CLINICAL STUDY PROTOCOL

A Phase II, Randomized, Double Blind, Placebo-Controlled Clinical Trial to Investigate the Anti-oxidant Activity of Heptex in Patients with Apparent Risk Factors of Nonalcoholic Steatohepatitis (NASH)

STUDY NUMBER: NW_PHYLLANTEX_17052018

STUDY NAME: PHYLLANTEX

Version Number:	Final V1.1
Date:	30-Dec-2019

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1 PROTOCOL SYNOPSIS

Title	A Phase II, Randomized, Double Blind, Placebo-Controlled Clinical Trial to Investigate the Anti-oxidant Activity of Heptex in Patients with Apparent Risk Factors of Nonalcoholic Steatohepatitis (NASH)
Study Number	NW_PHYLLANTEX_17052018
Study Sponsor	Natural Wellness Egypt
Product Name	Heptex
Study Drug	Dukung Anak 200 mg + Milk Thistle 100 mg Dukung Anak is a Powdered Extract from Aerial Parts of *Phyllanthus niruri*, Milk Thistle is a Powdered Extract from Fruits of *Silybum marianum*.
Indication	Anti-oxidant (Liver support)
Trial Location(s)	• Tropical Medicine Department, Faculty of Medicine, Ain Shams University, Abbasiya, Cairo, Egypt. • Tropical Medicine Department, Faculty of Medicine, Helwan University, Helwan, Egypt. • National Hepatology and Tropical Medicine Research Institute (NHTMRI), Fom Al Khalig Sq, El-Sayeda Zainab, Giza, Egypt.
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Medical and/or technical department(s) for Fibroscan and Ultrasound	**For FibroScan:** • Tropical Medicine Department, Ain Shams University Specialized Hospital, Khalifa El-Maamon st, Abbasiya sq., Cairo, Egypt. • Tropical Medicine Department at NHTMRI, Fom Al Khalig Sq, El-Sayeda Zainab, Giza, Egypt. • Air Force Specialized Hospital, New Cairo, Cairo, Egypt.

	For Ultrasound: • Tropical Medicine Department, Ain Shams Hospital, Khalifa El-Maamon st, Abbasiya sq., Cairo, Egypt. • Tropical Medicine Department at NHTMRI, Fom Al Khalig Sq, El-Sayeda Zainab, Giza, Egypt. • Air Force Specialized Hospital, New Cairo, Cairo, Egypt.
Study Objective(s)	**Primary Objectives:** • To explore the anti-oxidant activity of Heptex as assessed by improvement in serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in patients with apparent risk factors of NASH. **Secondary Objectives:** • To explore the optimum dose that is safe in patients with apparent risk factors of NASH giving the most significant effect among two different doses of Heptex. • To explore the hepatoprotective effect of Heptex as assessed by the change in Fibrosis score. • To explore the hepatoprotective effect of Heptex as assessed by the occurrence of hepatic complications. **Exploratory Objectives:** • To explore the lipid-lowering effect of Heptex as assessed by the change in lipid profile.
Study Design	This is a phase II, randomized, double blind placebo-controlled, three-arm, parallel-group, interventional clinical trial evaluating anti-oxidant activity of Heptex; a herbal medicinal product of Aerial Parts of *Phyllanthus niruri* (Dukung Anak) and Fruits of *Silybum marianum* (Milk Thistle) in patients with apparent risk factors of NASH.

	Study duration: 12 weeks of recruitment and 36 weeks of treatment. This period will include 2 washout periods (at Week 13 & Week 25)[1]. Sample Size: 47 subjects per arm, 141 in total. Participants in trial will be patients between 18 and 65 years consenting to participate in this study, who have elevated liver enzymes[34], controlled attenuation parameter (CAP)-confirmed hepatic steatosis, and Fib score of F1 and F2 assessed by the FibroScan liver stiffness measurement. FibroScan will be done for all patients at screening visit and those with F1 and F2 will be considered for inclusion in the study. FibroScan will be repeated for all patients after 6 months and at the end of treatment duration and results will be compared. Screening of patients will be continued for about 12 weeks. Eligible patients will be randomized in a 1:1:1 allocation ratio, into one of the three treatment groups, to receive either placebo or one of the two doses of Heptex. Randomization will be done using interactive response technology.
Main Selection Criteria	**Inclusion Criteria:** 1) Male or female aged between 18 and 65 years. 2) Both male and female patients who have childbearing potential must agree to practice an acceptable method of birth control during the study and for at least 6 months after the cessation of treatment; such contraceptive methods must include at least one barrier method. 3) Controlled Attenuation Parameter (CAP)-confirmed hepatic steatosis.

1 According to ICH M3 R2 *(ICH Harmonised Tripartite Guideline. 2009. Guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals. Dated 11 June 2009, Accessed at: http://www.ich.org/).* Despite the long-term tolerability of the marketed product, the conducted repeated dose studies will be related to the proposed duration of the clinical trial by adding suitable washout periods throughout the clinical study duration.

	4) Patients with elevated liver enzymes (alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST)) [34]. 5) Liver fibrosis stage F1-F2 as diagnosed by the FibroScan liver stiffness measurement of 5-10 kPa. 6) Liver condition according the following criteria; o Serum albumin > 3 g/dl o INR < 2 o No ascites on ultrasound o No documented or suspected hepatic encephalopathy 7) Willing to stop any other liver support and hepatoprotective medications throughout study duration. 8) Able and willing to provide written informed consent. 9) Able and willing to complete all study visits and procedures, including compliance with the requirements and restrictions listed in the consent form.
	Exclusion Criteria: 1) Pregnant or lactating women. 2) Patients with BMI > 40 Kg/m^2 or BMI < 18.5 Kg/m^2. 3) Serum creatinine > 1.5 x ULN OR creatinine clearance (GFR) < 60 mL/minute. 4) Platelet count < 75,000/mm^3. 5) Uncontrolled diabetes mellitus as evident by HbA1c ≥ 8.5%. 6) Patients who are currently receiving Thiazolidinediones. 7) Patients with ischemic heart disease (IHD). 8) History of parenteral nutrition. 9) History of liver transplant.

	10) Viral hepatitis, drug-induced liver injury, metabolic liver disease or auto-immune liver disease. 11) Liver cancer or serum alpha-fetoprotein (AFP) >100ng/ml. Patients with an AFP between 50 and 100ng/ml may be included as long as a liver ultrasound within 3 months of screening, or at screening, shows no evidence of potential hepatocellular cancer. 12) Use of drugs known to induce steatosis (valproate, amiodarone or prednisone) or to affect body weight and carbohydrate metabolism. 13) Use of drugs known to alter liver enzymes. 14) Allergy or allergic history to any of the drug components. 15) History of alcohol abuse as assessed by the investigator within the past 2 years, or an alcohol use pattern that may interfere with the patient's study compliance. Patients must have abstained from alcohol for at least 6 months prior to study start. 16) Patients with history of clinically-significant illness or any other major medical disorder that may interfere with subject treatment, assessment, or compliance with the protocol. 17) Receipt of an investigational drug within 6 months prior to screening, or active enrolment in another investigational medication or device trial. 18) Patients with any chronic illness or prior treatment which in the opinion of the investigator should preclude participation in the trial. 19) Inability to understand and cooperate with the investigators or to give valid consent.
Total expected number of patients:	141 patients (47 patients per arm)
Expected number of sites:	3 Sites

<table>
<tr><td>Investigational product; (dosing and mode of administration)</td><td>Heptex Capsule (size 1) consisted of the following;

Dukung Anak is a Powdered Extract 200 mg (from Aerial Parts of Phyllanthus niruri),

Milk Thistle is a Powdered Extract 100 mg (from Fruits of Silybum marianum).

Mode of administration:
Each patient will take 2 capsules size 1 of the study medication (either Placebo or Heptex) orally (PO) three times daily (TID) on empty stomach (15 min before meals or 1 hour after meals) with plenty of water (240 ml water, or full glass of water). The Placebo and Heptex capsules will be identical in physical appearance and mode of administration.

Control Arm 1:
Placebo (Rice bran) in 2 capsules size 1, administered PO TID on empty stomach with plenty of water.

Experimental Arm 2:
The contents of one capsule of Heptex is equally distributed and inserted into 2 capsules size 1, administered PO TID on empty stomach with plenty of water.

Experimental Arm 3:
The contents of two capsules of Heptex is equally distributed and inserted into 2 capsules size 1, administered PO TID on empty stomach with plenty of water.</td></tr>
<tr><td>Assessment Schedule</td><td>This phase II study will last for 36 weeks in addition to screening period, and it will include 2 washout periods (at Week 13 & Week 25). A total of 6 visits will be performed as shown in the table below;
<table>
<tr><th>Visit 1</th><th>Visit 2</th><th>Visit 3</th><th>Visit 4</th><th rowspan="2">Washout period (Week 13)</th><th>Visit 5</th><th rowspan="2">Washout period (Week 25)</th><th>Visit 6</th></tr>
<tr><td rowspan="2">Screening (12 weeks)</td><td>Baseline</td><td>Follow up</td><td>Follow up</td><td>Follow up</td><td>Follow up (EOS)</td></tr>
<tr><td>Week 0</td><td>Week 8</td><td>Week 12</td><td>Week 24</td><td>Week 36</td></tr>
</table></td></tr>
<tr><td>Data to be collected</td><td>Screening visit:</td></tr>
</table>

	During screening visit, data on informed consent process, inclusion and exclusion criteria, demographics, medical and surgical history, concomitant medications, physical examination, vital signs, FibroScan liver stiffness measurement (KPa), abdominal ultrasound, controlled attenuation parameter (CAP), lab tests (serum pregnancy test for females, PCR; HCV RNA, HAV IgM antibody test, HBsAg test, alpha fetoprotein, CBC, sodium, potassium, serum fibrinogen, albumin, bilirubin, prothrombin time, PTT, INR, ALT, AST, kidney function tests, lipid profile, and HbA1c) will be collected. Then Fib-4 score will be calculated. **Baseline (week 0):** At baseline visit, data on concomitant medications, physical examination and vital signs will be collected. Then occurrence of adverse events and serious adverse events including hepatic complications will be collected. Data on the dispensed drug will be recorded. **Week 8 and week 12:** At week 8 and week 12, data on concomitant medications, physical examination, vital signs, controlled attenuation parameter (CAP), lab tests (serum pregnancy test for females, CBC, serum fibrinogen, albumin, bilirubin, prothrombin time, PTT, INR, ALT and AST) will be collected. Then Fib-4 score will be calculated and occurrence of adverse events and serious adverse events including hepatic complications will be collected. The drug accountability for the dispensed drug will be recorded. **Week 24:** At week 24, data on concomitant medications, physical examination, vital signs, FibroScan liver stiffness measurement (KPa), controlled attenuation parameter (CAP), lab tests (serum pregnancy test for females, CBC, sodium, potassium, serum fibrinogen, albumin, bilirubin, prothrombin time, PTT, INR, ALT, AST, kidney function tests, lipid profile and HbA1c) will be collected. Then Fib-4

	score will be calculated and occurrence of adverse events and serious adverse events including hepatic complications will be collected. The drug accountability for the dispensed drug will be recorded. **Week 36:** At week 36, data on concomitant medications, physical examination, vital signs, FibroScan liver stiffness measurement (KPa), controlled attenuation parameter (CAP), lab tests (serum pregnancy test for females, CBC, sodium, potassium, serum Fibrinogen, albumin, bilirubin, prothrombin time, PTT, INR, ALT, AST, kidney function tests, lipid profile and HbA1c) will be collected. Then Fib-4 score will be calculated and occurrence of adverse events and serious adverse events including hepatic complications will be collected. Study completion form will be filled.
Criteria For Evaluation	During interim analysis (at 6 months of treatment) and final analysis at the end of treatment, the following endpoints will be evaluated: **Primary Endpoints:** • Compare the mean relative change in ALT & AST levels between the experimental arms and the control arm. • Compare proportions of patients with normal ALT & AST at end of treatment between the experimental arms and the control arm. The cut-off values of ALT were 0-43 IU/L and of AST were 0-40 IU/L[2]. **Secondary Endpoints:** • Comparing the mean change in Fib-4 score between the experimental arms and the control arm. • Comparing the frequency and percentage of patients experiencing hepatic

2 Uslusoy H, Nak S, Gülten M, Bıyıklı Z. Non-alcoholic steatohepatitis with normal aminotransferase values. World Journal of Gastroenterology. 2009;15(15):1863.

	complications between the experimental arms and control arm. **<u>Exploratory Endpoints:</u>** • Compare the mean relative change in lipid profile levels between the experimental arms and the control arm.
Statistical methodologies	**<u>Statistical Power and Sample Size Justification</u>**[3] Reference to Sanyal AJ, et al. there was a decrease in Alanine aminotransferase level among placebo arm of **-20 (U/liter)** among the placebo arm while for the active arm, a **double** of this improvement will be of clinical value. Accordingly, with an alpha error of **5%** using one-sided 95% CI of Mann–Whitney U test, a sample power of **80%** and an effect size between placebo and lowest active dose of **0.63**, a sample of **43 patients** for each arm will be required plus an expected **drop-out rate of 10%** during 9 months' study duration. Thus, a sample of **47 patients** per each treatment arm will be appropriate and the total same of the 3 arms will be **141 patients**. **<u>Randomization method:</u>** The arms randomization will be performed using Interactive web response system (IWRS). **<u>Statistical methodologies:</u>** The statistical analysis of this study will consist of reporting the individual data listings, providing descriptive statistics for parameters of interest, and statistical testing of the primary and secondary variables. The final statistical analyses will be performed when the patients on Heptex and placebo complete 9 months of treatment. Detailed analysis methods will be provided in the statistical analysis plan.

3 Gpower 3.1

	Descriptive analysis: Percent (%) distribution for all categorical variables and mean with (SD) and median (Minimum: Maximum) for continuous variables according their distribution. **Primary analysis:** The main objectives of this phase II study is to explore the anti-oxidant activity of Heptex as assessed by improvement in serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels by measuring: • The mean relative change in ALT & AST levels between the experimental arms and the control arm from baseline till end of treatment using T-test (Mann-Whitney test in case of non-parametric data). This analysis will be a comparative analysis and will be done on eligible population of patients without protocol violation and who have at least one treatment dose and an evaluable primary endpoint (AST and ALT). • Number of patients with normal ALT & AST at end of treatment between the experimental arms and the control arm. The cut-off values of ALT were 0-43 IU/L and of AST were 0-40 IU/L[4] using chi square (Fischer test for non-parametric data). This analysis will be comparative analysis and will be conducted on the eligible population of patients without protocol deviation, who have at least one treatment dose and an evaluable primary endpoint (ALT and AST). **Secondary Analysis:** • The mean change in Fib-4 score between the experimental arms and the control arm from baseline till end of treatment using T-test (Mann-Whitney test in case of non-parametric data). This analysis will be a

4 Uslusoy H, Nak S, Gülten M, Bıyıklı Z. Non-alcoholic steatohepatitis with normal aminotransferase values. World Journal of Gastroenterology. 2009;15(15):1863.

	comparative analysis and will be done on eligible population of patients without protocol violation and who have at least one treatment dose and an evaluable primary endpoint (AST and ALT). • Number of patients experiencing hepatic complications between the experimental arms and control arm at the end of the treatment using chi-square (Fischer for non-parametric data). This analysis will be a comparative analysis and will be done on eligible population of patients without protocol violation and who have at least one treatment dose and an evaluable primary endpoint (AST and ALT). **Exploratory Analysis:** • The mean relative change in lipid profile levels between the experimental arms and the control arm from baseline till end of treatment using T-test (Mann-Whitney test in case of non-parametric data). This analysis will be a comparative analysis and will be done on eligible population of patients without protocol violation and who have at least one treatment dose and an evaluable primary endpoint (AST and ALT). All tests will be performed on the 5% level of significance. Procedure for accounting for missing, unused, and spurious data will be explained in the statistical analysis plan. Any deviation(s) from the original statistical plan will be described in the final report). **Interim analysis:** Interim analysis will be performed when all patients complete 6 months of treatment. If a statistically significant difference is observed between placebo and active arm(s), the study will be terminated for the benefit of patients who

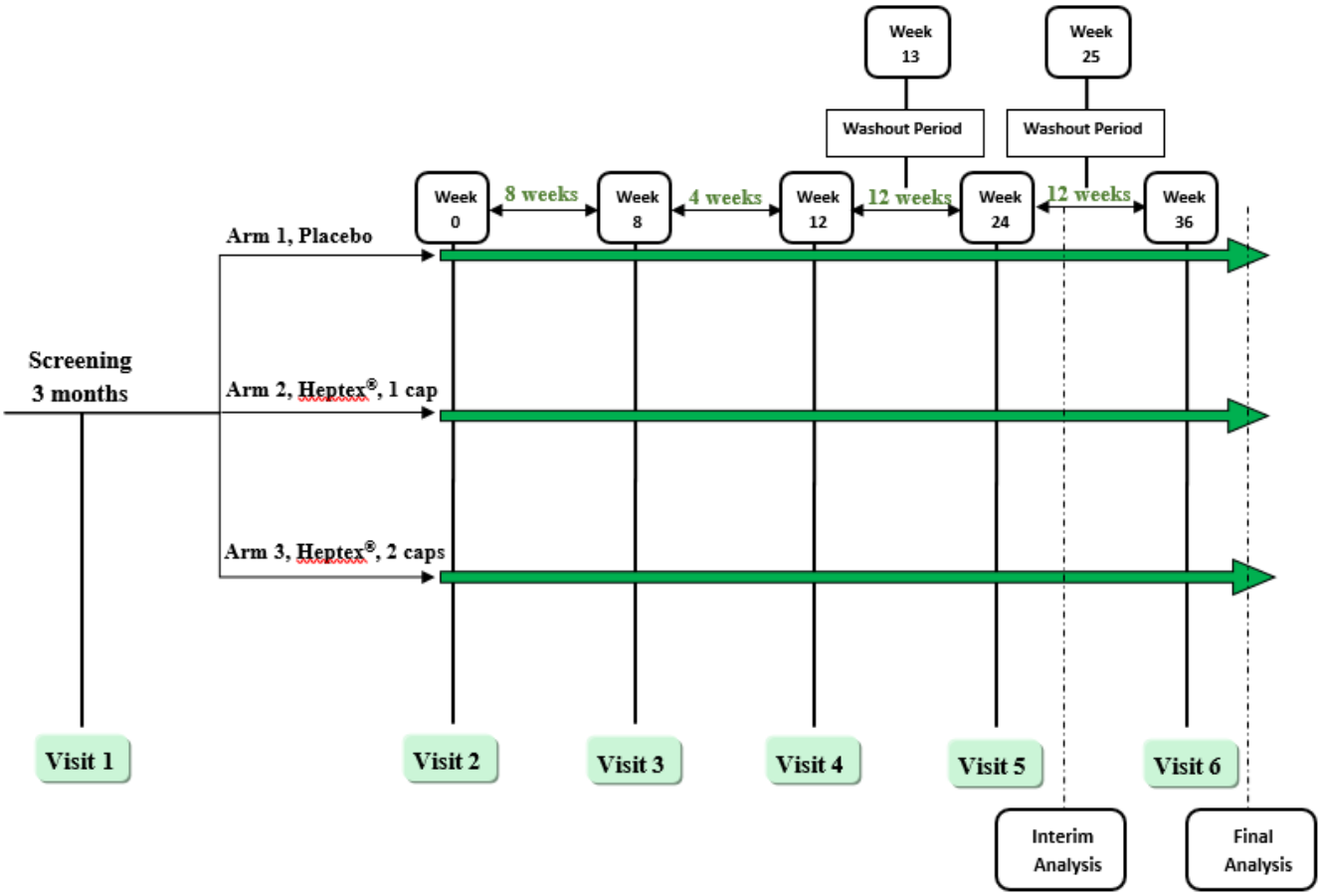
	were randomly assigned to placebo arm and to rapidly disseminate evidence supporting a treatment benefit to the broader community[5].
Estimate Duration of the Study	• **Protocol Planned Date: October 2018** • **First Patient In (FPI): December 2018** • **Last Patient In (LPI): March 2019** • **Last Patient Out (LPO): December 2019** • **Estimated enrollment duration: 3 Months** • **Estimate treatment duration: 9 Months** • **Database lock planned date: January 2020** • **Estimated Report date: February 2020**

5 Zannad F, et al. Development of Therapeutics for Heart Failure. When to Stop a Clinical Trial Early for Benefit: Lessons Learned and Future Approaches. Circulation: Heart Failure. 2012; 5: 294-302. doi: 0.1161/CIRCHEARTFAILURE.111.965707

2 ASSESMENT SCHEDULE

Data to be collected	Study Schedule*					
	V1 screening	V2 Week 0	V3 Week 8	V4 Week 12	V5 Week 24	V6 Week 36
Informed consent	X					
Inclusion/ exclusion criteria	X					
Demographics	X					
Medical/ Surgical history	X					
Concomitant medications	X	X	X	X	X	X
Physical examination	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X
Fibroscan liver stiffness measurement	X				X	X
Fib-4	X		X	X	X	X
Abdominal Ultrasound	X					
Controlled Attenuation Parameter (CAP)	X		X	X	X	X
Serum pregnancy test for females	X		X	X	X	X
PCR	X					
Alpha fetoprotein	X					
HCV RNA	X					
HAV IgM	X					
HBsAg	X					
CBC	X		X	X	X	X
Sodium	X				X	X
Potassium	X				X	X
Serum Fibrinogen	X		X	X	X	X
Liver Profile tests - Albumin, bilirubin, - PT, PTT, INR, - ALT and AST	X		X	X	X	X
Kidney function tests	X				X	X
Lipid Profile	X				X	X
HbA1c	X				X	X
Study drug dispensed		X	X	X	X	
Drug Accountability			X	X	X	X
Adverse Events Serious Adverse Events Hepatic complications		X	X	X	X	X
Study Completion Form						X
* Washout periods at week 13 and week 25.						

3 GRAPHICAL STUDY DESIGN



4 LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	Adverse Events
AESI	Adverse Event of Special Interest
AFP	Alpha-Fetoprotien
ALT	Alanine Transaminase
ANOVA	Analysis of Variance
AST	Aspartate Transaminase
BMI	Body Mass Index
CAP	Controlled Attenuation Parameter
CBC	Complete Blood Count
CI	Confidence Interval
CRF	Case Report Form
CRO	Clinical Research Organization
DBP	Diastolic Blood Pressure
DVP	Data Validation Plan
eCRF	electronic Case Report Form
EDC	Electronic Data Capture
EIU	Exposure In-Utero
ESC	European Society of Cardiology
ESH	European Society of Hypertension
FPI	First Patient In
g/dl	gram per deciliter
GFR	Glomerular Filtration Rate
HbA1c	Glycated hemoglobin
HAV	Hepatitis A Virus
HCV	Hepatitis C Virus
HDPE	High-density polyethylene
ICH	International Conference on Harmonisation
ID	Identification number
IEC	Independent Ethics Committee
IgM	Immunoglobulin M
IHD	Ischemic Heart Disease
IND	Investigational New Drug

Abbreviation	Definition
INR	International Normalized Ratio
IP	Investigational Product
IRB	Institutional Review Board
IWRS	Interactive Web Response System
kPa	Kilopascal
LPI	Last Patient In
LPO	Last Patient Out
MedDRA	Medical Dictionary for Regulatory Activities
mg/dl	milligram per diciliter
mm^3	cubic millimeter
mmHg	Millimeter of mercury
NAFLD	Non-Alcoholic Fatty Liver Disease
NASH	Non-Alcoholic Steato-Hepatitis
ng/ml	Nanogram per milliliter
PCR	Polymerase Chain Reaction
PO	Orally
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
QC	Quality Control
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
SD	Standard Deviation
SDV	Source Data Verification
SOP	Standard Operating Procedure
SVR	Sustained Viral Response
TID	Three Times per day
ULN	Upper Limit of Normal
WHO	World Health Organization

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6 MEDICAL BACKGROUND& RATIONALE

6.1 MEDICAL BACKGROUND

Nonalcoholic fatty liver disease (NAFLD) is a chronic condition in which excessive fat is accumulated in the liver without alcohol causing it [1].

There are two types of NAFLD; simple fatty liver and nonalcoholic steatohepatitis (NASH). The difference between these two is that NASH includes inflammation and liver cell damage along with fat accumulation in the liver [2]. NASH is the advanced form of NAFLD, which can progress to fibrosis, cirrhosis and then hepatocellular carcinoma [3 - 5].

Although the mechanisms involved in the occurrence and progression of fatty liver is unclear, many studies have proposed the pathogenesis of NAFLD. The "multiple parallel hits" hypothesis was mainstreamed. In 1998, Day et al. proposed that "the first hit", either high-fat diet or diabetes-induced steatosis, will sensitize the liver to other risk factors related to oxidative stress (OS) and induce severe lipid peroxidation "the second hit" [6].

Afterwards, NASH was found to be closely associated with insulin resistance (IR), obesity and type 2 diabetes [7, 8], along with (OS) that was presumed to contribute to hepatic injury in patients with the disease [8, 9]. Hence, therapy targeting IR and OS could be beneficial in patients with NASH.

Many studies have been conducted to investigate the efficacy of antioxidants such as vitamin E in normalizing liver enzymes (alanine aminotransferase "ALT" & aspartate aminotransferase "AST") in patients with NASH [10 - 14].

Hasegawa T et al. reported a decrease in serum ALT level in NASH patients during the 1- year α- tocopherol treatment along with improvement in the histological findings, such as steatosis, inflammation and fibrosis, while α- tocopherol had no effect on serum ALT in patients with NAFL [10].

In 2003, Harrison SA et al. conducted a prospective, double-blind, randomized, placebo-controlled trial where all NASH patients were randomized to receive either vitamins E and C or placebo daily for 6 months. Despite there was no improvement seen in necroinflammatory activity or ALT, the combination was found to be effective in improving fibrosis scores (specifically for patients with diabetes). For AST, no differences were noted between groups or within groups [11].

Another double-blind, randomized, placebo-controlled trial that was conducted in 2004 found that ursodeoxycholic acid (UDCA) in combination with vitamin E twice daily significantly improved serum ALT & AST levels and liver histology of patients with NASH. It was also found that patients received the combination had less steatosis and a lower activity index at the end of the 2 years of treatment [12].

For a very long time, *Silybum marianum,* commonly known as milk thistle, is used in the treatment of liver diseases [15, 16]. The lipophilic extract from plant seeds is the active form of milk thistle, this composed of a complex mixture of isomer flavonolignans [15].

Many studies (in vitro and in vivo) have shown the beneficial value of silymarin when it comes to its effects in liver diseases, whether acting as antioxidant, anti-inflammatory, and anti-fibrotic [17 - 21].

A meta-analysis was previously conducted to assess the therapeutic effect of silymarin in the treatment of patients with NAFLD. It included eight randomized clinical trials (RCTs) with a total of 587 patients. It showed that patients receiving silymarin (whether monotherapy or in combination) had significantly lower AST and ALT levels than those in control patients ($p = 0.0002$ & $p = 0.1$ respectively) [22].

A randomized, placebo-controlled trial that was conducted in Iran evaluated the efficacy of silymarin in the treatment of NASH. Eighty patients were assigned to either receive placebo or silymarin (with 210 mg/ day) orally for 8 weeks. It showed that patients receiving silymarin had significantly lower AST & ALT levels than patients receiving placebo ($p = 0.38$ & $p = 0.026$ respectively) [23].

Another RCT was conducted in Iran to assess the effect of silymarin in patients with NASH. It included 100 patients that were randomized either to receive placebo (Group A) or 280 mg of silymarin (Group B). Treatment was continued for 24 weeks, and patients were evaluated every 4 weeks. When compared to baseline, a significant reduction was reported in both ALT & AST levels in patients receiving silymarin ($p = 0.001$ & $p = 0.006$ respectively). On the contrary, no significant reduction was observed in patients receiving placebo ($p = 0.237$ & $p = 0.343$ respectively) [24].

In the same trial, a significantly higher percentage of patients with normal ALT & AST levels was observed in the silymarin-receiving group when compared to the placebo-receiving group ($p = 0.001$ & $p = 0.0001$ respectively) [24].

Phyllanthus niruri is also a widely used herb in traditional medicine. Many studies showed that *Phyllanthus* species could protect the liver from damages caused by OS and lipid peroxidation by antagonizing the first and preventing the second [25 - 27].

Many laboratory studies investigated its potential antioxidant, antimicrobial, anti-inflammatory, and hepatoprotective properties in liver diseases [28 - 32].

However, one pilot study was conducted in Malaysia to examine the safety and efficacy of *Phyllanthus niruri* in patients with NASH (randomized, double-blind, parallel-group, placebo-controlled trial with a total of 52 NASH patients who were randomized (1:1) to receive *Phyllanthus niruri* ($n = 25$) or placebo ($n = 27$) three times daily for 48 weeks) [33].

Although ALT & AST levels were decreased in patients on *Phyllanthus niruri*, there were no significant differences in ALT ($p > 0.95$) & AST ($p = 0.39$) levels between the two groups. Thus, this study was unable to demonstrate the clinical benefits of *Phyllanthus niruri* in patients with NASH. This study was limited by the small sample size that might contributed to the differences in baseline characteristics between the two groups [33].

This information along with the very promising preclinical data encouraged us to conduct further research (on a larger scale) to investigate both safety and efficacy of *Phyllanthus niruri* combined with *Silybum marianum* in patients suffering from NASH.

6.2 RATIONALE

Data about the efficacy of a combination of *Phyllanthus niruri* (Dukung Anak) and

Silybum marianum (Milk Thistle) in patients with NASH are still lacking. Therefore, this study was designed to evaluate both on a larger clinical scale.

7 STUDY OBJECTIVES

7.1 PRIMARY

- To explore the anti-oxidant activity of Heptex as assessed by improvement in serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in patients with apparent risk factors of NASH.

7.2 SECONDARY

- To explore the optimum dose that is safe in patients with apparent risk factors of NASH giving the most significant effect among two different doses of Heptex.
- To explore the hepatoprotective effect of Heptex as assessed by the change in Fibrosis score.
- To explore the hepatoprotective effect of Heptex as assessed by the occurrence of hepatic complications.

8 STUDY DESIGN

8.1 DESCRIPTION OF THE STUDY DESIGN

This is a phase II, randomized, double blind placebo-controlled, three-arm, parallel-group, interventional clinical trial evaluating anti-oxidant activity of Heptex; a herbal medicinal product of Aerial Parts of *Phyllanthus niruri* (Dukung Anak) and Fruits of *Silybum marianum* (Milk Thistle).

Study duration: 12 weeks of recruitment and 36 weeks of treatment. This period will include 2 washout periods (at Week 13 & Week 25).

Sample Size: 47 subjects per arm, 141 in total.

Participants in trial will be patients between 18 and 65 years consenting to participate in this study, who have elevated liver enzymes [34], controlled attenuation parameter (CAP)-confirmed hepatic steatosis, and Fib score of F1 and F2 assessed by the FibroScan liver stiffness measurement.

FibroScan will be done for all patients at screening visit and those with F1 and F2 will be considered for inclusion in the study. FibroScan will be repeated for all patients after 6 months and at the end of treatment duration and results will be compared.

Screening of patients will be continued for about 12 weeks. Eligible patients will be randomized in a 1:1:1 allocation ratio, into one of the three treatment groups, to receive either placebo or one of the two doses of Heptex. Randomization will be done using interactive response technology.

8.2 DURATION OF STUDY PARTICIPATION

This phase II study will last for 36 weeks in addition to a screening period, and it will include 2 washout periods (at Week 13 & Week 25). A total of 6 visits will be performed as shown in the table below;

Visit 1	Visit 2	Visit 3	Visit 4		Visit 5		Visit 6
Screening (12 weeks)	Baseline	Follow up	Follow up	**Washout period (Week 13)**	Follow up	**Washout period (Week 25)**	Follow up (EOS)
	Week 0	Week 8	Week 12		Week 24		Week 36

8.3 ESTIMATED STUDY DATES

- Protocol Planned Date: October 2018
- First Patient In (FPI): December 2018
- Last Patient In (LPI): March 2019
- Last Patient Out (LPO): December 2019
- Estimated enrollment duration: 3 Months
- Estimate treatment duration: 9 Months
- Database lock planned date: January 2020
- Estimated Report date: February 2020

8.4 DATA TO BE COLLECTED AT DIFFERENT STUDY INTERVALS

Screening visit:

During screening visit, data on informed consent process, inclusion and exclusion criteria, demographics, medical and surgical history, concomitant medications, physical examination, vital signs, FibroScan liver stiffness measurement (KPa), abdominal ultrasound, controlled attenuation parameter (CAP), lab tests (serum pregnancy test for females, PCR; HCV RNA, HAV IgM antibody test, HBsAg test, alpha fetoprotein, CBC, sodium, potassium, serum fibrinogen, albumin, bilirubin, prothrombin time, PTT, INR, ALT, AST, kidney function tests, lipid profile, and HbA1c) will be collected. Then Fib-4 score will be calculated.

Baseline (week 0):

At baseline visit, data on concomitant medications, physical examination and vital signs will be collected. Then occurrence of adverse events and serious adverse events

including hepatic complications will be collected. Data on the dispensed drug will be recorded.

Week 8 and week 12:

At week 8 and week 12, data on concomitant medications, physical examination, vital signs, controlled attenuation parameter (CAP), lab tests (serum pregnancy test for females, CBC, serum fibrinogen, albumin, bilirubin, prothrombin time, PTT, INR, ALT and AST) will be collected. Then Fib-4 score will be calculated and occurrence of adverse events and serious adverse events including hepatic complications will be collected. The drug accountability for the dispensed drug will be recorded.

Week 24:

At week 24, data on concomitant medications, physical examination, vital signs, FibroScan liver stiffness measurement (KPa), controlled attenuation parameter (CAP), lab tests (serum pregnancy test for females, CBC, sodium, potassium, serum fibrinogen, albumin, bilirubin, prothrombin time, PTT, INR, ALT, AST, kidney function tests, lipid profile and HbA1c) will be collected. Then Fib-4 score will be calculated and occurrence of adverse events and serious adverse events including hepatic complications will be collected. The drug accountability for the dispensed drug will be recorded.

Week 36:

At week 36, data on concomitant medications, physical examination, vital signs, FibroScan liver stiffness measurement (KPa), controlled attenuation parameter (CAP), lab tests (serum pregnancy test for females, CBC, sodium, potassium, serum Fibrinogen, albumin, bilirubin, prothrombin time, PTT, INR, ALT, AST, kidney function tests, lipid profile and HbA1c) will be collected. Then Fib-4 score will be calculated and occurrence of adverse events and serious adverse events including hepatic complications will be collected. Study completion form will be filled.

9 SELECTION OF PATIENTS

9.1 SAMPLE SIZE

It is planned to recruit 141 patients (47 patients per arm), in 2 centers in Egypt.

9.2 INCLUSION CRITERIA

1) Male or female aged between 18 and 65 years.
2) Both male and female patients who have childbearing potential must agree to practice an acceptable method of birth control during the study and for at least 6 months after the cessation of treatment; such contraceptive methods must include at least one barrier method.
3) Controlled Attenuated Parameter (CAP)-confirmed hepatic steatosis.
4) Patients with elevated Liver enzymes (ALT and/or AST) [34].
5) Liver fibrosis stage F1-F2 as diagnosed by the FibroScan liver stiffness measurement of 5-10 kPa.
6) liver condition according the following criteria;
    - Serum albumin > 3 g/dl
    - INR < 2
    - No ascites on ultrasound
    - No documented or suspected hepatic encephalopathy
7) Willing to stop any other liver support and hepatoprotective medications throughout study duration.
8) Able and willing to provide written informed consent.
9) Able and willing to complete all study visits and procedures, including compliance with the requirements and restrictions listed in the consent form.

9.3 EXCLUSION CRITERIA

1) Pregnant or lactating women.
2) Patients with BMI > 40 Kg/m2 or BMI < 18.5 Kg/m2.

3) Serum creatinine > 1.5 x ULN OR creatinine clearance (GFR) < 60 mL/minute.
4) Platelet count < 75,000/mm3.
5) Uncontrolled diabetes mellitus as evident by HbA1c ≥ 8.5%.
6) Patients who are currently receiving Thiazolidinediones.
7) Patients with ischemic heart disease (IHD).
8) History of parenteral nutrition.
9) History of liver transplant.
10) Viral hepatitis, drug-induced liver injury, metabolic liver disease or auto-immune liver disease.
11) Liver cancer or serum alpha-fetoprotein (AFP) >100ng/ml. Patients with an AFP between 50 and 100ng/ml may be included as long as a liver ultrasound within 3 months of screening, or at screening, shows no evidence of potential hepatocellular cancer.
12) Use of drugs known to induce steatosis (valproate, amiodarone or prednisone) or to affect body weight and carbohydrate metabolism.
13) Use of drugs known to alter liver enzymes.
14) Allergy or allergic history to any of the drug components.
15) History of alcohol abuse as assessed by the investigator within the past 2 years, or an alcohol use pattern that may interfere with the patient's study compliance. Patients must have abstained from alcohol for at least 6 months prior to study start.
16) Patients with history of clinically-significant illness or any other major medical disorder that may interfere with subject treatment, assessment, or compliance with the protocol.
17) Receipt of an investigational drug within 6 months prior to screening, or active enrolment in another investigational medication or device trial.
18) Patients with any chronic illness or prior treatment which in the opinion of the investigator should preclude participation in the trial.
19) Inability to understand and cooperate with the investigators or to give valid consent.

10 TREATMENT OF TRIAL PARTICIPANTS

10.1 RANDOMIZATION

Before randomization, fulfillment of the inclusion/exclusion criteria should be assured and the signed informed consent obtained. After that, eligible patients will be randomized in a 1:1:1 allocation ratio, into one of the three treatment groups, to receive either placebo or one of the two doses of Heptex. Randomization will be done using interactive response technology.

Control Arm 1:
Placebo (Rice bran) in 2 capsules size 1, administered PO TID on empty stomach with plenty of water.

Experimental Arm 2:
The contents of one capsule of Heptex is equally distributed and inserted into 2 capsules size 1, administered PO TID on empty stomach with plenty of water.

Experimental Arm 3:
The contents of two capsules of Heptex is equally distributed and inserted into 2 capsules size 1, administered PO TID on empty stomach with plenty of water.

10.2 INVESTIGATIONAL PRODUCT

Heptex Capsule (Size 1) consists of the following;

Dukung Anak is a Powdered Extract 200 mg (from Aerial Parts of *Phyllanthus niruri)*, **Milk Thistle** is a Powdered Extract 100 mg (from Fruits of *Silybum marianum)*.

Sponsor will provide the study treatment for the patients during the whole treatment duration according to regulation of Local Health Authorities involved in the product supply. Clinical Supplies will be pre-prepared for a sufficient number of patients entering double-blind treatment.

10.3 LABELLING AND PACKAGING

Investigational products will be packed in HDPE (High-density polyethylene) white bottles. Bottles will be packed in white carton boxes. A label will identify the patient's initials, patient stratification, randomization number, site number, visit date, visit number, batch number, no. of capsules, sponsor's name, dosage form, expiry date,

storage instructions and instructions to keep out of the reach of children in addition to a statement that this medication is for clinical trial use only. Investigational products will be stored at ambient temperature (not to exceed 30°c). During each study visit (from visit 2 to visit 6), each patient will receive a number of bottles containing the amount of medications sufficient for 8 or 12 weeks (according to visit interval).

10.4 MODE OF ADMINISTRATION:

Each patient will take 2 capsules size 1 of the study medication (either Placebo or Heptex) orally (PO) three times daily (TID) on empty stomach (15 min before meals or 1 hour after meals) with plenty of water (240 ml water, or full glass of water) for 9 months. The Placebo and Heptex capsules will be identical in physical appearance and mode of administration.

10.5 ACCOUNTABILITY OF STUDY TREATMENT:

The investigator or officially delegated pharmacist will be responsible for recording the receipt, administration and return of all drug supplies, and for ensuring the supervision of the storage and allocation of these supplies.

It is essential that the patients are able to accurately account for all supplies issued to them (i.e. Patient Event Log records of study drug taken should match drug supplies returned).

The investigator may withdraw any patient from the study if missing drug supplies cannot be satisfactorily accounted for, or if drug accountability records are consistently inaccurate, e.g. a patient may be discontinued if he has been cautioned to account correctly for medication issued and on a subsequent visit has failed to record two or more doses taken.

Any quality issue noticed with the receipt or use of an Investigational Product (deficient IP in condition, appearance, pertaining documentation, labeling, expiry date, etc.) should be promptly notified to the Sponsor, who will initiate a complaint procedure.

Under no circumstances will the Investigator supply Investigational Product to a third party, allows the Investigational Product to be used other than as directed by this Clinical Trial Protocol, or dispose of Investigational Product in any other manners.

11 CONCOMITANT MEDICATIONS

Throughout the study, investigators may prescribe any concomitant medications or treatments deemed necessary to provide adequate supportive care except other liver support, hepatoprotective medications and any medications prohibited as per the eligibility criteria. If these are required, the participant will be withdrawn. Any medication, other than the study medication taken during the study will be recorded in the CRF.

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins and/or herbal supplements) that the subject is receiving during participation in the study must be recorded in the electronic case report form. The investigator should review all concomitant medications for any potential interactions. During the Post-Treatment Period, all medications will be collected.

12 SOURCE DATA

Source documents are original documents, data, and records from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarized into the CRF), clinical and office charts, laboratory and pharmacy records…etc.

CRF entries will be considered source data if the CRF is the site of the original recording (e.g., there is no other written or electronic record of data).

All documents will be stored safely in confidential conditions. On all study-specific documents, other than the signed consent, the participant will be referred to by the study participant number/code, not by name.

Direct access to source documents will be granted to DATACLin CRO monitoring team as authorized representatives from the sponsor and to regulatory authorities to permit trial-related monitoring, audits and inspections.

13 CLINICAL DATA MANAGEMENT

13.1 DATA MANAGEMENT SYSTEM

Data will be collected and managed using Electronic Data Capture (EDC) technology. The system to be used is “OpenClinica, version 3.14”.

13.2 DATA ENTRY

Data entry and updates will be performed by authorized site staff (the investigator or offially delegated personnel). All personnel to be involved in the data entry will have the proper levels of access, grants and privileges.

Data entry screens development, validation rules programming and maintenance of study database will be the responsibility of DATACLin CRO.

13.3 DATA VALIDATION AND DISCREPANCIES MANAGEMENT BY CRO

The computerized handling of data by DATACLin CRO may generate additional queries automatically through pre-programmed and tested validation rules. Validation rules will be detailed in the Data validation Plan (DVP).

In addition to automatic valifdation rules, manual/ medical review of data may generate further queries that will be raised on the system as well. Site staff will be responsible for resolving automatic and manual queries by confirming or modifying the data questioned through the EDC system.

13.4 CLINICAL DATA CODING

DATACLin CRO will be reposnible for coding of safety data using the latest available version of MedDRA (Medical Dictionary for Regulatory Activities).

Collected trade names of drugs (e.g. concomitant medications) will be also coded to the relevant generic names.

14 QUALITY CONTROL AND QUALITY ASSURANCE

14.1 QUALITY CONTROL

14.1.1 In process quality control of biometrics

As per DataClin CRO SOPs, quality control by an independent qualified reviewer is performed on all biometrics related documents developed internally. This includes study protocol, case report form, informed consent form and study plans. In addition to these documents, database QC is performed automatically (programmed edit checks) and manually (manual review of line listings) to ensure that data is complete and consistent.

14.1.2 Monitoring and site data quality control

Study monitors will perform ongoing source data verification to confirm that criticalprotocol data entered into the eCRFs by authorized site personnel are accurate, complete, and verifiable from source documents.

100 % Source Data Verification (SDV) will be performed only for Critical Variables.

If specific issues are identified in a certain site, the percentage of Quality Control in the concerned siteand/or in all sites must be appropriately increased and corrective actions must be set up.

14.2 QUALITY ASSURANCE

All study procedures will be subject to pre-planned internal audit(s) by DataClin CRO quality assurance team. This covers biometrics phases, clinical operations and overall project management.

15 PHARMACOVIGILANCE

For safety reference; this study will be conducted consistent with The International Conference on Harmonization guideline E2A and 21 CFR Part 312 and WHO Guidelines.

Safety assessments will consist of monitoring and recording adverse events; including serious and non-serious adverse events, measurement of protocol-specified safety laboratory assessments, measurement of protocol-specified vital signs and other protocol-specified measures that are deemed critical to the safety evaluation of the study.

15.1 ADVERSE EVENTS MONITORING

All AEs regardless of seriousness or relationship to Investigational Product (IP), spanning from the signature of the informed consent form, until the end of the study as defined by the protocol for that patient, are to be recorded on the corresponding page(s) included in the CRF and reported as described in the following sections.

For all adverse events, the investigator must pursue and obtain information adequate both to determine the outcome of the adverse event and to assess whether it meets the criteria for classification as a serious adverse event requiring immediate notification to Sponsor or its designated representative.

For all adverse events, sufficient information should be obtained by the investigator to determine the causality of the adverse event. The investigator is required to assess causality. For adverse events with a causal relationship to the Investigational Product, Follow-Up by the investigator is required until the event or its sequelae resolve or stabilize at a level acceptable to the investigator, and Sponsor concurs with that assessment. This may imply that observations will continue beyond the last planned visit per protocol, and that additional investigations may be requested by the Monitoring Team up to as noticed by the sponsor

Whenever possible, diagnosis or single syndrome should be reported instead of symptoms. The Investigator should specify the date of onset, intensity, action taken with respect to IP, corrective treatment/therapy given, additional investigations performed, outcome and his/her opinion as to whether there is a reasonable possibility that the AE was caused by the IP.

15.2 REPORTING PERIOD

For serious adverse events, the reporting period to Sponsor or its designated representative begins from the time that the subject provides informed consent, which is obtained prior to the subject's participation in the study, i.e. prior to undergoing any study-related procedures and/or receiving IP, through and till the end of the Follow-Up Period as specified in the study protocol.

Any serious adverse event occurring any time after the reporting period must be promptly reported if a causal relationship to investigational product is SUSPECTED.

15.3 DEFINITIONS

Adverse Event (AE)

An adverse event is any untoward medical occurrence in a clinical investigation subject administered a product or medical device; the event need not necessarily have a causal relationship with the treatment or usage.

Examples of Adverse Events include but are not limited to:

- Abnormal Lab Test Findings Of Clinical Concerns
- Clinically Significant Symptoms and Signs
- Changes In Physical Examination Findings
- Hypersensitivity
- Progression/Worsening Of Underlying Disease

Additionally, they may include the signs or symptoms resulting from:

- Drug Overdose
- Drug Withdrawal
- Drug Abuse
- Drug Misuse
- Drug Interactions
- Drug Dependency
- Exposure During Pregnancy
- Drug Quality Defect That Has Impact On The Patient Safety

15.4 ADVERSE EVENT OF SPECIAL INTEREST

An Adverse Event of Special Interest (AESI) is an AE (Serious or Non-Serious) of scientific and medical concern specific to the Sponsor's product or program, for which ongoing monitoring and rapid communication by the Investigator to the Sponsor may be appropriate. Such events may require further investigation in order to characterize and understand them. AESIs may be added or removed during a study by Protocol Amendment.

In this study, no Adverse Event of Special Interest will be collected.

15.5 SERIOUS ADVERSE EVENTS

A serious adverse event or serious adverse drug reaction is any untoward medical occurrence at any dose that:

- Results In Death
- Is Life-Threatening (Immediate Risk Of Death)
- Requires Inpatient Hospitalization or Prolongation of Existing Hospitalization
- Results In Persistent Or Significant Disability/Incapacity
- Results In Congenital Anomaly/Birth Defect
- Important Medical Event

Medical and scientific judgment should be exercised in determining whether an event is an important medical event. An important medical event may not be immediately life-threatening and/or result in death or hospitalization. However, if it is determined that the event may jeopardize the subject and/or may require intervention to prevent one of the other adverse event outcomes, the important medical event should be reported as serious.

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

15.6 ADVERSE REACTION (ADVERSE DRUG REACTION)

An adverse reaction means any AE caused by a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event. This would include all noxious and unintended responses to the Investigational Product related to any dose.

15.7 SUSPECTED ADVERSE REACTION

Suspected adverse reaction means any AE for which there is a reasonable possibility that the drug caused the AE. A suspected adverse reaction implies a judgment by the clinical investigator or study sponsor that there is a reasonable possibility that the AE has a causal relationship with the Investigational Product, though with a lesser degree of certainty about causality, compared to the use of the term “adverse reaction” (or “adverse drug reaction”) as defined above

For the purposes of U.S. IND safety reporting, the meaning of ‘Reasonable Possibility’ is clarified in FDA’s recent changes to CFR 312 by the following examples of types of evidence that would suggest a causal relationship between the drug and the AE, such as:

- A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema)
- One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture)
- An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

15.8 UNEXPECTED ADVERSE DRUG REACTION

An AE or suspected adverse reaction is considered “Unexpected” if it is not listed in the approved product label (for regulatory-approved, commercially available products) or in the investigator brochure, or is not listed at the specificity or severity that has been observed in a serious adverse event at hand; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the available information about the Investigational Product. "Unexpected," as used in this context, also refers to AEs or suspected adverse reactions that are mentioned in the investigator brochure as occurring with the given class of drugs or may be anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular Investigational Product under investigation.

15.9 ABNORMAL TEST FINDINGS

Treatment-emergent laboratory test abnormalities considered to be of clinical concern should be recorded as AEs in the CRF

The criteria for determining whether an abnormal objective test finding should be reported as an adverse event are as follows:

1. Test result is associated with accompanying symptoms, and/or
2. Test result requires additional diagnostic testing or medical/surgical intervention, and/or
3. Test result leads to a change in study dosing or discontinuation from the study, significant additional concomitant drug treatment, or other therapy, and/or
4. Test result is considered to be an adverse event by the investigator or sponsor.

Merely repeating an abnormal test, in the absence of any of the above conditions, **does not** constitute an adverse event.
Any abnormal test result that is determined to be **an error** does not require reporting as an adverse event.

15.10 HOSPITALIZATION

Adverse events reported from studies associated with hospitalization or prolongations of hospitalization are considered serious. Any initial admission (even if less than 24 hours) to a healthcare facility meets these criteria. Admission also includes transfer within the hospital to an acute/intensive care unit (e.g., from the psychiatric wing to a medical floor, medical floor to a coronary care unit, neurological floor to a tuberculosis unit).

Hospitalization does not include the following:

- Rehabilitation facilities
- Hospice facilities
- Respite care (e.g., caregiver relief)
- Skilled nursing facilities
- Nursing homes
- Routine emergency room admissions
- Same day surgeries (as outpatient/same day/ambulatory procedures).

Hospitalization or prolongation of hospitalization in the absence of a precipitating, clinical adverse event is not in itself a serious adverse event. Examples include:

- Admission for treatment of a preexisting condition not associated with the development of a new adverse event or with a worsening of the preexisting condition (e.g., for work-up of persistent pre-treatment lab abnormality)
- Social admission (e.g., subject has no place to sleep)
- Administrative admission (e.g., for yearly physical exam)
- Protocol-specified admission during a study (e.g., for a procedure required by the study protocol)
- Optional admission not associated with a precipitating clinical adverse event (e.g., for elective cosmetic surgery)
- Pre-planned treatments or surgical procedures should be noted in the baseline documentation for the entire protocol and/or for the individual subject.

Diagnostic and therapeutic non-invasive and invasive procedures, such as surgery, should not be reported as adverse events. However, the medical condition for which the procedure was performed should be reported if it meets the definition of an adverse event. For example, an acute appendicitis that begins during the adverse event reporting period should be reported as the adverse event, and the resulting appendectomy should be recorded as treatment of the adverse event.

15.11 SEVERITY ASSESSMENT

The severity of each AE and laboratory abnormality is to be assessed by the investigator according to following criteria

- Mild (Grade 1): AE or laboratory abnormality that is transient or is easily tolerated on continuation of study drug.
- Moderate (Grade 2): AE or laboratory abnormality that causes the patient discomfort and causes interference with the patient's usual activities.
- Severe (Grade 3): AE or laboratory abnormality that is incapacitating and causes considerable interference with the patient's usual daily activities, and/or may be life-threatening if it worsens.
- Life-Threatening (Grade 4): The AE or laboratory abnormality is life threatening as it exists (i.e., no worsening is required for the abnormality to be life-threatening).
- Death Related AE (Grade 5)

Note the distinction between the severity and the seriousness of an adverse event. A severe event is not necessarily a serious event. For example, a headache may be severe (interferes significantly with subject's usual function) but would not be classified as serious unless it met at ease one of the 6 seriousness criteria.

15.12 CAUSALITY ASSESSMENT

The investigator's assessment of causality must be provided for all adverse events (Serious and Non-Serious); the investigator must record the causal relationship in the CRF, as appropriate, and report such an assessment in accordance with the serious adverse reporting requirements if applicable. An investigator's causality assessment is the determination of whether there exists a reasonable possibility that the investigational product caused or contributed to an adverse event. If the investigator's causality assessment is "unknown but not related to investigational product", this should be clearly documented on study records.

In addition, if the investigator determines a serious adverse event is associated with study procedures, the investigator must record this causal relationship in the source documents and CRF, as appropriate, and report such an assessment in accordance with the serious adverse event reporting requirements, if applicable.

WHO-UMC Causality Categories

Causality Term	Assessment Criteria
Certain	• Event or laboratory test abnormality, with plausible time relationship to drug intake • Cannot be explained by disease or other drugs • Response to withdrawal plausible (pharmacologically, pathologically) • Event definitive pharmacologically or phenomenologically (i.e. an objective and specific medical disorder or a recognized pharmacological phenomenon) • Rechallenge satisfactory, if necessary
Probable / Likely	• Event or laboratory test abnormality, with reasonable time relationship to drug intake • Unlikely to be attributed to disease or other drugs • Response to withdrawal clinically reasonable • Rechallenge not required
Possible	• Event or laboratory test abnormality, with reasonable time relationship to drug intake • Could also be explained by disease or other drugs • Information on drug withdrawal may be lacking or unclear
Unlikely	• Event or laboratory test abnormality, with a time to drug intake that makes a relationship improbable (but not impossible) • Disease or other drugs provide plausible explanations

Causality Term	Assessment Criteria
Conditional/Unclassified	• Event or laboratory test abnormality • More data for proper assessment needed, or • Additional data under examination
Unassessable/ Unclassified	• Report suggesting an adverse reaction • Cannot be judged because information is insufficient or contradictory • Data cannot be supplemented or verified

15.13 EXPOSURE DURING PREGNANCY

For Investigational Products and for marketed products, an exposure during pregnancy (also referred to as Exposure In-Utero [EIU] occurs if:

1. A female becomes, or is found to be, pregnant either while receiving or having been directly exposed to the Investigational Product (e.g., environmental exposure), or the female becomes, or is found to be, pregnant after discontinuing and/or being directly exposed to the Investigational Product (maternal exposure).
2. A male has been exposed, either due to treatment or environmental, to the Investigational Products prior to or around the time of conception and/or is exposed during his partner's pregnancy (paternal exposure).

If any study subject or study subject's partner becomes or is found to be pregnant during the study subject's treatment with the Investigational Products, the investigator must submit this information to the Sponsor.

This must be done irrespective of whether an adverse event has occurred and within 24 hours of awareness of the pregnancy.

The information submitted should include the anticipated date of delivery.

Follow-up is conducted to obtain pregnancy outcome information on all Exposure during pregnancy reports with an unknown outcome. The investigator will follow the pregnancy until completion or until pregnancy termination (i.e., induced abortion) and then notify the Sponsor of the outcome. The investigator will provide this information as a follow up to the initial Exposure in Utero Pregnant Form. The reason(s) for an induced abortion should be specified.

An EIU report is NOT created when an ectopic pregnancy report is received since this pregnancy is not usually viable. Rather, a serious adverse event case is created with the event of ectopic pregnancy.

If the outcome of the pregnancy meets the criteria for immediate classification as a serious adverse event (i.e., spontaneous abortion, stillbirth, neonatal death, or congenital anomaly [Including that in an aborted fetus, stillbirth or neonatal death]), the investigator should follow the procedures for reporting serious adverse events.

In the case of a live birth, the "normality" of the newborn can be assessed at the time of birth (i.e., no minimum follow-up period of a presumably normal infant is required before an Exposure in Utero Pregnant Form can be completed).

The "normality" of an aborted fetus can be assessed by gross visual inspection, unless pre-abortion test findings are suggestive of a congenital anomaly.

Additional information about pregnancy outcomes that are classified as serious adverse events follows:

1. Spontaneous abortion; includes miscarriage and missed abortion.
2. All neonatal deaths that occur within 1 month of birth should be reported, without regard to causality, as serious adverse events. In addition, any infant death after 1 month that the investigator assesses as possibly related to the exposure during pregnancy to the investigational medication should be reported.

Additional information regarding the exposure during pregnancy may be requested by the investigator. Further follow-up of birth outcomes will be handled on a case-by-case basis (e.g. follow-up on preterm infants to identify developmental delays).

15.14 WITHDRAWAL DUE TO ADVERSE EVENTS

Withdrawal due to adverse event should be distinguished from withdrawal due to Insufficient Response, according to the definition of adverse event noted earlier, and recorded on the appropriate adverse event CRF page.

When a subject withdraws due to a serious adverse event, the serious adverse event must be reported in accordance with the reporting requirements defined below.

15.15 ELICITING ADVERSE EVENT INFORMATION

A consistent methodology of non-directive questioning should be adopted for eliciting adverse event information at all patient evaluation time points.

Examples of non-directive questions include the following:

- "How have you felt since your last clinic visit?"
- "Have you had any new or changed health problems since you were last here?"

15.16 PROCEDURES FOR RECORDING ADVERSE EVENTS

Investigators should use correct medical terminology/concepts when recording adverse events on the Adverse Event CRF. Avoid colloquialisms and abbreviations.

Only one adverse event term should be recorded in the event field on the Adverse Event Form.

15.17 REPORTING REQUIREMENTS

General Instructions

Investigators will seek information on adverse events at each patient contact.
All adverse events, whether reported by the patient or noted by study personnel, will be recorded in the patient's medical record and on the Adverse Event Form in CRF.

After prescription of Investigational Product, all adverse events, regardless of relationship to study drug, will be reported.

Each adverse event is to be assessed to determine if it meets the criteria for serious adverse events. If a serious adverse event occurs, expedited reporting will follow local and international regulations, as appropriate.

Reporting of Serious Adverse Event

If a serious adverse event occurs, Sponsor or its representative is to be notified within 24 HOURS OF AWARENESS OF THE EVENT by the investigator or delegate on behalf. In particular, if the serious adverse event is fatal or life-threatening, notification to

Sponsor or its representative must be made IMMEDIATELY, irrespective of the extent of available adverse event information.

This timeframe also applies to additional new information (Follow-Up) on previously forwarded serious adverse event reports as well as to the initial and follow-up reporting of Exposure during pregnancy cases.

In the rare event that the investigator does not become aware of the occurrence of a serious adverse event immediately (e.g., if an outpatient study subject initially seeks treatment elsewhere), the investigator is to report the event within 24 hours after being aware of it and document the time of his/her first awareness of the adverse event.

For all serious adverse events, the investigator is obligated to pursue and provide information to Sponsor or its representative in accordance with the timeframes for reporting specified above. In addition, an investigator may be requested by Sponsor or its representative to obtain specific additional follow-up information in an expedited fashion.

In general, this information will include a description of the adverse event in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causality. Information on other possible causes of the event, such as concomitant medications and illnesses must be provided. In the case of a subject death, a summary of available autopsy findings must be submitted as soon as possible to Sponsor or its representative or its designated representative.

Reporting of Non-Serious Adverse Event

All Non-Serious Adverse Events will be reported on the adverse event page(s) of the CRF and reported **WITHIN 180 CALENDAR DAYS** to the local regulatory authority.

15.18 INSTRUCTIONS FOR REPORTING SERIOUS ADVERSE EVENTS FOR STUDY TEAM

In the case of occurrence of a SAE, the Investigator must immediately:

1. SEND the signed and dated corresponding page(s) from the CRF to the representative of the monitoring team whose Name, Fax Number and E-mail Address appear on the Study Protocol

2. ATTACH a photocopy of all examinations carried out and the dates on which these examinations were performed. Care should be taken to ensure that the patient's identity is protected and the patient's identifiers in the Clinical Trial are properly mentioned on any copy of source document provided to the Sponsor. For laboratory results, include the laboratory normal ranges;
3. All further documentation should be sent to the Monitoring Team within 1 working day of knowledge. In addition, any effort should be made to further document each SAE that is fatal or life threatening within the week (7 days) following initial notification.

If the **CRA** is Not Available, Please Contact:

Name	Dr. Mina Saeed
Title	Pharmacovigilance Manager
Mobile	(+2) 01022242873
E-mail	mina.saeed@dataclin.com
Tel	(+202) 3761-7301/2
Fax	(+202) 3345 0061

And sponsor's representative:

Name	Dr. Reem Alaa
Title	Clinical Research Associate
Mobile	(+2) 01202728990
E-mail	research.associate2@mynaturalwellness.com
Tel	(+202) 22720135
Fax	(+202) 2275 2709

15.19 SPONSOR REPORTING REQUIREMENTS TO REGULATORY AUTHORITIES

During the course of the study, the Sponsor will report All Adverse Events to the Local Health Authorities, in accordance with the timeframes for reporting specified above.

15.20 BREAKING RANDOMIZATION CODE

In case of an Adverse Event, the code must be broken only in exceptional circumstances when knowledge of the Investigational Product is essential for treating the patient. If possible, a contact should be initiated with the Monitoring Team before breaking the code.

If the blind is broken, the Investigator will document the date, time of day and reason for code breaking in the CRF.

16 STATISTICAL CONSIDERATIONS

16.1 SAMPLE SIZE CALCULATION[6]

Reference to Sanyal AJ, et al. There was a decrease in Alanine aminotransferase level among placebo arm of -20 (U/liter) among the placebo arm while for the active arm, a double of this improvement will be of clinical value. Accordingly, with an alpha error of 5% using one-sided 95% CI of Mann–Whitney U test, a sample power of 80% and an effect size between placebo and lowest active dose of 0.63, a sample of 43 patients for each arm will be required plus an expected drop-out rate of 10% during 9 months' study duration. Thus, a sample of 47 patients per each treatment arm will be appropriate and the total same of the 3 arms will be 141 patients.

16.2 ANALYSIS POPULATIONS

The obtained clinical data will be analyzed on an intention-to-treat basis. Primary analysis will be done by intention-to-treat including all eligible enrolled patients with at least one treatment dose and post first dose assessment (ALT & AST) attending any post treatment visit and efficacy variables will be analyzed using the last-observation-carried-forward convention (LOCF).

16.3 STUDY ENDPOINTS

During interim analysis (at 6 months of treatment) and final analysis at the end of treatment, the following endpoints will be evaluated:

16.3.1 Primary Efficacy Endpoint:

- Compare the mean relative change in ALT & AST levels between the experimental arms and the control arm.
- Compare proportions of patients with normal ALT & AST at end of treatment between the experimental arms and the control arm. The cut-off values of ALT were 0-43 IU/L and of AST were 0-40 IU/L[7].

16.3.2 Secondary Efficacy Endpoints:

- Comparing the mean change in Fib-4 score between the experimental arms and the control arm.
- Comparing the frequency and percentage of patients experiencing hepatic complications

6 Gpower 3.1

7 Uslusoy H, Nak S, Gülten M, Bıyıklı Z. Non-alcoholic steatohepatitis with normal aminotransferase values. World Journal of Gastroenterology. 2009;15(15):1863.

between the experimental arms and control arm.

16.3.3 Exploratory Endpoint:

- Compare the mean relative change in lipid profile levels between the experimental arms and the control arm.

16.4 STATISTICAL ANALYSES

The statistical analysis of this study will consist of reporting of individual data listings, providing descriptive statistics for parameters of interest, and statistical testing of the primary and secondary variables.

The final statistical analyses will be performed when the patients on Heptex and placebo complete 9 months of treatment.

Detailed analysis methods will be provided in the statistical analysis plan.

16.4.1 Descriptive analysis:

- Percent (%) distribution for all categorical variables and mean with (SD) and median (Minimum: Maximum) for continuous variables according their distribution.

16.4.2 Primary analysis:

- The main objectives of this phase II study is to explore the anti-oxidant activity of Heptex as assessed by improvement in serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels by measuring:
- The mean relative change in ALT & AST levels between the experimental arms and the control arm from baseline till end of treatment using T-test (Mann-Whitney test in case of non-parametric data). This analysis will be a comparative analysis and will be done on eligible population of patients without protocol violation and who have at least one treatment dose and an evaluable primary endpoint (AST and ALT).
- Number of patients with normal ALT & AST at end of treatment between the experimental arms and the control arm. The cut-off values of ALT were 0-43 IU/L and of AST were 0-40 IU/L8 using chi square (Fischer test for non-parametric data). This analysis will be comparative analysis and will be conducted on the eligible population of patients without protocol deviation, who have at least one treatment dose and an evaluable primary endpoint (ALT and AST).

All tests will be performed on the 5% level of significance.

8 Uslusoy H, Nak S, Gülten M, Bıyıklı Z. Non-alcoholic steatohepatitis with normal aminotransferase values. World Journal of Gastroenterology. 2009;15(15):1863.

Procedure for accounting for missing, unused, and spurious data will be explained in the statistical analysis plan.

Any deviation(s) from the original statistical plan will be described in the final report).

16.4.3 Secondary analysis:

- The mean change in Fib-4 score between the experimental arms and the control arm from baseline till end of treatment using T-test (Mann-Whitney test in case of non-parametric data). This analysis will be a comparative analysis and will be done on eligible population of patients without protocol violation and who have at least one treatment dose and an evaluable primary endpoint (AST and ALT).
- Number of patients experiencing hepatic complications between the experimental arms and control arm at the end of the treatment using chi-square (Fischer for non-parametric data. This analysis will be a comparative analysis and will be done on eligible population of patients without protocol violation and who have at least one treatment dose and an evaluable primary endpoint (AST and ALT).

16.4.4 Exploratory analysis:

- The mean relative change in lipid profile levels between the experimental arms and the control arm from baseline till end of treatment using T-test (Mann-Whitney test in case of non-parametric data). This analysis will be a comparative analysis and will be done on eligible population of patients without protocol violation and who have at least one treatment dose and an evaluable primary endpoint (AST and ALT).

16.4.5 Interim analysis:

- Interim analysis will be performed when all patients complete 6 months of treatment. If a statistically significant difference is observed between placebo and active arm(s), the study will be terminated for the benefit of patients who were randomly assigned to placebo arm and to rapidly disseminate evidence supporting a treatment benefit to the broader community[9].

9Zannad F, et al. Development of Therapeutics for Heart Failure. When to Stop a Clinical Trial Early for Benefit: Lessons Learned and Future Approaches. Circulation: Heart Failure. 2012; 5: 294-302. doi: 10.1161/CIRCHEARTFAILURE.111.965707

16.5 RANDOMIZATION

During screening visit, each patient will be allocated to a Patient Identifier (Patient ID) number. This will be a 4-digit number where the first digit identifies the study site and the last 3 digits (sequential numbers within the site) will identify the patient at a given study center. The subject ID will be retained throughout the study and will be used to uniquely identify each patient. Even patient who are not eligible to participate in the study will be assigned a patient ID and will be recorded in the screening log.

For patients eligible to receive double-blind treatment, a randomization number will be allocated at baseline visit (Visit 2/ Week 0) at the time of randomization. This randomization number will be used to identify and dispense the drug kits allocated to each subject.

Note that the subject identifier number (Subject ID) which uniquely identifies the subject is different from the randomization number (uniquely identifies a drug kit). It is the investigator's responsibility to ensure that each subject receives the study medication allocated to him.

Randomization of patients to one of the 3 treatment arms will be done through "Interactive response system".

The double-blind supplies will be provided to the investigator along with individually sealed envelopes containing the randomization (assigned drug) for each patient. This allows the investigator to break the treatment code for an individual patient in the event of an emergency. These sealed randomization envelopes must be kept in a secure place for inspection by Sponsor and/or its designated representative from time to time during the course of study and will be collected by the Clinical Research Associate at the end of the study. Even if a pharmacy is used to store and/or dispense the medication, the sealed copy of the randomization will remain with the investigator.

17 ETHICAL AND REGULATORY STANDARDS

17.1 ETHICAL PRINCIPLES:

This study will be conducted in accordance with the 18th World Medical Assembly (Helsinki, 1964) and all subsequent amendments and ICH guidelines for Good Clinical Practice (ICH-E6). Study team will ensure all necessary regulatory submissions (e.g.: IRB/IEC) are performed in accordance with local regulations including local data protection regulations.

17.2 LAWS AND REGULATIONS:

This Clinical Trial will be conducted in compliance with all applicable international laws and regulations, and national laws and regulations of Egypt in which the Clinical Trial is performed, as well as any applicable guidelines.

17.3 INFORMED CONSENT:

The Investigator (according to applicable regulatory requirements), or a person designated by the Investigator, and under the Investigator's responsibility, should fully inform the patient of all pertinent aspects of the Clinical Trial including the written information giving approval/favorable opinion by the Ethics Committee (IRB/IEC). All participants should be informed to the fullest extent possible about the study, in language and terms they are able to understand.

Prior to a patient's participation in the Clinical Trial, the written Informed Consent Form must be signed, name filled in and personally dated by the patient or by the patient's legally acceptable representative and by the person who conducted the informed consent discussion. A copy of the signed and dated written Informed Consent Form will be provided to the patient.

The Informed Consent Form used by the Investigator for obtaining the patient's informed consent must be reviewed and approved by the Sponsor prior to submission to the appropriate Ethics Committee (IRB/IEC) for approval/favorable opinion.

17.4 INSTITUTIONAL REVIEW BOARD/INDEPENDENT ETHICS COMMITTEE (IRB/IEC)

The Investigator or the Sponsor must submit this Clinical Trial Protocol to the appropriate Ethics Committee (IRB/IEC). Written dated approval/favorable opinion signed by the Chairman with Ethics Committee (IRB/IEC) composition must be obtained.

Investigational Product will not be released at the study site and the Clinical Trial will not start until a copy of this written and dated approval/favorable opinion has been received by the Sponsor.

During the Clinical Trial, any amendment or modification to the Clinical Trial Protocol should be submitted to the Ethics Committee (IRB/IEC). It should also be informed of any event likely to affect the safety of patients or the continued conduct of the Clinical Trial, in particular any change in safety.

If requested, a summary of the Clinical Trial's outcome at the end of the Clinical Trial is sent to the Ethics Committee (IRB/IEC).

18 DISCONTINUATION/ WITHDRAWAL OF PARTICIPANTS FROM STUDY

Each participant has the right to withdraw from the study at any time. In addition, the investigator may discontinue a participant from the study at any time if the investigator considers it necessary for any reason including:

1) Ineligibility (either arising during the study or retrospective having been overlooked at screening)
2) Significant protocol deviation
3) Significant non-compliance with treatment regimen or study requirements
4) Disease progression which requires discontinuation of the study medication or results in inability to continue to comply with study procedures
5) Consent withdrawn.
6) Lost to follow up

The reason for withdrawal will be recorded in the CRF.

If the participant is withdrawn due to an adverse event, the investigator will arrange for follow-up visits or telephone calls until the adverse event has resolved or stabilized.

19 PREMATURE DISCONTINUATION OF THE STUDY OR PREMATURE CLOSE-OUT OF A SITE

The sponsor may discontinue the study or close-out a site in some cases e.g.;

- If the information on the product leads to doubt as to the benefit/risk ratio;
- If the Investigator has received from the Sponsor all Investigational Product, means and information necessary to perform the Clinical Trial and has not included any patient after a reasonable period of time mutually agreed upon;
- If results of interim analysis show a statistically significant difference between placebo and active arms (in favor of active arm), the study will be terminated for the benefit of patients who were randomly assigned to placebo arm and to rapidly disseminate evidence supporting a treatment benefit to the broader community;
- If the aim of the Clinical Trial has become outdated or is no longer of interest;
- In the event of breach by the Investigator of a fundamental obligation under this agreement, including but not limited to breach of the Clinical Trial Protocol, breach of the applicable laws and regulations or breach of the ICH guidelines for Good Clinical Practice.

In any case, the Sponsor will inform the appropriate Ethics Committee(s) (IRB/IEC) and Health Authorities. In addition, investigators should be notified by written notice.

20 RESPONSIBILITIES OF INVESTIGATORS AND SPONSORS

20.1 RESPONSIBILITIES OF THE INVESTIGATORS

The Investigator is responsible to strictly follow the study protocol and all applicable regulations and guidelines.

Investigator's responsibilities include recording precise and accurate data pertinent to the study in the electronic CRF in addition to obtaining written informed consents from patients prior to inclusion in the study.

The investigator will be responsible for taking all appropriate measures to ensure patient's safety all over the study duration.

Study documents will be appropriately retained until the end of the study. In addition the Investigator should comply with local regulations with regards to record retention.

It is recommended that the Investigator retains the study documents at least five years after the completion or discontinuation of the study.

The Investigator agrees to allow and help with the performance of sponsor auditors/ regulatory authorities' inspectors to have direct access to study records, all necessary data, documents and facilities. Once the investigator is notified of an inspection, he shall inform the sponsor and authorize sponsor to participate in this inspection. The confidentiality of the data verified and the protection of the patients should be respected during these inspections. The investigator will make sure that any results and information resulting from the inspections will be immediately communicated to sponsor. It will be the investigator's responsibility to take corrective actions for any issues raised during the audits or inspections.

Names and responsibilities of coordinating investigator(s) and the other participating investigators will be documented prior to the start of the trial. All investigators will be given instructions on following the protocol, complying with a uniform set of standards for the assessment of clinical and laboratory findings, and completing the CRFs. Communication between investigators should be facilitated.

20.2 RESPONSIBILITIES OF SPONSOR

The sponsor will be responsible for providing adequate resources to ensure the proper conduct of the study according to study protocol and all applicable laws and regulations.

The sponsor will delegate a CRO to conduct the study.

Sponsor/ delegated CRO is responsible for all regulatory submission(s). In addition, sponsor/ delegated CRO will contract insurance for all participating patients during study participation.

Delegated CRO will be responsible for ongoing reporting of adverse events and serious adverse events to local regulatory authorities.

21 CONFIDENTIALITY AND DATA PROTECTION

21.1 CONFIDENTIALITY

All study related material, information and unpublished documents provided to the investigator are the exclusive property of sponsor.

These materials or information cannot be disclosed by any person to unauthorized persons without the prior formal written approval from sponsor.

The Investigator will consider all received information or resulted data as confidential and will take all necessary steps to ensure that there is no break of confidentiality, other than for information to be disclosed by law.

21.2 DATA PROTECTION

Patient's personal data (name, phone number, address …etc.) shall be kept confidential all over the study participation period and after study completion as well.

Upon archiving or processing patients' personal data, sponsor shall take all appropriate measures to prevent access to this data by any unauthorized third party.

22 PUBLICATIONS

All study investigators give full authority to the sponsor for primary publication of results. No other publication is allowed before the primary publication. Any subsequent publications by a study participant must be approved by the sponsor and make reference to the study and the primary publication. Sponsor may request that his name and/or names of one or several of its employees appear or do not appear in such publications.

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