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Original Research Article

A randomized, double-blind, parallel, placebo-controlled study to evaluate efficacy and safety of a synergistic multi-herbal extract blend KaraHeart™ in supporting healthy cholesterol levels

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ABSTRACT

Background: Hyperlipidemia is a condition involving abnormally high levels of lipids in the blood. Hyperlipidemia is a major risk factor for cardiovascular diseases and refers to either high levels of triglycerides (TGL) or cholesterol. Herbal supplements have been used in the management of cholesterol levels in Ayurveda, a complete medical system originating in India. KaraHeart™ is a multi-herbal extract synergistic blend that may help in the management of healthy cholesterol levels. The current study tested the efficacy, tolerability, and safety of KaraHeart™ versus a placebo in the management of cholesterol levels of patients with mild hyperlipidemia.
Methods: This was a randomized, double-blind, parallel, and placebo-controlled study. A total of 100 patients were divided into two groups. One group was given KaraHeart™ and the other group was given a placebo for 120 days. Treatment results were assessed by checking the lipid profile parameters such as total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), and TGL.
Results: The study found that the herbal supplement KaraHeart™ significantly reduced levels of LDL, VLDL, TGL, and total cholesterol, while increasing the levels of HDL in the blood. Additionally, the study concluded that KaraHeart™ was safe to use.
Conclusions: KaraHeart™ was shown to be safe and effective in the management of cholesterol levels.

Keywords: Healthy cholesterol, Hyperlipidemia, Herbal supplement, Natural treatment, Randomized Clinical trial, Lipid profile

INTRODUCTION

Hyperlipidemia is a common cause of mortality worldwide. The most common form of hyperlipidemia is hypercholesterolemia - a total cholesterol level above 200 mg/dl. Approximately one third of all ischemic heart diseases (IHDs) in the world are caused by hypercholesterolemia.[1] Globally, as reported by the World Health Organization (WHO), increased cholesterol levels contribute to about approximately 2.6 million deaths

(4.5% of total) and 29.7 million disability adjusted life years (DALYs).[1] In 2008, among adults in western countries, about 39% males and 40% females had high cholesterol levels.[2]

Hyperlipidemia is a metabolic abnormality leading to elevated levels of cholesterol and/or triglycerides. This disorder occurs due to the elevation of "bad cholesterol" (total cholesterol/TC, low-density lipoprotein (LDL-C), very-low-density lipoprotein (VLDL)) and triglyceride (TGL) concentrations above the normal range and a decrease of "good cholesterol" (high-density lipoprotein (HDL-C) cholesterol) below the normal range.[3,4] Commonly, the higher the levels of bad cholesterol and triglycerides in the blood above the normal ranges, the greater the risk of cardiovascular diseases (CVD).[5]

Hyperlipidemia is classified into primary and secondary forms. Primary hyperlipidemia is hereditary, while secondary hyperlipidemia is caused by other underlying diseases, dietary factors and/or medications/drugs.[6] Hyperlipidemia can lead to symptomatic vascular diseases such as coronary artery disease (CAD), stroke, and peripheral arterial disease (PAD). It is thus important to appropriately manage hyperlipidemia through better diet, more exercise, and medications. Concerns about short and long-term side effects of medications for hyperlipidemia, coupled with their high costs, may hinder their long-term use. Use of alternative treatments and natural supplements may reduce such treatment burden and may help to better and more safely manage hyperlipidemia in the general population.[7]

This study was conducted to test the efficacy, tolerability, and safety of KaraHeart™ in managing cholesterol levels compared to a placebo control.

METHODS

Overview and ethical approvals

This was a randomized, double-blind, placebo-controlled study conducted in Shetty's Hospital, Bangalore, India from August 2020 to December 2020. Reporting of the study was done according to Consolidated Reporting of Randomized Controlled Trials (CONSORT) guidelines. A CONSORT flow diagram 2010 is shown in Figure 1. The study was performed in accordance with the current version of the Declaration of Helsinki. The trial was conducted in agreement with the International Conference on Harmonisation (ICH) guidelines on Good Clinical Practice (GCP) and the applicable rules and regulations of India. The study was performed under strict compliance with the requirements of Indian regulations for carrying out the herbal and Ayurveda clinical trials and Ayurveda, Siddha, and Unani good clinical practices (ASU-GCP). ICH guidelines for Good Clinical Practice (ICH-GCP) issued by the U.S. Department of Health and Human Services were followed wherever applicable. Informed consent was obtained from all participants. The trial was registered with Clinical Trials Registry (CTRI), hosted at the ICMR's National Institute of Medical Statistics as per the mandate of Drugs Controller General of India (DCGI). The trial was also registered on July 29th, 2020 with WHO under registration number.

Participants

Sample size was calculated using analysis of covariance (ANCOVA) using the primary objectives. The number of measures pre-randomization and post-randomization were 1 and 4 respectively, assuming an anticipated standard effect size of 0.4 and interclass correlation of 0.5. Estimating a drop-out rate of approximately 25%, a minimum of 47 patients in each arm were needed to be recruited to obtain a power rate of more than 80%. Hence a total of 100 participants, 50 in each arm were recruited in the study.

Inclusion criteria

Healthy adult men and women between the ages of 20–60 years with a confirmed case of mild to moderate hyperlipidemia. As per ATP III guidelines; baseline LDL ranging >100 mg/dl, TC >200 mg/dl, TGL between 150-199 mg/dl, VLDL-Cholesterol >40 mg/dl, HDL-cholesterol: Men-<40 mg/dl, and women- <50 mg/dl. Subjects with at least one or more of the diagnostic criteria mentioned above were selected for the study and with normal BMI but abnormal lipid profile. Subjects who were able to understand the risks/benefits of the protocol and were willing to give written informed consent.

Exclusion criteria

Subjects who: were using concurrent lipid-lowering medications like statins or fibrates, or dietary supplements within 30 days prior to screening; had hyperlipidemia due to other medications (eg. Glucocorticoids); had chronic diseases requiring continuous use of vasoactive diuretics or lipid-lowering drugs; were intractably obese or who had experienced any recent, unexplained weight loss or gain; had a history of major illness or cardiovascular diseases (example: Angina pectoris, myocardial infarction, etc.) or a history of a thyroid disorder (TSH- levels of <0.4 or >10 µg/dl), renal disorder, cholelithiasis, polycystic ovary syndrome (PCOS), Type I or II diabetes,, abnormal liver or kidney function test (ALT or AST) two times the upper limit of normal or elevated creatinine (male 125 µmol/L, female 110 µmol/L), a positive HIV test, a history of smoking and/or high alcohol intake (2 standard drinks per day); a history of psychiatric disorders that may impair the ability of subjects to provide written informed consent; females who were pregnant, breast feeding, or planning to become pregnant during the study. Also excluded, were subjects with any other condition that, in the opinion of investigator, would adversely affect the subject's ability to complete the study or its measures. Finally, subjects with a known allergy to KaraHeart™ constituents or ingredients were also excluded from the study.

Intervention

KaraHeart™ is a synergistic herbal formula consisting of well-known herbs, such as extract of Commiphora mukul, Allium sativum, Camellia sinensis, Trigonella foenum-graecum, Zingiber officinale, Cinnamomum verum which have traditionally been used for managing hyperlipidemia with Ayurvedic medicine.[8-13] Both KaraHeart™ and placebo were in the form of 500 mg capsules. Daily dosage for both products was 1000 mg (i.e., 2 capsules/day).

Trial design

A total of 122 subjects were screened for a final sample size of 100 randomized subjects. Eligible subjects were randomly allocated to either of the study arms in accordance with the randomization code found on the study product containers' label. The same was documented into the randomization record. Identical and sealed packed bottles of KaraHeart™ and placebo capsules were provided to the clinical sites. Investigators prescribed the allocated number of bottles of either KaraHeart™ or placebo in a blinded manner to the subjects on a first come, first served basis.

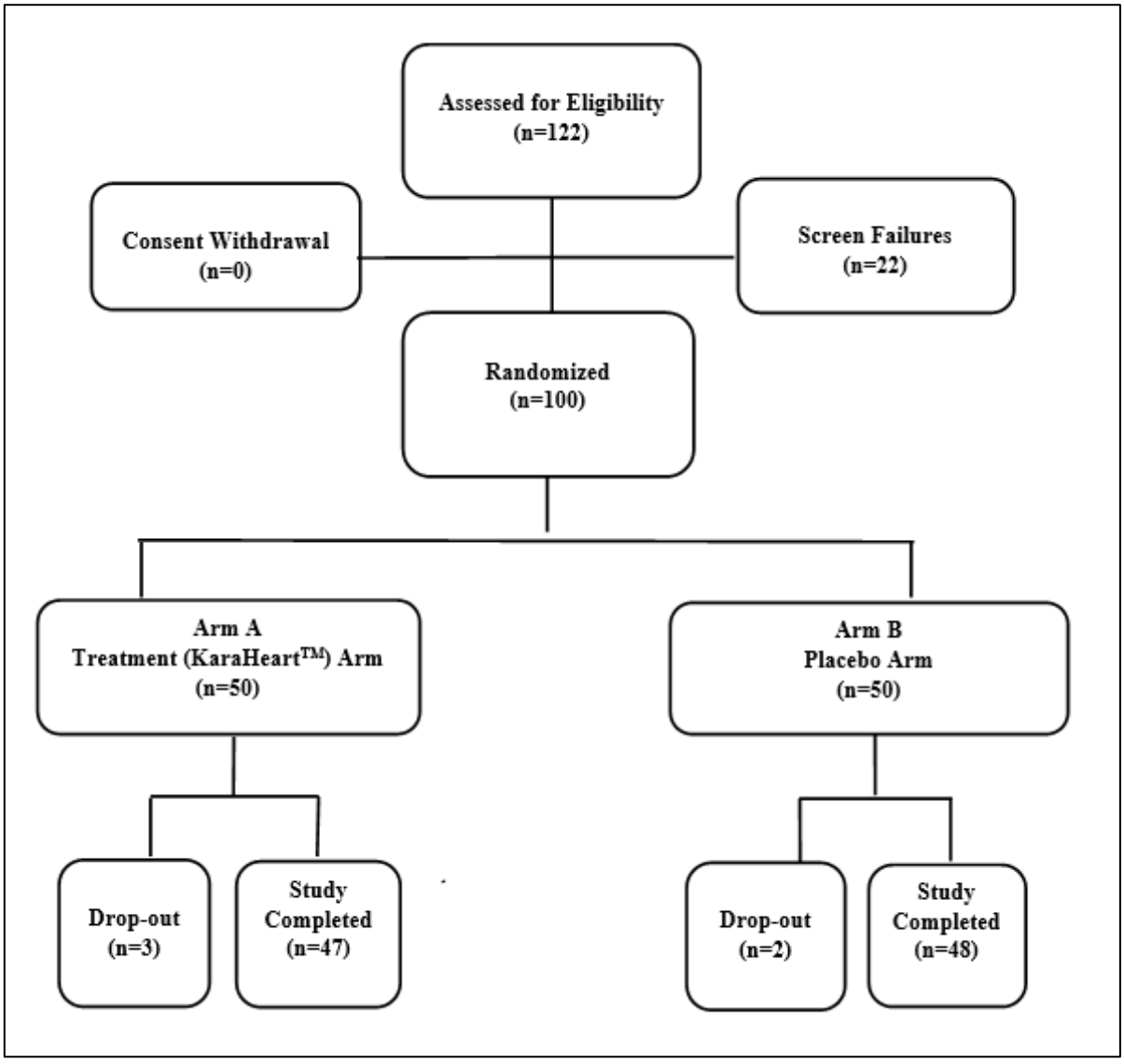

Figure 1: Trial design showing distribution of subjects in the study.

A total of 100 subjects (50 subjects in each arm) were recruited randomly into the two study arms: Group A - KaraHeart™ and Group B - placebo. Duration of the study was 120 days with 6 scheduled clinical visits (screening visit, baseline, 30 days, 60 days, 90 days, and 120 days). Each visit had a window period of +3 days (Figure 1).

Subjects were given assigned medication at visit 2 (day 1) and asked to take 1 capsule orally, twice daily (after breakfast and dinner). Subjects were given supplements to last until the next visit (visit 3, day 30±3) and asked to record daily consumption in the diaries and compliance cards provided to them. Subjects were also asked to walk

for 30 minutes daily and record adverse events, if any. With the exception of the biostatistician, all others (the sponsor's designee, investigator, subjects, and CRO's designee) were kept blinded to the investigational product (IP) provided to each participant. Similarly, all others (the sponsor's designee, investigator, subjects and CRO's designee) were kept blinded about the Investigational Product (IP) provided to each participant. The screening visit included obtaining the informed consent, demographic details of the participants, physical examination, recording of vital signs, collecting medical history from the patients, and laboratory examinations. Height, weight, and BMI of subjects were recorded during the screening visit. Each subject underwent clinical laboratory tests at screening and follow-up visits. Urine for urinalysis and blood for hematology, biochemistry, and serology were collected during screening and at the end of the study visit. For the hematology, biochemistry, and serology laboratory tests, blood samples were collected by direct venipuncture of peripheral veins for clinical laboratory tests at the screening visit (V1), follow-up visits, and the final visit (V6). A total of approximately 40 to 45 ml of blood was collected over the course of this study for clinical laboratory evaluations. Blood and urine samples were collected from each prospective participant to analyze and assess the inclusion criteria for fasting/random blood sugar (FBS/RBS), HbA1C, C-Reactive Protein, ECGs, HIV, liver function tests, kidney/renal function tests and urinalysis were performed during the course of the study. In all female subjects of child-bearing potential, a urine pregnancy test was performed during visits V1-6. Negative results were recorded in the source document to confirm the non-pregnant status of participants in order to confirm eligibility for enrolment and/or continuation in the study.

Each follow-up visit (days 30, 60, 90, and 120±3) involved distribution of the supplement, assessments of lipid parameters, and collection of safety and tolerability information. At no point was the code broken, or un-blinded study product administered to any subject. The investigator had the right to break the blind in special situations such as for treatment of emergent serious adverse events (SAE) or to protect the safety of the patient, but it was not necessary for any participant over the duration of the study.

Compliance and adverse events

Any unused or extra medication was returned to the investigators to confirm that the correct number of capsules had been taken. The investigator verified the subjects' daily diary and compliance cards and reconciled the supplement use to subjects. This reconciliation was logged on the IP reconciliation form. Proper care was made to record all adverse events (AEs) in source documents and case report forms (CRF).

AE were recorded for severity and relationship to the consumption of the study supplement. All AEs were followed until they were resolved or stabilized or until they were no longer considered clinically significant by the investigator. All reported AEs were mild to moderate in nature, thus, no additional measurements or evaluations were done to investigate the nature of an AE. There were no severe AEs (SAEs) reported during the study.

Withdrawal and dropout

Subjects who did not meet inclusion/exclusion criteria were considered screen failures. Participating subjects could withdraw at any time without the need to justify his/her decision, even after undergoing consenting process (consent withdrawal). No subject was discontinued from the study due to non-compliance with medication, protocol violation, worsening of disease or tolerability, AEs, or SAEs. A total of five subjects (from treatment and placebo groups) dropped out from the study at different intervals due to personal reasons. None of these subjects dropped out due to any AE. Data from these subjects were used to examine safety, but not efficacy. The withdrawal of these subjects was prior to the final outcome assessments; therefore, their data was excluded from the main analysis. In case of statistics on the ITT population, missing values were replaced using the last observation carried forward (LOCF) method and efficacy assessments were completed.

Outcome measures

Primary outcome measures: Change in the following lipid profile parameters from baseline to end of treatment period at the following time points: Baseline, Day 30 (±3), Day 60 (±3), Day 90 (±3) and Day 120 (±3). Total Cholesterol (TC): This is a sum of the blood cholesterol content. The average level of TC should be below 200mg/dl.[14] High-Density Lipoprotein (HDL): This is called "good" cholesterol because it helps carry away LDL, thus keeping arteries open and blood flowing more freely. The average level of HDL should be above 40mg/dl.[14] Low-Density Lipoprotein (LDL): This is called "bad" cholesterol. Too much of it in your blood causes a build-up of fatty deposits (plaques) in the arteries (atherosclerosis), which reduces blood flow. These plaques sometimes rupture and can lead to a heart attack or stroke. The average level of LDL should be less than 100mg/dl.[14] Triglycerides (TGL): Triglycerides are a type of fat in the blood. The body converts calories it doesn't need into triglycerides, which are stored in fat cells. High triglyceride levels are associated with being overweight, eating sweets or drinking too much alcohol, smoking, sedentary lifestyle, or diabetes with elevated blood sugar levels. The average levels of triglycerides should be less than 150 mg/dl.[14] Very-Low-Density Lipoprotein (VLDL): The liver makes VLDL and releases it into the bloodstream. VLDL particles mainly carry triglycerides to the tissues. Elevated levels of VLDL can increase a person's risk of developing heart diseases. Normal VLDL should be less than 30 mg/dl (0.1 to 1.7 mmol/l).[14] Total HDL-Cholesterol Ratio: The ratio of TC/HDL. The optimal ratio is between 3.5 and 1. A higher ratio indicates an increased risk of heart disease.

Secondary outcome measures

Change from Baseline to end of study period (Day 120) in: Serum Apolipoprotein A1: Apolipoproteins are proteins that bind lipids together to form lipoproteins. Their main function is transportation of lipids (and fat-soluble vitamins) in blood, cerebrospinal fluid, and lymph fluid. The 2 major apolipoproteins responsible for lipid transport are ApoA1 and ApoB.[15] Decreases in the concentration of ApoA1 levels along with increases in the concentration of ApoB are associated with increased risk of cardiac diseases. The ApoA1 is the major protein component of HDL and is associated with fat efflux from tissue to liver for excretion. In patients suffering from CAD, ApoA1 levels serve as a better diagnostic tool than HDL levels as they have higher sensitivity and specificity.[16] HbA1C: To control and monitor the glycemic index in diabetic patients, the HbA1C test is routinely performed. Factors such as sugar intake, exercise, and adherence to medications can affect the levels of HbA1C. Studies have reported that HbA1c can be utilized as a possible biomarker for predicting dyslipidemia and cardiovascular diseases (CVD). A study published in 2017 found that the ideal HbA1c level for people without diabetes is in the 5.0% to 6.0% range. Beyond 6.0%, the risk of death from CVDs rises significantly.[17] C-reactive protein (CRP): CRP is an inflammatory marker. Inflammation is a major factor in any atherothrombotic disease. Levels of high-sensitivity C-reactive protein (hs-CRP), a marker of systemic inflammation and a mediator of atherothrombotic disease, are potential risk factors for cardiovascular disease. Currently, CRP is recognized as an indicator of vascular inflammation. CRP may be used as a predictor of cardiovascular conditions secondary to atherosclerosis and is a strong predictor of cardiovascular events when compared with low-density lipoprotein cholesterol (LDL-C). The evaluation of serum CRP together with the lipid pattern can be very useful in the early identification of type 2 diabetic individuals who are at high risk of developing CVD.[18]

Statistical analysis

Study data collected was assessed using Statistical analysis software (SAS) 9.4 package. Descriptive analysis for baseline summary statistics, including mean, medians, and standard deviation for demographic data and proportion of males and females was performed.

The intention to treat (ITT) efficacy analysis set consisted of subjects who took at least 1 dose of IP and have at least 1 post-baseline assessment. ITT efficacy analysis was provided only for the primary end point. Per protocol set population (PP) analysis set was a subset of the ITT population, consisting of subjects who had no major protocol violations affecting the primary efficacy variables. A total of 95 subjects completed the study and were included in the PP population analysis.

Data are expressed as mean ± standard deviation (SD). P values were calculated using paired Students t-tests to compare time points within the same group, ANOVA was performed to compare groups at same time point, or ANCOVA using baseline measurement as a covariant when comparing baseline to V6 across groups. Missing post-baseline observations were imputed using last observation carried forward approach (LOCF). All hypotheses were tested at a significance level of .05 and 95% confidence interval.

RESULTS

In total, five subjects discontinued the study: one dropped out in V4 from the placebo group, two subjects dropped out in V5 from the treatment (KaraHeart™) group and two subjects dropped out in V6 from the placebo group; these subjects were included in data analysis as ITT population through LOCF method. However, all efficacy analysis were performed using PP population.

Table 1: Statistical analysis for TC (per protocol population).

Variable	KaraHeart™ (N=47)	Placebo (N=48)	P value[a]	ANCOVA, P value[c]
TC at day 0 (mg/dl)	206.3 (33.026)	207.1 (25.004)	0.8935	0.1435
TC at day 30 (mg/dl)	201.1 (31.719)	204.6 (22.979)	0.5405	
Mean difference	-5.26	-2.56		
CI	(-7.434, -3.077)	(-5.882, 0.757)		
P value[b]	<0.0001	0.1271		
Day 60				
TC at day 0 (mg/dl)	206.3 (33.026)	207.1 (25.004)	0.8935	0.0022
TC at day 60 (mg/dl)	195.9 (29.829)	206.3 (23.195)	0.0617	
Mean difference	-10.4	-0.85		
CI	(-14.55, -6.260)	(-6.287, 4.579)		
P-value[b]	<0.0001	0.7532		
Day 90				
TC at day 0 (mg/dl)	206.3 (33.026)	207.1 (25.004)	0.8935	0.0213
TC at day 90 (mg/dl)	190.1 (29.109)	199.9 (25.887)	0.0878	
Mean difference	-16.2	-7.25		
CI	(-22.11, -10.27)	(-13.70, -0.803)		

Continued.

Variable	KaraHeart™ (N=47)	Placebo (N=48)	P value[a]	ANCOVA, P value[c]
P-value[b]	<0.0001	0.0283		
Day 120				
TC at day 0 (mg/dl)	206.3 (33.026)	207.1 (25.004)	0.8935	0.0397
TC at day 120 (mg/dl)	184.7 (30.446)	195.7 (30.743)	0.0812	
Mean difference	-21.7	-11.4		
CI	(-29.50, -13.82)	(-19.25, -3.540)		
P-value[b]	<0.0001	0.0054		
Note: P Value[a]: Two sample t-test. P value[b]: Paired t-test. P value[c]: ANCOVA P value				

Table 2: Statistical analysis for HDL-C (per protocol population).

Variable	KaraHeart™ (N=47)	Placebo (N=48)	P value[a]	ANCOVA P value[c]
HDL-C at day 0 (mg/dl)	43.15 (8.715)	43.38 (10.342)	0.9086	0.0074
HDL-C at day 30 (mg/dl)	43.81 (7.459)	41.56 (10.683)	0.2373	
Mean difference	0.66	-1.81		
CI	(-0.339, 1.659)	(-3.390, -0.235)		
P value[b]	0.1904	0.0252		
Day 60				
HDL-C at day 0 (mg/dl)	43.15 (8.715)	43.38 (10.342)	0.9086	0.0001
HDL-C at day 60 (mg/dl)	44.23 (7.429)	40.92 (10.465)	0.0779	
Mean difference	1.09	-2.46		
CI	(-0.017, 2.187)	(-3.943, -0.974)		
P value[b]	0.0534	0.0017		
Day 90:				
HDL-C at day 0 (mg/dl)	43.15 (8.715)	43.38 (10.342)	0.9086	0.0004
HDL-C at day 90 (mg/dl)	44.45 (7.762)	40.56 (10.320)	0.0412	
Mean difference	1.30	-2.81		
CI	(-0.054, 2.650)	(-4.755, -0.870)		
P value[b]	0.0596	0.0055		
Day 120				
HDL-C at day 0 (mg/dl)	43.15 (8.715)	43.38 (10.342)	0.9086	0.0005
HDL-C at day120 (mg/dl)	45.17 (7.707)	41.06 (10.873)	0.0363	
Mean difference	2.02	-2.31		
CI	(0.472, 3.571)	(-4.281, -0.344)		
P value[b]	0.0117	0.0223		
Note: P value[a] : Two sample t-test. P value[b]: Paired t-test P Value[c]: ANCOVA P value				

Table 3: Statistical analysis for HDL-C (per protocol population) in different sub-groups.

Category	Variable	KaraHeart™ Group (N=47)	Placebo group (N=48)	P value[a]	ANCOVA P value[c]
HDL above 45 mg/dl	N	15	18		
	Baseline	53.13 (6.435)	53.67 (9.299)	0.7115	0.3369
	V6	51.80 (8.117)	50.33 (9.804)	0.6477	
	Mean difference	-1.33	-3.33		
	CI	(-3.565, 0.898)	(-7.483, 0.817)		
	P-value[b]	0.2208	0.1084		
HDL 40 to 45 mg/dl	N	14	11		
	Baseline	42.71 (1.541)	41.91 (1.514)	0.3103	0.0033
	V6	44.93 (3.731)	38.91 (4.085)	0.0015	
	Mean difference	2.21	-3.00		
	CI	(0.238, 4.191)	(-5.585, -0.415)		
	P-value[b]	0.0309	0.0271		
HDL below 40 mg/dl	N	18	19		
	Baseline	35.17 (3.746)	34.47 (2.342)	0.5018	0.0089
	V6	39.83 (5.182)	33.53 (7.741)	0.0065	
	Mean difference	4.67	-0.95		
	CI	(1.698, 7.636)	(-4.098, 2.203)		
	P-value[b]	0.0041	0.5355		
Note: P value[a] : Two sample t-test. P value[b]: Paired t-test P value[c]: ANCOVA P value.					

Table 4: Statistical analysis for LDL-C (per protocol population).

Variable	KaraHeart™ (N=47)	Placebo (N=48)	P value[a]	ANCOVA P value[c]
LDL-C at day 0 (mg/dl)	124.8 (28.912)	120.6 (23.005)	0.4345	0.5277
LDL-C at day 30 (mg/dl)	126.1 (27.902)	125.7 (24.644)	0.9414	
Mean difference	1.27	5.07		
CI	(-5.126, 7.656)	(-0.652, 10.792)		
P value[b]	0.6921	0.0811		
Day 60				
LDL-C at day 0 (mg/dl)	124.8 (28.912)	120.6 (23.005)	0.4345	0.0979
LDL-C at day 60 (mg/dl)	123.5 (26.180)	128.3 (24.723)	0.3649	
Mean difference	-1.35	7.61		
CI	(-7.797, 5.102)	(0.370, 14.854)		
P value[b]	0.6760	0.0398		
Day 90				
LDL-C at day 0 (mg/dl)	124.8 (28.912)	120.6 (23.005)	0.4345	0.2221
LDL-C at day 90 (mg/dl)	118.7 (26.606)	122.2 (27.437)	0.5378	
Mean difference	-6.11	1.52		
CI	(-13.00, 0.784)	(-6.395, 9.444)		
P value[b]	0.0810	0.7003		
Day 120				
LDL-C at day 0 (mg/dl)	124.8 (28.912)	120.6 (23.005)	0.4345	0.0095
LDL-C at day 120 (mg/dl)	112.3 (28.107)	123.4 (26.663)	0.0504	
Mean difference	-12.6	2.79		
CI	(-20.00, -5.108)	(-5.751, 11.334)		
P value[b]	0.0014	0.5141		

Note: P value[a] : Two sample t-test. P value[b]: Paired t-test P value[c]: ANCOVA P value.

Table 5: Statistical analysis for VLDL-C (per protocol population).

Variable	KaraHeart™ (N=47)	Placebo (N=48)	P value[a]	ANCOVA P value[c]
VLDL-C at day 0 (mg/dl)	34.10 (11.488)	39.04 (14.997)	0.0752	0.0137
VLDL-C at day 30 (mg/dl)	31.15 (9.318)	37.29 (11.541)	0.0054	
Mean difference (mg/dl)	-2.95	-1.75		
CI	(-4.455, -1.443)	(-4.116, 0.624)		
P value[b]	0.0003	0.1450		
Day 60				
VLDL-C at day 0 (mg/dl)	34.10 (11.488)	39.04 (14.997)	0.0752	<0.0001
VLDL-C at day 60 (mg/dl)	28.19 (7.551)	37.10 (11.587)	<0.0001	
Mean difference	-5.91	-1.93		
CI	(-8.203, -3.619)	(-5.299, 1.432)		
P value[b]	<0.0001	0.2536		
Day 90				
VLDL-C at day 0 (mg/dl)	34.10 (11.488)	39.04 (14.997)	0.0752	<0.0001
VLDL-C at day 90 (mg/dl)	26.95 (7.442)	37.15 (12.835)	<0.0001	
Mean difference	-7.15	-1.89		
CI	(-9.529, -4.769)	(-5.671, 1.896)		
P value[b]	<0.0001	0.3207		
Day 120				
VLDL-C at day 0 (mg/dl)	34.10 (11.488)	39.04 (14.997)	0.0752	<0.0001
VLDL-C at day 120 (mg/dl)	27.20 (8.583)	38.95 (14.306)	<0.0001	
Mean difference	-6.90	-0.08		
CI	(-9.658, -4.138)	(-3.761, 3.594)		
P value[b]	<0.0001	0.9638		

Note: P value[a] : Two sample t-test. P value[b]: Paired t-test P value[c]: ANCOVA P value

Table 6: Statistical analysis for VLDL-C (per protocol population) in different sub-groups.

Category	Variable	KaraHeart™ Group (N=47)	Placebo group (N=48)	P value[a]	ANCOVA P value[c]
VLDL above 40 mg/dl	N	12	20		
	Baseline	48.97 (6.655)	53.75 (8.929)	0.1193	0.0020
	V6	32.67 (7.008)	48.61 (12.840)	0.0001	
	Mean difference	-16.30	-5.14		
	CI	(-20.61, -11.99)	(-11.08, 0.796)		
	P value[b]	<0.0001	0.0858		
VLDL 32 to 40 mg/dl	N	18	14		
	Baseline	34.56 (2.206)	34.13 (1.954)	0.5727	0.0962
	V6	27.64 (8.792)	33.74 (10.987)	0.0911	
	Mean difference	-6.91	-0.39		
	CI	(-11.14, -2.685)	(-7.108, 6.336)		
	P value[b]	0.0031	0.9032		
VLDL below 32 mg/dl	N	17	14		
	baseline	23.12 (7.047)	22.93 (7.373)	0.9425	0.0132
	V6	22.87 (7.304)	30.37 (11.236)	0.0329	
	Mean difference	-0.25	7.44		
	CI	(-3.176, 2.682)	(1.422, 13.464)		
	P value[b]	0.8603	0.0192		
Note: P value[a] : Two sample t-test. P value[b]: Paired t-test P value[c]: ANCOVA P value.					

Table 7: Statistical analysis for total cholesterol/ HDL-C ratio (per protocol population).

Variable	KaraHeart™ (N=47)	Placebo (N=48)	P value[a]	ANCOVA P value[c]
TC/HDL-C at day 0	4.92 (1.097)	5.03 (1.308)	0.6650	0.0004
TC/HDL-C at day 30	4.67 (0.864)	5.22 (1.318)	0.0202	
Mean difference	-0.24	0.19		
CI	(-0.386, -0.103)	(-0.044, 0.420)		
P value[b]	0.0011	0.1095		
Day 60				
TC/HDL-C at day 0	4.92 (1.097)	5.03 (1.308)	0.6650	<0.0001
TC/HDL-C at day 60	4.51 (0.838)	5.33 (1.320)	0.0006	
Mean difference	-0.40	0.30		
CI	(-0.567, -0.242)	(0.039, 0.561)		
P value[b]	<0.0001	0.0251		
Day 90				
TC/HDL-C at day 0	4.92 (1.097)	5.03 (1.308)	0.6650	<0.0001
TC/HDL-C at day 90	4.38 (0.899)	5.22 (1.371)	0.0007	
Mean Difference	-0.54	0.19		
CI	(-0.732, -0.348)	(-0.108, 0.495)		
P value[b]	<0.0001	0.2028		
Day 120				
TC/HDL-C at day 0	4.92 (1.097)	5.03 (1.308)	0.6650	<0.0001
TC/HDL-C at day 120	4.19 (0.917)	5.08 (1.350)	0.0003	
Mean Difference	-0.73	0.06		
CI	(-0.981,-0.487)	(-0.212, 0.327)		
P value[b]	<0.0001	0.6689		
Note: P Value[a] : Two sample t-test. P value[b]: Paired t-test P Value[c]: ANCOVA P Value.				

Table 8: Statistical analysis for triglyceride (per protocol population).

Variable	KaraHeart™ (N=47)	Placebo (N=48)	P value[a]	ANCOVA P value[c]
Triglycerides at day 0 (mg/dl)	171.0 (57.249)	195.2 (74.984)	0.0812	0.0114
Triglycerides at day 30 (mg/dl)	155.7 (46.590)	186.5 (57.706)	0.0054	
Mean Difference (mg/dl)	-15.3	-8.73		
CI	(-22.65, -7.900)	(-20.58, 3.120)		
P value[b]	0.0001	0.1450		
day 60				
Triglycerides at day 0 (mg/dl)	171.0 (57.249)	195.2 (74.984)	0.0812	<0.0001
Triglycerides at day 60 (mg/dl)	140.9 (37.756)	185.5 (57.935)	<0.0001	
Mean difference (mg/dl)	-30.1	-9.67		
CI	(-41.41, -18.76)	(-26.49, 7.160)		
P value[b]	<0.0001	0.2536		
Day 90				
Triglycerides at day 0 (mg/dl)	171.0 (57.249)	195.2 (74.984)	0.0812	<0.0001
Triglycerides at day 90 (mg/dl)	134.7 (37.209)	185.8 (64.177)	<0.0001	
Mean Difference (mg/dl)	-36.3	-9.44		
CI	(-48.08, -24.47)	(-28.35, 9.479)		
P value[b]	<0.0001	0.3207		
Day 120				
Triglycerides at day 0 (mg/dl)	171.0 (57.249)	195.2 (74.984)	0.0812	<0.0001
Triglycerides at day 120 (mg/dl)	134.3 (40.114)	194.8 (71.532)	<0.0001	
Mean Difference (mg/dl)	-36.7	-0.42		
CI	(-49.76, -23.68)	(-18.80, 17.970)		
P value[b]	<0.0001	0.9638		

Note: P value[a] : Two sample t-test. P value[b]: Paired t-test P value[c]: ANCOVA P value.

Table 9: Statistical analysis for triglyceride (per protocol population) for different sub groups.

Category	Variable	KaraHeart™ Group (N=47)	placebo group (n=48)	P value[a]	ANCOVA P value[c]
Triglycerides above 200 mg/dl	N	12	20		0.0020
	Baseline	244.8 (33.275)	268.8 (44.646)	0.1193	
	V6	163.3 (35.041)	243.1 (64.202)	0.0001	
	Mean difference	-81.50	-25.70		
	CI	(-103.0, -59.97)	(-55.38, 3.979)		
	P value[(b)]	<0.0001	0.0858		
Triglycerides 160 TO 200 mg/dl	N	19	14		0.0361
	Baseline	172.3 (10.954)	170.6 (9.771)	0.6636	
	V6	133.7 (35.195)	168.7 (54.937)	0.0330	
	Mean difference	-38.58	-1.93		
	CI	(-54.86, -22.29)	(-35.54, 31.682)		
	P value[(b)]	<0.0001	0.9032		
Triglycerides below 160 mg/dl	N	16	14		0.0159
	Baseline	114.2 (35.900)	114.6 (36.863)	0.9729	
	V6	113.3 (37.423)	151.9 (56.182)	0.0331	
	Mean difference	-0.94	37.21		
	CI	(-16.60, 14.724)	(7.109, 67.319)		
	P value[(b)]	0.9002	0.0192		

Note: P value[a] : Two sample t-test. P value[b]: Paired t-test P value[c]: ANCOVA P value.

Statistical analysis of total cholesterol (TC) (PP Population) revealed that at baseline there were no significant differences in the values between the KaraHeart™ and placebo groups (p>0.05). An independent Students t-test was performed (Table 1) and was non-significant (p=0.8935) at baseline, confirming that the total cholesterol at baseline between the groups were essentially identical at the beginning of the study and thus, results at the end of study were comparable. ANCOVA was performed to test different effects by

eliminating unwanted variance on the outcome variable. ANCOVA analysis did not show a difference at Day 30 between the groups (p>0.05).

However, TC in KaraHeart™ group was significantly different at Day 30 as compared to baseline (p<0.0001), unlike the placebo group. These results suggest that KaraHeart™ helped reduce TC within 30 days of treatment. KaraHeart™ continued to show statistically significant reductions in the level of TC when compared to baseline at Day 60 (5%; ANCOVA p=0.0022), Day 90 (7.9%; ANCOVA, p=0.0213) and Day 120 (10.5%; ANCOVA p=0.0397) when compared to the placebo group. By Day 120, the KaraHeart™ group demonstrated approximately twice the reduction in TC compared to that of the placebo group. The placebo group did not show any statistically significant improvement until Day 90, whereas the KaraHeart™ group began showing statistically significant decreases in TC starting at Day 30 (Table 1).

The HDL level was well maintained in the KaraHeart™ group with no statistical difference observed at Day 30 from Baseline. In contrast, the placebo group demonstrated a statistically significant reduction in HDL. At Day 120, the KaraHeart™ group had a statistically significant increase in HDL of 4.7% whereas the placebo group showed a statistically significant decrease in HDL of 5.32%. These data indicate that, without active treatment, the patients' HDL levels were deteriorating (Table 2). The ANCOVA P values were significant at all time points (Days 30, 60, 90, and 120) indicating that KaraHeart™ increased HDL levels. In a sub-group analysis of high-risk category patients (baseline HDL below 40 mg/dl), HDL levels in the KaraHeart™ treated group demonstrated an even greater increase than the entire KaraHeart™ group in HDL compared to the placebo group. In this sub-group analysis (Table 3), a significant increase of HDL (4.67 mg/dl, 13.27%) was observed in the KaraHeart™ group from the baseline to the end of study indicating that KaraHeart™ improved HDL levels. In contrast, there was a decrease of 0.9 mg/dl (2.7%) observed in the placebo group (sub-group analysis) from baseline to the end of study. The ANCOVA P value (0.0089) is significant in the sub-group analysis of HDL levels indicating that KaraHeart™ is effective at increasing HDL, whereas the placebo group experienced deteriorating HDL levels. The paired Students t-test (p=0.004) was significant for KaraHeart™ group, but not for the placebo group (p=0.5355) indicating that that treatment group improved significantly from baseline, but the placebo group did not.

At day 120, the KaraHeart™ group had a tendency toward a decrease in LDL compared to the placebo group, as demonstrated by a nearly 13 mg/dl decrease in mean LDL level compared (10% decrease) to the placebo group (approximately 3 mg/dl increase in mean LDL, a 2.3% increase) ANCOVA (p=0.095) (Table 4).

The KaraHeart™ group had a statistically significant reduction in VLDL levels, as compared to baseline, from Day 30 through Day 120. The KaraHeart™ group had statistically significant reductions in mean VLDL of 3 mg/dl (9% reduction) and 7 mg/dl (20% reduction) at Day 30 and Day 120, respectively. In contrast, there was no statistically significant reduction observed in VLDL in the placebo group at any time point compared to baseline. ANCOVA p values for days 30, 60, 90, and 120 were all less than 0.05 (Table 5). In a sub-group analysis of high-risk patients (Baseline VLDL above 40 mg/dl), there was a significant decrease (p<0.0001) of 16.3 mg/dl (33.28%) observed indicating a positive effect of KaraHeart™. There was no statistically significant change (p>0.05) observed in the level of VLDL in placebo group from baseline to the end of the study. The ANCOVA p value was significant (p=0.0020), which was due to reduction of VLDL in KaraHeart™ group (Table 6).

The KaraHeart™ group had a statistically significant reduction of mean TC/HDL-C at Day 30 (5% decrease), Day 60 (8% decrease), Day 90 (11% decrease), and Day 120 (15% decrease) compared to baseline. The placebo group showed no statistically significant decrease during any of the time point. ANCOVA P-values were less than 0.05 at all measurement times (Table 7).

The KaraHeart™ group had a statistically significant reduction in triglycerides at all time points compared to baseline, whereas the placebo group had no significant reduction at any time point. At Day 30, the KaraHeart™ group had a mean 15.3 mg/dl unit decrease (9% decrease), and by Day 120, the group had nearly a 37 mg/dl decrease (21% decrease) of triglycerides. ANCOVA p values were less than 0.05 at all measurement times (Table 8). In a sub-group analysis of high-risk category patients (baseline triglycerides above 160 mg/dl), the KaraHeart™ had an even greater decrease in triglycerides at all time points compared to baseline with a decrease of 81.5 mg/dl (33.2%) in KaraHeart™ group from the baseline to the end of the study period. In contrast, the placebo group did not have a statistically significant change in triglycerides from baseline to end of study in the high-risk sub-group (p=0.0858). ANCOVA p value (0.0020) and P-value between the two groups (0.0001) were significant indicating that KaraHeart™ was more effective at reducing triglyceride level than the placebo. In the category of patients with baseline TGL values between 160 to 200 mg/dl, a decrease of 38.6 mg/dl (22%) was observed in the KaraHeart™ group and a negligible non-statistically significant decrease of 1.9 mg/dl (1.1%) was observed in the placebo group from baseline to the end of the study. The ANCOVA p value was significant (0.0361) indicating a difference between the groups and supporting a role for KaraHeart™ in decreasing triglycerides in the blood (Table 9).

Average HbA1C at baseline in the KaraHeart™ group was 5.37 (SD=0.349) and was 5.42 (SD=0.410) in the placebo group. The mean of two groups was statistically

comparable (p=0.536) at Day 0. The level of HbA1C increased 0.17 units from baseline to Day 120 in the KaraHeart™ group (p<0.0001) and it increased by 0.24 units in the placebo group (p<0.0001).

Mean C-reactive protein (CRP) in the KaraHeart™ group was 6.54 (SD=1.518) mg/L and mean CRP in the placebo group was 6.22 (SD=1.278) mg/L at the Baseline visit. CRP decreased by 0.59 units at Day 120 from Baseline in KaraHeart™ group (p=0.0463) and decreased by 0.07 units in placebo group (p=0.7717).

Serum Apolipoprotein A1 (ApoA1) in the KaraHeart™ group was 136.81mg/dl (SD=23.237) and in placebo group was 138.81mg/dl (SD=26.285) at the Baseline visit. In the KaraHeart™ group, ApoA1 increased by 5.37 units at Day 120 compared to Baseline (p=0.0122) and decreased by 1.37 units in the placebo group (p=0.6678). The normal range of ApoA1 for men is 110-180 mg/dl and 250 mg/dl for women.[19] Higher levels of ApoA1 is considered beneficial for cardiac health and can be considered independently of HDL levels. KaraHeart™ increased the ApoA1 levels in the present study suggesting that it is beneficial for cardiac health.

Adverse events

There were no serious adverse events observed in this study. KaraHeart™ was well tolerated with few mild to moderate side effects which were equally distributed between the KaraHeart™ and placebo groups (3 cases in the KaraHeart™ group, 4 cases in placebo group).

DISCUSSION

The therapeutic goal for treating hyperlipidemia and associated CVD is to manage the level of cholesterol in the blood. Cholesterol is managed by increasing HDL and decreasing LDL, VLDL, and TGL in the blood. Currently, there are medications available for managing cholesterol, though the side-effects and costs associated with these medications can be detrimental to the patient. The primary AE with statins, which were originally derived from fungi, are the statin-induced myopathies.[20] The recent SAMSON trial, however, indicated that in a significant number of patients, this could be interpreted as a nocebo effect.[21] AE for the fibrates, another class of drug used to treat hyperlipidemia, include nausea, pain, cholelithiasis, cholecystitis, hepatic disorders and clotting disorders.[22] These results notwithstanding, a natural alternative to the available medications could be a lower-cost option with fewer and milder side-effects. One study showed that Citrus Bergamia polyphenols and Cynara cardunculus extracts could work together effectively to help support dyslipidemic patients.[23] Furthermore, in 2017, the ILEP (International Lipid Expert Panel) recommended that phytosterols and red yeast should be considered as useful options for cholesterol management. [24]

Currently, there is no supplement proven to be safe and effective in treating hyperlipidemia. In the present study, we show that KaraHeart™ (a supplement with a proprietary herbal composition) is safe and effective in treating hyperlipidemia. Supplementation with KaraHeart™ increased HDL and reduced the levels of LDL, VLDL, TGL and TC in the blood. This study also showed that supplementation with 1000 mg/day of KaraHeart™ was safe, as there were no serious adverse side effects. Thus, KaraHeart™ can be considered safe and effective in helping patients manage their cholesterol levels. In conclusion, this study demonstrated that KaraHeart™, a synergistic herbal extract blend, helped manage cholesterol levels by normalizing lipid parameters. KaraHeart™ did not alter the vital signs of the patients and did not cause any serious adverse side effects, making it a safe and effective treatment option for patients with mild to moderate hyperlipidemia.

Our current study had a few limitations. Firstly, it was conducted on 100 patients. It would be helpful to conduct a follow up study on a larger population size covering multiple geographic locations to make an even more conclusive determination about the effectiveness of KaraHeart™. Secondly, our study was four months long. It would be helpful to do longer term studies to make a more conclusive determination about the long-term effectiveness of KaraHeart™.

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