consumption of food containing 1% extract, the mice will be getting a daily dose of DMC-2 of 0.002 X 1000mg/kg = 2 mg/kg. We have done gavage studies at 500 mg/kg doses which translates to a dose of DMC-2 of 1mg/kg. The amount of DMC-2 in the 5011-Nutrasorb is 0.1-0.2%. Based on these calculations, we would estimate that current dosing for humans would be 10 grams. However, we would like to evaluate low, middle and high dose for safety, tolerability, adverse effects and tolerability, our suggested dosing would be 0.3, 1, 3, 10, 20, and 30 grams for the SAD studies. Dosing for the MAD studies will be based on results for the most effective dose and side effect profile from the SAD study. As outlined in other sections of the proposal, dosing for the MAD studies will be determined based on PK analysis in SAD study in addition to assessment of adverse events and biologic response. Based on these parameters, we will have comprehensive data to suggest dosing for the MAD studies and frequency of dosing as to once daily, twice daily or three times daily.

5.2 Handling of Study Interventions

The product for the clinical studies will be obtained from the Botanical Core Lab of the Rutgers Component of the Botanical Research Center. Working in collaboration with Dr. John Finley at

the Louisiana State University Food Science department, the final product will be delivered to the Pennington Biomedical Research Center where the clinical study will be performed.

The placebo will be the soy protein that has not been co-dried with the *Artemisia dracunculus* tea and will be modified using common food handling procedures (such as toasting or adding food colorant) so that it will have a similar appearance to the test material.

5.3 Concomitant Interventions

5.3.1 Allowed Interventions

As individuals will not be diabetic or have severe hyperglycemia, there is no suggested rescue therapy for glucose. Subjects will be allowed to be on medications such as ACE inhibitors, statins and other blood pressure medications if they have been administered in a stable dosage during the preceding 6 months and dosage will continue unchanged during the study.

Over the counter medications taken as PRN such as Tylenol, antacids, aspirin are acceptable if not taken more than twice weekly. The use of stool softeners is acceptable. Use of bulking agents, if required, should remain constant.

5.3.2 Required Interventions

The only required intervention will be the study medication, soy-protein isolate Russian tarragon (*Artemisia dracunculus* L.) complex (5011-Nutrasorb).

5.3.3 Prohibited Interventions

Subjects who are on concomitant therapy with glucocorticoids (except topical or inhalant glucocorticoids) will not be included. Subjects who are currently on thiazolidinediones (rosiglitazone or pioglitazone) or who have taken these agents in the previous 12 weeks or on medications known to affect glucose metabolism, i.e. metformin, will not be allowed. Subjects who have chronic use of laxatives or cathartics will not be enrolled.

5.4 Adherence Assessment

Assessing Botanical Levels/Compliance: Levels of the bioactive components will be assessed by determining concentrations of the component in blood plasma (circulating levels) and urine (excretion) on inpatient clamp days. Subjects will be given their randomized intervention (5011-Nutrasorb or placebo) two hours prior to start of OGTT (0 hour) and will have plasma obtained at 0, 30 minutes, 1, 2, 3, and 4 hours post ingestion. For the SAD study, additional plasma will be obtained at 12 and 24 hours post ingestion. After initial void, urine will be collected over a 6 hour interval. These specimens will be measured in the Botanical Core lab.

6. STUDY PROCEDURES

See **Figures 1 and 2** for this information

6.1 Schedule of Evaluations (SEE FIGURE 1, page 13 and FIGURE 2, page 16)

6.2 Description of Evaluations

Specific Methods:

Glucose Tolerance: A 75–g oral glucose tolerance test will be used for screening and to assess for glucose tolerance and insulin secretory function in human studies according to the guidelines of the American Diabetes Association. Time points for assessment will include -15, 0, 30, 60, 90, 120, and 180 minutes.

Insulin Resistance and Insulin Secretion:

Calculations to assess insulin resistance and insulin secretion:

Insulin sensitivity: Insulin sensitivity has been identified as a primary clinical parameter for which PMI-5011 has an effect. We will estimate insulin sensitivity by the homeostasis assessment model of insulin resistance (HOMA-IR). HOMA-IR will calculated by the formula HOMA-IR = fasting insulin (μU/ml) X fasting glucose (mmol/l) / 22.5.

Insulin secretion: Insulin secretion will be estimated by the homeostasis assessment model of β-cell function and the insulinogenic index. HOMA β-cell will be calculated by the formula HOMA β-cell = [20 X fasting insulin (μU/ml)] / [fasting glucose (mmol/l)]. The insulinogenic index (a measure of insulin secretory capacity) will be defined as the ratio of the increment of insulin to the increment of glucose during the first 30 minutes after an oral glucose load is administered.

Fasting Lipid Profile: Subjects will be evaluated with fasting lipid profile to assess potential changes in lipid parameters including triglycerides, LDL and HDL levels.

Body Composition: Fat-free mass, fat mass and body fat % will be measured by dual-energy X-ray absorptiometry (DEXA).

Plasma Bioactives: Selective ion monitoring using the molecular masses for the bioactive compounds will be used to determine the relative abundance in the plasma and urine derived from the consumption of the extract. Amounts of bioactives will be calculated from their respective peak areas and normalized with the use of internal standards added before the chromatography.

Proposed Bioactives to be assessed: From a series of studies conducted by our group, 5 bioactive compounds were identified from PMI-5011 using *in vitro* activity guided fractionation.[23] As with all botanical preparations, the identification of bioactive compounds does not preclude the presence of additional bioactive compounds that have not been isolated. DMC-2 was identified as the most universally active compound since it was active in all *in vitro* assays and is considered the marker compound for the extract. DMC-2 was also chemically synthesized and validated as active *in vivo*, using an acute hypoglycemic assay in mice.[23] The bioactive compounds identified from the extract were also identified from the plasma of animals treated with PMI-5011 as shown in **Figure 3** below published by Ribnicky.[23] The identification of the compounds requires hydrolysis treatment of the plasma using β glucuronidase in order to detect them, suggesting that they are present in the plasma as glucuronide conjugates, a common

metabolic form of many botanical compounds and drugs. The compounds were present in the plasma over a 6 h period. The compounds could never be detected at 24 h (data not shown).

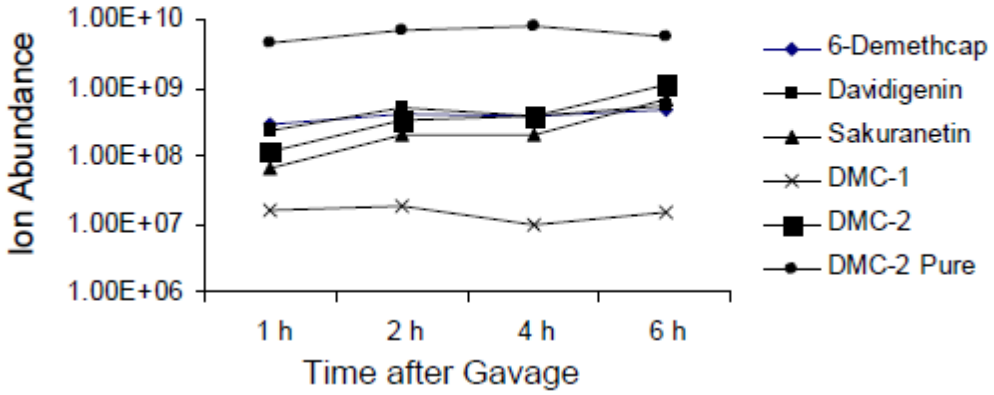


Figure 3. Relative abundance of the active compounds of PMI-5011 as measured by LC-MS in the blood plasma of C57 mice treated with Pure DMC-2 (2′, 4′–dihydroxy-4-methoxydihydrochalcone) formulated with 66% Labrasol or PMI-501l formulated with 66% Labrasol over a 6 h period. DMC-2, Demethcap (6-demethoxycapillarison), DMC-1 (2′, 4–dihydroxy-4′-methoxydihydrochalcone) and sakuranetin are components of PMI-5011.

In a recent study that is currently being submitted for publication by Ribnicky et al., the glucuronide conjugate of DMC-2 was identified in unhydrolyzed plasma from mice treated with the pure DMC-2. This prepublication is based on studies using 5011-Nutrasorb, which is the preparation of the Artemisia that will be used in present clinical study. The results of the studies showed that Nutrasorb-5011 has improved bioaccessibility characteristics using the TIM-1 digestive unit, improved bioavailability using hydrolyzed plasma levels of DMC-2 and improved bioactivity based on glucose measurements in mice. DMC-2 was also synthesized in gram quantities for use as a chemical standard in current and future studies.

In the present clinical study, bioavailability and pharmacokinetic data will be collected from plasma samples obtained from subjects (see treatment schedule) using the protocols described above. DMC-2 will be specifically quantified by LC-MS from hydrolyzed plasma samples based on a standard curve for DMC-2. An internal standard (to be determined) will be used to adjust for recovery. The other active compounds (see figure above) will also be analyzed by LC-MS relative to the DMC-2 because no chemical standards are available for the other compounds. Analysis of the unhydrolyzed plasma will be performed, as described for the mice treated with pure DMC-2, in order to determine if the DMC-2 in the plasma of clinical subjects is also glucuronidated. The plasma conjugate, however, may not be detectable from treatments with the PMI-5011 preparations having low levels of DMC-2 as it is not detectable in mice. Since the compounds were detectable in plasma hydrolyzed with glucuronidase in the previous clinical study, they are likely to be present in the plasma as glucuronides.

6.2.1 Screening Evaluation

These evaluations will occur to determine if the candidate is eligible for the study.

Consenting Procedure

Before any screening procedure is performed, informed consent will be obtained. There will be a single informed consent form that describes both the screening and study procedures. The study coordinator will conduct the consent process and the consent will be provided to the study subject in advance of the visit to allow time to read and address any questions. The medical investigator or Principal Investigator will also be available if any questions or clarifications are required. A copy of the consent form will be given to the subject and consent form placed in study source documents and medical records.

Screening

All screening evaluations to determine eligibility must be completed and reviewed by the individual providing medical supervision or Principal Investigator prior to study start. Generally, all labs to assess entry criteria must be completed no less than 10 days before subject enters study. Screening evaluations are outlined in **Figure 1**.

6.2.2 Enrollment, Baseline, and/or Randomization

Enrollment and Baseline Assessments

All subjects will undergo an outpatient screening evaluation to assess their eligibility prior to entrance into the study. After informed consent, the following procedures will be conducted:

- A complete medical history and physical examination will be performed**.**
- Blood samples will be taken for a complete blood chemistry, blood counts, thyroid hormone panel and urinalysis.
- Subjects with diabetes will be excluded.
- Subjects will be assessed for dietary intake data and instructed on weight maintenance diets.

Baseline Assessments

See above combined Enrollment and Baseline Assessments section

Randomization

After completing initial physiological sequence, each subject will be randomized by Dr. Johnson (study biostatistician). Subjects will be randomized to the 5011-Nutrasorb or placebo. The 5011-Nutrasorb used in human studies will be standardized using QA/QC concentration parameters established by the Botanical Core.

6.2.3 Blinding/Unblinding

Dr. William Johnson, Botanical Research Center biostatistician, will be responsible for all blinding procedures and for randomization. Randomization will be blinded to the staff performing the physical measures, metabolic testing, performance of assays, entering or calculating data related to the study and study investigators to assure minimal potential sources of bias. Sealed

randomization envelopes will be sent to the pharmacist for emergency unblinding. The randomization envelopes will be maintained in a secure location with access limited to authorized personnel.

Blinding is critical to the integrity of this clinical trial. However, in the event of a medical emergency or pregnancy in an individual subject, in which knowledge of the investigational product is critical to the subject's management, the blind for that subject may be broken by the medical investigator of the trial. This will be done by a request to the pharmacist to break the blind and report the treatment assignment to the medical investigator.

Before breaking the blind of an individual subject's treatment, the investigator should have determined that the information is necessary, i.e., that it will alter the subjects' immediate management. In many cases, particularly when the emergency is clearly not investigational product-related, the problem may be properly managed by assuming that the subject is receiving active product without the need for unblinding.

In case of an emergency, the investigator may open the emergency unblinding envelope to reveal the identity of the medication for that subject. If such unblinding occurs, the investigator shall notify the DSMB, NCCAM and IRB immediately. This information, including the reason for the blind being broken must be recorded in the participant chart and regulatory files.

6.2.4 Follow-up Visits

Follow-up visits will occur as outlined in **Figures 1 and 2**

6.2.5 Completion/Final Evaluation

Study design for both single and multiple dose studies are listed in detail in **Figures 1 and 2**

7. SAFETY ASSESSMENTS

7.1 Specification of Safety Parameters

The plant has been used as a medicinal botanical or food for centuries and continues to be cultivated in Europe. PMI-5011 has been the primary study focus of our NIH Botanical Research Center since 2005 and a study focus at Rutgers University for at least 10 additional years. Extensive safety testing has been conducted with the botanical extract including complete Ames analysis, a 14-day repeated-dose oral toxicity study in rats and an oral subchronic 90-day toxicity study in rats, none of which showed any indication of toxicity.

7.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

The study team including coordinator and investigators will inquire regarding adverse events while minimizing the chance for bias when detecting AEs and/or SAEs. The study team will employ open-ended and non-leading verbal questioning of the subject as the preferred method to inquire about AE occurrence. For examples, appropriate questions include: "How are you feeling?", "Have you had any (other) medical problems since your last visit/contact?", or "Have

you taken any new medicines, other than those provided in this study, since your last visit/contact?".

Recording of AEs and SAEs
It is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) relative to the event when an AE/SAE occurs. The investigator will then record all relevant information regarding an AE/SAE in the appropriate data collection tool.

We define an **adverse event (AE)** as any unfavorable and unintended diagnosis, symptom, sign (including an abnormal laboratory finding), syndrome or disease which either occurs during the study, having been absent at baseline, or if present at baseline, appears to worsen. Adverse events are to be recorded regardless of their relationship to the study intervention.

We will define a **serious adverse event (SAE)** as any untoward medical occurrence that results in death, is life threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or in a congenital anomaly.

It is the responsibility of the investigator to attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis will be documented as the AE/SAE and not the individual signs/symptoms.

Evaluating AEs and SAEs

Assessment of Intensity
An assessment of intensity for each AE and SAE reported during the study will be provided by the investigator and the investigator will assign it to one of the following categories:

- Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that is sufficiently discomforting to interfere with normal everyday activities.
- Severe: An event that prevents normal everyday activities.

An AE that is assessed as severe will not be confused with an SAE and both AEs and SAEs can be assessed as severe. Severe Adverse Event is a category utilized for rating the intensity of an event when it meets at least one of the pre-defined outcomes as described in the definition of an SAE.

Assessment of Causality
The investigator is obligated to assess the relationship between study treatment and the occurrence of each AE/SAE. A "reasonable possibility" is meant to convey that there are facts/evidence or arguments to suggest a causal relationship, rather than a relationship cannot

be ruled out. The investigator will use clinical judgment to determine the relationship. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the study treatment will be considered and investigated.

7.3 Adverse Events and Serious Adverse Events

An AE is any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. We define AE as any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product.

Events meeting the definition of an AE **include**:

- Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECGs, radiological scans, vital signs measurements), including those that worsen from baseline, and felt to be clinically significant in the medical and scientific judgment of the investigator.
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after study treatment administration even though it may have been present prior to the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication (overdose per se will not be reported as an AE/SAE).

Events that **do not** meet the definition of an AE include:

- Any clinically significant abnormal laboratory findings or other abnormal safety assessments that are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition.
- The disease/disorder being studied, or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject's condition.
- Medical or surgical procedure (e.g., endoscopy, appendectomy); the condition that leads to the procedure is an AE.
- Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

Definition of Serious Adverse Events

If an event is not an AE, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease, etc.).

An SAE is any untoward medical occurrence that, at any dose, results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalization, results in disability/incapacity or a congenital anomaly/birth defect. We define an SAE as one associated with liver injury **and** impaired liver function defined as: ALT □ 3xULN, and total bilirubin □ 2xULN or INR > 1.5.

7.4 Reporting Procedures

Once the investigator determines that an event meets the protocol definition of an SAE, the SAE will be reported to the IRB and Chair of Data Safety Monitoring Committee within 24 hours and notification will be sent to program official at NCCAM/ODS. If the investigator does not have all information regarding an SAE, the investigator will not wait to receive additional information before notifying the responsible parties of the event and completing the appropriate forms. An assessment of causality at the time of the initial report will be provided. Email transmission of the SAE data collection tool will be the preferred method to transmit this information followed by notification by telephone and/or fax. A copy of the SAE report will also be sent to the institution officials and via overnight mail to NCCAM/ODS officials. Initial notification via the telephone does not replace the need for the investigator to complete and sign the SAE data collection tool within the designated reporting time frames.

7.5 Follow-up for Adverse Events

After the initial AE/SAE report is completed and sent, the investigator is required to proactively follow each subject at subsequent visits/contacts. As currently practiced, it is planned that all AEs and SAEs will be followed in clinic until: 1) resolution; 2) the condition stabilizes; 3) the event is otherwise explained; or 4) the subject is lost to follow-up.

Additional measurements and/or evaluations as may be indicated will be conducted by the investigator/research team in order to elucidate as fully as possible the nature and/or causality of the AE or SAE. These evaluations/measurements may include additional laboratory tests or investigations, histopathological examinations, consultation with other health care professionals or radiologic examinations. In the event that a subject dies during participation in the study or during a recognized follow-up period, the investigator will provide the IRB/DSMB with a copy of any post-mortem findings, including histopathology.

New or updated information will be recorded in the originally completed data collection tool. The investigator will submit any updated SAE data within the designated reporting time frames.

7.6 Safety Monitoring

As is required of all protocols conducted at Pennington Biomedical Research Center, Human Subjects research studies will undergo independent monitoring. Additional monitoring by NCCAM and ODS Program Officials will be expected to provide additional specific guidelines to the PI for the study.

8. INTERVENTION DISCONTINUATION

Subject Completion: A completed subject is one who has completed all phases of the study including the follow-up visit. The end of the study is defined as the last subject's last visit.

Subject Withdrawal Criteria: A subject may withdraw from study treatment at any time at his/her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioral or administrative reasons.

Subject Withdrawal Procedures

Subject Withdrawal from Study: Every effort will be made to follow-up subjects who withdraw or are withdrawn from the study prematurely.

- If a subject withdraws or is withdrawn from the study at a scheduled visit after dosing, complete assessments for the scheduled visit and any other assessments specified for the follow-up visit that are not included in the regularly scheduled visit, where possible.
- If a subject withdraws or is withdrawn from the study between visits after randomization, complete assessments for follow-up visit, where possible.
- At a minimum, the following assessments should be performed for any subject who is withdrawn from the study; clinical safety labs and AE assessment.

Subjects who terminate their participation due to adverse events should be followed up as appropriate in order to determine the final outcome.

Subject Withdrawal from Study Treatment: Subjects may withdraw from the study at any time and for any reason. They are not obliged to state the reason for withdrawal. However, the reasons for withdrawal, or failure to provide a reason, must be documented by the physician on the case report form.

Treatment After the End of the Study: Subjects will not receive any additional treatment after completion of the study because only healthy volunteers are eligible for study participation for the safety and tolerability studies and will not be on glucose lowering medication if they have obesity or insulin resistance.

Screen and Baseline Failures: Data for screen and baseline failures will be collected in source documentation at the site.

9. STATISTICAL CONSIDERATIONS

9.1 General Design Issues

The trials described in this protocol are the first stage of testing *Artemisia* sp. extract in humans. The studies will be conducted in (1) an ascending single dose evaluation of doses 0.3, 1, 3, 10, 20, and 30 g; and (2) a multiple oral dose evaluation of 3 doses chosen from the first evaluation and denoted doses 1, 2, and 3 as described in Section 3, Study Design. The single dose evaluation will be conducted by studying 6 doses (amounts) of extract and a placebo in a series of 3-treatment, 3-period crossover trials. The 3 treatments (placebo or one of two doses of extract) will be given at weekly intervals each in a single dose followed by a 7 day washout period. Each crossover trial will consist of 3 sequences of treatments across the weekly intervals [each sequence being a different order for receiving a triad of 2 doses of extract and a placebo (dose 1, dose 2, placebo; dose 3, dose 4, placebo; dose 5, dose 6, placebo). The placebo can be taken in period 1, period 2 or period 3 and the extract is taken in the other two periods where the lower dose of extract is always taken in a period that precedes that of the higher dose]. The primary objective is to assess the safety and tolerability of ascending amounts of the extract for each amount given in a single dose. If no adverse effects are observed with a given pair of extract doses, the dose escalates (e.g. dose 1, dose 2, placebo escalates to dose 3, dose 4, placebo which escalates to dose 5, dose 6, placebo) and a new cohort of subjects is studied in a new crossover trial with each escalation.

The multiple dose evaluation will be conducted similarly by studying 3 doses (amounts) of extract and a placebo in a series of 3-treatment, 3-period crossover trials. The 3 treatments (placebo or one of two doses of extract) will each be given once daily across a 21 day period followed by a 7 day washout period. Each crossover trial will consist of 3 sequences of treatment (each sequence being a different order for receiving 2 doses of extract and a placebo where the lower dose of extract always precedes the higher dose). The primary purpose is to further investigate safety, tolerability and bioavailability of once daily consumption of the extract over a 7 day period.

9.2 Sample Size and Randomization

As comparative efficacy of the extracts is not the primary issue in this protocol, power to detect efficacious effects is not specifically evaluated to justify sample sizes. A sample of 27 subjects (3 subjects in each of 3 treatment sequences in each of 3 cohorts) will be enrolled in the ascending single dose evaluation. Thus, all 9 subjects in Cohort 1 will receive the placebo and both dose 1 and dose 2 of the extract; similarly all 9 subjects in Cohort 2 will receive the placebo and both dose 3 and dose 4 of the extract; and all 9 subjects in Cohort 3 will receive the placebo and both dose 5 and dose 6 of the extract. Before the next cohort is commenced, subjects in the preceding cohort must have received all 3 treatments and the safety and tolerability data must have been assessed showing good tolerability; and information must be available in a safety summary of adverse events, complete hematologic and biochemistry profile in blood, and measures of glucose and insulin response to glucose tolerance.

The 9 subjects in each cohort for the SAD study will be enrolled with 3 subjects in each of 3 strata (normal weight – BMI =18.5-24.9 kg/m2; overweight -- BMI = 25.0-29.9 kg/m2; obese – BMI = 30.0-32 kg/m2). The randomization will be carried out within each weight stratum so that one normal weight, one overweight, and one obese subject will be assigned to each sequence.

A sample of 27 subjects (3 subjects in each of 3 treatment sequences in each of 3 cohorts) will be enrolled in the multiple oral dose evaluation. Thus, all 9 subjects in Cohort 1 will be receive the placebo and both dose 1 and dose 2 of the extract; similarly, all 9 subjects in Cohort 2 will receive the placebo and both dose 2 and dose 3 of the extract; and all 9 of the subjects in Cohort 3 will receive both dose 1 and dose 3 of the extract. Randomization for the multiple dose evaluation will be carried out within each weight stratum so that each cohort once completed will have one normal weight, one overweight, and one obese subject will be assigned to each sequence. Specifically, the 9 subjects in each cohort will be enrolled with 3 subjects in each of 3 strata (normal weight – BMI =18.5-24.9 kg/m2; overweight -- BMI = 25.0-29.9 kg/m2; obese – BMI = 30.0-40.0 kg/m2). The randomization will be carried out within each weight stratum so that one normal weight, one overweight, and one obese subject will be assigned to each sequence

Both randomizations will be performed using computer generated pseudo-random number generators. Because each subject receives each treatment in both studies, among treatment comparisons will be made using within-subject data. Thus, the main purpose of stratifying treatment assignment by weight category is to ensure good representation of subjects with diverse weights in the evaluation of safety and tolerability.

9.3 Definition of Populations

For **the single and multiple dose studies** to assess safety and tolerability, we will evaluate **healthy subjects**. Deviations from inclusion criteria for these studies are not allowed because they can potentially jeopardize the scientific integrity of the study or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

A subject will be deemed as “healthy” and eligible for inclusion in this study only if all of the following criteria apply:

- Healthy as assessed on a medical evaluation including medical history, physical examination, laboratory tests and cardiac monitoring.
- Males and females between 18 and 65 years of age inclusive at the time of signing the informed consent.
- Women of child-bearing potential must agree to use one of the approved contraception method [(i.e., barrier method, intrauterine and cervical devices, oral contraceptives,
- hormonal injections (Depo Provera®), condoms with spermicidal gel or foam, contraceptive patch (Ortho Evra), diaphragm, or abstinence)], prior to the start of dosing
- to sufficiently minimize the risk of pregnancy at that point. Female subjects must agree
- to use contraception until the follow-up visit.

- BMI within the range 18.5 – 32 kg/m^2 (rounding up or down as appropriate is permitted for calculation), for both males and females for the single dose studies, and a BMI of
- 18.5 – 40.0 kg/m^2 for the multiple dose study.

*Capable of giving written informed consent, which includes compliance with the requirements and restrictions listed in the consent. (*See Section 4, page 16-18 for exclusions).

9.4 Interim Analyses and Stopping Rules

No Interim analysis is planned.

Stopping Rules
Dosing within a cohort will be stopped and further dosing will be halted until unblinded safety information can be reviewed in the event that:

- A death occurs
- Two or more subjects experience the same SAE following administration of study drug.
- Two or more subjects in one dose panel experience the same severe drug-related AE following administration of study drug
- Based on AEs, laboratory findings, clinical findings or ECGs the Investigator or DSMB determine that review of pertinent safety information is required.

Dosing may only resume if, after review of safety information, both the Investigator, DSMB and IRB agree that it is safe to proceed.

9.5 Outcomes

9.5.1 Primary Outcome

The primary outcome will be to determine the effect of the selected 5011-Nutrasorb complex on safety and tolerability based on clinical and biochemical assessment.

9.5.2 Secondary Outcomes

The secondary outcomes will consist of assessing possible mechanisms of action with use of OGTT to assess insulin action and insulin secretory effects. Plasma appearance of metabolites will be obtained to assess metabolism.

9.6 Data Analyses

Statistical comparisons will focus on comparisons between subjects randomized to the selected *Artemisia* sp. extract and the placebo group with respect to baseline to follow-up change in: (a) a descriptive summary of adverse events, complete hematologic and biochemistry profile in blood, and measures of glucose and insulin response to glucose tolerance will be compiled for both single and multiple dose studies; (b) measurements assessing lipid metabolism, i.e. fasting lipid profile; (c) physiologic assessments of insulin action (HOMA-IR) and insulin secretion; and (d) body weight/body composition. For each outcome, the equality of the outcome means for 5011-Nutrasorb versus placebo will be tested against a two-directional alternative, controlling the type 1 error at $\alpha = 0.05$. The analyses will be conducted using mixed effects linear statistical

models with repeated measures to conduct analysis of variance for crossover designs. Post-hoc tests will be conducted for pairwise comparisons of extract doses and placebo employing the Tukey method for controlling the global comparison-wise significance at 0.05 level. Logarithms or other transformations will be analyzed for data seriously deviating from normality; alternatively, non-parametric analyses may be conducted.
Handling of missing data; The data will be analyzed using the procedures PROC MIXED and PROC GLIMMIX SAS (v 9.3 or later). Thus, mixed effects models for repeated measures will be employed to analyze all available data irrespective of missing or incomplete data attributable to dropouts or other phenomena. Our experience in previous studies that employed similar study designs resulted in little if any missing data. Nevertheless, in anticipation of the possibility of such we plan to conduct sensitivity analyses that compare our findings against protocol completer analyses and last observation carried forward analyses in addition to investigating characteristics of participants who fail to protocol.

10. DATA COLLECTION AND QUALITY ASSURANCE

10.1 Data Collection Forms

Data will be collected on case report forms at baseline/screening, inpatient visit and outpatient visits by trained research staff. Randomization will be blinded to the research staff performing the physical measures, metabolic testing, performance of assays, entering or calculating data related to the study and study investigators to assure minimal potential sources of bias.

Data collection forms will be created and developed. Specifically, source documents will be tailored to each clinical assessment data output for data entry or data upload including: history, physical, lab results, MRS scanning, clamp results, dietary intake.

10.2 Data Management

All volunteers are assured of their confidentiality both verbally and in the informed consent form. The clinical facilities are strictly limited to the staff of the research institution and to research volunteers. This is accomplished by a variety of stringent security measures. All medical records are stored in locked areas. Access to these areas is limited to the clinical support staff, director of the clinical facilities, and the PI's. Volunteers' medical records are filed according to ID numbers. All forms on the chart, with the exception of consent form, display only the ID number. Electronic data storage is similarly restricted with only the PI's and authorized persons having access to databases containing confidential clinical records, i.e. those containing name OR other identifying information.

All data are confidentially collected from study participants and are only used for research purposes. All records are kept in locked file cabinets, and participant data can be identified only by number. Data are used only in aggregate, and no identifying characteristics of individuals are published or presented.

Each participant will be issued an ID number that will be utilized throughout the study. A secure master file linking names, addresses and ID numbers will be maintained in a confidential

computer file accessible only to the investigators. Access to data files can be made only with permission of the Principal Investigator. Privacy in the context of this study includes confidentiality of data and personal information. During interviews and measurements, the study staff will ensure full privacy of participants and will ensure that the data are stored in a secured area. All study staff must be HIPAA certified. The Pennington Biomedical Research Center's Biostatistics Core will cooperate with the data manager who will manage the data entry into the clinical database to ensure quality of data. The Pennington Biomedical Research Center's Data Management Core will have the prime responsibility for database design and implementation, programming, coordination and manual data entry of the study data into the study database.

Data for common endpoints will be entered directly into the research computing (RCU) data management system per the RCU's procedures. All data that are Pennington Biomedical Research Center specific will be housed in the Pennington Biomedical Research Center Clinical Database. Data from most instruments are directly uploaded into the Clinical Database. Data that must be hand entered is entered twice via an established data entry system. Prior to analysis, all data will be evaluated for outlying and extreme values. All data manually entered must be entered by two clerks before it is considered valid for use in research analysis. Data manually entered into the database include anthropometry (height, weight, BMI, circumferences), vital signs and adverse events. Data to be manually entered is collected on standard case report forms that match the flow of the computerized data entry screens. These standardized case report forms are created by the software development team and provided to the clinical research staff. Manual double data entry applications are created to include data verification checks, numeric value range checks and computerized record comparisons between first and second entry. The data entry applications will not allow a data record to be first and second entered by the same data entry clerk. The data entry clerk's logon id is stored in the database with the data record and flagged as either first entry or second entry. Only records that are flagged as second entry and verified are considered valid for research use. Once a record is verified this data becomes view only to authorized personnel using the customized Pennington Biomedical data entry/viewing applications. Data entry clerks must be trained and certified by a member of the software development team before they are granted the necessary computer network rights needed to access the data entry applications.

10.3 Quality Assurance

10.3.1 Training

The study personnel and staff working on this study are highly qualified with extensive research backgrounds and experience conducting trials involving human participants, both here at the Pennington Biomedical Research Center, and at previous institutions. Dr. Rood as PI will oversee all aspects of the research project. The Pennington Biomedical Research Center Recruiting Core will oversee recruitment for the trial and clinical aspects of screening and testing. In addition, Dr. Rood will be engaged in day-to-day scientific decisions and problem solving. Dr. William Johnson is the Director of the Pennington Biomedical Research Center's Biostatistics and Data Management Core. He supervises a staff that includes PhD and master's

level biostatisticians and skilled programmers, and data entry personnel. Dr. Johnson will be responsible for statistical analyses relevant to this funding.

Dr. Rood will work closely with the co-investigators and staff to ensure that the study is executed with the utmost scientific integrity. Dr. Rood will also provide oversight of the protocol to ensure adherence.

All staff has completed GCP training by CITI and will be trained before the study starts on protocol interventions and the consent process.

10.3.2 Quality Control Committee

This study will utilize a data and safety monitoring board (DSMB). The DSMB will receive quarterly reports via email. A minimum of 2 meetings each year will be conducted in person or via conference call and additional meetings may be called if necessary. Prior to the start of recruitment the DSMB will give formal approval of the study protocol and informed consent.

- **Size and Composition.** The DSMB will consist of 4-5 members both internal and external to the Pennington Biomedical Research Center. The planned composition is as follows: Biostatistician (1), Pharmacologist (1), Clinician-Scientist/Trialist(1-2), and Layperson (1-2)

  The Major Responsibilities of Members are as follows: 1. Sign and abide by a statement of confidentiality; 2. Disclose any actual or potential conflicts of interest; 3. Be familiar with research protocol and plans for safety monitoring; 4. Oversee safety of participants to include review of adverse events; 5. Review reports of related studies, as appropriate; 6. Review major proposed modifications; and 7. Monitor recruitment and adherence.

- **Reports.** Following each meeting, the DSMB will provide written documentation regarding findings for the study as a whole and any relevant recommendations related to continuing, changing, or terminating the study. All DSMB recommendations will be submitted to the Principal Investigator and/or his designee, with a copy provided to the Pennington Biomedical Research Center IRB and Chair of the Pennington Medical Staff Committee. Reports will be made available to NIH Project Officer or designee.

- **Qualifications and Responsibilities of the Safety Officer.** The Safety Officer for this trial will be familiar with the adverse event definitions and reporting requirements for the study. The Safety Officer will review reports sent by the study coordinator as they occur and will determine whether there is any corrective action or stopping rule violation. The safety officer will send written documentation of the decision to the PI and NIH.

10.3.3 Metrics

All data will be entered directly into a database maintained by the Pennington's Data Management Core. The core will be responsible for quality control of the database and will

generate data queries both at the point of entry and in batch, as well as additional checks for data consistency within or across forms.

10.3.4 Protocol Deviations

All major protocol deviations/violations must be reported by the investigator to the IRB within five (5) working days of learning of the deviation/violation. If it is necessary to make a permanent change to the study procedures in order to avoid harm to other subjects, then a protocol modification will be submitted as soon as possible by the investigator. If appropriate to maintain safety of the subjects, new subject enrollment will be temporarily stopped by the investigator until the modification is approved.

The investigator will report no matter who discovers a major protocol deviation/violation (e.g., sponsor or their agent during a monitoring visit), the investigator is responsible for reporting it to the IRB.

10.3.5 Monitoring

The data safety monitoring board as described will assess adverse events, subject complaints, unanticipated problems, recruiting analysis, interim safety reviews and data collection. The results of the DSMB meeting will be given to the IRB and/or any other institutional committees required to review the report.

11. PARTICIPANT RIGHTS AND CONFIDENTIALITY

11.1 Institutional Review Board (IRB) Review

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the Pennington Biomedical Research Center IRB responsible for oversight of the study. The consent form will be separate from the protocol document.

11.2 Informed Consent Forms

A signed consent form will be obtained from each participant. The population being recruited for this study allows for only subjects who can consent themselves. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A signed copy will be given to each participant and this fact will be documented in the participant's record.

11.3 Participant Confidentiality

All volunteers are assured of their anonymity and confidentiality both verbally and in the informed consent form. The clinical facilities are strictly limited to the staff of the research institution and to research volunteers. This is accomplished by a variety of stringent security measures. All medical records are stored in locked areas. Access to these areas is limited to the clinical support staff, director of the clinical facilities, and the PI's. Volunteers' medical records are filed according to ID numbers. All forms on the chart, with the exception of consent form, display only the ID number. Electronic data storage is similarly restricted with only the PI's and

authorized persons having access to databases containing confidential clinical records, i.e. those containing name, Social Security Number or other identifying information. Data, including body weight and body composition, will be collected from participants. Data are confidentially collected from study participants and are only used for research purposes. All records are kept in locked file cabinets, and participant data can be identified only by number. Data are used only in aggregate, and no identifying characteristics of individuals are published or presented.

11.4 Study Discontinuation

The study may be discontinued at any time by the IRB, the institution, NCCAM/ODS, the OHRP or other government agencies as part of their duties to ensure that research participants are protected.

12. COMMITTEES

The study will employ a Data Safety and Monitoring Committee. The PI will update the monitoring committee at the specified intervals on recruitment, progress to date, adverse events and other details as required to fully evaluate progress and safety of study. The report to the DSMB will also be sent to the Medical Staff Committee of the Pennington Biomedical Research Center at each quarter. The Medical Staff Committee of Pennington consists of all medical staff including physicians, clinical researchers, clinical research staff and IRB official who evaluate research activities of the center including evaluating adverse events and reporting of ongoing studies.

13. PUBLICATION OF RESEARCH FINDINGS

Being an investigator initiated study, there is no Publication committee and results of study will be published by PI and co-investigators.

The study will be registered on ClinicalTrials.gov. Results will be reported on the site following data analysis and completion of study procedures.

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15. SUPPLEMENTS/APPENDICES

Appendix A: IND NOT REQUIRED letter from Department of Health & Human Services, Food and Drug Administration 8/21/12

Appendix B: Targeted/Planned Enrollment Table

Appendix A

DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
Silver Spring, MD 20993

PIND 116198

IND NOT REQUIRED

William T. Cefalu, M.D.
Associate Executive Director of Clinical Research
Pennington Biomedical Research Center
6400 Perkins Road
Baton Rouge, LA 70808

Dear Dr. Cefalu:

This letter responds to your inquiry dated August 12, 2012, concerning the need for an Investigational New Drug Application (IND) for your clinical investigation titled "Evaluation of Soy-protein Russian Tarragon *(Artemisia dracunculus L.)* complex as a dietary supplement to improve carbohydrate metabolism" using Soy-protein and Russian Tarragon complex.

The intent of your study appears to be limited to evaluating the effect of a dietary supplement on the structure or function of the body. Therefore, use of this product in the manner described in the information you submitted does not require an IND.

If you modify the protocol to evaluate the ability of this product to diagnose, cure, mitigate, treat or prevent a disease, or to administer a substance that meets the definition of a drug, you should inquire again about the need for an IND.

We remind you that you should comply with requirements ofyour local Institutional Review Board, obtain patient informed consent and conform to the guidelines on Good Clinical Practice. You can obtain a copy of the "Good Clinical Practice: Consolidated Guidance" by calling 301-796-3400 or online at
www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryinformation/Guidances/UCM073122.pQf.

If you have any questions regarding this action, please contact Mehreen Hai, Ph.D., Acting Chief, Project Management Staff, at 301-796-5073.

Sincerely yours,

{Electronic signature page attached}

Mary H. Parks, M.D.
Director
Division of Metabolism and Endocrinology Products
Office of Drug Evaluation II
Center for Drug Evaluation and Research

Reference ID: 3177443

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

MEHREEN HAI
08/21/2012

Appendix B

Targeted/Planned Enrollment Table

This report format should NOT be used for data collection from study participants.

Study Title: Evaluation of soy-protein isolate Russian tarragon (Artemisia dracunculus L.) complex as a dietary supplement to improve carbohydrate metabolism; Dosing, safety and tolerability

Total Planned Enrollment: 54

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	Sex/Gender		
	Females	Males	Total
Hispanic or Latino			
Not Hispanic or Latino	29	25	
Ethnic Category: Total of All Subjects *			54
Racial Categories			
American Indian/Alaska Native			
Asian	1	1	
Native Hawaiian or Other Pacific Islander			
Black or African American	9	7	
White	19	17	
Racial Categories: Total of All Subjects *	29	25	54

• The "Ethnic Category: Total of All Subjects" must be equal to the "Racial Categories: Total of All Subjects."