Effect of seaweed (Ecklonia cava extract) on Postprandial blood glucose and insulin level on pre-diabetic patients: A double-blind randomized-controlled trial

(SW2020)

Date: 25/09/2020

IRB Approval No:

E-24-4249

PROTOCOL TEMPLATE

Instructions to User:

1. **Sections and text that are in regular font and that have not been highlighted in grey** represent standard language. In general, these sections should be present in your final protocol and the language should not be changed. However, every protocol is unique and changes to standard sections and language may be necessary to meet the needs of your protocol. Please review the language carefully to make sure that it is accurate for your study.
2. **Sections that are highlighted in grey, but that have regular font**, represent sections or information that needs to be customized as applicable to your study, but the language that is present is generally considered to be standard if that section (or procedure) applies to your protocol.
3. **Sections that are highlighted in grey, and where the text is italicized**, represent instructions with some example text. All require complete customization for your study.
4. As you customize each section of the protocol, **remove the highlighting and restore the font to regular (from italics)** to denote that section as having been completed.
5. When your protocol is complete, **review** it to ensure that all highlighting and italics have been removed.

Clinical Research Protocol
SEAWEED STUDY

Protocol Number:	SW2020
Version Date:	25 / 09 / 2020
Investigational Product:	Brown Seaweed (Ecklonia cava)
IND Number:	
Development Phase:	Phase 3
Sponsor:	Name: Malak Almutairi Address: 22 King Abdullah st, Alrayan, Almethnab 56526, Qassim, KSA City, State Almethnab, Qassim, KSA
Funding Organization:	King Saud University
Principal Investigator:	Name: Malak Almutairi Telephone: +966544479135 Fax: E-mail: malak.rog@gmail.com
Medical Monitor:	Name: Telephone: Fax: E-mail:
Coordinating Center:	Clinical trial Unit at King Khalid University Hospital

Approval:

PI or Sponsor Signature (Name and Title) Date

This confidential information about an investigational product is provided for the exclusive use of investigators of this product and is subject to recall at any time. The information in this document may not be disclosed unless federal or state law or regulations require such disclosure. Subject to the foregoing, this information may be disclosed only to those persons involved in the study who have a need to know, with the obligation not to further disseminate this information.

PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study subjects enrolled under my supervision and providing Malak Almutairi the PI with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Number: SW2020

Protocol Title: SEAWEED STUDY

Protocol Date: 25/09/2020

	25 / 09 / 2020
Investigator Signature	Date

Print Name and Title

Site # _____

Site Name _____

Address _____

_____

_____

_____

Phone Number _____

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LIST OF ABBREVIATIONS

AE	adverse event
CFR	Code of Federal Regulations
CRF	case report form
DMC	Data Monitoring Committee
DSMB	Data Safety Monitoring Board
FBG	Fasting Blood Glucose
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICF	informed consent form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
PI	Principal Investigator
PPBG	Postprandial Blood Glucose
PPIL	Postprandial Insulin Level
SAE	serious adverse experience

PROTOCOL SYNOPSIS

TITLE	Effect of sea weed (Ecklonia cava extract) on blood glucose and insulin level on pre-diabetic patients: A double-blind randomized-controlled trial (SEAWEED STUDY)
SPONSOR	Malak Almutairi
FUNDING ORGANIZATION	King Saud University
NUMBER OF SITES	One site
RATIONALE	There is a noticeable interest and enthusiasm toward brown seaweed anti-diabetic effects. Many vivo studies have shown strong inhibition activity of polyphenolic-rich brown seaweed on a-amylase and a-glucosidase enzymes (12-15). For example, Heo et al. have displayed a significant inhibitory activity of a new phlorotannin, diphlorethohydroxycarmalol (DPHC) isolated from brown seaweed called Ishige okamurae on a-glucosidase and a-amylase significantly as well as showed suppression of postprandial blood glucose level in diabetic and normal mice (14). This inhibitory activity on a-amylase and a-glucosidase were similar to an anti-diabetic commercial drug called acarbose (7). similarly, Lee et al, have claimed in their study that the IC50 value for phlorotannin derivatives extracted from brown algae, E. cava, against a-glucosidase were higher than the IC50 value for the drug acarbose (15). In regard to human trials, investigation of polyphenolic-rich brown seaweed effect on glycemic control did not yield consistent evidence, yet (4,5,8,9).. However, desirable effects have been shown in some clinical studies (11, 12, 13). Lee et al, have conducted a randomized clinical trial on 73 male and female participants with fasting blood glucose (FBG) between 100 to 180 mg dL−1 (11). Participants were randomly divided into two groups given 500 mg of AG-dieckol three times a day for 12 weeks. The result shows acute significant reduction in postprandial blood glucose (PPBG) ($p < 0.05$) after 12 weeks intervention. However, non-significant postprandial insulin level (PPIL) reduction was seen in group who consumed AG-dieckol compared to placebo group. Nonetheless, within group reduction was significantly observed in terms of insulin level compared to baseline (11). Moreover, a study has investigated the acute effects of brown algae extracts (Ascophyllum nodosum and Fucus vesiculosus) on postprandial glucose and insulin concentration (12). participants were given 508 mg brown algae extract 30 minutes prior to 110 g carbohydrates consumption. Despite of healthy individuals and different seaweed source, containing only 10% polyphenols, used in this study, acute inulin concentration was significantly decreased ($p < 0.05$) in brown algae groups compared to placebo group (12). Also, non-significant postprandial glucose concentration was found to be decreased in brown algae extract ($p >$

0.05). In contrast, non-significant result was reported in a clinical study examining high dose (2000 mg), low dose (500 mg) of polyphenolic-rich brown algae (Fucus vesiculosus) compared to placebo in healthy people. The study concludes non-significant result in terms of acute PPBG and PPIL (14). As pointed out by Margaret et al, in their systematic review that the source of seaweed extract and its polyphenolic content is very important in triggering antidiabetic effect (15). Thus, they strongly suggest that more clinical studies are required to determine consistent pattern of dose-response and to identify the effectiveness dose in a high-risk population (15). According to side effect, no study has reported any significant differences between brown seaweed and placebo in terms of measuring adverse effect (9-14). Lee et al, have reported similar incidence of adverse event between groups (22.2 vs 16.2) for brown algae group compared to placebo, respectively (11). Similarly, paradis et al, find that 13 subjects in each group have reported adverse effect with minimal intensity (1 in a scale of 0 to 3 where 0 absent and 3 means sever intensity) being the most reported for most of measured symptoms (12). Despite many promising results in vitro and vivo studies (animal studies), the effects of polyphenol-rich brown seaweed on blood glucose and insulin level in human have not been effectively investigated. Thus, more investigational studies are needed particularly on the interaction of brown seaweed and its polyphenols with human cellular systems which may provide better understanding on parameters such as bioavailability, mechanisms of actions of in glucose homeostasis in humans. This can be translated into long-term health benefits. Further research would widen the likelihood of screening more biologically proficient polyphenols and its derivatives. Results of such studies could provide us with resourceful drug substitute to reduce or regulate diet-linked chronic malfunctions such as diabetes. In terms of the type of seaweed intended to be used, Kunihisa, et al have claimed that "this type of brown seaweed (E. cava) polyphenolic-rich extract is an important functional food component that could be used in reducing diabetes related symptoms" (16). Also, Margert et al, on their systematic review investigating the effect of algal polyphenols on antidiabetic, anti-hyperlipidaemia or anti-inflammatory effects in humans, have stated that " E. cava is currently the most extensively investigated source of marine polyphenols for anti-hyperglycaemic effects and has shown the most promise in this area" (8). Indeed, they have concluded that " Marine polyphenols, particularly from E. cava, show potential as natural functional ingredients for the prevention and management of type 2 diabetes and CVD. However further high quality RCTs are required to determine the most effective dose, treatment schedule and macroalgal source to produce consistent effects on glycaemia and dyslipidaemia in humans" (8). Moreover, Previous human studies have mainly performed on healthy individuals using different types of seaweed (4,5). Therefore, this proposal is meant to address the gap in this scientific area, using Ecklonia cave on

	prediabetes patient which hypothesized to have beneficial result regarding blood glucose and insulin level (8). Although, the methodology in this study focuses on the acute effect of E. cava on glycaemic control, it can be a promising result for future studies including long-term studies. Result in this study would give indications for future research regarding type of seaweed, dose and targeted population. More research in this area may benefits us in different aspect including prevention and clinical use of seaweed extract, diabetic patients' compliance, and cost spent in health care systems as seaweed is an abundant and sustainable naturally existing source of active biologic compounds, which makes it one of the best proposed choices for tackling diabetes.
STUDY DESIGN	randomized, double-blind, placebo-controlled phase 3 study.
PRIMARY OBJECTIVE	Investigate the effectiveness of seaweed (E. cava) in reducing postprandial blood glucose and plasma. insulin level (to determine insulin sensitivity) in pre-diabetic patients, compared to placebo.
SECONDARY OBJECTIVES	Investigate the safeness and potential adverse effect of applying sea weed (E. cava) as a therapeutic diet composition on patient with pre-diabetes, compared to placebo.
NUMBER OF SUBJECTS	30 subjects
SUBJECT SELECTION CRITERIA	Inclusion Criteria: Participants will be eligible to participate in this study if they are: • diagnosed in their medical history as pre-diabetic patient with fasting plasma glucose (FPG) between 100 and 125 mg dL−1. • aged between 18 and 65 years. • blood pressure within the normal range (systolic blood pressure ≤ 140 mmHg, diastolic blood pressure ≤ 90 mmHg). • having no other health complications. Exclusion Criteria: Participants will be excluded if they are: • taking any treatment with either insulin or anti-diabetic drugs. • any other natural health products known to impact blood sugar, or polyphenol absorption (e.g. fish oil). • Smoker. • pregnant or lactation

	• having liver, thyroid, or significant gastrointestinal disorders.
TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	Product brown seaweed (ecklonia cava) will be given at 580 mg dose Product will be administered only one time at the only one visit to investigate the acute effect of the investigational product. administration will be orally.
CONTROL PRODUCT, DOSE AND ROUTE OF ADMINISTRATION	Product plain dextrin as a placebo at 500 mg dose Product will be administered only one time at the only one visit to investigate the acute effect of the investigational product. administration will be orally.
DURATION OF SUBJECT PARTICIPATION AND DURATION OF STUDY	Subjects will be on study for only one time for only 3 hours Screening: one visit Treatment: one time at the only one visit. Follow-up: one day The total duration of the study is expected to be 4 months. 2 months for subject recruitment and 2 months for final subject follow-up as they will visit in different days.
CONCOMMITANT MEDICATIONS	• **Allowed**: - any medication that are not listed in prohibited list. • **Prohibited:** - any treatment with either insulin or anti-diabetic drugs. - any other natural health products known to impact blood sugar, or polyphenol absorption (e.g. fish oil). - Any liver, thyroid, or gastrointestinal disorders medications.
EFFICACY EVALUATIONS	NA
PRIMARY ENDPOINT	• Plasma glucose concentration will be determined immediately by blood finger-prick sample following standard procedure using Accu-Chek® aviva (Roche Diagnostics, Rotkreuz, Switzerland), Whereas, plasma insulin will be measured at the laboratory department at KKUH using enzyme-linked immunosorbent assay (ELISA)
SECONDARY ENDPOINTS	• Participant\s will be asked to indicate whether side effects are absent, mild, moderate or severe by giving scores as 0,1,2,3, respectively. The side effect questionnaire, will be required to be completed within 24 hours after intervention or placebo ingestion.
OTHER EVALUATIONS	NA
SAFETY	Change in blood glucose level from baseline to more than 240 mg /dl or

EVALUATIONS	less than 70 mg /dl Adverse events such as: Headach, Bloating or Minor abdominal pain
PLANNED INTERIM ANALYSES	When approximately 50% of patients have completed the study through Visit 2, an interim analysis for safety will be conducted by an independent data monitoring committee. Serious adverse events will be monitored by the committee on an ongoing basis throughout the study.
STATISTICS **Primary Analysis Plan**	Mean (standard deviation) will be reported for normally distributed data, whereas median (interquartile range) will be reported for not normally distributed data. Either independent t-test or Mann-Whitney test, based on normality of data, will be used to determine the difference between the two groups for both PPBG and PPIL at a significant level of $p < 0.05$. Mann-Whitney test will be used to determine differences for symptoms of intolerance between groups. Incremental area under the curve (iAUC), time to peak and peak blood concentration assessment will be used to assess postprandial responses of plasma glucose and insulin level.
Rationale for Number of Subjects	Sample size was calculated based in similar study (24) using sample size determination equation $\frac{2SD^2(Z_{a/2} + Z_B)^2}{d^2}$ $n = 2(32)^2(1.96+0.84)^2 / 38^2$ $= 2(1024)(2.8)^2 / 1444 = 2048*7.84 / 1444$ $= 16056.32 / 1444$ $= 11.11 \approx 12$ subjects/group whereas: - $Z_{a/2}$ = standard normal variate for level of significant, at 5% type 1 error, (if $p < 0.05 = 1.96$). - Z_B = Standard normal variate for power (for 80% power = 0.84, for 90% power = 1.28). - SD= Standard Deviation (determined from the literature). - d= effect size –glucosidases, thus improving diabetic related response (8). In general, Seaweeds, also called difference between mean values (determined from the literature).

1 BACKGROUND

In general, Seaweeds, also called marine algae, is considered high-quality healthy food as it contains diversified bioactive compound which exhibits various beneficial biological effects (5). Seaweed, particularly brown seaweed, is excellent source of polyphenolic antioxidants such as catechines, flavonoids which are well-known as functional foods components (9). In addition, the only seaweed that contain phlorotannins is the brown colored seaweed, thus it is the most studied type of seaweed. Phlorotannins is a subgroup of tannins that produced completely by polymerization of phloroglucinol. Ecklonia cava species is expected to have high content of phlorotannins. The most studied seaweed polyphenols-rich extracted from Ecklonia cava (E. cava) are phloroglucinol, phloroglucinol tetramer, eckol, phlorofucofuroeckol A, dieckol, and 8,80-bieckol, dioxinodehydroeckol. Recent evidence found that phlorotannins has exhibited various biological activities in vivo and vitro. E. cava polyphenolic-rich extract is an important functional food component that could be used in reducing diabetes related symptoms

1.1 Overview of Non-Clinical Studies

The effect of different types of phloroglucinol oligomers from E. bicyclis, a brown seaweed, on the activity of different types of glucosidase has been studied in a vitro study using the viscera of turan shell (Turbo cornutus). As resu lt, phlorofucofuroeckol A, dieckol, and 8,80-biecko have shown strong inhibition effect on α-fucosidase, α-galactosidase, and α-mannosidase (1). Phloroglucinol derivatives from E. bicyclis namely 2-phloroeckol, eckol and dieckol have also showed high anti-diabetic activity by the inhibition of α-amylase as well as glycation (2). Okada et al, have reported the percentage of a-amylase inhibition as 89.5%, 87.5% and 97.5% by 2-phloroeckol, eckol and dieckol, respectively (3). Zhang et al, have found that ethanolic extract of Ascophyllum nodosum reduced liver glycogen levels in diabetic mice by inhibition of intestinal α-glucosidase with IC50 (half maximal inhibitory concentration) value of 77 mg/ml. ecklonia stolonifera is a brown alga species that contain high amount of polyphenols (estimated to be phlorotannins) (4). The ethanolic extract E. stolonifera (MEE) shows a reduction in plasma glucose peroxidation level in non-insulin dependent diabetic mice model KK-Ay model (4). Also, dieckol extracted from E. cava demonstrated significant activity for with IC50 value of 10.8mmol/l a-amylase and a-glucosidase and 124.9mmol/l (5). Also, phlorofucofuroeckol A from Ecklonia stolonifera showed similar anti-diabetic result when applied on diabetic-complication such asangiotensin converting enzyme (ACE), advanced glycation end products (AGE), rat lens aldose reductase (RLAR), reactive oxygen species (ROS) in vitro study (6). Heo et al. also have displayed a significant inhibitory activity of a new phlorotannin, diphlorethohydrox-ycarmalol (DPHC) isolated from Ishige okamurae on a-glucosidase and a-amylase significantly as well as showed suppression of postprandial blood glucose level indiabetic and normal mice (7). This inhibitory activity on a-amylase and a-glucosidase were similar to an anti-diabetic commercial drug called acarbose (5). similarly, Lee et al, have claimed in their study that the IC50 value for phlorotannin derivatives extracted from brown algae, E. cava, against a-glucosidase were higher than the IC50 value for the therapeutic drug acarbose (8). They found that dieckol high inhibitory activity against α- glucosidase and α-amylase, with IC50 values at 10.97

and 124.98 μmol L−1, respectively. This suggest that E. cava polyphenolic extract is an important functional food component that could be used in reducing diabetes related symptoms (5). In different study, dieckol isolated from E. cava showed reduced α-glucosidase and α- amylase activity alongside with postprandial hyperglycemia reduction in diabetic male mice (9). There was no cytotoxicity reported in this experiment (19). Another brown seaweed extract, Ishige okamurae, anti-diabetic effects on postprandial glucose and insulin level were investigated in male diabetic mice. The study showed that improvement in insulin resistance and fasting blood glucose reduced HbA1c level in the diabetic mice group treated with Ishige okamurae extract compared to diabetic control mice group (10).

1.2 Overview of Clinical Studies

In general, human studies that investigated effect of marine algae (seaweed) on glycaemic control are limited. However, desirable effects have been shown in some clinical studies (11, 12, 13). Lee et al, have conducted a randomized clinical trial on 73 male and female participants with fasting blood glucose between 100 to 180 mg dL−1 (11). Participants were randomly divided into two groups given 500 mg of AG-dieckol three times a day for 12 weeks. The result shows acute significant reduction in postprandial glucose ($p < 0.05$) after 12 weeks intervention. However, non-significant postprandial insulin reduction was seen in group who consumed AG- dieckol compared to placebo group. Nonetheless, within group reduction was significantly observed in terms of insulin level compared to baseline (11). It worth to mention that Lee et al study was the only long-term study investigating the effect of marine algae on blood sugar and insulin level (11). However, randomization, bindingness, and participants' compliance measurement were not reported in this study, which may impact the quality of the study. Moreover, a study has investigated the acute effects of brown algae extracts (Ascophyllum nodosum and Fucus vesiculosus) on postprandial glucose and insulin concentration (12). participants were given 508 mg brown algae extract 30 minutes prior to 110 g carbohydrates consumption. Despite of healthy individuals and different seaweed source, containing only 10% polyphenols, used in this study, acute inulin concentration was significantly decreased ($p < 0.05$) in brown algae groups compared to placebo group (12). Also, non-significant postprandial glucose concentration was found to be decreased in brown algae extract ($p > 0.05$). One of early studies on sea weed effect on acute FBG and PPBG was conducted in Mexico (13). They found that FBG and PPBG were significantly decreased among group who received seaweed extract (not specified) compared to placebo group (13). Researchers have referred the reduction of FBG and PPBG to the increase in fibres intake, which were found to be 2.5 times in sea weed group compared to placebo groups (13). This could be another important research point related to the benefits of seaweed needed further investigation in different clinical population. In addition, Non-consistent evidence was reported in a clinical study examining high dose (2000 mg), low dose (500 mg) of polyphenolic-rich brown algae (Fucus vesiculosus) compared to placebo in healthy people. The study concludes non-significant result in terms of acute PPBG and IL (14). As pointed out by Margaret et al, in their systematic review that the source of seaweed extract and its polyphenolic content is very important in triggering antidiabetic effect (15). Thus, they strongly suggest that more clinical studies are required to determine consistent pattern of dose-response and to identify the effectiveness dose in a high-risk population

(15). According to the knowledge of researcher, no study has reported any significant differences between brown algae and placebo in terms of measuring adverse effect (9-14). Lee et al, have reported similar incidence of adverse event between groups (22.2 vs 16.2) for brown algae group compared to placebo, respectively (11). Similarly, paradis et al, find that 13 subjects in each group have reported adverse effect with minimal intensity (1 in a scale of 0 to 3) being the most reported for most of measured symptoms (12).

2 STUDY RATIONALE

There is a noticeable interest and enthusiasm toward brown seaweed anti-diabetic effects. Many vivo studies have shown strong inhibition activity of polyphenolic-rich brown seaweed on a-amylase and a-glucosidase enzymes (12-15). For example, Heo et al. have displayed a significant inhibitory activity of a new phlorotannin, diphlorethohydroxycarmalol (DPHC) isolated from brown seaweed called Ishige okamurae on a-glucosidase and a-amylase significantly as well as showed suppression of postprandial blood glucose level in diabetic and normal mice (14). This inhibitory activity on a-amylase and a-glucosidase were similar to an anti-diabetic commercial drug called acarbose (7). similarly, Lee et al, have claimed in their study that the IC50 value for phlorotannin derivatives extracted from brown algae, E. cava, against a-glucosidase were higher than the IC50 value for the drug acarbose (15). In regard to human trials, investigation of polyphenolic-rich brown seaweed effect on glycemic control did not yield consistent evidence, yet (4,5,8,9).. However, desirable effects have been shown in some clinical studies (11, 12, 13). Lee et al, have conducted a randomized clinical trial on 73 male and female participants with fasting blood glucose (FBG) between 100 to 180 mg dL−1 (11). Participants were randomly divided into two groups given 500 mg of AG-dieckol three times a day for 12 weeks. The result shows acute significant reduction in postprandial blood glucose (PPBG) ($p < 0.05$) after 12 weeks intervention. However, non-significant postprandial insulin level (PPIL) reduction was seen in group who consumed AG-dieckol compared to placebo group. Nonetheless, within group reduction was significantly observed in terms of insulin level compared to baseline (11). Moreover, a study has investigated the acute effects of brown algae extracts (Ascophyllum nodosum and Fucus vesiculosus) on postprandial glucose and insulin concentration (12). participants were given 508 mg brown algae extract 30 minutes prior to 110 g carbohydrates consumption. Despite of healthy individuals and different seaweed source, containing only 10% polyphenols, used in this study, acute inulin concentration was significantly decreased ($p < 0.05$) in brown algae groups compared to placebo group (12). Also, non-significant postprandial glucose concentration was found to be decreased in brown algae extract ($p > 0.05$). In contrast, non-significant result was reported in a clinical study examining high dose (2000 mg), low dose (500 mg) of polyphenolic-rich brown algae (Fucus vesiculosus) compared to placebo in healthy people. The study concludes non-significant result in terms of acute PPBG and PPIL (14). As pointed out by Margaret et al, in their systematic review that the source of seaweed extract and its polyphenolic content is very important in triggering antidiabetic effect (15). Thus, they strongly suggest that more clinical studies are required to determine consistent pattern of dose-response and to identify the effectiveness dose in a high-risk population (15). According to side effect, no study has reported any significant differences between brown seaweed and placebo in terms of measuring adverse effect (9-14). Lee et al, have reported similar incidence of adverse

event between groups (22.2 vs 16.2) for brown algae group compared to placebo, respectively (11). Similarly, paradis et al, find that 13 subjects in each group have reported adverse effect with minimal intensity (1 in a scale of 0 to 3 where 0 absent and 3 means sever intensity) being the most reported for most of measured symptoms (12). Despite many promising results in vitro and vivo studies (animal studies), the effects of polyphenol-rich brown seaweed on blood glucose and insulin level in human have not been effectively investigated. Thus, more investigational studies are needed particularly on the interaction of brown seaweed and its polyphenols with human cellular systems which may provide better understanding on parameters such as bioavailability, mechanisms of actions of in glucose homeostasis in humans. This can be translated into long-term health benefits. Further research would widen the likelihood of screening more biologically proficient polyphenols and its derivatives. Results of such studies could provide us with resourceful drug substitute to reduce or regulate diet-linked chronic malfunctions such as diabetes. In terms of the type of seaweed intended to be used, Kunihisa, et al have claimed that "this type of brown seaweed (E. cava) polyphenolic-rich extract is an important functional food component that could be used in reducing diabetes related symptoms" (16). Also, Margert et al, on their systematic review investigating the effect of algal polyphenols on antidiabetic, anti-hyperlipidaemia or anti-inflammatory effects in humans, have stated that " E. cava is currently the most extensively investigated source of marine polyphenols for anti-hyperglycaemic effects and has shown the most promise in this area" (8). Indeed, they have concluded that " Marine polyphenols, particularly from E. cava, show potential as natural functional ingredients for the prevention and management of type 2 diabetes and CVD. However further high quality RCTs are required to determine the most effective dose, treatment schedule and macroalgal source to produce consistent effects on glycaemia and dyslipidaemia in humans" (8). Moreover, Previous human studies have mainly performed on healthy individuals using different types of seaweed (4,5). Therefore, this proposal is meant to address the gap in this scientific area, using Ecklonia cave on prediabetes patient which hypothesized to have beneficial result regarding blood glucose and insulin level (8). Although, the methodology in this study focuses on the acute effect of E. cava on glycaemic control, it can be a promising result for future studies including long-term studies. Result in this study would give indications for future research regarding type of seaweed, dose and targeted population. More research in this area may benefits us in different aspect including prevention and clinical use of seaweed extract, diabetic patients' compliance, and cost spent in health care systems as seaweed is an abundant and sustainable naturally existing source of active biologic compounds, which makes it one of the best proposed choices for tackling diabetes.

2.1 Risk / Benefit Assessment

NA

3 STUDY OBJECTIVES

3.1 Primary Objective

To Investigate the effectiveness of seaweed (E. cava) in reducing postprandial blood glucose and plasma insulin level (to determine insulin sensitivity) in pre-diabetic patients, compared to placebo.

3.2 Secondary Objectives

Investigate the safeness and potential adverse effect of applying sea weed (E. cava) as a therapeutic diet composition on patient with pre-diabetes, compared to placebo.

4 STUDY DESIGN

4.1 Study Overview

This is a single center, double-blind, placebo-controlled, randomized study. 30 (number) of subjects are planned. Because the study only investigating the acute effect of the study interventional product, each subject will be administered a single dose of either study dietary supplement or placebo only one time. Subjects will be assigned to the treatments in random order. Evaluations will be taken at baseline and every 30 min following the ingestion of carbohydrates for the following 2 hours at the interval of 30, 60, 90, 120 min.

Screening data will be reviewed to determine subject eligibility. Subjects who meet all inclusion criteria and none of the exclusion criteria will be entered into the study.

The following treatment regimens will be used:

- A dietary supplement E.cava extracted capsule (seaweed) - at dose of 580 mg.
- Placebo (dextrin) – at a dose of 500 mg.

Total duration of subject participation will be six weeks. Total duration of the study is expected to be 12 weeks.

5 CRITERIA FOR EVALUATION

5.1 Primary Efficacy Endpoint

Reduction in postprandial blood glucose and insulin level compare to baseline.

As the dietary supplement (seaweed) is expected to have an effect on a-amylase and a-glucosidase enzymes that are responsible for breaking down carbohydrates which. Thus, postprandial blood glucose level and postprandial insulin level are best available way to see the effect of the product as it is expected to directly affect blood sugar enzymes that may lead to glycemic index control. As it is mentioned previously, this study is investigating the acute effect of the investigational product which will only compare change in PPBG and PPIL to baseline which will last only for one 3 hours in only one visit.

5.2 Secondary Efficacy Endpoints

In order to assess side effects of study intervention, measurement of side effects will be obtained by using an arbitrary scale, similar to that used by Paradis et al (12), that allow participants to rate symptoms of intolerance by indicating whether each side effect was absent (0), of mild intensity (1), of moderate intensity (2), or of severe intensity (3). The side effect questionnaire, will be required to be completed within 24 hours after intervention or placebo ingestion. The side effect will include headache, energy levels, appetite, gastrointestinal symptoms, unusual pain or sensations, cardiac palpitations, balance disorders, and anxiety.

5.3 Safety Evaluations

Change in blood glucose level from baseline to more than 240 mg /dl or less than 70 mg /dl. Adverse events such as: Headach, Bloating or Minor abdominal pain Other Evaluations.

6 SUBJECT SELECTION

6.1 Study Population

Subjects with a diagnosis of pre-diabetic who meet the inclusion and exclusion criteria will be eligible for participation in this study.

6.2 Inclusion Criteria

1. Male or female ≥ 18 years of age at Visit 1.
2. Documentation of a pre-diabetic diagnosis as evidenced by one or more clinical features consistent with and one of the following criteria:
   - fasting plasma glucose (FPG) between 100 and 125 mg dL−1 as a set criterion by American Diabetic Association (16),
3. Written informed consent (and assent when applicable) obtained from subject or subject's legal representative and ability for subject to comply with the requirements of the study.
4. blood pressure within the normal range (systolic blood pressure ≤ 140 mmHg, diastolic blood pressure ≤ 90 mmHg).
5. having no other health complications.

6.3 Exclusion Criteria

1. Pregnant, breastfeeding, or unwilling to practice birth control during participation in the study.
2. Presence of a condition or abnormality that in the opinion of the Investigator would compromise the safety of the patient or the quality of the data.
3. taking any treatment with either insulin or anti-diabetic drugs or any other natural health products known to impact blood sugar, or polyphenol absorption (e.g. fish oil).

4. Smoker.
5. having liver, thyroid, or significant gastrointestinal disorders.

7 CONCURRENT MEDICATIONS

All subjects should be maintained on the same medications throughout the entire study period, as medically feasible, with no introduction of new chronic therapies.

7.1 Allowed Medications and Treatments

Standard therapy for pre-diabetic is allowed except for treatments noted in the exclusion criteria described above and as noted in the prohibited medications section below.

7.2 Prohibited Medications and Treatments

The following medications are prohibited during the study and administration will be considered a protocol violation:

- any treatment with either insulin or anti-diabetic drugs.
- any other natural health products known to impact blood sugar, or polyphenol absorption (e.g. fish oil).
- Any liver, thyroid, or gastrointestinal disorders medications.

8 STUDY TREATMENTS

8.1 Method of Assigning Subjects to Treatment Groups

Up to 30 eligible patients will be randomly assigned to the dietary supplement seaweed or placebo treatment groups in a 1:1 ratio using a SAS-based computer-generated randomization scheme developed by a study investigator who will not be involved in data collection and analysis. Participants and other investigators will be blinded to which supplement is consumed each testing occasion for each participant until data analysis completed. At screening visit investigators will complete patient required data as detailed in the Study Manual, and fax it to the investigator who is responsible for randomization. The investigator who responsible for randomization will send back participants data encoded with numbers and supplements (intervention & placebo) with letters (A, B). Study intervention or placebo will be encapsulated in identical capsules and will be labelled with the letter A and B to conceal which supplement is given to each participants.

8.2 Blinding

Due to the objectives of the study, the identity of test and control treatments will not be known to investigators, research staff, or patients. The following study procedures will be in place to ensure double-blind administration of study treatments.

- Access to the randomization code will be strictly controlled.
- Packaging and labeling of intervention and placebo treatments will be encapsulated identically to maintain the blindness.

The study blind will be broken on completion of the clinical study and after the study database has been locked. Investigators will be aware of their subjects' treatment assignment after study statistical analysis completed.

During the study, the blind may be broken only in emergencies when knowledge of the patient's treatment group is necessary for further patient management. When possible, the Investigator should discuss the emergency with the Medical Monitor prior to unbinding. The pharmacist who is dispensing the study treatment will be available during study conduction and in case of any emergency, the pharmacist will disclose the blindness to medical monitor as it will be written in dispensing log in the research pharmacy.

8.3 Formulation of Test and Control Products

8.3.1 Formulation of Test Product

The intervention product used in this study is a dietary supplement extracted from seaweed called Ecklonia cava called "Seoul" that contain 13% pholoratannic polyphenol per capsule as stated by the manufacture company (Seanol inside, 4215 95th St SW Lakewood, WA 98499 USA). Other ingredients are dextrin, magnesium stearate and silica (in neglected percentage). The intervention supplement is encapsulated in vegetable cellulose that contains 500 Ecklonia cava extract (Seanol). This dose was selected to be similar to previous studies that shows no harm or sever adverse effect on participants (12, 14). The encapsulated product is brown-colored powder that requires no reconstitution.

Table X: Formulation and Measured pH of Dietary supplement (Seanol) and Placebo

	Dietary supplement (Seanol)	Placebo
Active Ingredient, mg/mL	13% phlorotanninic polyphenol (include eckols, dieckol, 6,6'-bieckol, 8,8'-bieckol, phlorofucofuroeckol-A)	Dextrin
Other ingredient, mg/mL	80 % Dextrin magnesium stearate and silica (in neglected amount)	Non
pH		0.5 – 7.5

8.3.2 Formulation of Control Product

The placebo will be dextrin (BETA CYCLODEXTRIN, NF) will be ordered and provided by the study PI from a pharmaceutical company "MEDISCA" (https://www.medisca.co.uk/). Dextrin was selected to account for the similar complex carbohydrate content of the intervention supplement. Placebo will be encapsulated in vegetable cellulose capsules that is identical in size and coulure to the intervention capsules

8.3.3 Packaging and Labeling

Packaging: Study drug is supplied in a bottle containing 60 single use capsules. The capsules will be packaged in enclosed within a laminated foil pouch bottle.

Labeling: Each bottle of study drug will be labeled with the required FDA warning statement, the protocol number, a treatment number, the name of the principle investigator, and directions for patient use and storage. direction for both study test and placebo will be the same as both has the same direction which will ensure blinding in the study.

8.4 Supply of Study Drug at the Site

The principle investigator will ship Study Drug to the investigational sites. The initial study drug shipment will be shipped after site activation. Subsequent study drug shipments will be made after site request for resupply.

8.4.1 Dosage/Dosage Regimen

The study test is a dietary supplement called brown seaweed (Ecklonia cava) which will be given at 580 mg dose. Seaweed will be administered only one time at the only one visit to investigate the acute effect of the investigational product. administration will be orally.

The placebo is a plain dextrin will be given at 500 mg dose. Placebo will be administered only one time at the only one visit to investigate the acute effect of the investigational product. administration will be orally.

8.4.2 Dispensing

The pharmacists at research pharmacy at KKUH will be the only pharmacist at hospital who are responsible for study treatment or placebo dispensing.

8.4.3 Administration Instructions

The study dietary supplement (seaweed) or placebo will be administered to patient at the study site only one time at a supervision of study investigator and medical monitor where the patient will be instructed to administer the study capsules orally.

8.5 Supply of Study Drug at the Site

Study dietary supplement will be supplied to the pharmacy of research at study site (KKUH) by the principle investigator of the study. If more participants needed as a replacement of withdrawn participants, study treatment will be stored at the pharmacy of research at KKUH which will be available for the new participants.

8.5.1 Storage

Study dietary supplement should be stored by the study site at controlled room temperature, 15 to 30ºC (59 to 86ºF). If the temperature of study drug storage in the pharmacy exceeds or falls below this range, this will be reported to the study PI or designee and captured as a deviation. the supplement will be stored in original packaging (foil pouch and protected from light) at room temperature according to the instructions outlined on the Drug Administration Instructions.

8.6 Study Drug Accountability

An accurate and current accounting of the dispensing and return of study drug for each subject will be maintained on an ongoing basis by a member of the study site staff. The number of study drug dispensed and returned by the subject will be recorded on the Investigational Drug Accountability Record. The study monitor will verify these documents throughout the course of the study.

8.7 Measures of Treatment Compliance

Not applicable due to that study dietary supplement (seaweed) or placebo will be administered to patient at the study site only one time at a supervision of study investigator.

9 STUDY PROCEDURES AND GUIDELINES

A Schedule of Events representing the required testing procedures to be performed for the duration of the study is diagrammed in Appendix 1.

Prior to conducting any study-related activities, written informed consent and the Health Insurance Portability and Accountability Act (HIPAA) authorization must be signed and dated by the subject If appropriate, assent must also be obtained prior to conducting any study-related activities.

9.1 Clinical Assessments

9.1.1 Concomitant Medications

All concomitant medication and concurrent therapies will be documented at Baseline/Screening. Dose, route, unit frequency of administration, and indication for administration and dates of medication will be captured.

9.1.2 Demographics

Demographic information (date of birth, gender, race) will be recorded at Screening.

9.1.3 Medical History

Relevant medical history, including history of current disease, other pertinent respiratory history, and information regarding underlying diseases will be recorded at Screening.

9.1.4 Physical Examination

A complete physical examination will be performed by either the investigator or a subinvestigator who is a physician at screening. Qualified staff (MD, NP, RN, and PA) may complete the abbreviated physical exam at all other visits. New abnormal physical exam findings must be documented and will be followed by a physician or other qualified staff at the next scheduled visit.

9.1.5 Vital Signs

Body temperature, blood pressure, pulse and respirations will be performed after resting for 5 minutes at all Visits.

9.1.6 Adverse Events

Information regarding occurrence of adverse events will be captured throughout the study. Duration (start and stop dates), severity outcome, treatment and relation to study drug will be recorded on the case report form (CRF).

9.2 Pharmacokinetic Measurements

NA

9.3 Research Laboratory Measurements

9.3.1 Blood profile

Blood will be obtained and sent to the site's clinical chemistry lab for determination of random glucose, fasting blood glucose and insulin level.

10 EVALUATIONS BY VISIT

10.1 Visit 1 (Day1 /Week 1/Month 1)

1. Review the study with the subject (subject's legal representative) and obtain written informed consent.
2. Assign the subject a unique screening number.
3. Record demographics data.
4. Record medical history, including a history of pre-diabetic, diagnosis date.
5. Perform a complete physical examination.
6. Perform and record vital signs.
7. Perform and record results of blood pressure testing.
8. Collect blood for clinical laboratory tests (baseline of blood glucose level).
9. Schedule subject for Visit 2 in different days.
10. List all additional procedures, such as Randomize subject

10.2 Visit 2

1. Perform abbreviated physical examination.
2. Perform and record vital signs.
3. Collect blood for clinical laboratory tests (baseline of blood glucose and insulin levels).
4. Administer either study dietary supplement (seaweed) or placebo based on blinded randomization.
5. Perform carbohydrates ingestion
6. Collect blood for clinical laboratory tests (blood glucose and insulin levels 30 min after administration of study treatment).
7. Collect blood for clinical laboratory tests (blood glucose and insulin levels 60 min after administration of study treatment).
8. Collect blood for clinical laboratory tests (blood glucose and insulin levels 90 min after administration of study treatment).
9. Collect blood for clinical laboratory tests (blood glucose and insulin levels 120 min after administration of study treatment).

10.3 Early Withdrawal Visit

1. Record any Adverse Experiences
2. Perform complete physical examination.
3. Perform and record vital signs.

11 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

11.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical investigation of a patient administered a pharmaceutical product and that does not necessarily have a causal relationship with the treatment. An AE is therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product. An unexpected AE is one of a type not identified in nature, severity, or frequency in the current Investigator's Brochure or of greater severity or frequency than expected based on the information in the Investigator's Brochure.

The Investigator will probe, via discussion with the subject, for the occurrence of AEs during each subject visit and record the information in the site's source documents. Adverse events will be recorded in the patient CRF. Adverse events will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study drug, or if unrelated, the cause.

11.1.1 AE Severity

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. The modified criteria can be found in the study manual. If the experience is not covered in the modified criteria, the guidelines shown in Table 1 below should be used to grade severity. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 1. AE Severity Grading

Severity (Toxicity Grade)	Description
Mild (1)	Transient or mild discomfort; no limitation in activity; no medical intervention or therapy required. The subject may be aware of the sign or symptom but tolerates it reasonably well.
Moderate (2)	Mild to moderate limitation in activity, no or minimal medical intervention/therapy required.
Severe (3)	Marked limitation in activity, medical intervention/therapy required, hospitalizations possible.
Life-threatening (4)	The subject is at risk of death due to the adverse experience as it occurred. This does not refer to an experience that hypothetically might have caused death if it were more severe.

11.1.2 AE Relationship to Study Drug

The relationship of an AE to the study drug should be assessed using the following the guidelines in Table 2.

Table 2. AE Relationship to Study Drug

Relationship to Drug	Comment
Definitely	Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is unlikely to be explained by the known characteristics of the subject's clinical state or by other interventions.
Possibly	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to that suspected drug; but that could readily have been produced by a number of other factors.

Unrelated	An event that can be determined with certainty to have no relationship to the study drug.

11.2 Serious Adverse Experiences (SAE)

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention to prevent one of the outcomes listed.

11.2.1 Serious Adverse Experience Reporting

Study sites will document all SAEs that occur (whether or not related to study drug) per UCSF CHR Guidelines. The collection period for all SAEs will begin after informed consent is obtained and end after procedures for the final study visit have been completed.

In accordance with the standard operating procedures and policies of the local Institutional Review Board (IRB)/Independent Ethics Committee (IEC), the site investigator will report SAEs to the IRB/IEC.

11.3 Medical Monitoring

Insert Medical Monitor Name should be contacted directly at these numbers to report medical concerns or questions regarding safety.

Phone: (966)505682918
E-mail: aalguwaihes@ksu.edu.sa

12 DISCONTINUATION AND REPLACEMENT OF SUBJECTS

12.1 Early Discontinuation of Study Drug

A subject may be discontinued from study treatment at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for study treatment discontinuation:

- Subject withdrawal of consent (or assent)
- Subject is not compliant with study procedures
- Adverse event that in the opinion of the investigator would be in the best interest of the subject to discontinue study treatment

- Protocol violation requiring discontinuation of study treatment
- PI request for early termination of study

If a subject is withdrawn from treatment due to an adverse event, the subject will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

All subjects who discontinue study treatment should come in for an early discontinuation visit as soon as possible and then should be encouraged to complete all remaining scheduled visits and procedures.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents Refer to Section 10 for early termination procedures.

12.2 Withdrawal of Subjects from the Study

A subject may be withdrawn from the study at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents. As noted above, subjects who discontinue study treatment early (i.e., they withdraw prior to 1) should have an early discontinuation visit. Refer to Section 10 for early termination procedures. Subjects who withdraw after Visit 1 but prior to Visit 2 should be encouraged to come in for a final visit (and the procedures to be followed would include those for their next scheduled visit).

12.3 Replacement of Subjects

Subjects who withdraw from the study treatment will not be replaced.

Subjects who withdraw from the study will not be replaced.

13 PROTOCOL VIOLATIONS

A protocol violation occurs when the subject, investigator fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

- Failure to meet inclusion/exclusion criteria
- Use of a prohibited concomitant medication

Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The PI will determine if a protocol violation will result in withdrawal of a subject.

When a protocol violation occurs, it will be discussed with the investigator and a Protocol Violation Form detailing the violation will be generated. This form will be signed by a Sponsor representative and the Investigator. A copy of the form will be filed in the site's regulatory binder and in the Sponsor's files.

14 STATISTICAL METHODS AND CONSIDERATIONS

Prior to the analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be written describing all analyses that will be performed. The SAP will contain any modifications to the analysis plan described below.

14.1 Data Sets Analyzed

All eligible patients who are randomized into the study and receive the only one dose of the study dietary supplement (the Safety Population) will be included in the safety analysis.

14.2 Demographic and Baseline Characteristics

- weight
- height
- BMI,
- age
- exercise level
- Collect blood for clinical laboratory tests (baseline of blood glucose level).

14.3 Analysis of Primary Endpoint

Mean (standard deviation) will be reported for normally distributed data, whereas median (interquartile range) will be reported for not normally distributed data. Either independent t-test or Mann-Whitney test, based on normality of data, will be used to determine the difference between the two groups for both PPBG and PIL at a significant level of $p < 0.05$. Incremental area under the curve (iAUC), time to peak and peak blood concentration assessment will be used to assess postprandial responses of for plasma glucose and insulin level. Safety and tolerability data will be summarized by treatment group.

14.4 Analysis of Secondary Endpoints

Mann-Whitney test will be used to determine differences for symptoms of intolerance between groups.

Adverse events will be tabulated by treatment group and will include the number of patients for whom the event occurred, the rate of occurrence, and the severity and relationship to study drug.

14.5 Interim Analysis

When approximately 50% of patients have completed the study through Visit 2, an interim analysis for safety will be conducted by an independent data monitoring committee. Serious adverse events will be monitored by the committee on an ongoing basis throughout the study.

14.6 Sample Size and Randomization

Sample size was calculated based in similar study (24) using sample size determination equation:

$$\frac{2SD^2(Z_{a/2} + Z_B)^2}{d^2}$$

$n = 2(32)^2(1.96+0.84)^2 / 38^2 = 2(1024)\,(2.8)^2 / 1444 = 2048*7.84 / 1444$

$= 16056.32 / 1444$

$= 11.11 \approx 12$ subjects/group

whereas:

- $Z_{a/2}$ = standard normal variate for level of significant, at 5% type 1 error, (if $p < 0.05 = 1.96$).
- Z_B = Standard normal variate for power (for 80% power = 0.84, for 90% power = 1.28).
- SD= Standard Deviation (determined from the literature).
- d= effect size – difference between mean values (determined from the literature).

15 DATA COLLECTION, RETENTION AND MONITORING

15.1 Data Collection Instruments

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject treated with the study drug.

Study personnel at the site will enter data from source documents corresponding to a subject's visit into the protocol-specific paper CRF when the information corresponding to that visit is available. Subjects will not be identified by name in the study database or on any study documents to be collected by the PI (or designee), but will be identified by a subject number

If a correction is made on a CRF, the study staff member will line through the incorrect data, write in the correct data and initial and date the change.

The Investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator.

15.2 Data Management Procedures

The data will be entered into a validated database. The Data Management group will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed.

All procedures for the handling and analysis of data will be conducted using good computing practices meeting FDA guidelines for the handling and analysis of data for clinical trials.

15.3 Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. Query reports (Data Clarification Requests) pertaining to data omissions and discrepancies will be forwarded to the Investigators and study monitors for resolution. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented.

15.4 Archival of Data

The database is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be maintained. Databases are backed up by the database administrator in conjunction with any updates or changes to the database.

At critical junctures of the protocol (e.g., production of interim reports and final reports), data for analysis is locked and cleaned per established procedures.

15.5 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the monitor, other authorized representatives of the Sponsor (or designee), IRB/IEC, and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each subject must be maintained that includes the signed Informed Consent, HIPAA Authorization and Assent Form and copies of all source documentation related to that subject. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (patient files, signed informed consent forms, copies of CRFs, Study File Notebook, etc.) must be kept secured for a period of two years following marketing of the investigational product or for two years after centers have been notified that the IND has been discontinued. There may be other circumstances for which the Sponsor is required to maintain study records and, therefore, the Sponsor should be contacted prior to removing study records for any reason.

15.6 Monitoring

Monitoring visits will be conducted by representatives of the Sponsor according to the U.S. CFR Title 21 Parts 50, 56, and 312 and ICH Guidelines for GCP (E6). By signing this protocol, the Investigator grants permission to the Sponsor (or designee), and appropriate regulatory authorities to conduct on-site monitoring and/or auditing of all appropriate study documentation.

15.7 Subject Confidentiality

In order to maintain subject confidentiality, only a site number, subject number and subject initials will identify all study subjects on CRFs and other documentation submitted to the Sponsor. Additional subject confidentiality issues (if applicable) are covered in the Clinical Study Agreement.

16 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. All study records will be kept in a locked file cabinet and code sheets linking a patient's name to a patient identification number will be stored separately in another locked file cabinet. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

16.1 Protocol Amendments

Any amendment to the protocol will be written by PI. Protocol amendments cannot be implemented without prior written IRB/IEC approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRBs are notified within five working days.

16.2 Institutional Review Boards and Independent Ethics Committees

The protocol and consent form will be reviewed and approved by the IRB/IEC of each participating center prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB/IEC in accordance with the standard operating procedures and policies of the IRB/IEC, and the Investigator will keep the IRB/IEC informed as to the progress of the study. The Investigator will obtain assurance of IRB/IEC compliance with regulations.

Any documents that the IRB/IEC may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB/IEC. The IRB/IECs written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. The IRB/IECs unconditional approval statement will be transmitted by the Investigator to PI prior to the shipment of study supplies to the site. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB/IEC approval except when necessary to eliminate immediate hazards to

the patients or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB/IEC and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB/IEC must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

16.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

The Investigator will prepare the informed consent form, assent and HIPAA authorization and provide the documents to the Sponsor or designee for approval prior to submission to the IRB/IEC. The consent form generated by the Investigator must be acceptable to the Sponsor and be approved by the IRB/IEC. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonisation and will also comply with local regulations. The Investigator will send an IRB/IEC-approved copy of the Informed Consent Form to the Sponsor (or designee) for the study file.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects must be given ample opportunity to inquire about details of the study. If appropriate and required by the local IRB/IEC, assent from the subject will also be obtained. If a subject is unable to sign the informed consent form (ICF) and the HIPAA authorization, a legal representative may sign for the subject. A copy of the signed consent form (and assent) will be given to the subject and the original will be maintained with the subject's records.

16.4 Publications

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the study Sponsor and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

16.5 Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when to protect the safety, rights or welfare of subjects.

2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Report to the Sponsor or designee any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
5. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
6. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee).
7. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
8. Promptly report to the IRB and the Sponsor (or designee) all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports).
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.
10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

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APPENDIX 1.

	VISIT 1 (Day/Week/Month #)[a]	**VISIT 2 (Day/Week/Month #)[a]**
Informed Consent	**X**	
Medical History	**X**	
Complete Physical Exam	**X**	
Height	**X**	**X**
Weight	**X**	**X**
Vital Signs	**X**	**X**
Exercise Level	**X**	
Baseline Fasting Blood glucose Level	**X**	**X**
Postprandial Blood glucose Level		**X**
Baseline Insulin Level		**X**
Postprandial Insulin Level		**X**
ESR	**X**	
Randomization	**X**	
Dispensing or Administration of Study Drug		**X**

Concomitant Medication Review		**X**
Adverse Experiences		**X**

[a] ±2 days

Required Components of Informed Consent

Informed consent is not a single event or just a form to be signed; it is an educational process that takes place between the investigator and the prospective subject. The basic elements of the consent process include:

full disclosure of the nature of the research and the participant's involvement,

adequate comprehension on the part of the potential participant, and

the participant's voluntary choice to participate.

It is the investigator's responsibility to document that the informed consent process has taken place, and an informed consent form is the standard for documenting the process for research projects involving human participants.

Consent forms must contain all the required components of informed consent as defined in SOP 9: Informed Consent Options, Processes, and Documentation and summarized below. The consent form must be written in language that is easy for a potential participant to understand and assures that individual's comprehension. Therefore, avoiding technical terms and complex sentences, even for the educated layperson, is very important.

When the participant population is not homogeneous, different consent documents may be required for different groups of people. If the research population will include participants under 18 years of age, then the IRB will expect investigators to use an assent form and a parental permission form instead (see SOP 11: Informed Consent, Enrollment, and Other Considerations for Research Involving Children). Similarly, research with cognitively or decisionally impaired individuals will require documented consent from another party—namely that person's legally authorized representative (see SOP 9, section 8 and/or SOP 10: xxx).

The IRB also recognizes that there are instances when documenting written informed consent is not appropriate to a research project. Alternatives to using a signed form for documenting informed consent are detailed in SOP 9.

Regardless of the method of documenting informed consent, however, the process of obtaining informed consent should always contain the same required components.

Helpful guidelines for constructing an effective consent form:

Use common, ordinary language instead of technical, academic terms. Ideally we would like consent forms to be written an 8th grade reading level. A helpful gage is to consider if one's 13 year-old cousin would be able to understand the research after reading the consent form.

Try to keep the sentences as short and simple as possible. Write in the second person using you/your pronouns. For example: "You are being asked to participate in a research

project…," "If you have questions later, you may contact…," or "You will be given a copy of this form to keep for your records."

Do not use assumptive statements such as "You understand that ..." or "You have been told that...."

Use adequate white space so that the form is easy to read, and avoid using small fonts to squeeze all the text onto one page.

Headings for paragraphs are helpful and make the form easier to read and understand.

Required Elements of Informed Consent Forms:

1. A clear, concise explanation of the purposes of the research, including the name of the study and prominent use of the term "research." (Note: the IRB can waive this element if the study requires deception. In such cases, a debriefing statement should also be used to inform participants at an appropriate time after their involvement in the study.)

2. An explanation of what will be happening to the participant during the study, and an indication of the participant's time commitment for each component.

3. Description of the risks, side effects or discomforts of the study procedures. For instance, even though it is not considered a risky procedure, a needle stick to draw blood may cause brief pain or discomfort. For social science and behavioral research, though risks usually do not extend beyond the possible loss of confidentiality and/or mild emotional distress, these should also be made clear to prospective participants.

If it appears that there are no real risks to participation, state, "We do not anticipate any risks to you participating other than those encountered in daily life." Please see our Sample Consent Form for an example of appropriate wording of a risk statement.

4. Description of any potential benefits from participating.

For individual participants, these should be limited to direct benefits: information about better coping skills, awareness of available support or resources, or any other personal gain other than financial rewards. (Learning about how experiments are conducted, receiving a gift, or earning extra credit for being a research participant are NOT recognized as benefits. Gifts, extra credit for courses, and reimbursement for expenses are considered compensation.) If there are no direct benefits, simply indicate that there are none.

For indirect benefits to society or scientific knowledge, statements such as "…information from this study may benefit other people now or in the future…" or "…we hope to learn more about _____ …" are appropriate.

5. A statement that the participant's involvement is voluntary, the participant may refuse to participate before the study begins, discontinue at any time, or skip any questions that may make him/her feel uncomfortable, with no penalty to him/her, and no effect on the compensation earned before withdrawing, or their academic standing, record, or relationship with the university.

6. A statement that the participant is allowed to ask questions concerning the study, both before agreeing to be involved and during the course of the study. See required contact information in #11 below.
7. A description of how the participant's confidentiality will be protected.
8. A description of what will be done with the data once the study is completed.
9. An indication that recording devices, audio or visual, are being used (when applicable).

Be sure to describe what will be done with the any video or audio tapes upon the completion of the study (destroyed, erased, archived, etc.), and when (after transcription, 3 years, 5 years, etc.).

Also, provide a separate signature line on the consent form for the participant to agree to be video/audio taped or photographed, if the recording is optional for participation. For example:

Please sign below if you are willing to have this interview recorded on tape (specify audio or video). You may still participate in this study if you are not willing to have the interview recorded.

I am willing to have this interview recorded on tape:

Signed: _____

Date: _____

10. An indication that the participant shall receive a copy of the signed and dated consent form.
11. The name(s) of the investigator(s) and contact information.
12. An indication that the participant may contact the Institutional Review Board for Human Participants (IRB) with any concerns or complaints. Additionally, a statement indicating that participants can report their concerns or complaints anonymously through.
13. A "statement of consent" and the name and signature of the participant.
14. The name signature of the person obtaining consent.
15. At the bottom of the form the following statement: "This consent form will be kept by the researcher for at least three years beyond the end of the study and was approved by the IRB on [date]."