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RESEARCH

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The activity of a herbal medicinal product of Phyllanthus niruri and Silybum marianum powdered extracts (Heptex®) in patients with apparent risk factors for nonalcoholic steatohepatitis: a phase II, multicentered, randomized, double-blind, placebo-controlled clinical trial

Mohamed Kamal Shaker[1*], Mohamed Hassany[2], Basem Eysa[2], AbdulMoneim Adel[2], Ahmed Zidan[2] and Shahnaz Mohamed[3]

Abstract

Background Nonalcoholic steatohepatitis (NASH) is a severe form of nonalcoholic fatty liver disease (NAFLD) characterized by damage and inflammation of hepatocytes. Some medicinal plants have shown antioxidant and anti-inflammatory effects on liver cells. We aimed to investigate the hepatoprotective effect of Heptex® capsules containing 200 mg of Dukung Anak (a powdered extract from aerial parts of *Phyllanthus niruri*) and 100 mg of Milk Thistle (a powdered extract from fruits of *Silybum marianum*) in patients with an apparent risk factor for NASH.

Methods This was a phase II, randomized, double-blind, placebo-controlled, three-arm, interventional clinical trial. Patients were randomized in a 1:1:1 ratio to receive placebo, low dose (one capsule) of Heptex®, or high dose (two capsules) of Heptex®. After 36 weeks, liver enzymes, Fib-4 score, lipid profile, CAP score, and kPa score were evaluated. Patients were monitored for safety throughout the treatment duration.

Results A total of 146 patients were enrolled in the study. A significant decrease was observed in ALT levels in the low-dose group (57 IU/L to 40 IU/L, $p=0.026$) and the high-dose group (61 IU/L to 47.5 IU/L, $p<0.0001$) and in AST levels in the high-dose group (43.5 IU/L to 32 IU/L, $p=0.001$), with no significant difference between the relative percent change in ALT ($p=0.465$) or AST ($p=0.632$) between the three groups. No significant difference was revealed between the three groups regarding the median change in Fib-4 score at the end of treatment ($p=0.985$). No significant change in the lipid profile was observed in any of the three groups except for the total cholesterol level, which significantly decreased from 210 IU/L to 187 IU/L, $p=0.031$ in the low-dose group.

*Correspondence:
Mohamed Kamal Shaker
mohamedshaker@med.asu.edu.eg; dr_shaker@hotmail.com;
mohamed.k.shaker@gmail.com
Full list of author information is available at the end of the article

Conclusion Heptex® capsules were safe and well tolerated over a treatment period of 36 weeks. However, the hepatoprotective effect in patients at risk of NASH still needs further assessment using more accurate investigation tools, a larger sample size, and/ or higher doses of the combination.

Trial registration Retrospectively registered (registration date: 25/04/2022; trial registration number: NCT05343780)

Keywords NASH, NAFLD, Silymarin, Phyllanthus niruri, Silybum marianum, Hepatoprotection

Introduction

Nonalcoholic fatty liver disease (NAFLD), also known as metabolic associated fatty liver disease (MAFLD), is the most common chronic liver disease with a prevalence of almost 25% worldwide and 31.8% in all adults in the Middle East and North Africa regions [1, 2]. NAFLD is defined as hepatic fat accumulation in ≥ 5% of hepatocytes according to histology without alcohol consumption, long-term use of a steatogenic medication, or monogenic hereditary disorders [3, 4]. Non-alcoholic steatohepatitis (NASH) is a subtype of NAFLD characterized by hepatocyte damage and inflammation. NASH can progress to cirrhosis, liver failure, and hepatocellular cancer, with an estimated death rate of 11.77 out of 1,000 persons per year [1, 5, 6].

The etiology of NASH has been linked to several risk factors, such as body obesity, insulin resistance, and oxidative stress [7–9]. Since there is no primary therapy, modifying risk factors such as lifestyle interventions, increasing insulin sensitivity, lowering cholesterol levels, and using antioxidants are recommended to manage NASH [10, 11]. Several studies examined the effectiveness of antioxidants in NASH patients, such as vitamin E, thiazolidinedione derivatives, and glucagon-like peptide-1 (GLP-1) analogs; however, different guidelines have not agreed on the impact of any of them [12, 13].

Consideration may be given to complementary therapies like herbal medicines since they have shown promising effects in treating many diseases [14]. One of those herbs is *Silybum marianum*, known as milk thistle, which has been used for many years to treat liver diseases [15, 16]. In vivo, silymarin —an active ingredient of milk thistle— has exhibited hepatoprotective activity against damage caused by numerous toxic substances, where it inhibits lipid peroxidation and reduces the cellular uptake of xenobiotics. All of which have anti-inflammatory, anti-fibrotic, and antioxidant effects [17–19]. Moreover, evidence from placebo-controlled randomized clinical trials (RCTs) showed that silymarin significantly reduced levels of liver transaminase enzymes (aspartate aminotransferase [AST] or alanine aminotransferase [ALT]) in NAFLD and NASH patients [20, 21]. A 2017 meta-analysis that included eight RCTs involving 587 patients with NASH also reported that silymarin successfully reduced transaminases levels in this population [22].

In addition, *Phyllanthus niruri* (Dukung Anak) is frequently used in herbal medicine. Several studies showed the possible antioxidant, anti-microbial, anti-inflammatory, and hepatoprotective effects of Phyllanthus species in treating liver diseases by preventing oxidation and lipid peroxidation [23–29]. One pilot study that was conducted in Malaysia to examine the safety and efficacy of *Phyllanthus niruri* in patients with NASH showed decreased ALT and AST levels. However, this reduction was not statistically significant, which may be attributed to the small sample size [30]. In contrast, the hepatoprotective activity of *Phyllanthus niruri* was significant both in vitro (in rat and human hepatic cells) and in vivo in rat animal models. In vitro, pretreatment *Phyllanthus niruri* aqueous extract successfully maintained normal AST, ALT, glutathione (GSH), and superoxide dismutase levels in both rat liver normal cells and human liver hepatoma cells, upon inducing cytotoxicity by treatment with carbon tetrachloride (CCl_4). Similarly, CCl_4 injections induced hepatotoxicity in male Wistar rats, presenting as 2.5- and 6-fold increase of AST and ALT levels, respectively, accompanied with GSH depletion and 1.5-fold increase in reactive oxygen species. These elevated levels were significantly restored in rats pretreated with a minimum dose of 100 mg kg^{-1} day^{-1} of the aqueous extract of *Phyllanthus niruri* [31]. Similar results were reported in another study on albino mice in 2007, where pretreatment of *Phyllanthus niruri* protected hepatic tissues against oxidative toxicity [27].

Consistently, *Silybum marianum* and *Phyllanthus niruri* combination (Heptex®) was found to decrease CCl_4 induced hepatoxicity in rats — expressed as increased serum levels of AST, ALT, alkaline phosphatase and lactate dehydrogenase enzymes. Additionally, pretreatment with the combination inhibited the oxidative stress inflected by CCl_4 toxicity in a dose-related manner. Pretreatment of rats with *Silybum marianum* and *Phyllanthus niruri* combination followed by CCl_4 injection also modulated the inflammatory marker TNF-alpha expression compared to rats receiving CCl_4 injections only. More data on this preclinical study is available in Additional file 1: Investigator's Brochure.

Safety and genotoxicity of the combination were evaluated in vivo in rats treated with 250, 500 and 1000 mg/kg Heptex® for 14 days, and their liver and ovary/testis

tissues were analyzed for histopathological alterations. The safety and lack of toxicity of the combination were established as no signs of genotoxicity or pathological abnormalities were detected [32].

Given the results of preclinical studies, we aimed to further evaluate the safety and effectiveness of this combination in human subjects at risk of hepatocyte damage associated with NASH. To our knowledge, no previous clinical studies were conducted on the *Silybum marianum* and *Phyllanthus niruri* combination in patients with apparent risk factors for NASH.

This was an exploratory study with the primary objective of exploring the antioxidant activity of the combination of *Silybum marianum* and *Phyllanthus niruri* (Heptex®) as assessed by improvement in serum AST and ALT levels in patients with apparent risk factors for NASH. The secondary objectives included 1) exploring the optimum dose that is safe in patients with apparent risk factors for NASH giving the most significant effect among two different doses of *Silybum marianum* and *Phyllanthus niruri* combination, 2) exploring the hepatoprotective effect of the combination as assessed by the change in Fibrosis score, and 3) exploring the safety of the combination as assessed by the occurrence of hepatic complications. An exploratory objective was to explore the lipid-lowering effect of the combination as assessed by the change in lipid profile.

Subjects and methods

Study population

Inclusion criteria

We included both genders aged between 18 and 65 years old, with elevated liver enzymes (cut-off values of ALT were 0–43 IU/L and of AST were 0–40 IU/L), controlled attenuation parameter (CAP)-confirmed steatosis, and Fib-4 scores of F1 and F2 assessed by the FibroScan liver stiffness (kPa) measurement. In addition, eligible patients had serum albumin > 3 g/dl, international normalized ratio (INR) < 2, no ascites on ultrasound, and no documented or suspected hepatic encephalopathy. All assessments were done by designated gastroenterologists. Males and females with childbearing potential agreed to use contraceptives during the study and after 36 weeks of treatment. Patients willed to stop any liver support or hepatoprotective medications during the study.

Exclusion criteria

Patients were excluded if they were underweight (body mass index [BMI] < 18.5 kg/m^2), extremely obese (BMI > 40 kg/m^2), pregnant or lactating, had a history of alcohol abuse, had comorbidities such as uncontrolled diabetes mellitus (HbA1c ≥ 8.5%), ischemic heart disease or any history of or current chronic illness that may have interfered with the study. In addition, patients with kidney abnormalities (Serum creatinine > 1.5 × upper normal serum creatinine or creatinine clearance [GFR] < 60 mL/minute) or liver abnormalities (Platelet count < 75,000/mm^3, viral hepatitis; drug-induced liver injury; metabolic liver disease; auto-immune liver disease; liver transplantation; liver cancer or serum alpha-fetoprotein [AFP] > 100 ng/ml; or AFP between 50 and 100 ng/ml with liver ultrasound within three months of screening, or at screening, showed evidence of potential hepatocellular cancer) were excluded. Moreover, during the study period, patients who received drugs such as thiazolidinediones, drugs known to induce liver steatosis or alter liver enzymes or affect body weight and carbohydrate metabolism, or had a history of taking parenteral nutrition or drug allergy were excluded. If patients had any investigational drug within six months before screening, active enrolment in another investigational medication or device trial, or prior medication that may affect the study were also excluded.

Study discontinuation and exclusion from assessment

Participants in the study had the right to withdraw voluntarily at any time. Additionally, the investigators had the authority to discontinue participants for any of the reasons indicated in the study protocol, including instances of ineligibility arising during the study or retrospectively identified following oversight during screening, significant protocol deviations, significant non-compliance with treatment regimen or study requirements, disease progression that necessitated discontinuation of the study medication or hindered compliance with study procedures, withdrawal of consent, or being lost to follow-up.

Randomization and treatment

This was a phase II, randomized, double-blind, placebo-controlled, three-arms, parallel-groups, interventional clinical trial. Patients and investigators were blinded, where both were unaware of the treatment allocations; additionally, placebo and Heptex capsules were identical in physical appearance and mode of administration. Blinding was maintained throughout the study period. The site pharmacist was delegated by the investigators to generate allocation codes using interactive web response technology (IWRS). Patients fulfilling the eligibility criteria were randomized in a 1:1:1 ratio to take either placebo (rice bran in 2 capsules size 1), low-dose Heptex® (one capsule of the drug equally distributed and inserted into two capsules size 1), or high-dose Heptex® (two capsules of the drug equally distributed and inserted into two capsules size 1). A nutritionist was available onsite to provide dietary advice to all patients as part of routine practice;

this was not part of the study intervention and was not included in the analysis. Study data were collected at the Tropical Medicine Department at the National Hepatology and Tropical Research Institute (NHTMRI), while laboratory tests were performed in the designated central laboratory "Al-Mokhtabar". Fibroscan and ultrasound were also done at the Tropical Medicine Department at NHTMRI.

Each capsule of Heptex® contained 200 mg of Dukung Anak (powdered extract from aerial parts of *Phyllanthus niruri)* and 100 mg of *Milk Thistle* (powdered extract of *Silybum marianum* fruits). The capsules were taken orally three times daily on an empty stomach (15 min before meals or 1 h after meals) with plenty of water (240 ml water or a full glass) for 36 weeks. This period included 2 washout periods (at Week 13 & Week 25), in accordance with ICH guidelines to align the repeated dose studies previously conducted with the proposed duration of the trial. The detailed analytical data supporting the standardization of the physicochemical and microbial controls for Heptex®, including chromatograms and test results, are provided in Additional File 2.

Study endpoints

The primary endpoints were 1) comparing the relative change in ALT & AST levels between the experimental arms and the control arm and 2) comparing the proportions of patients with normal ALT & AST at the end of treatment between the experimental arms and the control arm with cut-off values of 0–43 IU/L for ALT and 0–40 IU/L for AST. The secondary endpoints included 1) comparing the change in Fib-4 score between the experimental arms and the control arm and 2) comparing the frequency and percentage of patients experiencing hepatic complications between the experimental arms and control arm. An exploratory endpoint was comparing the mean relative change in lipid profile levels between the experimental arms and the control arm.

Sample size calculation

The primary objective of our study was to assess the efficacy and the safety of the combination of 200 mg of *Phyllanthus niruri* and 100 mg *Silybum marianum* (Heptex®) as measured by improvement in the mean change of AST and ALT levels after 36 weeks in patients with apparent risk factors for NASH. In reference to Sanyal AJ et al., there was a decrease in ALT level among the placebo arm of -20 (U/liter), while for the active arm (pioglitazone), double of this improvement was of clinical value [33]. Accordingly, with an alpha error of 5% using a one-sided 95% CI of Mann–the Whitney U test, 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 was required plus an expected drop-out rate of 10% over the 36-week study duration. Thus, a sample of 47 patients per treatment arm was appropriate, resulting in a total of 141 patients across the three arms.

Statistical analysis

Analysis population

The primary analysis followed the intention-to-treat principle, including all eligible enrolled patients with at least one treatment dose and post-first dose assessment (ALT & AST) attending any post-treatment visit. Efficacy variables were analyzed using the last-observation-carried-forward (LOCF) method.

Descriptive analysis

We summarized normally distributed quantitative data using mean and standard deviation (SD), non-normally distributed quantitative variables using median and interquartile range (IQR), and categorical variables using counts and percentages.

Comparative analysis

The median relative percent change of ALT, AST, Fib-4, and lipid profile levels between the experimental arms and the control arm were analyzed using the Mann–Whitney test and the Kruskal–Wallis test. Wilcoxon signed-rank test was used to compare the median values in each treatment arm at screening and Week 36. The numbers of patients with normal levels of ALT and AST and those who experienced hepatic complications at the end of treatment were compared between the experimental and the control arms using the Chi-square test. These analyses were comparative and were 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). Additionally, we conducted comparative analysis to assess the relationships between variables. Specifically, we employed two ordinary least squares (OLS) regression models. One model used the FibroScan-AST (FAST) score as the outcome variable, while the other model used ALT as the outcome variable. For both models, screening measurements for ALT and FAST score were included as covariates, respectively, to account for their potential influence on the outcome variables. Additionally, diabetes and hypertension (combined into a single confounder to minimize reduction in degrees of freedom) and body weight were also included as covariates in both models to explore their potential impact.

Trial registration

The trial was registered under the title "Clinical Trial to Investigate the Anti-oxidant Activity of Heptex in

Patients With Apparent Risk Factors of NASH (PHYLLANTEX)" with the full protocol accessible through ClinicalTrials.gov website [34]. Trial registration number: NCT05343780

Ethical considerations

Ethics committee approval was obtained from the Tropical Medicine Department at the Faculty of Medicine, Ain-Shams University, and the National Hepatology and Tropical Medicine Research Institute (NHTMRI). All patients provided written informed consent before conducting any study-related procedures.

Results

Study Population

Recruitment of eligible patients took place from June 10th, 2019 to July 8th, 2021. A total of 167 patients were screened for enrollment in the study (safety population). Of these, 146 patients were randomized into the three treatment groups. While a sample size of 141 patients was intended, more patients were recruited to account for potential dropouts due to the study taking place during the COVID-19 pandemic. After randomization, ten patients were lost to follow-up and eight other patients were excluded from the efficacy analysis for various reasons, as shown in Fig. 1. A total of 128 patients were included in the efficacy analysis, including 43 patients in the placebo group, 41 patients in the low-dose Heptex® group, and 44 patients in the high-dose Heptex® group. More details are provided in Fig. 1.

Baseline characteristics and medical history

Most of the study patients were females ($n=84$; 65.6%), married ($n=122$; 95.3%), unemployed ($n=78$; 60.9%), and received primary/basic education ($n=90$; 70.3%). Nine patients (7.0%) had diabetes mellitus type 2, three had hypertension (2.3%), and one patient reported having dyspepsia (0.8%). Table 1 shows details of the baseline characteristics and medical history of the study population.

Efficacy analysis

Change in liver enzymes

The median ALT level significantly decreased from 59 IU/L in the screening visit to reach 38 IU/L at Week 36 in the placebo group ($p=0.006$), from 57 IU/L to 40 IU/L in the low-dose group ($p=0.026$), and from 61 IU/L to 47.5 IU/L in the high-dose group ($p<0.0001$). However, the median relative percent reduction in ALT level did not significantly differ between the three study groups ($p=0.465$). At the end of treatment, 58.1% of patients in the placebo group, 51.2% of patients in the

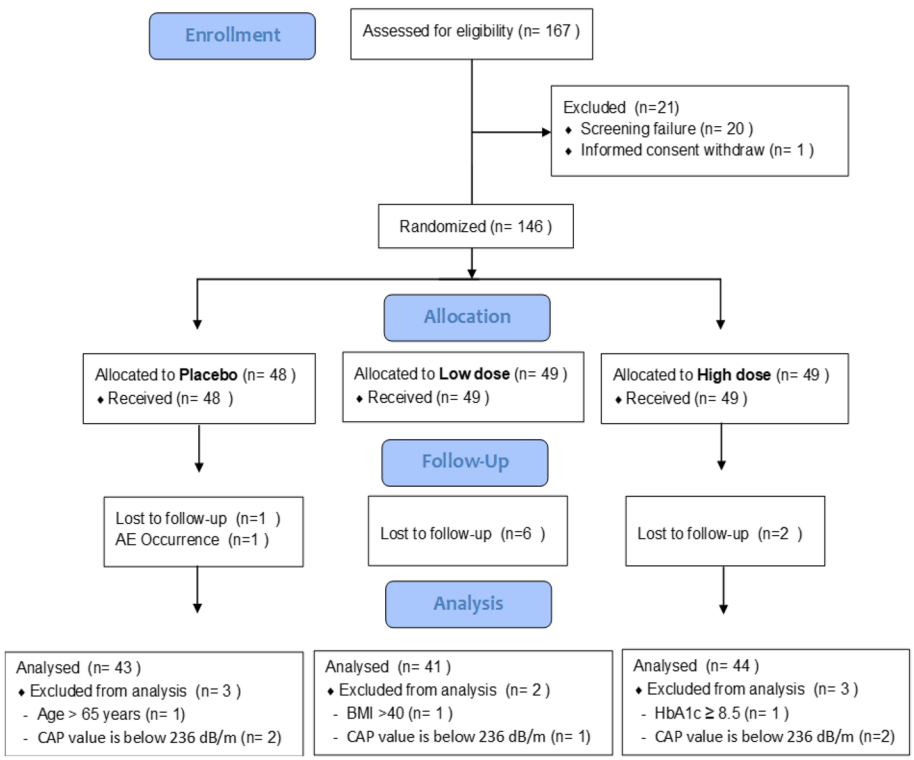

Fig. 1 Flow diagram of the study patients

Table 1 Baseline characteristics and medical history of the study population (*N* = 128)

	Placebo (*N* = 43)	**Low dose (*N* = 41)**	**High dose (*N* = 44)**
Characteristic			
Age (years)—*mean ± SD*	40 ± 9	40.5 ± 9.7	40.5 ± 9.6
BMI (Kg/m^2)—*mean ± SD*	33.9 ± 3.6	34.4 ± 4.1	34.1 ± 4.6
Gender – *no. (%)*			
Males	13 (30.2)	16 (39)	15 (34.1)
Females	30 (69.8)	25 (61)	29 (65.9)
Marital Status – *no. (%)*			
Single	3 (7)	3 (7.3)	-
Married	40 (93)	38 (92.7)	44 (100)
Education – *no. (%)*			
None	-	-	-
Basic/Primary	31 (72.1)	29 (70.7)	30 (68.2)
Secondary	6 (14)	6 (14.6)	6 (13.6)
College graduate/higher	6 (14)	6 (14.6)	8 (18.2)
Employment – *no. (%)*			
Employed	13 (30.2)	16 (39)	21 (47.7)
Unemployed	30 (69.8)	25 (61)	23 (52.3)
Residence – *no. (%)*			
Rural	-	1 (2.4)	1 (2.3)
Urban	43 (100)	40 (97.6)	43 (97.7)
Heart Rate (beats/min)—*mean ± SD*	78.7 (4.5)	80 (4.1)	78 (5.2)
FibroScan liver stiffness (KPa)—*mean ± SD*	6.7 (1.2)	6.6 (1.2)	6.7 (1.1)
FIB-4 score—*mean ± SD*	1.03 (0.6)	0.9 (0.6)	0.9 (0.4)
Controlled Attenuation Parameter (CAP), dB/m—*mean ± SD*	333.8 (40.1)	325.8 (45.4)	340.8 (34.8)
Medical History			
Does the patient have any co-morbidities? – *no. (%)*			
Yes	5 (11.6)	4 (9.8)	2 (4.5)
Diabetes Mellitus Type II	5 (11.6)	2 (4.9)	2 (4.5)
Hypertension	2 (4.7)	1 (2.4)	-
Gastrointestinal disorders (Dyspepsia)	-	1 (2.4)	-
No	38 (88.4)	37 (90.2)	42 (95.5)
Does the patient take any concomitant medications? – *no. (%)*			
Yes	5 (11.6)	4 (9.8)	2 (4.5)
Antihypertensives	2 (4.7)	1 (2.4)	-
Analgesics	1 (2.3)	-	-
Anti-diabetics (Oral, Insulin)	5 (11.6)	2 (4.9)	2 (4.5)
PPIs	-	1 (2.4)	-
Anti-emetics	-	1 (2.4)	-
No	38 (88.4)	37 (90.2)	42 (95.5)

low-dose group, and 45.5% of patients in the high-dose group reached normal ALT levels, with no statistically significant difference in the proportions of patients who reached normal ALT level in the three groups ($p = 0.495$).

In addition, the median AST level significantly decreased from 44 IU/L in the screening visit to reach 34 IU/L at Week 36 in the placebo group ($p = 0.037$) and from 43.5 IU/L to 32 IU/L in the high-dose group ($p = 0.001$), while no statistically significant reduction was observed in the low-dose group ($p = 0.054$). Similar to ALT, no statistically significant difference was observed between the three groups regarding the median relative percent reduction in AST ($p = 0.632$). At the end of treatment, 65.1% of patients in the placebo group, 65.9%

of patients in the low-dose group, and 68.2% of patients in the high-dose group reached normal AST levels, with no statistically significant difference in the proportions of patients who reached normal AST level in the three groups ($p = 0.951$). More details about the change in ALT level and AST level are provided in Table 2.

Changes in CAP, KPa, FIB-4, and FAST scores

The median CAP score significantly decreased from 335 dB/m to 320 dB/m in the placebo group ($p = 0.014$), from 343 dB/m to 315 dB/m in the high-dose group ($p < 0.0001$), while no significant change was observed in the low-dose group ($p = 0.064$). On the other hand, no significant change in KPa scores was observed in any of the study groups ($p > 0.05$). In addition, no significant difference was revealed between the three groups regarding the median change in Fib-4 score at the end of treatment ($p = 0.985$). Table 3 shows additional details about the change in CAP score, KPa score, and Fib-4 score. Similarly, the mean FAST score decreased across all the three study arms. Where at screening, mean baseline FAST score was 0.492, 0.436, and 0.476 in the placebo group, low-dose group, and high-dose group, respectively, i.e., greater than the cut-off point for lower likelihood of NASH (0.35 or less). At Visit 6, FAST scores decreased to 0.311 in the low-dose group and 0.340 in the high-dose group. Additional details on the change in FAST scores are also available in Table 3.

Change in lipid profile

The lipid profile was assessed at the screening visit and the end of treatment. No statistically significant change was observed in HDL, LDL, or triglyceride levels in any of the study groups ($p > 0.05$). However, the median total cholesterol in the low-dose group significantly decreased from 210 IU/L in the screening visit to reach 187 IU/L at Week 36 ($p = 0.031$), while no significant difference was observed in the placebo group ($p = 0.845$) or the high-dose group ($p = 0.577$). Table 4 shows the change in lipid profile in each study group.

Table 2 Comparing the change in ALT and AST in the Three Groups

	Placebo (*N* = 43)		**Low dose (*N* = 41)**		**High dose (*N* = 44)**	
	Screening	**Week 36**	**Screening**	**Week 36**	**Screening**	**Week 36**
ALT (IU/L)*						
Count	43	43	41	41	44	44
Median (IQR)	59 (45)	38 (38)	57 (28)	40 (39)	61 (36)	47.5 (41.8)
Mean ± SD	75.3 ± 38.6	61.3 ± 62.2	66.8 ± 31.2	54.6 ± 42.9	73.1 ± 41.0	54.6 ± 35.2
P-value**	0.006		0.026		< 0.0001	
Median (IQR) relative % change	-37.5 (56.6)		-25.3 (67.2)		-22.7 (46.1)	
Normal ALT at the end of the treatment – *no. (%)* *The cut-off values are 0–43 IU/L*	25 (58.1)		21 (51.2)		20 (45.5)	
Mann–Whitney test to compare the median relative percent reduction in placebo and low-dose Heptex revealed p = 0.247, Mann–Whitney test to compare the median relative percent reduction in placebo and high-dose Heptex revealed p = 0.368, and Kruskal Wallis One Way ANOVA test to compare the median relative percent reduction in the three arms revealed p = 0.465. *Chi-square test to compare the proportion of patients with normal ALT levels in the three groups revealed; p = 0.495.*						
AST (IU/L)*						
Count	43	43	41	41	44	44
Median (IQR)	44 (30)	34 (35)	45 (26)	29 (21.5)	43.5 (22.3)	32 (23.5)
Mean ± SD	54.8 ± 32.3	47.5 ± 40.5	45 ± 18.8	38 ± 22.7	47.7 ± 22.7	36.9 ± 19.3
P-value**	0.037		0.054		0.001	
Median (IQR) relative % change	-32.3 (67)		-17.8 (66.8)		-26.3 (44.1)	
Normal AST at the end of the treatment – *no. (%)* *The cut-off value is 0–40 IU/L*	28 (65.1)		27 (65.9)		30 (68.2)	
Mann–Whitney test to compare the median relative percent reduction in placebo and low-dose Heptex revealed p = 0.442, Mann–Whitney test to compare the median relative percent reduction in placebo and high-dose Heptex revealed p = 0.737, and Kruskal Wallis One Way ANOVA test to compare the median relative percent reduction in the three arms revealed p = 0.632. *Chi-square test to compare the proportion of patients with normal AST levels in the three groups revealed; p = 0.951.*						

** Last-observation-carried-forward convention (LOCF) is considered in the study endpoint*
*** Wilcoxon signed rank test to compare the median at screening and week 36 in each arm*
*Relative % change formula = (LOCF value-Screening Value)/screening value *100*
N.B: One patient had a missing ALT value in the screening visit, and post assessment visit was considered instead. And one patient had a missing AST value in the screening visit, and post assessment visit was considered instead

Table 3 Change in CAP score, KPa score, FIB-4 score, and FAST score in the three groups

	Placebo ***(N=43)***		**Low dose** ***(N=41)***		**High dose** ***(N=43)***	
	Screening	**Week 36**	**Screening**	**Week 36**	**Screening**	**Week 36**
CAP (dB/m)						
Count	43	37	41	34	42	38
Median (IQR)	335 (62)	320 (94)	320 (73.5)	315.5 (65.5)	343 (51.5)	315 (50)
Mean± SD	333.8± 40.1	305.3± 94	325.8± 45.4	308.6± 42.5	340.8± 34.8	315± 42.3
P-value*	0.014		0.064		<0.0001	
Median Change (IQR)	-24 (89.5)		-27.8 (78)		-23 (67.8)	
Mann-Whitney test to compare the median changes in placebo and low-dose groups revealed p=0.547, Mann-Whitney test to compare the median changes in placebo and high-dose groups revealed p=0.932, and Kruskal Wallis One Way ANOVA test to compare the median changes in the three arms revealed p=0.742.						
FibroScan liver stiffness score (kPa)						
Count	43	37	41	34	43	38
Median (IQR)	6.5 (1.2)	6.3 (1.6)	6.3 (1.6)	6.1 (1.7)	6.4 (1.9)	6.1 (1.9)
Mean± SD	6.6± 1.2	6.6± 2.1	6.3± 1.2	7± 4.7	6.7± 1.1	6.6± 1.8
P-value*	0.164		0.150		0.213	
Median Change (IQR)	-0.7 (2.2)		-0.4 (2.1)		-0.4 (2.3)	
Mann-Whitney test to compare the median change in placebo and low-dose groups revealed p=0.760, Mann-Whitney test to compare the median changes in placebo and high-dose groups revealed p=0.675, and Kruskal Wallis One Way ANOVA test to compare the median changes in the three arms revealed p=0.794.						
Fib-4 score						
Count	43	39	41	34	42	36
Median (IQR)	1 (0.77)	1 (0.45)	0.8 (0.54)	0.7 (0.55)	0.8 (0.47)	0.7 (0.69)
Mean± SD	1± 0.6	1± 0.6	0.9± 0.6	0.9± 0.5	0.9± 0.4	0.8± 0.5
Median Change (IQR)	-0.02 (0.4)		-0.02 (0.6)		-0.04 (0.4)	
Mann-Whitney test to compare the median changes in placebo and low-dose groups revealed p=0.886, Mann-Whitney test to compare the median changes in placebo and high-dose groups revealed p=0.978, and Kruskal Wallis One Way ANOVA test to compare the median changes in the three arms revealed p=0.985.						
FAST score						
Count	43	37	41	34	41	35
Mean (SD)	0.492 (0.176)	0.347 (0.231)	0.436 (0.179)	0.311 (0.221)	0.476 (0.148)	0.340 (0.211)
Median [Min, Max]	0.497 [0.137, 0.855]	0.266 [0.0195, 0.781]	0.453 [0.0932, 0.718]	0.257 [0.0170, 0.835]	0.461 [0.148, 0.761]	0.328 [0.00595, 0.841]

*Wilcoxon signed rank test to compare the median at screening and week 36 in each arm

Change in BMI as an associated risk factor

Patients' BMI was assessed at the screening visit and the end of treatment. The median BMI significantly decreased in the placebo group from 33.4 kg/m^2 to 32.3 kg/m^2 after 36 weeks ($p=0.020$). However, no significant decrease was observed from the screening to 36 weeks visit in the low-dose group ($p=0.458$) and in the high-dose group ($p=0.443$).

Linear regression

After adjusting for screening covariates (ALT levels and FAST scores) as well as comorbidities (diabetes, hypertension, and body weight), no relationship was detected between ALT levels nor FAST scores and the treatment arms. Additional details are available in Table 5.

Safety analysis

Out of 167 patients (the safety population), seven patients (4.2%) experienced seven adverse events (AEs). Five of these patients were in the placebo group (10.4%), one was in the low-dose group (2.04%), and one patient was in the high-dose group (2.04%). The reported AEs included gastrointestinal disorders ($n=3$; 1.7%), nervous system disorders ($n=2$; 1.2%), a musculoskeletal disorder ($n=1$; 0.6), and a skin disorder ($n=1$; 0.6%). All reported AEs were mild and non-serious. No hepatic complications

Table 4 Comparing the change in lipid profile in the three groups

	Placebo (*N* = 43)		Low dose (*N* = 41)		High dose (*N* = 43)	
	Screening	**Week 36**	**Screening**	**Week 36**	**Screening**	**Week 36**
HDL (mg/dl)						
Count	43	39	41	34	44	37
Median (IQR)	41 (16)	41 (10)	45 (16)	43 (15)	39 (13)	38 (10)
Mean ± SD	44.3 ± 12.1	42.4 ± 10	46.1 ± 15	43 ± 11.3	42 ± 15.6	41.5 ± 16.6
P-value*	0.290		0.189		0.177	
Median Change (IQR)	0 (13)		-0.5 (12.5)		-1 (8.5)	
Mann–Whitney test to compare placebo and low-dose Heptex median relative change revealed p = 0.550, Mann–Whitney test to compare placebo and high-dose Heptex median relative change revealed p = 0.655, and Kruskal Wallis One Way ANOVA test to compare the three arms median relative change revealed p = 0.827.						
LDL (mg/dl)						
Count	43	39	41	34	43	36
Median (IQR)	121 (48)	116 (50)	124 (56)	120 (60)	109 (46)	111 (30)
Mean ± SD	126.9 ± 45.9	117.2 ± 35.3	131.3 ± 45.5	121.1 ± 51.8	117.9 ± 48.1	110.5 ± 33.8
P-value*	0.645		0.072		0.413	
Median Change (IQR)	1 (49)		-12 (37.8)		-3 (55)	
Mann–Whitney test to compare the median changes in placebo and low-dose groups revealed p = 0.196, Mann–Whitney test to compare the median changes in placebo and high-dose groups revealed p = 0.935, and Kruskal Wallis One Way ANOVA test to compare the median changes in the three arms revealed p = 0.413.						
Total Cholesterol (mg/dl)						
Count	43	39	41	34	44	37
Median (IQR)	198 (55)	191 (38)	210 (71)	187 (67.8)	188 (41)	187 (43.5)
Mean ± SD	204 ± 50.2	195.5 ± 39.5	216 ± 54.2	205.3 ± 52.8	196.6 ± 58.3	187.6 ± 41.8
P-value*	0.845		0.031		0.577	
Median Change (IQR)	2 (40)		-7 (31.8)		2 (46.5)	
Mann–Whitney test to compare the median changes in placebo and low-dose groups revealed p = 0.182, Mann–Whitney test to compare the median changes in placebo and high-dose groups revealed p = 0.921, and Kruskal Wallis One Way ANOVA test to compare the median changes in the three arms revealed p = 0.335.						
Triglyceride (mg/dl)						
Count	43	39	41	34	44	37
Median (IQR)	159 (131)	166 (105)	164 (125)	175 (120.5)	163 (125)	170 (127.5)
Mean ± SD	202.6 ± 193	197.9 ± 178.8	193.6 ± 96.7	227.9 ± 151.6	196.8 ± 136.5	170 ± 89.2
P-value*	0.906		0.149		0.994	
Median Change (IQR)	3 (85)		12 (133)		5 (89)	

Mann–Whitney test to compare the median changes in placebo and low-dose groups revealed $p = 0.365$, Mann–Whitney test to compare the median changes in placebo and high-dose groups revealed $p = 0.783$, and Kruskal Wallis One Way ANOVA test to compare the median changes in the three arms revealed $p = 0.393$

* Wilcoxon signed rank test to compare the median at screening and week 36 in each arm

were reported for patients in all study groups. Table 6 shows details of the AEs, and a listing of the reported AEs with their description as per the investigator is provided in Additional file 3.

Discussion

The extracts of *Silybum marianum* and *Phyllanthus niruri* have gained interest for their potential to be effective therapeutic options for NAFLD. Silymarin, found in the extract of Silybum marianum, was found to lower hepatocyte oxidative stress and regulate lipid metabolism in a mouse model of NAFLD [35]. In addition, *Phyllanthus niruri* extract has a hepatoprotective effect against NAFLD as it improves insulin signaling inside the liver, reducing fat accumulation and inflammation [36]. The current study evaluated the hepatoprotective effect of two doses of Heptex® capsules (containing powdered extracts of *Phyllanthus niruri* aerial parts and *Silybum marianum* fruits) in patients with an apparent risk factor for NASH.

Regarding the impact of the combination on liver enzymes, our study showed a statistically significant

Table 5 Linear regression

Characteristic	Beta	95% CI[a]	*p*-value
FAST score as the outcome			
FAST score at screening	0.48	0.24, 0.72	< 0.001
Treatment arm			
Placebo	—	—	
Low-dose Heptex	-0.02	-0.12, 0.08	0.6
High-dose Heptex	-0.01	-0.11, 0.09	0.9
Diabetes or Hypertension			
No	—	—	
Yes	0.05	-0.11, 0.21	0.5
Weight at baseline	0.00	-0.01, 0.00	0.13
Weight at end of treatment	0.00	0.00, 0.01	0.057
ALT as the outcome			
ALT at baseline	0.83	0.64, 1.0	< 0.001
Treatment arm			
Placebo	—	—	
Low-dose Heptex	-3.2	-21, 15	0.7
High-dose Heptex	-9.1	-28, 9.3	0.3
Diabetes or Hypertension			
No	—	—	
Yes	-15	-43, 14	0.3
Weight at baseline	-0.34	-1.3, 0.63	0.5
Weight at end of treatment	0.41	-0.35, 1.2	0.3

[a] *CI* = Confidence Interval

improvement after 36 weeks of treatment in ALT ($p < 0.0001$) and AST ($p = 0.001$) levels in the high-dose group as well as an improvement in ALT level in the low-dose group ($p = 0.026$). However, there were no significant differences between the experimental and control groups in terms of median relative percent change in liver enzymes ($p > 0.05$). Correspondingly, upon calculation of FAST score, which was recently adopted to report on the risk for NASH progression [37], a decrease in risk was observed across all the study arms; however, the observed change was statistically insignificant, which require further investigations given that longitudinal data on changes in FAST score over time, and functional outcomes accompanying these changes, are still limited [38]. Additionally, a linear regression analysis was employed to consider potential influence of confounding factors. The findings of linear regression analysis suggested that the treatment arms, as well as the screening FAST score and the presence of diabetes or hypertension, did not significantly influence the functional outcomes measured by the FAST score. Similar results were observed upon employing ALT as the outcome variable. This warrants further investigation to better understand the potential relationship between other variables, e.g., dietary habits and obesity, and functional outcomes in this study population.

Our study was preceded by some studies comparing the effect of *Phyllanthus niruri* alone [32], silymarin alone [39–42], or combined silymarin with *Phyllanthus niruri* and choline [43, 44]. Previous studies showed inconsistent findings on the impact on liver enzymes after treatment.

Despite *Phyllanthus niruri*'s proven enzyme-lowering action observed in rats [45], in agreement with our study, a pilot study conducted in Malaysia on 52 patients showed that the changes in AST ($p = 0.39$) and ALT ($p = 0.95$) levels did not significantly differ between the Phyllanthus and placebo groups after 48 weeks of treatment [32]. Similar results were observed for silymarin in an RCT conducted on 78 patients that showed no significant differences in the levels of ALT ($p = 0.08$) and AST ($p = 0.39$) between the low-dose silymarin group (420 mg/day), high-dose silymarin group (700 mg/day) and the placebo group [39]. In addition, in another RCT ($n = 99$), a high dose of silymarin (700 mg, given three times daily for 48 weeks) did not show a significant decrease in the levels of ALT and AST ($p = 0.467$) [40].

On the other hand, several studies conducted on silymarin alone showed a promising hepatoprotective effect after using it. In a Pakistani study of 200 patients with NAFLD, the treatment group received two daily tablets (200 mg) of silymarin and showed significantly decreased levels of ALT and AST. In contrast, the control group showed statistically insignificant changes in both enzymes after 12 weeks [41]. In addition, a double-blinded RCT was conducted to assess the efficacy of silymarin in morbidly obese patients with fatty liver compared to a placebo. The study showed a significant difference between the two groups in terms of AST/ALT ratio ($p = 0.04$), sonographic grades ($p = 0.004$), and BMI ($p = 0.02$) [42].

Other trials that investigated the effect of combined therapies on liver enzymes revealed an additive effect of a combination. An RCT on 101 patients showed a significant improvement in hepatic parameters of the combination group (350 mg silymarin + 675 mg *Phyllanthus niruri* + 180 mg of choline) compared to the silymarin group (450 mg silymarin per day) [43]. Another randomized study in 86 patients for 180 days with high doses had similar results [44]. This proves that the combination is more efficient than silymarin alone in hepatic disorders of different etiologies, including NASH. However, this effect may be due to the high dose of *Phyllanthus niruri* or the added choline, as choline deficiency was reported to be associated with liver injuries (hepatic steatosis) [46].

The conflicting results of the impact of previous herbal treatments could be attributed to the

Table 6 Incidence and attack rate of AEs and their specifications and characteristics

Incidence rate - n (%)	**Safety Population** ***(N=167)***	**Randomized Population** ***(N=146)***		
		Placebo ***(N=48)***	**Low dose** ***(N=49)***	**High dose** ***(N=49)***
Number of patients	7 (4.2)	5 (10.4)	1 (2.04)	1 (2.04)
Frequency of AEs	7 (4.2)	5 (10.4)	1 (2.04)	1 (2.04)
AE Specification – no. (%)				
Gastrointestinal disorders	3 (1.7)	2 (4.2)	1 (2.04)	-
Musculoskeletal and connective tissue disorders				
Arthralgia	1 (0.6)	1 (2.1)	-	
Nervous system disorders				
Hypoesthesia	1 (0.6)		-	1 (2.04)
Headache	1 (0.6)	1 (2.1)		
Skin and subcutaneous tissue disorders				
Pigmentation[a]	1 (0.6)	1 (2.1)	-	-
Characteristics of AEs				
Intensity				
Mild	7 (100)	5 (100)	1 (100)	1 (100)
Moderate	-	-	-	-
Severe	-	-	-	-
Relatedness to Study Medication				
Related	1 (12.5)	1 (6.6)	-	-
Possibly Related	1 (12.5)	1 (16.6)	-	-
Unrelated	5 (75)	3 (66.6)	1 (100)	1 (100)
Seriousness Criteria				
Serious	-	-	-	-
Not Serious	7 (100)	5 (100)	1 (100)	1 (100)

[a] The patient came in for an unscheduled visit on 10 December 2019. During the visit, a dermatologist consultation and biopsy were performed, revealing that the persistent elevated bilirubin and liver enzymes (AST, ALT) since the start of the study on 15 July 2019 are due to an autoimmune disease (cholangitis) and not related to NASH. Therefore, it was decided that the patient no longer needs to continue with our investigational product and will be referred to a dermatologist for treatment

non-sensitivity of ALT and AST in assessing treatments' efficacy. A study carried out at Mayo clinic showed that AST and ALT levels could normalize spontaneously in some patients with NAFLD. However, this improvement is independent of disease progression demonstrated in histopathological features [47]. Hence, the histopathological features are critical in interpreting studies with changes in enzyme levels as a primary endpoint. Also, the previously mentioned study showed a reduction in fibrosis in the silymarin group, which was not seen in the placebo group, with a statistically significant difference between the groups ($p=0.026$), while this difference was not revealed regarding ALT and AST levels or FIB-4 score ($p>0.05$) [40]. Moreover, a study conducted on alcoholic hepatitis patients treated with *Phyllanthus niruri* (1000 mg daily for four weeks) showed a statistically significant increase in the level of total antioxidants ($p=0.034$) with an additional appetite stimulant activity ($p=0.03$) in 4 weeks [1]. Accordingly, using ALT and AST may not be optimal for evaluating therapeutic efficacy alone and should be accompanied by a liver biopsy to confirm the positive outcome. However, since it is an invasive procedure that cannot be used in clinical trials, other non-invasive trustworthy means, such as total antioxidants, hepatorenal index, and liver stiffness, could be used instead.

Results of the current study revealed no significant differences between the three groups in terms of the median change in Fib-4 score ($p=0.985$), KPa score ($p=0.794$), or CAP score ($p=0.742$). This is consistent with the results of the RCT ($n=99$) that showed no significant change in the Fib-4 score between the control group and the high-dose silymarin group ($p=0.113$) [40]. In addition, in the double-blinded RCT assessing the efficacy of silymarin in morbidly obese patients with fatty liver, no significant changes were revealed in terms of FibroScan stages ($p=0.057$), FIB-4 score ($p=0.90$) or NAFLD score ($p=0.075$) [42].

Regarding the effect of the combination on lipid profile, the current study showed no significant changes between

the treatment and control groups in terms of HDL, LDL, total cholesterol, and triglyceride levels change. A prior placebo-controlled trial ($n=100$) showed no significant change in the lipid profile levels between the experimental and control groups [21]. Several studies revealed the same results [32, 40]. Interestingly, silymarin has an antihyperlipidemic effect in rat models by regulating lipid metabolism [35]. Therefore, more specific tests are needed to further investigate the effect of the combination on lipid metabolisms, such as liver biopsy, ApoB/ApoA1 ratio, and Lipoprotein (a) [48].

The combination of *Silybum marianum* and *Phyllanthus niruri* proved its safety for a period of 36 weeks. Two mild, non-serious adverse events were only reported in the treatment groups. The Malaysian study showed that all adverse events were mild or moderate and non-severe [32]. Another study on silymarin approved no significant difference between the control group and the experimental group [39].

However, we couldn't conclude the drug's efficacy as the current study could be limited by its small sample size. In addition, depending only on noninvasive sonography and hematological tests is considered a limitation of the study. Accordingly, assessing the histological findings and/or the direct antioxidant activity may reveal more promising effects for this combination [32, 39].

Conclusion

Results of the current study suggest that Heptex® is safe and well tolerated for 36 weeks. However, its hepatoprotective effect in patients at risk of NASH still needs further assessment using other non-invasive tests to accurately investigate the histopathological features of NAFLD. In addition, further studies are needed to examine the efficacy of higher doses of this combination in patients suffering from the whole spectrum of NAFLD, starting from simple steatosis to the more severe form of steatohepatitis, along with diet control and exercise as complementary factors across all study groups.

Supplementary Information

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Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

Authors' contributions

MS contributed to study design, data analysis, and writing the manuscript; MH contributed to data collection, data interpretation, and writing the manuscript; BE contributed to data analysis and critical review of the manuscript; AA contributed to data collection and writing the manuscript; AZ contributed to data analysis and critical review of the manuscript; and SM contributed to study design and critical review of the manuscript. All authors have read and approved the final manuscript.

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Data availability

The datasets supporting the conclusions of this article are available upon request from the corresponding author.

Declarations

Ethics approval and consent to participate

All experiments were performed in accordance with relevant international and local guidelines and regulations. The study protocol was approved by the Egyptian Ministry of Health and Population (MoHP) Research Ethics Committee (code: 3-2020/13), the National Hepatology and Tropical Medicine Research Institute (NHTMRI) Research Ethics Committee (code: ITH00001), and Ain Shams University Research Ethics Committee (code: P02c/2019-2022). Informed consent was obtained from all participants prior to conducting any study-related procedures.

Consent for publication

Not applicable.

Competing interests

Shahnaz Mohamed is an employee of Natural Wellness. The rest of the authors declare no conflicts of interest.

Author details

[1]Department of Tropical Medicine, Faculty of Medicine, Ain Shams University, Cairo, Egypt. [2]National Hepatology and Tropical Medicine Research Institute (NHTMRI), Cairo, Egypt. [3]School of Pharmaceutical Sciences, University Sains Malaysia, Gelugor, Malaysia.

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