BIOMEDICAL RESEARCH ON INSTITUTIONAL COMMITTEE IN HUMANS

RESEARCH PROJECT EVALUATION FORMAT

CIIBH Registration No.: GAS-1107-13 / 15-1 Version 3: December 10, 2020

1. Title of the project

"Prospective, randomized and controlled study for the evaluation of the effectiveness of a supplement of tequilana Agave Weber var. Blue vs Psyllium fiber psyllium derivatives in Patients With functional constipation "

2. Researchers

2a. Identification

Name, signature and affiliation of every one of the Researchers participations. The main researcher must be a professional Affiliated to the Institution (basic medical trainee or researcher) and not a student, resident, intern, apprentice, etc.)

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2b. Relevance of the Group of Researchers with regards to the project

Briefly, the researcher group qualifications described with regards to the scientific research process in General and with regards to the Proposed project. (Eg academic degree, work experience, research member of the INS system, SNI, etc.)

- Enrique Coss Adame, MD, Internal Medicine specialty, sub-specialty Gastroenterology, Affiliated to the Department of Gastroenterology and the Gastrointestinal Motility Laboratory at INCMNSZ.

- Maria Lorena Cassis Nosthas, MS, Chemist, Affiliated to the Department of Food Science and Technology

at INCMNSZ.

- Fernando Tuz-Dzib, Chemist, Affiliated to the Infectious Disease Department at INCMNSZ.
- Alicia Chavez Sofía Villar, MD, postgraduate degree in Gastrointestinal Motility, Gastrointestinal Motility Laboratory, Gastroenterology Department at INCMNSZ.
- Maria Fernanda García Cedillo, Nutritionist, Affiliated to the Gastroenterology Department at INCMNSZ.
- Emmanuel Martinez Hernandez, Nutritionist, Affiliated to the Gastroenterology Department at INCMNSZ.
- Irina Melinee Tapia MD Gámez Social Service Intern in the Gastroenterology Department at INCMNSZ.
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- Juan Rosales Guevara, MS Chemical Sciences Faculty, La Salle University.
- Fernando Parra Garcia, Master of Engineering, Chemical Sciences Faculty, La Salle University.
- Gustavo Armendariz Bustillo, MD, Bustar Alimentos SAPI de CV, legal representative.

3. Participating Institutions

Name and address of the participating Institutions and a brief description of how They will be involved. For multicenter studies, add the information of the coordinating center.

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4. Sponsorship

4A. Sponsoring Institutions

Name, address and telephone number of the Institutions, Organizations That Will Provide laboratories or funds.

Bustar Alimentos SAPI de CV
Address: Miguel Angel # 16 Col. Real Vallarta, CP.45020, Zapopan, Jalisco.
Telephone: 3338142031

4b. Specify if the Researchers receive payment (monetary or in kind) for Their participation in the specific research.

If this is the case, Please describe.

The Researchers will not receive any kind of payment.

5. Abbreviations.

Definition of abbreviations used in the document.

° C	Degree Celsius
µL	microliters
SCFA	Short chain fatty acids
cm	centimeters
DNA	deoxyribonucleic acid
FC	Functional constipation
G	grams
h / hrs	hours
INCMNSZ	National Institutes of Medical Sciences and Nutrition Salvador Zubirán
KCl	potassium chloride
KH2PO4	monopotassium phosphate
mg	milligrams
min	minutes
mL	milliliters
mM	millimoles
mmHg	Millimeters of mercury
Na2HPO4	sodium phosphate
NaCl	sodium chloride

PAC-QOL	Quality of Life Questionnaire for the evaluation of the patient With constipation
PCR	Polymerase chain reaction
RNAr	Ribosomal ribonucleic acid
SF36	Quality of life questionaire
SII	Irritable Bowel Syndrome
SmartPill ©	Wireless Motility Capsule
BP	Blood Pressure
CFU / g	Colony Forming Units per gram

6. Theoretical framework

Explain in detail the foundations available to date in Which the Proposed study (biological sense, data About animals or in experiments in humans):
a) background
b) problem definition
c) justification

1. Theoretical framework (It is completely Reorganized)

constipation

Introduction

Constipation is one of the gastrointestinal disorders MOST frequent That Affects around 20% of the western population, being more frequent in the female gender (M1.5: H1). Constipation can be primary, When causes constipation associated With (medication, mechanical obstruction, spinal cord injury, etc.) and secondary, When other factors Have Been diagnosed, more Frequently in population With onset or worsening of constipation after the 50 years of age1. Functional constipation defined by the ROME III criteria requires the presence of symptoms 3 days in a month for 3 months, continuous or discontinuous, Whose symptom onset has-been in the last 6 months: 1) Increase in pushing, 3) hard stool, 3) feeling of obstruction / blockage During evacuation, 4) use of digital maneuvers,

Besides age and genre, other risk factors are associated With constipation: race, physical inactivity, low socio-economical level, chronic use of medication, low intake of fiber and water and psychological comorbidity (eating disorders, depression, etc.). 1

Treatments

There are multiple therapeutic options for the management of constipation, changes in lifestyle Including, the intake of fiber, laxatives and pharmacological treatment and in selected cases (colonic inertia) surgery. The first option in the therapeutic management for Patients With constipation are changes in lifestyle, Among Which is the type of diet (increase in fiber intake, Which Has Been Associated With an improvement in the fecal matter and the evacuation frequency), liquid intake and exercise5 .

The dietary fiber has a role in the Very Important gastrointestinal tract, esta happens With any non-digestible carbohydrate (CH) That gets to the color, ferments intestinal bacteria Where it partially or completely, for short chain fatty acids (SCFA) and gases. Dietary fibers can be associated gastrointestinal symptoms With, mainly flatulence, abdominal distention and pain, as well as meteorism, due to the anaerobic bacteria in the fermentation of fiber colon6.

Many types of fiber Have Been Used, Plantago Psyllium being The One with more scientific evidence backing it up, starting at dose of 10g per day, at Least for 4 Weeks with an average response of 57%. Plantago psyllium is a long chain Considered moderately fermentable CH; it is a type of mucilage That Provides viscosity to stool through formation of gels I related to a peristaltic action in the intestine and Causing Increase in the intestinal motility. It is one of the agents of fecal matter forming Most Used for constipation control7-8. Among the contraindications for ITS use are: intestinal obstruction and sub-occlusion, abdominal pain, nausea, hypersensitivity, flatulence, abdominal distension and severe meteorism9. ACCORDING to theAmerican Gastroenterological Association Medical Position Statement on Constipation (AGA) They recommend Plantago Psyllium With a supplementation of 15g / day in Patients With Functional constipation10.

The genre, Agave Tequilana Webber var. Blue stands out as an agronomically and economically crop in Mexico Important Which has relevance in the national and the international market, Besides its usefulness in the sugar and soluble dietary fibers, for ITS prebiotic fructans and Its beneficial effect ability in health. About 60-85% of the carbohydrates in agave are fructans and the type of link β (2-1) presents it causes the salivary, gastric and enteric enzymes not to be hydrolyzed by humans, Which causes them to arrive intact to the intestine and can be subject to fermentation by the colon microbiota, especially bifidobacterial by. It Has Been Reported That Can Regulate the intake ITS lipid metabolism through the synthesis of triglycerides, and thanks to it soluble fiber percentage, beneficial effect It provides on the gastrointestinal physiology,

Thanks to its branched structure That Confers ITS prebiotic effect at the same time it is classified That as soluble fiber, Which is fermented in the colon by bacteria endogenous, giving rise to metabolic and energetic substrates. Due to ITS osmotic potential and Its excessive fermentation, some secondary effects Caused by ITS Intakes are flatulence, meteorism, abdominal pain and discomfort, as well as diarrhea17,18.

It has-been associated in vitro and in vivo preliminary studies, with the Increase bifidobacteria and lactobacillus of the population When They are Compared with a placebo. In the same way, there is a trend of

Presenting an improvement in the consistency of evacuations, as well as a decrease in the feeling19,20 constipation.

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***Agave tequilana*Weber var. blue**

The family of Agavaceae is endemic of the American Continent; it is grown in semi-arid areas in the North and the Center. Its origin and greater diversity are in Mexico, Where it is grown in 26 states. Besides, the 75% of the total of the species in the Americas are in our country and gown 55% are endemic11,23.

The main product of the agave fructans are photosynthesis, are defined as That fructose polymers linked by β-fructofuranosyl bond, soluble in water and to a terminal glucose molecule by. These are synthesized and stored mainly in the heart of the agave plant and Its main role is like a book carbohydrate.14,24.

There Have Been Reported more than one type of agave fructans in, and it has-been noticed esta That depends on the species. At the same time, the complexity and heterogeneity of These molecules are Attributed to the presence of glucosyltransferases That Have activity and specificity owners giving as result of fructans composition and distribution in each species14,23.

Fructans can be classified to the type of ACCORDING bond, structure and the position of the glucose residue in:

1. **inulin:** bond β (2-1) straight chain.
2. **Inulin Neoseries:** With two straight chains β bonds (2-1) and a sucrose molecule Between them.
3. **Levans:** bond β (2-6) to straight chain and terminal residue sucrose.
4. **Levans Neoseries:** With two straight chains β bonds (2-6) and a sucrose molecule Between them.
5. **Mixed fructans:** They have β bonds (2-1) and β (2-6), branched chains of glucose and a.

Of the total carbohydrates in the agave plant, Between 60 and 85% are fructans, Which Have Been Reported to have a varied Polymerization (number of monomers in Its chain) degree That goes from 3 to 35 fructose in STI structures, Besides 20% of bonds β (2-6) (2.7) 25.

Mechanisms

Fiber metabolism

The dietary fiber has an Important role in the gastrointestinal tract, This Happens With any non-digestible carbohydrate That gets to the color, Where it is partially or totally fermented by intestinal bacteria, in order to produce short chain fatty acids (SCFA) and gases like it is shown on the Figure6.

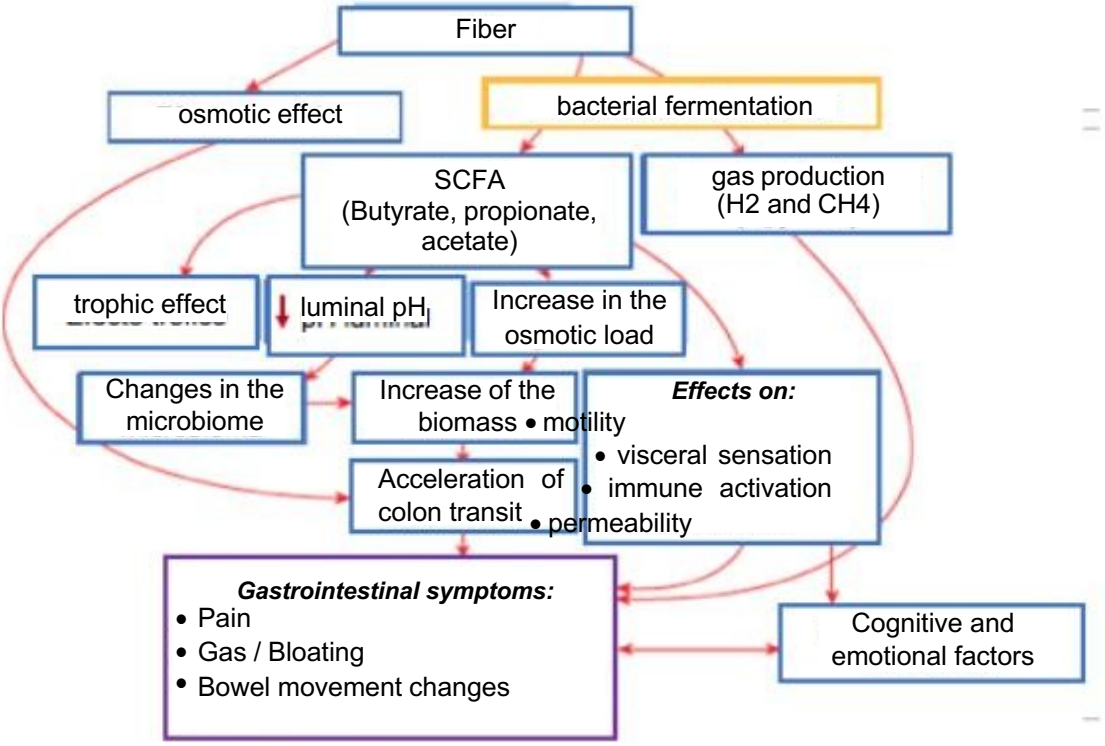


Figure one. Metabolism of fiber. Source from Shanti Eswaran (2013)

Dietary fibers can be related to gastrointestinal symptoms, mainly flatulence, bloating, abdominal pain and meteorism, due to fiber fermentation by the colon's anaerobic bacteria. These effects are attributable to fructooligosaccharides (FOS) and galactooligosaccharides to (GOS) 10. These are highly fermentable by microbiota When They get to the colon, Causing different types of gas, hydrogen and methane Such as predominantly. Which is why, in order to Achieve a relief of symptoms, it is recommended the fiber intake is That Carried out to get a better Gradually adaption of the gastrointestinal tract26.

Fructooligosaccharides' metabolism

The intake of carbohydrates Certain causes fermentation in the intestine, Which is a complex process dependent of the interaction of a group of bacterial enzymes. The end products of the fermentation include gas (hydrogen, CO2 and methane), short chain fatty acids (butyrate, acetate and propionate) and organic

acids. The fermentations of the different substrates by host microbiota is influences by conditions, the colon's physicochemical medium, the competition for the nutrients and the metabolic interactions Among bacteria27.

The fermentation in the proximal colon is intense, the short chain fatty acid production is high, the pH is acid (pH 5-6) and the bacterial growth is fast. In the distal colon, due to a lower substrate availability, the pH is neutral, the putrefaction procesos are quantitatively more Important and the bacterial growth is low.

The colonic microorganisms Participate in the vitamin (K, B12, biotin, folic acid and pantothenate) synthesis and amino acids from ammonia and urea. They Also for the calcium, magnesium and iron absorption. The ion absorption in the proximal colon is Favored by the carbohydrate fermentation and the short chain fatty acids production. Butyrate is completely absorbed by the intestinal epithelium and is the main source of energy for the colonocytes. It provides About 50% of the daily energy requirements of the gastrointestinal mucosa and it Participates in the cellular differentiation and proliferation28,29 mucosa. Acetate and propionate Provide energy to the brain, the muscles and the heart29. They are in circulation and website are metabolized by the liver Eventually (propionate) or by the peripheral tissue, especially in the muscle (acetate).

The Most Important role of These short chain fatty acids on the colonic Their physiology is trophic effect on the intestinal epithelium. The epithelial cell differentiation is by ITS AFFECTED interaction wit the resident microorganisms. The three short chain fatty acids Stimulate, in vivo, the proliferation and differentiation of the intestinal epithelium cells. In vitro, butyrate have antagonistic effects, since it is able to inhibit cell proliferation and neoplastic epithelial cells Stimulate the; at the same time, it can neoplastic cell reversion to Facilitate Normal cells28.

FOS are, probably, the MOST Studied prebiotics and confirmed by They are in vivo and in vitro studies for Their ability Within the microbiota, to selectively Stimulate the SPP29 Bifidobacterium genre. Gibson and Wang That Bifidobacterium infantis image information not only grows very well in presence of FOS, but it Present Inhibits E. coli and Clostridium perfingens. FOS and inulin Have Also Showed to be very selective in stimulating bifidobacteria in vitro complex symptoms, simulating the microbial diversity in the human intestine30,31.

Even though the in vitro models of the human intestinal microbiota are useful for the development of the prebiotic potency of the oligosaccharides Compared With the prebiotic capacity of different oligosaccharides under controlled laboratory conditions, the final stage With the prebiotic activity validation can be only derived from the human intake studies.

When inulin is ingested, it arrives in the intestine almost complete, That is, it is not hydrolyzed by digestive enzymes when it goes through the mouth, stomach and small intestine. Due to ITS solubility in water, the only effect That Has Been Observed in the small intestine is the fecal volume on the Increase. On the other hand,

by not being fermented, monosaccharides are not produced Which is why it can be useful for diabetic people, since the glucose levels in the blood do not increase32 The inulin fermentation process is Carried out in the large intestine, Where the fermentation products are short chain carboxylic acids such as: acetate, propionate, butyrate and L-lactate (oligosaccharides oxidation products), Which are Important substrates or intermediaries in the intestinal microbiota in the cell proliferation and metabolism; H2, CO2 and CH4 gases are FORMED Also, metabolically Which are unnecessary for the organism33.

The mechanism through Which the short chain carboxylic acids (propionate, lactate and acetate) are absorbed in a 90-95% through the intestinal wall, are incorporated to the site circulation and are taken to the liver for Their use, is unknown. However, of the absorbed acetate, About 25 to 50% since it is not metabolized into the muscle arrives Directly circulation through tissue. Butyrate does not pass to site circulation since this is the substrate That Provides energy to the microbiota (colonocytes) and for ITS proliferation34.

Inulin fermentation Decreases the colon's pH and feces as a consequence of the production of short chain carboxylic acids, it modifies the colonic microflora Significantly Increases and dry weight of the feces. As side effects, it causes intestinal distention at level produced by mainly due to a change oligosaccharides in the osmotic pressure, especially by an Increase of water in the colon, Which is translated as a possible laxative effect. Another side effect is flatulence due to the gas production and short chain fatty acid formation During fermentation34. That literature says people's sensitivity due to the fiber intake is variable.

People are not sensitive Those Who Can intake 40g / day or more without Having the aforementioned side effects. People are sensitive Those Who Can intake 10g / day without presenting unintended reactions, and people are very sensitive Those Who can present adverse effects from 10g / day.35

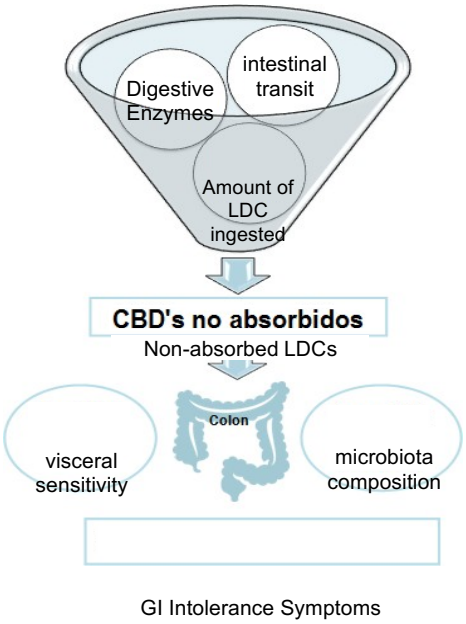
Tolerance and clinical adaptation to fructans intake

Low-digestible carbohydrates (LOQ) are absorbed in low or absent in the amount small intestine and pass to be fermented by bacteria Residing in the colon36. Due to the fructans' high fermentability, They are Frequently associated With the development of gastrointestinal symptoms: such as distention, Borborygmus and flatulence37,38.

In general LDCsare well tolerated, however, they can cause a reaction They dose-response linked to ITS osmotic nature and gastrointestinal symptoms Causing excess fermentation. The benefits fromLDCTheir are Limited by tolerance, since the fermentation process causes abdominal pain, gas and diarrhea. However, the symptoms presented by the intake of carbohydrates does not Represent any threat to the life of the person WHO Takes them.

The gastrointestinal symptoms are produced in a fast way and taken as They are side effects or intolerance. On the other hand, reference to the tolerance Makes development of "no response" Referring to SpecificallyLDCs, The tolerance Refers to the absence of gastrointestinal symptoms After their intake.

Tolerance is different in every subject and it is influenced by the different factors presented down (Fig. 1), furthermore, influenced by the They are amount ofLDC concentration, the amount of food consumed, the frequency of the consumption, consumption of other food (that Increase tolerance), and the presence of water (Decreases tolerance) 36.39.



Regular LDCintake can improve increase the intestinal microbiota ability to ferment carbohydrates's (bacterial adaptation), in Addition to decreasing diarrhea as symptoms Such (clinical adaptation). Additionally, the intake of small doses of These fructans Throughout the day can decrease the presence of symptoms, and With a regular intake, the dose can be Increased without Increasing the symtoms36,37,39.

toxicologic elimination

In order to determine if inulin / oligofructose and FOS are safe for ingestion in high Concentrations, toxicology safety studies Have Been Carried out in mice and rats expose to a diet With FOS (0, 3, 6 and 9 g / kg) (average DP 3.5) for 10 weeks. The results Showed That FOS do not Affect state of health or rats and mice Makes weight gain FOS When They are administered doses of 9g / kg. In another study Carried to evaluate- out in Wistar rats Subacute Toxicity (SPF) Where the dose 1.5, 3 and 4.5 FOS / kg dose was tested, for 6 weeks. On the 2nd, 4th and 6th week blood samples Were taken from the animals and after That They Were sacrificed in order to

Obtain the liver, kidney, brain, cerebellum, heart, lungs, spleen and the pituitary gland. The results revealed no abnormalities That Were Examined found in the organs, there is no concluding That toxicity related with the treatment in any of the groups Treated With FOS Until a 4.5g / kg dose for 6 weeks.

The genotoxicity studies of FOS (DP 3.5) Were tested in three assays: 1) mutation assays of inverse microbial strains on Salmonella typhimurium TA1535, TA1537, TA 1538, TA98 and TA100, and Escherichia coli WP2 uvr in A; 2) L5178Y assay of the mutation of mammalian cells lymphoma TK on mice and 3) not programmed induction of DNA synthesis in human epithelioid cells (HeLa S3). A wide range of test dose was used in every assay, with and without metabolic activation. The results Showed absence of genotoxic potential When FOS was used.

The test Concluded That there was no toxicity, carcinogenicity or genotoxicity Observed related to the treatment, from the doses higher than the expected human exposure.

Regarding use it in food, the actual problem is not safety, but gastrointestinal tolerance. Intolerance can Appear With Intakes signs over 20-30g. This is equivalent to a daily fiber value That Consumers Have Currently Achieving trouble.

Taking into account the current requirements for labeling of dietary fiber, will be the Consumers able to make adequate and personal decisions About the daily intake.

It is Concluded That type fructans inulin is safe for human consumption under conditions of use as dietary fiber.40

Breath Test

One of the MOST useful, non-invasive diagnostic tools in gastroenterology for dyspepsia, malabsorption, lactose intolerance evaluation, Among Others, is the Breath Test. Through Exhaled air, more than 2000 humans expel different substances, Where each one Gives us a clinical usefulness; The most Studied gasses are hydrogen (H_2) and carbon dioxide (CO_2).

The most frequent gastrointestinal Among Indications are the assessment of intolerance to carbohydrate absorption, bacterial overgrowth, orocecal transit estimate, etc. (41)

The Hydrogen breath test (H_2) Consist on the periodic measurement of H_2 and methane (CH_4), are produced by gasses That bacterial fermentation before non-absorbable carbohydrates. CO_2 is produced by bacteria, but only hydrogen and methane are measurable metabolic products Considered (42).

The malabsorption of carbohydrates is a common condition That presents symptoms at the abdominal level, ITS Which is why detection has an Important clinical impact (43).

The β (1,2) bonds of fructans -glycosudics preserves the molecule From its metabolic decomposition in the small intestine, it can arrive That Guaranteeing to complete the caecum; Place Where it is fermented by bacteria, producing oxygen, methane, CO2 and short chain fatty acids. The substance does not cause significant osmotic effects, not the intestinal transit Directly Affecting.

The advantages of ITS is esta substract low cost Compared With lactose. The hydrogen test WITH fructans is used for the transit orocecal assessment44.

Colonic transit time is Measured by Usually radiopaque markers; however, recent studies Have Demonstrated the efficacy of the use of the wireless motility capsule (SmartPill ©) for the evaluation of gastrointestinal motility in Patients With constipation. This capsule has pH sensors (0.05 to 9 pH units), temperature (25-49 ° C) and pressure (0-350 mmHg). In Patients With constipation it Has Been Reported That is slow gastrointestinal transit When Compared With healthy subjects, Obtaining results through the use like radiopaque markers of. Among the advantages of using the SmartPill © Is That esta study is performed under usual conditions psychological and physiological[44].

Quality of life

Recent studies show gastrointestinal disorders That Patients With (especially chronic constipation and irritable bowel syndrome) Often experience a lower quality of life than the overall population. To Establish esta relationship, the SF-36 questionnaire has-been used Frequently, Which Evaluates the physical function and limitation, the limitation due to emotional aspects, emotional well-being, the sensation of pain and energy as well fatigue as, the social function and the perception of health in General and thanks to this, a lower quality of life has Been Demonstrated, with a noticeable effect mainly in the psychosocial spheres and in the perception of pain45,46.

Finally, there are different compounds in the market made with different combinations of fibers for use Promoted That are in Patients With constipation, lack scientific evidence however MOST to support Their prescription and use.

background

Preclinic information

1. **Evidence from studies With inulinslos authors uacionr the actual effectiveness of each type of fiber used for the treatment of constipation clrada placebo**

In preliminary studies the prebiotic effect and tolerance of inulins in healthy subjects Have Been EVALUATED in changes in the composition of the microbiota, gastrointestinal signs and symptoms as well as in various diseases (cardiovascular and endocrine) Table 1.

Bruhwyler et al.(2009) gastrointestinal Compared tolerance in different doses (5, 10 and 20g) of fructan inulin, Where They Observed That the three groups dig a to Increase gastrointestinal symptoms (flatulence, borborygmus, distention, abdominal pain, abdominal cramps, nausea, evacuation frequency and stool consistency) Regardless of the dose, the average symptom score was 19mm with a maximum scale of 800mm, with the dose of 20g the intensity of the symptoms was reported almost moderate as with a significant difference When Compared with the other doses ($p < 0.001$) , however Patients Reported good tolerance With the 3 deuces used. 37

Bonnema et al.,(2010) determined to the gastrointestinal tolerance of inulin type fibers, short chain oligofructose and inulin native Were randomized in four groups: 1) 5g oligofructose, 2) 10g oligofructose, 3) 3.5g inulin, 4) 10g of inulin. These Were Compared with a Control That was 5 and 10g of maltodextrin, in the results They Observed That the 4 treatments Tends to Increase the score of gastrointestinal symptoms Compared to the Control Where the average score of the Control was of 2 Compared To Those Who Took 5 and 10g of oligofructose (2.8 and 3.6) and 5 and 10 g of inulin (2.5 and 2.7), however there was no significant difference Control Compared to the except for the 10 g of oligofructose ($p < 0.05$). The most frequent symptoms Among Were distention (38%) and flatulence (42%).48

Author / Sample	Design / Intervention	results
Bruhwyler, J, et al / 2009 Sample size = 84 healthy volunteers Age (18-45 years) BMI: 25.1 kg / m2 Total fiber intake 12 g	Randomized, double-blind, placebo-controlled, crossover clinical trial 1. Fibrulose F97 (5 and 20 g / day) 2. Instant Fibruline (5, 10 and 20 g / day) 3. Fibruline XL (10 g / day) vs. Placebo Five two-week periods: placebo treatment 1 wash With placebo, treatment 2 placebo withdrawal	**<u>Digestive symptoms:</u>** All three products Tended to Increase digestive symptoms (flatulence, Borborygmus, distention, abdominal pain, abdominal cramps, nausea, evacuation frequency and stool consistency) Regardless of the dose. Changes Were mild (maximum, +19 mm 800 mm on the scale) and significant ($p < 0.001$) for Instant Fibruline at 20 g / day. Significant Difference between Fibruline Instant and Fibrulose F97 ($P = 0.011$), with 20 g / day Presence of dose-effect relationship: both for Fibrulose F97 ($P > 0.05$) as for Fibruline Instant ($P = 0.042$). The rest of the treatments Were not significant.
Bonnema, A. et al 2010 Sample size = 26 13 men, BMI: 24 ± 2 13 women, BMI: 23 ± 3 Age: 18 to 60 years.	Double-blind, placebo-controlled, crossover study Treatment groups every week 10 I. 5g oligofructose II. 10g oligofructose III. 3. 5g inulin IV: 10g inulin V. Placebo: 5g maltodextrin 10g maltodextrin Intervention: 1 bagel (120 g), 28g cream cheese 296 mL orange juice. + 1 of the treatments in the orange juice	GI tolerance through assessment questionnaires: 0, 2, 4, 24 and 48 hours **<u>Gastrointestinal symptoms:</u>** Both inulin fibers tend to lightly Increase the symptoms. **<u>Most frequent symptoms:</u>** Flatulence followed by distention. 10g dose oligofructose Increase the GI symptoms vs Control Native inulin dose up to 10g / day and up to 5g / day of oligofructose Were well tolerated by the subjects. The symptoms Were not consistent Among the subjects; MOST of them did not Have When tolerance problems to inulin or oligofructose adding Their breakfast.

Telephone evaluation During five visits during breakfast	in each visit	

1. Evidence of studies With Agave

What is more, preliminary studies the use of proven Have Agave fructans as prebiotic effect in in vitro models, as well as healthy subjects. . Gomez et al, (2010), through and in vitro study, Observed the properties and the potential prebiotic activity of Agave fructans, Compared with 5 types of soluble fiber of commercial brands: Synergy, FOS-Raftilose, Predilife, Actilight, Inulin HP Cellulose and to control. The prebiotic and the short chain fatty acid production in Said Were EVALUATED study, through the comparison of discontinuous anaerobic cultures and Inoculated With human feces. The results Obtained Showed a significant Increase of bifidobacteria and lactobacillys ($p = <0.05$) in the fructan Compared With cellulose substrate.

Holscher et al., (2014), Through a randomized, double-blind, controlled, crossover clinical trial, EVALUATED the gastrointestinal tolerance in healthy subjects With an Agave inulin Where significant Increase of the symptoms: such as bloating, flatulence and borborygmus in subjects Who Took 5 and 7.5g of Agave inulin Compared to the controls (0g), with significant Differences in distention and Borborygmus ($p <0.05$) as well as an Increase in the subject's number of evacuations Who Took the 7.5g dose ($p <0.05$), however, the presence of symptoms was well tolerated by the healthy subjects. Conversely, was an Increase in the production of Observed Compared to the H2 statistically significant difference in the groups That Took Agave inulin (5 and 7.5g) vs controls ($p <0.001$) 38.

Holscher et al.(2015) Carried out a crossover study in three healthy subjects 29 periods Where Were Given 5 and 7.5g of agave inulin for 21 days with 7 days of washing Between periods; Their subjects recorded the daily dietary intake and fecal samples Which Were Collected underwent fermentation 16s Illumina sequencing and analysis. The results Obtained Showed That the subjects ingested 7.5g of WHO agave inulin did not report adverse effects and the subjects ingested WHO 5 and 7.5g of agave inulin the number of Significantly Increased faecal Bifidobacteria (basal = 1.7 vs 4.9 Final $p <0.01$). Compared to the controls did not ingest agave That inulin50.

In another crossover study (Ramnani et al., 2015), in Which 38 healthy subjects Were Recruited, 19 of Said subjects Were Given 5g of agave fructans (experimental) and the remaining 21 Were Given 5g of maltodextrin (control) for 3 weeks followed by 2 weeks of washing. From the results Obtained, it was found That the subjects WHO ingested the agave fructans Showed an Increase in the number of fecal Bifidobacteria (basal 9.2 log10 - Final 9.6 log10 ($p = 0.01$) and Lactobacillus (basal 7.3 log10 - Final 7.7 log10 ($p = 0.001$) Compared to the

WHO subjects ingested maltodextrin (Bifidobacteria: basal 9.2 log 10 - log 10 9.2 end; Lactobacillus: basal 7.3 log 10 - log 10 end 7.4) 0.51

Author / Sample	Design / Intervention	results
***Gomez E, et al*, 2009** **Sample size 5 fibers** one. Synergy1 2. FOS-Raftilose 95 3. Agave fructans (Predilife) Four. Actilight 5. HP Inulin **Fecal matter of the subjects:** 30-40 years	*In vitro study* Of fecal matter 1g 15 mL of fecal matter suspension Fermentation: 24 hours	**<u>This study PROVED:</u>** 1. Changes in the bacterial population: Agave fructans Predilife and Riftilose 95: Increase in the Bifidobacteria concentration (8.92±0.18) of the basal p <0.05. Cellulose: There was no statistical significance. Agave fructans (Predilife), Sinergy 1 and Raftilose 95: Increase in the Lactobacillus concentration (10h of fermentation) vs Placebo. There was no significant difference Between the different fructans. 2. **<u>Total production of SCFA vs Cellulose:</u>** like in all the fructans. ↑ Acetate production: more prevalent in all the subtracts Agave fructans (Predilife): (22.38 ± 9.19) (10 h) Cellulose: (4.55 ± 0.80), p <0.05 Followed by Raftilose 95 ↑ Propionate production: Agave fructans (Predilife): (18.32 ± 17.4) (24h) Cellulose: (1.61 ± 0.59) p <0.05 Followed by inulin HP ↑ Buyirate production:> Raftilose Agave fructans (Predilife): (0.98 ± 0.85) Cellulose: (1.61 ± 0.59) p <0.05
***Holscher HD, et al*., 2014** **Sample size:** 29 healthy volunteers **Age:**20-40 years	Randomized, double-blind, controlled, crossover clinical trial. Intervention: 21 Days with a wash period of 7 days Group 1: 0g (Control) Group 2: 5g (Agave inulin) Group 3: 7.5 g (Agave inulin)	**<u>Clinical symptoms</u>** Significant Increase in symptoms as bloating and flatulence Such Those subjects in WHO Were Given 5 and 7.5 g of the Agave inulin (p <0.05). The symptoms Were Reported mildly. Increase in the number of bowel movements and stool improvement in the consistency of WHO Were Those subjects administered 7.5g (p <0.05) **Gas concentration (H2)** The concentration of hydrogen Increased in Those Were Given subjects WHO 5 and 7.5g of Agave inulin Compared to the control (p <0.01)
***Holscher HD, et al*. 2015** **Sample size =** 29 healthy volunteers **Age:**20-40 years **BMI:**> 18.5 and <29.5 kg / m2	Randomized, double-blind, crossover clinical trial Intervention: 21 days with a period of 7 days wash Group 1: 0 g (control) Group 2: 5g (Agave fructans) Group 3: 7.5g (Agave fructans)	After Supplementation With fructans of Agave: Group 2 and Group 3 Increase in the count of bifidobacteria vs Group 1 (control) **1. Changes in the bacterial population (sequence%)** Group 1: 1.7 Group 2: 3.2 Group 3: 4.9 *P <0.01* **pH Levels:** Decreased after supplementation ***(P = 0.06)*** **2. Clinical changes** Were there no adverse effects with 7.5 g
Ramnani P, et al. (2015) **Sample size =**38 healthy volunteers	Randomized, double-blind, controlled, crossover clinical trial. ***Experimental group:***5g of *Agave tequilana* Weber var blue fructans	**<u>This study PROVED:</u>** **1. Changes in the bacterial population** Exp Group. Agave fructans: ↑ Bifidobacteria (9.6±0.4) p <0.01 ↑ Lactobacillus (7.7±0.8) p <0.001

18 -50 years ***BMI: 18-30 kg / m2***	***Placebo group*** **|:**5 gmaltodextrin	Placebo Group: Maltodextrin, There Were no significant Differences in the bacterial changes. **2. Total SCFA production:** Production of acetate, propionate and butyrate = Predilife and maltodextrin. **Presence of symptoms:** no significant Differences Were Observed in abdominal pain, bloating and flatulence as well as in stool consistency.

5. Problem definition

The initial treatment of Patients with Functional constipation is based on fiber. There are multiple fiber products in the market, which are Promoted as a supplement for constipation without any scientific evidence to support ITS use. Furthermore, the tolerability of These compounds limits long-term Their use in esta patient population. Scientific evidence is required to support the effectiveness in the use of These fiber supplements in Patients With functional constipation.

6. Justification

There are multiple fibers are in the market That Promoted as a beneficial supplement for Patients With constipation, DESPITE esta, there is not enough scientific evidence derived from randomized and controlled studies to support ITS use. THEREFORE, it is Necessary to Provide evidence to support the use of blue agave fructans Tequilana Weber in Patients With functional constipation.

7A. hypothesis

Defined as a verifiable statement About the relationship Between a variable-dependent and an independent variable. (Remember That the concepts of "null hypothesis", "alternate hypothesis" are related to the statistical analysis So They Should not be included in this item)

General Hypothesis:

Plantago Psyllium When Compared With, the blue agave fructans Weber Tequilana have a like effect or are more effective in Improving functional constipation in adult patients.

Objectives

PRIMARY

1. To Evaluate the efficacy of the blue agave fructans Tequilana Weber in Patients With functional constipation.

SECONDARY

1. To compare the efficacy and tolerability of 5 and 10g doses of blue agave fructans Tequilana Weber in Patients With functional constipation.
2. To Evaluate the impact of the consumption of blue agave fructans Tequilana Weber on the quality of life of the subjects WHO ingest it by Applying the quality of life questionnaire SF-36.
3. To determine the impact of the blue agave fructans of Weber Tequilana intake in the fasting glucose, glycosylated hemoglobin A1c and the lipid profile (metabolic profile) of the subjects WHO ingest it.
4. To Evaluate the presence of short chain fatty acids (butyrate) before vs after the treatment with blue agave fructans Tequilana Weber.
5. To determine the impact of the ingestion of blue agave fructans Tequilana Weber in the microbiota before vs after ingestion.
6. Evaluate to the tolerance to the ingestion of blue agave fructans Weber Tequilana through the use of breath test.
7. To evaluate- gastrointestinal transit in Patients With functional constipation and after ingestion of before blue agave Tequilana Weber fructans.

8. Methodology: General Design.
Describes the overall design of the study and, if relevant, specify the following points:
a) Study design: Describes if it is randomized / non-randomized, controlled, cohort, Blinding type (double-blind, single), type of controls (placebo, active medication), wash period.
b) Maneuver or intervention description.
c) Sample size (# of Patients to be included; justify the calculation)
d) Treatment allocation mechanism (random / open)
e) Treatment groups and
f) Duration of Individual follow-up

Study Design

This is a prospective, randomized, controlled and single blind study to determine the phase III efficacy of the blue agave fructans Tequilana Weber intake in subjects with functional constipation.

In order to Achieve so, it is planned to include over 18 years of Patients age Fulfill the WHO as well as inclusion criteria The Rome III criteria for functional constipation. (See table below) 2.

The phase of our study is designated based on article 66 of chapter II "On Pharmacological Research" of the Regulation of the General Law on Health in Research That defines PHASE III studies Follows as: It is the administration of a research drug to large groups of Patients (Usually external), to define ITS therapeutic utility and identification identify adverse reactions, interactions and external factors That May alter the pharmacological effect.

ROME III criteria

To Comply with the presence of symptoms for more than 6 months before diagnosis, Having Said symptoms for at Least 3 consecutive months or not, and:

1. Absence of abdominal pain or discomfort in the last 3 months.
2. Include 2 or more of the following symptoms.
   Excessive effort in more than 25% of the evacuations.
   Hard stool or "pellets" in more than 25% of evacuations.
   Sensation of incomplete evacuation 25% of evacuations.
   Sensation of blockage or blockage in 25% evacuations.
   Maneuvers to Facilitate expulsion in 25% of evacuations.
   Less than 3 evacuations per week.
3. Stool consistency decrease, rarely, without the use of laxatives.
4. Insufficient criteria of IBS.

Patients of the institute will be called after invitations to Participate in Placing a protocol for Patients With constipation. Patients Who Have Not Been registered and meet the inclusion criteria May Also Participate.

The study to be Carried out will be Explained and read and, where appropriate`, the informed consent will be signed by the Patient During the initial visit (Visit 1) of eligibility, Where the subject will be Given 2 questionnaires (see annexes), one They meet to determine if the ROME III criteria for functional constipation (FC) and another to ASSESS if the patient does not meet the criteria for irritable ROME III bowel syndrome (IBS) and a baseline breath test will be performed.

Having met the inclusion criteria, the patient will be EVALUATED by a graduate in nutrition with the objective of Evaluating the fiber intake in the subject's usual diet. The amount of daily fiber intake will be standardized and a diary will be Given to Evaluate Said intake (see annexes) as well as a diary Where the subject will keep a record of his / her evacuations (see annexes).

In Addition, the previous use of fiber supplements and laxatives, which must be suspended at Least 2 months prior to randomization, will be EVALUATED.

The subject will return to a second visit (Visit 2) (Which will depend on Whether the patient is taking laxatives or fiber supplements) for a complete physical evaluation and the fiber intake diary will be Assessed to determine the amount of fiber intake in the diet as well as the diary of evacuations and the SmartPill © will be Given and That way, the patient will return to the hospital to register the data after the capsule Having evacuated.

The patient will return a week later (Visit 3) on a 12 hour fast, the fiber intake and the evacuation diaries will be re-EVALUATED and will be EVALUATED They again by a nutritionist. A new breath test will be Carried out. The subject will be

Asked to complete the SF-36 Quality of Life Questionnaire. A stool sample will be Collected for ITS analysis in Addition to a blood sample for determination of fasting glucose, glycosylated hemoglobin A1c, Insulin and full lipid profile.

The subjects will be randomized to receive one of four treatments That can be Psyllium plantago 15g, blue agave Tequilana Weber fructans at doses of 5, 10g and blue agave Tequilana Weber fructans at a dose of 5 g + 10 g excipient for a concentration of 33 % of blue agave fructans Tequilana Weber.

The randomization will be computer-generated with a participation number and the assignment of the treatment will be consecutive.

The product will be supplied in identical bottles With the same amount of powder in a container That will only be identifiable With the name of the protocol as well as the patient's number.

The patient will be provided with a measuring spoon to administer the amount of product Necessary for each of the doses (5, 10 and 15g respectively) or with envelopes Containing the exact Amounts Weighed.

The patient will be instructed to keep a diary of the diet, of the evacuations and a sheet to ASSESS adverse effects. The subject will return (Visit 4) at the end of a month ± 5 days on a 12 hour fast and Their adherence will be Assessed by product weight in the container subtracting the number of grams required daily ACCORDING TO the assignment of treatment.

The fiber intake diaries will be EVALUATED again by the nutritionist, the evacuation will Also be EVALUATED diary. A medical evaluation will be Carried out That includes questioning and physical evaluation. Subsequently, a blood sample will be to determine fasting glucose Obtained, glycosylated hemoglobin A1c, insulin and lipid profile and a full sample of excrement will be Obtained and SmartPill® will be Given.

The patient will Continue with the supplement in the same container and will be scheduled for a month later. In the end-of-treatment visit visit (visit 5), the fiber will be EVALUATED journals consumption again by a nutritionist and the evacuation will be EVALUATED diary as well.

A medical evaluation will be Carried out That includes questioning and physical evaluation and Their adherence will be Assessed by weighing the product in the container, subtracting the number of grams That it requires daily ACCORDING to the treatment assignment or delivery by the patient of the empty envelopes used.

Subsequently, a blood sample will be to determine fasting glucose Obtained, glycosylated hemoglobin A1c, insulin and lipid profile and a full sample will be Collected stool and breath test at the end will be Carried out.

For end of the visit, Which Will Be Carried out one month after stopping the treatment, the evacuation will be EVALUATED diary and a stool sample will be Collected and with it the intervention in the part of the study will end. Total The follow-up duration for each patient will be 3 months, enough time to observe and compare the effect of the fibers to be used. It is Important to mention That in recent studies Carried out by the same team and with blue agave Tequilana Weber fructans,

effects on intestinal transit Have Been observed from the first week of intake, in fact this is one of the Reasons to focus the study on functional constipation and intestinal transit.

Breath test:

A breath test will be Carried out in order to measure hydrogen and methane Concentrations previousle Exhaled In Patients randomized in visits 1 and 5.

In order to carry out the breath test gas chromatography equipment will be used, brand Quintron Breath Tracker.

For That, The Patients will be Asked:

- Not to take antibiotics prior weeks At least to the test.
- Stop the intake of food rich in fibers and starches 24 h before the test.
- Not to take alcohol, or smoke
- Have a minimum of 8 hours fasting
- Perform oral hygiene before starting the test and during the performance of measurements.

This test lasts 3 hours, during which the patient can not leave the laboratory facilities motility, can not speak too much or sleep. Exhaled air samples are taken every 15 minutes during this period.

SmartPill®:

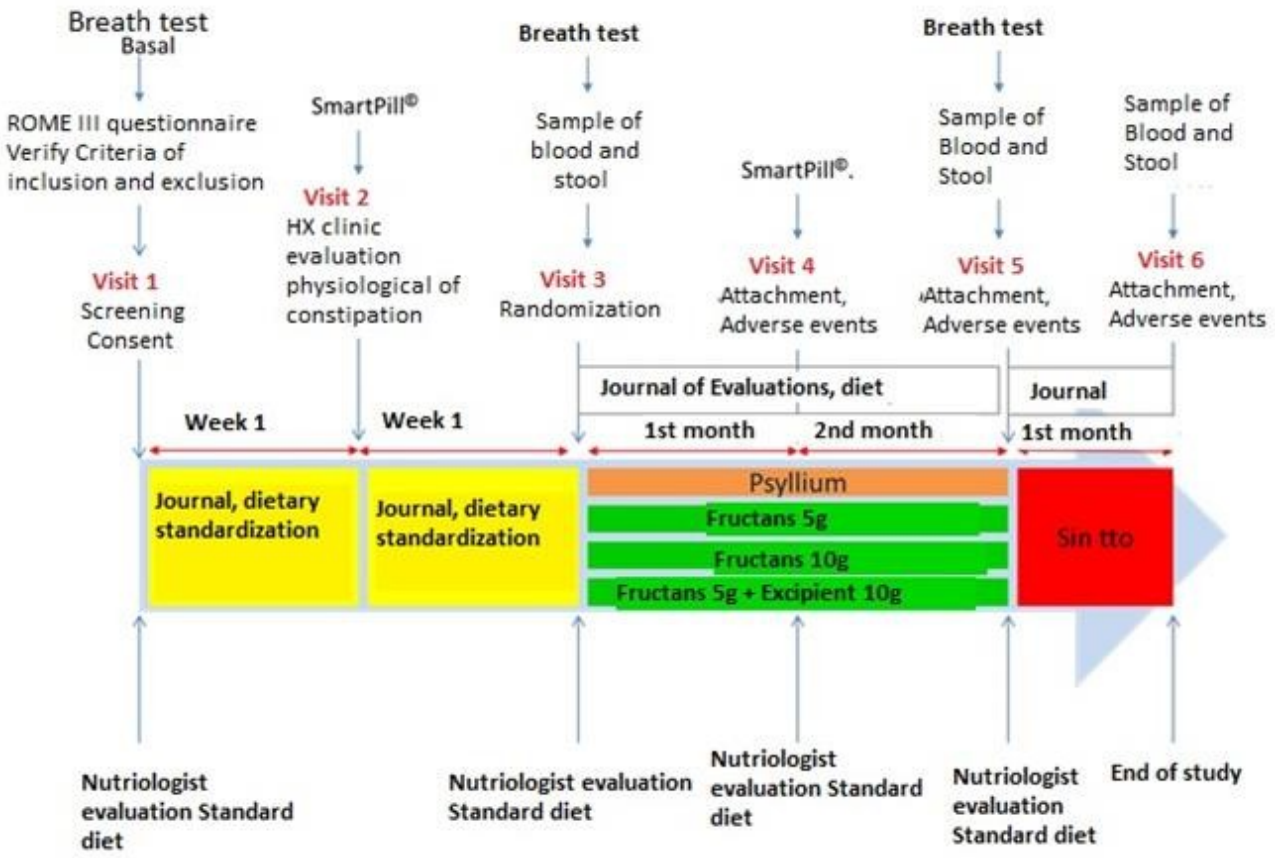
SmartPill® test will be conducted on visits 2 and 4.

colonoscopy:

Colonoscopy study is a tool which will be subject to scrutiny patients ≥55 years with newly diagnosed constipation or those where changes in bowel habits observed in this age group. This fundamental objective, rule out potentially serious diseases and not to delay the diagnosis of these during the research study. one colonoscopic study all patients who have constipation and any of the following characteristics will be used:

1. Diagnosis of constipation less than 1 year
2. Subjects ≥55 years who do not have colonoscopic evaluation in the last 3 years prior to study entry
3. Chronic but with alarm data (: Unexplained weight loss, recent transrectal bleeding <6 months, recent changes in the defecation pattern example) Constipation

Study Flow Chart



Patients include:

Studies with fiber supplements (Psyllium, Plums[8]) Have included 25 to 30 patients per group to find differences between groups.

In total it is planned to include 60 patients randomized as follows: 15 patients receive fructans blue agave Tequilana Weber dose of 5 g, 15 patients received the dose of 10 g, 15 patients received the dose of 5g of agave fructans blue Tequilana Weber + 10 g excipient and 15 patients received 15 g plantago psyllium.

9. Methodology Selection Criteria

a) Inclusion criteria (consider not participating in other research and contraception if necessary)

- Patients over 18 years old.
- functional constipation ROME III criteria as
- Subjects ≥55 years with recent colonoscopy (<3 years)

b) Exclusion criteria

- Taking supplements of fiber in the last 2 months
- Stimulant laxatives consumption chronically (Senna, Bisacodyl)
- decompensated comorbidities (example: uncontrolled diabetes mellitus HbA1c> 9%, decompensated Organ failure, decompensated hypertension (untreated or TA> 160 mmHg systolic and 95 mmHg diastolic)

- Using digital maneuvers or enemas to achieve evacuation
- Personal history of active or previous cancer
- abdominal surgery (except appendectomy, cholecystectomy, hysterectomy)
- Presence of alarm data in the last 6 months (trans-rectal bleeding, unexplained weight loss, abdominal pain)
- Use of drugs known promote constipation (opioid derivatives, clacioantagonistas, anticholinergics tricyclic antidepressants type)
- Prostration (immobility or poor mobility)
- Diseases such as untreated hypothyroidism with hormone replacement, and diseases associated with hypercalcemia that promote constipation
- Other causes of secondary constipation
- Meet criteria for constipation predominant irritable bowel
- Pregnancy.

c) Elimination criteria (considering pregnancy if necessary).

- Requiring individuals to withdraw their informed consent
- acute illness (eg appendicitis, medical-surgical requiring hospitalization intervention and follow-up)
- In our study provides no substitute for patients who have completed or prematurely abandoned the study. It is contemplated an analysis by intention to treat and the percentage of losses for each treatment group will be collected, clarifying the reason why the study was abandoned.

10. Methodology: Outcomes and variables
a) Variables / Main outcome measure
b) Variables / secondary endpoints measured
b) frequency measurements,
c) Criteria for success and failure, and if necessary
d) Strategy statistical analysis.
Where applicable should be specified and substantiated techniques, equipment and / or instruments to be used in the measurement (this includes mechanical / electronic / cyber special equipment, evaluation forms, questionnaires, matching tables, etc.), indicating the validity criteria , reproducibility and quality controls that are the same.

main outcome variable:

Effect of supplementation of fructans Tequilana Weber blue agave in increasing at least one complete evacuation from basal spontaneous.

spontaneous evacuation It is defined as an evacuation in the absence of use of laxatives, suppositories or enemas and complete evacuation is defined as spontaneous evacuation to that sensation of incomplete emptying of the rectal ampulla.

secondary measured variables:

I. Effect of supplementation of fructan Weber blue agave Tequilana in the form of bowel movements, assessed by the Bristol scale. It is defined as a change in the form of evacuations when presenting a change in the classification of Bristol, more than 50% according to the daily evacuations taking the average of the evacuations submitted within 15 days.

II. Effect of supplementation of fructan Weber blue agave Tequilana in the frequency of bowel movements. It shall be defined as responder presenting increased 50% in stool frequency compared with baseline, according to the daily bowel movements taking the average of evacuations presented at 15 days.

III. Evaluate the impact of the concentration of short-chain fatty acid, particularly butyrate, in the feces of subjects before and after addition of fructans Tequilana Weber blue agave. The butyrate concentration was determined by method of gas chromatography and the increase will be assessed in millimoles per liter.

IV. Evaluate the impact of supplementation fructans Weber blue agave Tequilana the microbiota by determining edges present in feces of the subjects before and after the supplementation fructan derivatives fiber; which are:
1) Actinobacterias (Bifidobacteriales)
2) Bacteroidetes (Prevotella, Paraprevotela, Ruminococcaaceae, Faecalibacterium)
3) Firmicutes (Veillonella, Anaerostipes, lactobacilli Clostridiales, Enterococcus)
4) Proteobacteria (Bilophila, Escherichia).
5) Euryarchaeota (Methanobacteriales)

V. To evaluate the impact of serum glucose concentration and HbA1c fasting serum of subjects before and after addition of fructans Tequilana Weber blue agave. HbA1c concentration is determined as it is intended to monitor the impact of fiber on glucose throughout the duration of the study (2 months) and in order to avoid the variability that fasting glucose.

Gas Chromatography: (determination of short-chain fatty acid AGCC)
AGCC for determining ion detector flame will be used. The following steps are performed:

1. ethylbutyrate - 1 g of faeces which is added 50 uL of 2 homogenizará.
2. 1 mL of hydrochloric acid was be added.
3. It is centrifuged at 3000 g for 10 minutes. The supernatant was removed.
4. Subsequently it is added 300 uL N-Methyl Nt metildibutilsililtrifluoroacetamida (MTBSTFA).
5. solution at 80 ° C for 20 min heat up.
6. It left undisturbed at room temperature for 24 hrs to ensure complete derivatization of lactic acid.
7. analyzing samples by gas chromatography according to method developed for specific samples will be performed.

Extraction of DNA from faeces, PCR amplification and bacterial 16S rRNA sequencing:
RRNA sequencing is the method of choice to determine high taxonomic relationships (gender up level). Because the 16S rRNA molecule contains highly variable regions, it is usually possible to find regions 20 to 30 bases that are completely unique to a single species of bacteria.

To perform the analysis, will collect in fecal specimens are sterile plastic containers. Immediately after collection, the samples will be transported to the laboratory in ice bucket 2 to 8 and then frozen at -73 ° C until processing.

DNA extraction was carried out following the instructions of the automated method NucliSENS® easyMAG® (BioMerieux, Durham, NC, USA) with a brief initial modification before automated process. Is weighed and suspended 200 mg of fecal matter of each sample in 0.3 mL of phosphate buffered saline solution-(8 mM Na2HPO4, 137 mM NaCl, 2.7 mM KCl, 1.5 mM KH2PO4 and) vigorous agitation for 30 seconds. 100 .mu.l of the suspension was placed in boiling 15 min and immediately frozen at 0 ° C on ice. Bacterial homogenate was centrifuged at 13,000 g for 10 min and the supernatant will be collected in tubes 500 .mu.l.

This specimen will be used for the isolation of DNA on automated equipment. PCR amplification real time 16S rRNA bacterial be carried out as described by previous reports (Berthelet et al., 1996 and Yuan et al., 2012) using a Light Cycler-(Roche) equipment.

Quantification of bacterial phyla in major categories described: 1) Firmicutes, 2) Bacteroidetes, 3) Actinobacterias, 4) Proteobacteria. It will be conducted by the method of quantitative PCR in real time using the primers and conditions described previously (Arboleya, 2012), pR amplify specific fragments of bacterial 16S rRNA population groups described. We will use primers called barcode 967 F (CNACGCGAAGAACCTTANC) and 1046R (CGACAGCCATGCANCACCT) to amplify the 16S rRNA fragments V6 bacterial V6.

Sequencing and analysis of the amplified fragments is by fluorescent sequencing equipment terminator 3130 / genetic analyzer (Applied Biosystems).

The PCR products were sequenced and analyzed and will be submitted to GenBank for species identification.

Extracting bacterial DNA and quantitation of bacterial species used to demonstrate the prebiotic effect of agave fiber, since selective growth of the population of bifidobacteria and lactobacilli and occurrence of short-chain fatty acid in stool is expected.
Thus in addition to the functional properties and their effects on constipation, you may check the prebiotic effect of agave fructans.

Data analysis:

a) Statistical analysis plan

The data obtained will be analyzed with SPSS version 24. For continuous variables the results are presented in mean ± standard deviation when the variables are normally distributed or medians and percentiles otherwise and as frequency and percentages when they were categorical. For basal and subsequent comparison to the intra intervention groups, Student's t-test for paired samples (variables with normal distribution) or the Mann-Whitney U (variables without normal distribution) is used in the case of continuous variables and X2 or the McNemar test in the case of categorical variables. For comparison between groups ANOVA (variables with normal distribution) or Kruskal-Wallis (non-normal distribution variables) used for continuous variables and the same percentage of change obtained when the variables are continuous.

Also an analysis of covariance (ANCOVA) was performed to control variables that might have an effect on the response variables.

b) Non-inferiority study

There are multiple fibers on the market that are promoted as beneficial supplement to patients with constipation; Despite this, there is insufficient scientific evidence from randomized controlled trials to support its use. Psyllium psyllium, is the first-line treatment used for such functional bowel disorder; fructans tequilana Weber blue variety are soluble fibers which are rich fructooligosacáridos role in prebiotic substance with the which is associated with a number of health benefits including intestinal health; therefore, as an alternative treatment in patients with constipation using these fructans it is raised to generate clinical evidence and demonstrate the therapeutic potential similar to plantago psyllium.

c) Sample size

The sample size was calculated from the formula mean differences for non-inferiority studies and data from 52 studies were used Holscher HD., Et a38, Erdogan A., et al 53 and R Closa-Monasterolo , et al 54. accuracy of 95% (0.05 probability of making an error or type I was usedα) And a force or power of 80% (0.20 probability of committing a type II error or β).

$n = \lambda/\Delta.$

Where:

n = sample size

λ= 10.91

Δ= 1

Where:

$\Delta = \frac{1}{(\sqrt{\sum \sigma})^2} \quad (\sum \sigma)$

σ_1= 0.01

σ_2= 0.12

σ_3= 0.43

σ_4= 0.27

$= \Delta \frac{1}{(0.82)}(0.82) = 1$

$= 10.91 \approx 11 n = 10.91/1$

Taking a 20% loss during the study sample size are:

n =13 ≈ 15

d) Statistical significance

It is considered each of the variables under study as the statistically significant with a value of p <0.05

e) Statistical analysis of the population

The study population are volunteers with functional constipation and statistically analyzed considering the per protocol population and intent-to treat.

f) statistical treatment

It does not, in our strategy of statistical analysis a power of 80% for our study with an alpha of 0.05 is contemplated to demonstrate non-inferiority. Therefore, a greater percentage of losses accepted 20% to give validity to our study.

11. List of materials to use resources.

Team	Model	Description
portable stadiometer	Seca213	Measuring range 20-205 cm Division 1 mm Brand: Seca
Impedance	RJLQIV	Impedance Analyzer Brand: RJL Systems Includes: analyzer, charger case, LEMO cables, resistor, 400 electrodes, software
Weighing machine	BBZ-420	Maximum capacity up to 140 Kg minimum division 100 g Zamac bar with matt chrome Pylons cursors zamac beige or white enameled Base 54 x 20 x 10.5 cm Scale weight: 17 kg authorization NOM Made from sheet metal Finished in baked enamel paint
SmartPill	REF. 50100400	The capsule measured 26.8x11.7 mm and transmits a radio frequency at 434 MHz to an electronic device that uses the patient. The capsule has a helmet containing polyurethane sensors measuring luminal pH, pressure, temperature and a battery with minimum of 120 h. After activation, the pH is measured every 5 sec for the first 24 hours, every 10 seconds from 24 to 48 hours and every 2.5 min after 48 hours; pH changes are detected with a sensitivity of ± 0.5 pH units. Pressure is measured from 0 to 350 mmHg and collected every 5 seconds for the first 24 hours and every second thereafter, the accuracy with which pressures are measured are ± 5 mmHg for pressures below 100 mmHg and ± 10% for pressures greater than 100 mmHg.
Headband	Seca201	Ergonomic measuring tape circumferences Brand: Seca
Food Replicas	WA29168HR	Pack food replica plate kit It Includes: 36 replications
Littmann stethoscope	Master Classic	Physical exploration
Software: NutriKcal	version 0	Analyzes the nutritional composition per serving recipes
Software: Statistical	SPSS version 20	Analysis of data
Software: Determination of fatty acids	GC / MSD Agilent Chemical Station	Determination of fatty acids with spectra library to search NISTC (National Standard Institute and Technology)
Gas chromatography coupled to mass detector	6890	Identification and quantification of fatty acids
PCR equipment	7500 Real Time Fast	Identification of bacteria Brand: Apple Brand BioSystems
Imperial incubator III	310 M	Incubator bacteria Brand: Lab-line
Auxiliary services	----------	The INCMNSZ has a laboratory in which corresponding studies of blood samples will be requested.
Emergency services	----------	INCMNSZ has the emergency room where participants will be referred research protocol if required by the intervention.

12. Risks and Benefits Study

a) Discomfort generated by the study (when blood samples, record the total number of punctures, the amount of blood by puncture and / or total and the frequency of punctures.)

b) Potential risks (presence of complications or side effects, drug interactions considered, considering psychological effects of evaluation methods, v.gr.: surveys sensitive subjects)

c) Detection methods anticipated risks.

d) Security measures for early diagnosis and prevention of risks.
e) Procedures for resolving risks if they arise.
f) Expected direct benefits.
g) Expected indirect benefits.
h) Overall weight of risks versus benefits of the proposed study.

Discomforts generated by the study:

Discomfort from the study are related to the puncture for blood collection (3). It is anticipated that the fiber, when containing fructans, generate a fermentation process with flatulence and bloating. However, reports of studies using the fiber, have shown that this effect is transient lasting 2 weeks.

Risks:

Regulation of the General Law of Health in Research for Health, said that obtaining biological samples represents a minimal risk in the investigation. The risks of blood sampling are: possibility of slight bleeding or bruising at the puncture site, dizziness or fainting and rarely arterial puncture may occur. Personnel draw the blood sample is trained for this, which will minimize the risks of complications.
If there are complications will be referred directly to your treating physician. It is anticipated in the informed consent we do not have resources in case complications arise.

Benefits:

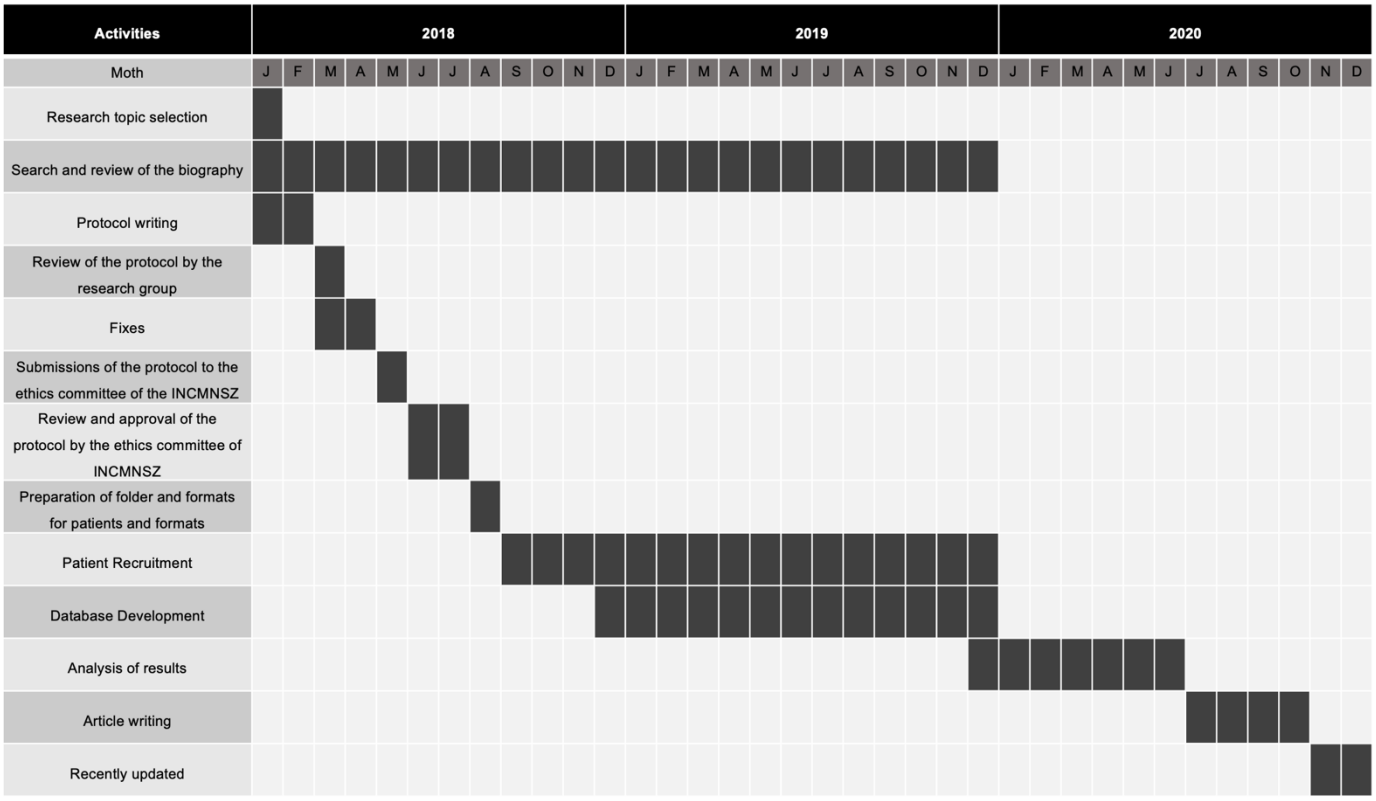
Administration fiber, it has shown to be healthy and beneficial for the treatment of functional constipation. Such supplementation, is the first line treatment of functional constipation. Patients entering this study will benefit from fiber start treatment within the diagnostic and therapeutic approach constipation.
Moreover, thanks to the participation of volunteers, your community can benefit significantly by finding new ways to address this medical complication.

Rescue measures:

If the patient has no spontaneous evacuation after 72h will prompt the patient to contact the research team, for instructions such as laxatives previously prescribed by your physician. If distended or flatulence, using anti detergents (simethicone) that is effective to treat this condition is allowed. For present colic, it can be used a conventional antispasmodic. each of the side effects will be recorded as well as the need for therapies rescues specified above.

13. Schedule of activities

The letter updating the research project is attached. (Annex 1)



14. costs

a) Specify (direct / indirect, monetary, time-sharing, visits / transfers) research costs generated for the study subjects. Specify whether consultations, laboratory tests / cabinet and medical / surgical treatments, generated on the occasion of the study will or not covered by the patient / research subject. Declaration on who will cover these costs.

b) Specify the compensation to be offered (replacement of expenses incurred by participation in the study; v.gr.: payment of transportation, food, accommodation, etc).

c) Specify the incentives to be offered if appropriate (incentive is understood as offers or influence compelled to perform an action without implying a significant deviation from our general plan of life; v gr.: give a book to have. participated)

Note: A compensation / incentive out of proportion is considered a coercive attitude.

1. The fructans Weber blue variety Agave Tequilana They will be provided by the sponsor Bustar Alimentos SAPI de CV
2. Peripheral blood takes performed to determine lipid profile, fasting glucose, glycosylated hemoglobin and insulin; They will be processed in the laboratory of INCMNSZ. The costs of these samples will be borne by the study sponsor Bustar Alimentos SAPI de CV
3. The determination of short-chain fatty acid will be carried out by gas chromatography in stool samples from patients. The samples will be processed in the laboratory of the Faculty of Chemistry at the University La Salle, Mexico City and will be paid for by the study sponsor Bustar Alimentos SAPI de CV
4. The determination of the intestinal microbiota will be analyzed in the stool samples of patients participating in the study and will be sent the Department of Infectious Diseases INCMNSZ for processing. The cost will be covered by the study sponsor, Bustar Alimentos SAPI de CV
5. The gas chromatography equipment brand Quintron Breath Tracker, are owned INCMNSZ.Inputs for performing the breath test will be covered by the study sponsor Bustar Alimentos SAPI de CV
6. Equipment and software interpretation SmartPill ©They are owned by the INCMNSZ. Inputs for testing gastrointestinal transit (wireless capsules, cereal bar) will be covered by the study sponsor Bustar Alimentos SAPI CV

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16. Informed Consent

It Informed Consent as all those actions that promote a process of communication and dialogue that facilitate a person to make decisions about an action, practice or product that affects your body, privacy or other living spaces means. It is transferred to the research subject information in order to enable you to make an independent decision regarding participation or not in a clinical research project. This process is implemented through dialogue with the subject and documented by an institutionalized written (on letterhead of the Institute) and taking into account the guidelines accepted for this:

16a. Patient report sheet to participate in the study (attach a separate sheet).

You must work it out in writing the principal investigator, using understandable language second person for the research subject (v.gr.: You suffer ... and for this reason we invite you to participate ... etc.). the use of multi-language understanding this as phrases to explain the same one level above and one level below the expected level of understanding for the subject to be included is recommended. Give you a copy. This sheet report should include, but not limited to, the following:

The clear description of the rationale and research objectives, procedures to be used, aspects of the study are experimental, discomfort or expected risks and benefits that may be obtained.

The alternative procedures or treatments.

The responsibilities of the patient and physician, including the guarantee to provide answers to any questions and / or clarification about the research protocol.

Compensation in terms of health, drugs, economic (must not be handled as compensation for the donation of an organ or tissue, but as a donation for participation as a research subject), etc., produced by their participation in the study and in case to present any adverse effects.
Participation costs, availability of medical treatment in cases that merit damage directly caused by the investigation are presented.
The guarantee of confidentiality, voluntary participation and to refuse to participate or withdraw at any time without losing their benefits as a patient of the Institute or be penalized.
The reasons that could end the study.
The name and telephone number of investigator in charge.
The name and telephone number of the person who can answer questions or further information about ethical issues.

16b. Informed Consent letter (attach a separate sheet).
This corresponds to the patient's statement regarding its participation in the project so it is made in person (v.gr.: I so and so am aware ofthe project .....etc.). It will run in duplicate, on letterhead of INCMNSZ, leaving a copy to the research subject or his legal representative and another held by the researcher. This letter should include, but not limited to, the following
The patient has received clear information in writing.
Who have attended all doubts about participation in the protocol (headlining, it is essential to note the precise protocol for each title).
Who knows the risks, benefits and responsibilities arising from their participation.
Which he agreed to participate voluntarily and that the confidentiality of the investigation will be guaranteed.
Which may be withdrawn at any time without losing their benefits as a patient of the Institute or be penalized.
The name and signature of the research subject or his legal representative.
The name and signature of two witnesses and the relationship they have with the research subject.
The name and signature of the investigator obtaining consent.
Date on which the informed consent was obtained.

It is attached for review

17. Declaration of researchers
Copy and print this statement on letterhead of the Institute.

Open File CIIBH 04 STATEMENT OF RESEARCHERS to print and be signed by each and every one of the participants in the proposed project. Attach the sheet to format printed Assessment delivered.

18. Resolution of the Committee
This section is only for knowledge of researchers: Projects will be reviewed by each of the members of the Committee. Formal assessment and its resolution shall be made by the Committee in plenary. The discussion is given can generate observations and / or objections same as inure to the Opinion which may be Approbatory, No Approbatory or Pending.

18a. Observations
They are points identified in the project that generate questions or warrant clarification but is considered NOT affect the scientific / ethical structure.

18b. Objections
They are points identified in the project that generate questions or warrant clarification and is judged to affect the scientific / ethical structure of it and deserve explanation, defense, clarification, amendment and / or justification for continuing evaluation and reach a resolution

18c. Opinion	
Approved Project Approval Letter which shall terminate its institutional registration and may be initiated will be delivered. If any comments should be answered by the principal investigator.	
Not approved The project presents a formal objection scientific or ethical issues that prevent approval. It may be amended and resubmitted by a new Request for Review.	
Pending or in progress The Committee did not reach a definite opinion because the project presents comments / objections that merit explanation, reply, clarification, amendment and / or justification for continuing evaluation and reach a resolution. a letter with comments / objections will be given and evaluation as they are answered will continue.	

Annex 1

INSTITUTO NACIONAL DE CIENCIAS MÉDICAS Y NUTRICIÓN SALVADOR ZUBIRÁN

2019
AÑO DEL CAUDILLO DEL SUR EMILIANO ZAPATA

CIUDAD DE MÉXICO A 10 DE DICIEMBRE DE 2019
REG. CONBIOÉTICA-09-CEI-011-20160627
OFICIO No. MCONTROL-1640/2019

DR. ENRIQUE COSS ADAME
INVESTIGADOR PRINCIPAL
DEPTO. DE GASTROENTEROLOGÍA
INSTITUTO NACIONAL DE CIENCIAS MÉDICAS Y NUTRICIÓN SALVADOR ZUBIRÁN
AV. VASCO DE QUIROGA NO. 15
COL. BELISARIO DOMÍNGUEZ SECCIÓN XVI
DEL. TLALPAN, C.P. 14080, CD. DE MÉXICO
P R E S E N T E

En respuesta a su atenta del pasado 21 de noviembre, con relación al Protocolo de Investigación Clínica, titulado:

"Estudio prospectivo, aleatorizado y controlado para la evaluación de la eficacia de un suplemento de fructanos derivados de la fibra de Agave tequilana Weber variedad Azul vs Psyllium plantago en pacientes con estreñimiento funcional"
Ref. 1107

Le informamos que se toma conocimiento del estado actual del estudio, así mismo se autoriza la re-aprobación anual con vigencia hasta el 10 de diciembre de 2020.

Sin otro particular, reciba un cordial saludo.

ATENTAMENTE,

DR. CARLOS ALBERTO AGUILAR SALINAS
PRESIDENTE
COMITÉ DE INVESTIGACIÓN

DR. ARTURO GALINDO FRAGA
PRESIDENTE
COMITÉ DE ÉTICA EN INVESTIGACIÓN

INS. NACIONAL DE CIENCIAS MEDICAS Y NUTRICIÓN "SALVADOR ZUBIRÁN"
10 DIC 2019
COMITÉ DE ÉTICA EN INVESTIGACIÓN

GAAS/AGF/MRG

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