International Journal of Basic & Clinical Pharmacology
Rajendran K et al. Int J Basic Clin Pharmacol. 2021 Oct;10(10):1170-1181
http://www.ijbcp.com
pISSN 2319-2003 | eISSN 2279-0780

DOI: https://dx.doi.org/10.18203/2319-2003.ijbcp20213747

Original Research Article

Assessment of safety and efficacy of a dietary supplement KaraLiv™ in supporting liver health: a double-blind, parallel, placebo-controlled randomized clinical trial

Krishna Rajendran[1]*, Rajendran Ramaswamy[2], Kudiganti Venkateshwarlu[3], Ravikumar Rajendran[2], K. Sunil Naik[4]

[1]Karallief Inc, Cambridge, Massachusetts, United States of America
[2]Green Chem, Bangalore, Karnataka, India
[3]AyurLife Health Solutions, Bangalore, Karnataka, India
[4]Government Medical College and Government General Hospital, Srikakulam, Andhra Pradesh, India

Received: 16 August 2021
Revised: 13 September 2021
Accepted: 14 September 2021

***Correspondence:**
Krishna Rajendran,
Email: krishna@karallief.com

ABSTRACT

Background: The liver is responsible for many critical functions within the body. If the liver becomes diseased or injured, loss of those critical functions can cause significant damage to the body. KaraLiv™ is a novel herbal formulation which contains a blend of different herbal extract ingredients. The current study tested the safety and efficacy of KaraLiv™ versus a placebo control in supporting liver function.
Methods: The study is a randomized, double-blind, parallel, and placebo-controlled study. A total of 60 patients were divided into 2 groups of 30 each. One group was given KaraLiv™ and the other group was given a placebo for a period of 56 days. Treatment results were assessed by evaluating the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, and alkaline phosphatase (ALP) in both groups.
Results: The herbal supplement KaraLiv™ significantly supported healthy liver function compared to the placebo following the 56 days of treatment. The treatment (KaraLiv™) group showed a statistically significant improvement in assessed liver enzyme levels compared to the placebo group.
Conclusions: The all-natural herbal supplement KaraLiv™ is a safe and effective product that can significantly help support healthy liver function.

Keywords: Liver, Herbal supplement, Clinical trial, Liver enzymes

INTRODUCTION

The liver is a critical organ of the human body and plays a key role in metabolism and excretion.[1,2] The liver performs many essential functions including the synthesis of cholesterol, triglycerides, proteins, blood clotting factors, glycogen, and bile.[3] Symptoms of liver disorders can include jaundice, swelling, abdominal pain, confusion, bleeding, fatigue, weakness, nausea, vomiting, and weight loss.[3] Alcohol can be toxic to the liver, especially in high doses. Long-term alcohol abuse is a common cause of liver disorders.[4]

Modern drugs do not provide many effective options for treating liver disorders.[5] The existing liver medications may also cause side effects that can exacerbate the liver condition.[6] Herbal formulations based on traditional uses may be a safer alternative to currently available

medications. Herbal remedies have been used for liver disorders in many different systems of traditional medicine including Ayurveda, Chinese, and European.[5] In the modern era, the quest to find herbal remedies for liver disorders has led to combining traditional knowledge with modern scientific evaluation: using rigorous, randomized, placebo controlled clinical trials to evaluate herbal products.[7]

In India, more than 87 medicinal plants are used in different combinations as herbal treatments for liver diseases; however, not all plants have been evaluated for pharmacological efficacy, even though many are reported to be hepatoprotective.[7] The present study was conducted to test the safety and efficacy of the herbal extract blend KaraLiv™ in supporting liver function.

METHODS

The study is a randomized, double-blind, parallel, placebo-controlled study. The study was conducted at Government Medical College and General Hospital, Srikakulam, Andhra Pradesh from September 2020 to December 2020. Reporting of the study was done according to consolidated reporting of randomized controlled trials (CONSORT) guidelines (Figure 1).

Selection criteria

Inclusion criteria

For this study, the subjects selected were between 18 and 70 years with mild to moderately elevated liver enzyme levels based on medical history, physical examination, and laboratory tests. These subjects were otherwise healthy. These subjects also had a ratio of aspartate aminotransferase (AST) to alanine aminotransferase (ALT) greater than 1.5. Also, the subjects' ALT and AST levels were greater than 1.5 times the upper limit of normal. Subjects also had to be able to provide written informed consent and be able to understand and be willing to comply with the requirements of the study.

Exclusion criteria

Patients with the following criteria were excluded from the trial: pregnant women and women of childbearing potential who are at risk of pregnancy; subjects with severe alcoholic hepatitis who have cirrhosis or life expectancy less than 3 months; subjects with severe renal impairment defined by a glomerular filtration rate below 60 ml/min per 1.73 m^2; subjects with hepatic disorders due to cardiac causes, inherited metabolic causes, hemochromatosis, or Wilson's disease; subjects with severe alcoholic hepatitis with cirrhosis; subjects with active viral hepatitis; subjects undergoing active treatment for alcohol withdrawal syndrome at study entry; subjects on hepatotoxic medications, such as antitubercular medication, antiviral medication, and paracetamol; subjects participating in another clinical trial with an active intervention, drug, or device with the last dose taken within 60 days; subjects with any other condition which, in the opinion of the investigator, would adversely affect the subject's ability to complete the study or its measures; and subjects who have a known allergy to the ingredients present in KaraLiv™.

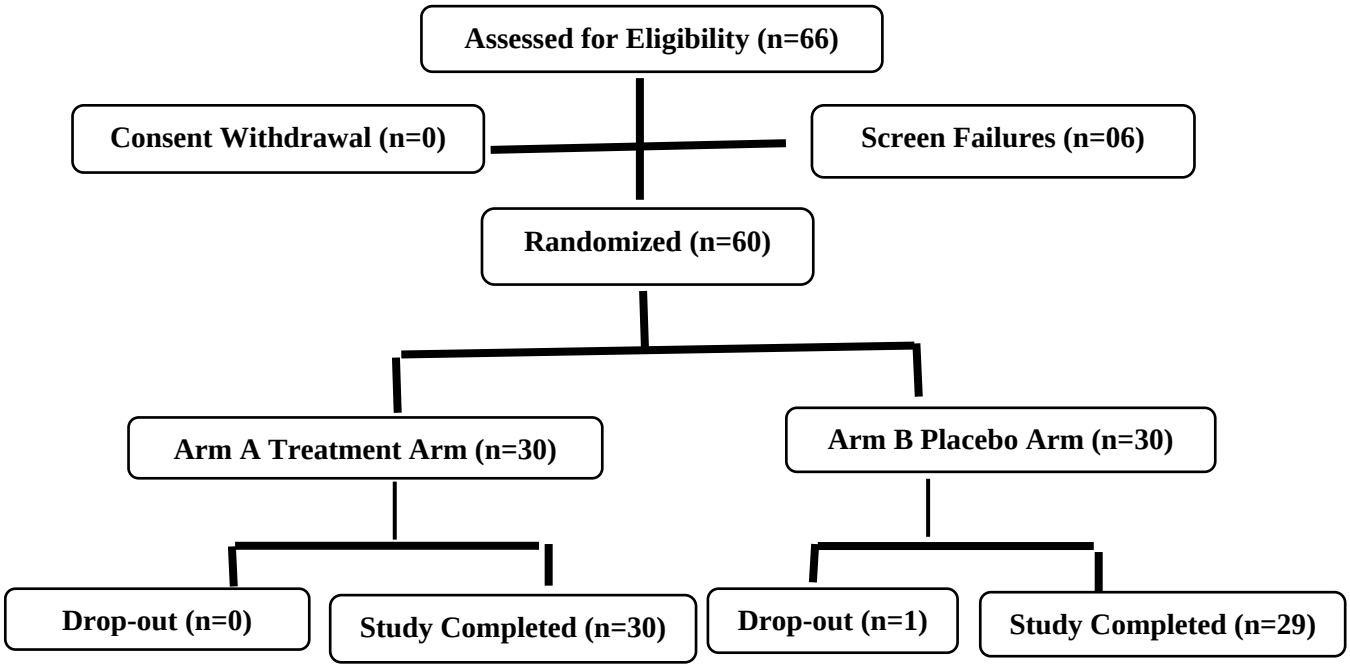

Figure 1: A total of 66 subjects were screened for the inclusion and exclusion criteria at the initial visit. Of those screened, 60 subjects were eligible to participate and signed the informed consent. 30 subjects were randomized in each treatment arm. One subject from Placebo arm dropped out due to personal reasons.

Participants

Sample size was calculated using repeated measure analysis of covariance keeping aspartate aminotransferase, also known as serum glutamic oxaloacetic transaminase (AST/SGOT), alanine aminotransferase (formerly called serum glutamic pyruvic transaminase; ALT/SGPT), alkaline phosphatase (ALP), and total serum bilirubin as primary objectives.

An anticipated standardized effect size of 0.4 and interclass correlation of 0.6 was assumed. Considering a drop-out rate of 15%, 30 subjects were recruited in each arm to obtain a power of more than 80% to meet the primary objective.

Intervention

KaraLiv™ is a proprietary blend of standardized herbal extracts of *Momordica charantia*, *Phyllanthus niruri*, *Andrographis paniculata*, *Brassica rapa*, *Asparagus racemosus*, and ginger.[8-13] Each of these herbal extracts have been standardized to specific active compounds.

Comparison with a placebo group was expected to provide information on the safety and efficacy of KaraLiv™ without the placebo effect. Each capsule contained 500mg of either KaraLiv™ or the placebo. Daily dosage was 1000 mg (i.e. two capsules/day).

Trial design

Prior to conducting the study, each subject was provided with a Subject Information Sheet describing detailed procedures, potential risks, and anticipated benefits. Participants were provided ample time to consider the information presented and were subjected to screening procedures after obtaining written informed consent. Eligible subjects who completed informed consent were randomly allocated to the treatment groups (KaraLiv™ or placebo).

Randomization of participants was performed through computer-generated randomization codes using permuted block design and block size selected were known only to the statistician until the analysis was completed. Allocation concealment was done using sequentially numbered opaque sealed envelopes; everyone involved in the study, except for the statistician, was blinded to medication assignments. 30 subjects were allocated to each group with a total of 60 participants: group A (n=30 subjects) investigation product (IP) KaraLiv™ and group B (n=30 subjects) placebo.

Duration of study was 56 days with 4 scheduled visits (screening visits, Randomization visit-day 1, day 28, and day 56). Each visit had a flexibility window of ±two days.

Study medications were packed according to an assigned randomization number. Sealed packs of KaraLiv™ were provided to the clinical site. Investigators who received the IP maintained inventory and reconciliation logs for individual supplies. Either KaraLiv™ or placebo was dispensed to the subjects on visit 2 (day 1) and visit 3 (day 28). KaraLiv™ or placebo capsules were taken orally twice daily half an hour after breakfast and half an hour after dinner, respectively, for 56 days.

Subjects recorded their consumption of supplements in diary cards. Investigators verified the subjects' diaries and compliance cards and reconciled the study medication to subjects. This reconciliation was logged onto the IP reconciliation form and signed and dated by the study team. The investigators performed the physical exam (measurement of vital signs, collection of concomitant medication, checks for illness, and collection of adverse events (AE) information) during screening visits and each subsequent study visit.

Complete medical histories were taken during screening and throughout the study at all visits. Medical histories were recorded for each subject on the case report form (CRF) which included past medical or surgical procedures and current conditions. Medical histories of subjects were noted, with respect to duration, description of intensity when there is no exacerbation, date of onset of present exacerbation, primary disease symptoms with intensity, dietary restriction, tobacco usage, and prior treatments. A complete physical examination was conducted at all visits. Each follow up visit involved the administration of supplements. Vital signs (heart rate, blood pressure, temperature, respiratory rate, and pulse rate) and weight were recorded at all visits.

Each subject underwent clinical laboratory tests at screening visits and at follow-up visits. Urine for urinalysis and blood for hematology and biochemistry were collected during screening visits and end of the study visits. Blood samples were collected by direct venipuncture for hematology, biochemistry, and serology laboratory tests.

Throughout the course of the study, all subjects, investigators, and sponsor's personnel remained blinded to the study medication assignment. The investigators were given the right to break the blinding in the following situations: treatment of emergent serious adverse events (SAE) and protecting the safety of the patient.

Compliance and adverse events

At each visit, excess medication was returned to investigators to confirm that the correct number of capsules had been taken. AEs (if any) were recorded in source documents and the CRF.

Information collected included the nature, date and time of onset, intensity, duration, causality, action taken, and outcome of the event. Details of medications given to the

subject (to abate the AEs) were recorded on the concomitant medication page by the investigator.

All AEs during the study were followed until resolution (returned to normal or baseline values), stabilization, or until judged to be no longer clinically significant by investigators. Since all AEs were mild to moderate in nature, no supplemental measurements, and no evaluations (such as laboratory tests, diagnostic procedures, or consultation with other healthcare professionals) were necessary to investigate the nature and/or causality of an AE.

There were no SAEs reported. The minor adverse events were evenly distributed in KaraLiv™ and placebo groups. These minor adverse events were self-limiting and subsided with use of concomitant medication or without any intervention. Thus, KaraLiv™ is safe for human consumption.

Withdrawal and dropout

Subjects who did not meet inclusion/exclusion criteria were considered screening failures. Participating subjects could withdraw from the study at any time without justification of his/her decision, even after undergoing consent. No subjects were discontinued due to non-compliance with medication, protocol violation, worsening of disease or tolerability, AE, or SAE. One subject in group B (placebo group) dropped out from the study due to personal reasons and not due to any AE.

Outcome measures

Primary objective

ALT/SGPT

ALT/SGPT helps with protein metabolism. When the liver is impaired, ALT can leak into the blood. Normal levels of ALT are below 45 IU/l in males, while these levels are somewhat lower in females and vary depending on age.[14]

AST/SGOT

AST is an enzyme found in many parts of the body including the heart, liver, muscles, and kidney. AST gets released into the blood when there is damage to any of the organs where it is present. Thus, elevated blood AST levels are not conclusive indicators of liver damage and AST is measured with ALT to make a more liver-specific diagnosis. Normal levels of AST are under 35 IU/l in adults.[14]

ALP

ALP is an enzyme mainly found in the liver but can also be found in other parts of the body such as bones and bile ducts. ALP gets released into the blood when there is damage to any part of the body containing ALP. Liver impairment, obstructed bile ducts, and bone related problems can all lead to raised ALP levels in the blood. Normal levels of ALP are between 30 and 120 IU/l.[14]

Total serum bilirubin

When red blood cells (RBCs) are broken down, a waste product called bilirubin is generated. When the liver is damaged, bilirubin cannot be cleared as effectively leading to elevated bilirubin levels in the blood. The normal range of serum bilirubin is 2 to 17 micromoles/l (0.12-1.0 mg/dl).[14]

Secondary objective

The secondary objectives were to change in quality of life (QOL) scores - physical health, *c*hange in QOL scores - psychosocial health, and to assess the safety and tolerability of KaraLiv™.

Changes from baseline to the end of the study period in these parameters were monitored to determine the overall safety and tolerability of KaraLiv™: malondialdehyde (MDA), superoxide dismutase (SOD), Gamma-glutamyl transferase (GGT), and sex hormone binding globulin (SHBG).

Ethics approval

The study was performed as per the principles of the declaration of Helsinki and conducted in agreement with international council for harmonisation (ICH) guidelines on good clinical practice (GCP). The study was carried out in compliance with Indian regulations for herbal and Ayurvedic clinical trials and Ayurveda Siddha Unani-GCP. ICH-GCP issued by the United States (US) department of health and human services was followed. The trial was registered with the clinical trials registry (GC/KL/2020/01) on the 08 March 2020 and hosted at Indian Council of Medical Research's (ICMR) National Institute of Medical Statistics as per the mandate of drugs controller general of India. The trial protocol was approved by the institutional ethics committee, Government Medical College, and Government General Hospital in Srikakulam, Andhra Pradesh.

Statistical analysis

Data collected from the study site were assessed using the statistical package for social sciences (SPSS) software version 21, SPSS Inc, Chicago III, USA. Significance was defined as $p<0.05$. Descriptive analysis for baseline summary statistics including mean, median, standard deviation for demographic data, and proportion of males and females were completed. Inferential statistics were performed with one-way analyses of variance (ANOVAs) with Tukey tests for primary outcome and biomarkers intragroup comparison.

Paired student t-tests were performed to analyze safety data. Unpaired student t-tests were performed for intergroup comparison. Missing observations were imputed using the last observation carried forward approach.

RESULTS

Of the 66 subjects who participated in the screening visit, six were screening failures. 60 subjects qualified for the study based on the inclusion/exclusion criteria and all signed the informed consent. Subjects were randomized to groups: group A received KaraLiv™ and group B received the placebo. One subject dropped out of the study from group B due to personal reasons. The final statistical analyses and results were depicted for 59 participants at the end of the study (Figure 1).

A summary of baseline demographic data of included subjects is shown in Table 1.

Analysis of primary outcomes

Changes in levels of ALT/SGPT, AST/SGOT, bilirubin, and ALP levels were measured at baseline, 28 days (V3), and at end of the study (56 days) (Table 2). Analysis between the groups at visit 1 (baseline) for all primary outcomes showed no statistical difference between the 2 groups ($p>0.05$) (Table 3). At visit 3, there was a statistically significant difference between the two groups for ALT/SGPT, AST/SGOT, and serum (S.) bilirubin ($p>0.05$). At visit 4, there was a statistically significant difference between the two groups for all primary parameters ($p>0.05$), showing that KaraLiv™ was significantly more effective than the placebo for all primary outcomes (Table 3).

There was a reduction in ALT/SGPT from a mean of 79.13 (baseline) to 61.63 (day 28) to 47.2 (day 56) in the KaraLiv™ group, resulting in a 40.35% reduction from baseline to the end of the study. The placebo group showed a reduction of mean ALT/SGPT from 76.53 (baseline) to 65.96 (day 28) to 61.62 (day 56), totaling a 19.48% reduction from baseline to the end of the study (Table 4).

There was a reduction in AST/SGOT in the KaraLiv™ group from a mean of 122.4 (baseline) to 93.26 (day 28) to 57.16 (day 56) totaling a 53.3% reduction from baseline to the end of the study. The placebo group had a reduction of mean AST/SGOT from 119.53 (baseline) to 102.31 (day 28) to 90.52 (day 56) totaling a 24.28% reduction from baseline to the end of the study (Table 4).

There was a reduction of mean bilirubin in the KaraLiv™ group from 1.22 (baseline) to 1.07 (day 28) to 0.97 (day 56) resulting in a 20.5% reduction from baseline to the end of the study. In the placebo group there was a reduction of mean bilirubin from 1.20 (baseline) to 1.11 (day 28) to 1.05 (day 56) resulting in a 12.5% reduction from baseline to the end of the study (Table 4).

There was a reduction of mean alkaline phosphatase in the KaraLiv™ group from 119.37 (baseline) to 105.3 (day 28) to 94.70 (day 56) totaling in a 20.67% reduction from baseline to the end of the study. There was a decrease in mean ALP in the placebo group from 117.30 (baseline) to 109.27 (day 28) to 105.44 (day 56) resulting in a 10.11% reduction from baseline to the end of the study (Table 4).

Analysis of blood cells

The KaraLiv™ group had a slight statistically significant increase in mean hemoglobin, RBC, and platelet count. This group also had a statistically significant decrease in the level of Eosinophil. Additionally, the blood urea nitrogen (BUN) and serum urea mean values had a statistically significant decrease from baseline to the end of the study (Table 5).

There was a statistically significant decrease in eosinophil and serum urea levels in the placebo group (Table 5).

Analysis of secondary outcomes

Malondialdehyde (MDA)

Lipid peroxidation is a chain of reactions in hepatocytes leading to oxidative stress and the formation of a toxic product called MDA. Higher values of MDA indicate oxidative stress.[15]

In the KaraLiv™ group, mean MDA levels decreased from 3.44±0.43 at baseline to 2.51±0.37 at the end of the study (Table 6). In the placebo group, mean MDA levels decreased from 3.33±0.58 at baseline to 3.31±0.5 at the end of the study (Table 7). KaraLiv™ resulted in a statistically significant decrease in MDA levels when compared to the placebo ($p<0.05$) (Table 8).

Superoxide dismutase (SOD)

SOD protects cells from oxidative stress and the toxic effects of endogenously generated superoxide radicals (free radicals). Disturbances in the antioxidant system (which neutralizes free radicals) may play a role in the pathogenesis of chronic liver disease.[16]

The release of reactive oxygen species occurs when products of free radical reactions are involved in pathogenesis and/or progression of medical cholestasis. When free radicals are released, the serum SOD increases to minimize the liver injury. Hence low levels of SOD may lead to more liver damage.

Mean SOD levels in group A were significantly increased from baseline (179.97±13.72) to the end of the study (216.13±20.84) ($p<0.05$) (Table 6). For group B, the baseline and end of the study values were 205.76±26.23 and 207.52±27.87 respectively; but this change was not statistically significant (Table 7).

Gamma-glutamyl transferase (GGT)

GGT is an enzyme found in high levels in the liver. Elevated serum GGT is a sign that the liver or bile ducts are impaired.[17] Mean GGT levels in group A at baseline and end of study were 69.03±11.64 and 54.43±10.59, respectively. The mean GGT in group B was 66.52±11.64 at baseline and 63.48±13.33 at the end of the study (Tables 6 and 7). Both groups A and B had a statistically significant decrease in mean GGT levels from baseline to the end of study ($p<0.05$).

Sex hormone binding globulin (SHBG)

SHBG binds to three sex hormones: estrogen, dihydrotestosterone, and testosterone. SHBG determines the amount of biologically available testosterone in the human body. High serum levels of SHBG non-specifically indicates liver impairment.

Mean SHBG levels in the KaraLiv™ group were 140.1±37.89 at baseline and 129±36.10 at the end of the study. Mean SHBG levels in the placebo group were 115.1±41.69 at baseline and 111.5±44.1 at the end of the study (Table 6 and 7). The KaraLiv™ group had a statistically significant decrease in mean SHBG whereas the placebo group did not ($p<0.05$).

Overall, the treatment group exhibited a statistically significant decrease in MDA, GGT, and SHBG levels from baseline to end of the study while the SOD levels exhibited a statistically significant increase (Table 6). The placebo group exhibited a statistically significant decrease in GGT level from baseline to the end of the study (Table 7). In contrast, there was no statistically significant change observed in MDA, SOD, and SHBG for the placebo group (Table 7).

Analysis of QOL

QOL was assessed through a pre- and post-questionnaire short form (SF) 36. The questionnaire had eight domains: physical functioning, limitations due to physical health, limitations due to emotional problems, energy/fatigue, emotional well-being, social functioning, pain, and general health. All covariate factors were adjusted to find the exact influence of liver disease on the domains. Higher scores for each domain indicate improvement in the QOL.

The QOL parameters were assessed for group A and B. Patients in group A exhibited significant improvement in all the parameters from visit 1 (V1) to visit 4 (V4), whereas in group B, except for the physical functioning parameter, there was no meaningful change seen from baseline to V4. At baseline, there was no statistically significant difference between the groups in any parameter ($p>0.05$). However, by the end of the study (V4), there was a statistically significant difference between group A and B in all parameters ($p<0.05$) (Table 9).

Table 1: Demographic information of subjects meeting the eligibility criteria and providing signed informed consent.

Variable	Statistics	Group A and B (N=60)	
Height at baseline (cm)	Mean (SD)	159.13 (8.14)	
	Min, max	143, 175	
Weight at baseline (kg)	Mean (SD)	58.50 (8.17)	
	Min, max	41,79	
BMI at baseline (kg/m^2)	Mean (SD)	23.12 (2.33)	
	Min, max	19.30,28.70	
		Group A (N=30)	**Group B (N=30)**
Gender			
Male	N (%)	16 (53.3%)	17 (56.7%)
Female	N (%)	14 (46.7%)	13 (43.3%)
Age at baseline	Mean (SD)	40.666 (12.56)	38.57 (10.22)
	Min, max	(19, 68)	(20, 64)

Table 2: Statistical summary of primary outcomes at different visits.

Variable	Baseline	Subsequent visits	Mean difference (visit 1 – subsequent visit)	P value difference
Group A - KaraLiv™				
ALT/SGPT (IU/l)	Visit 1 (baseline)	Visit 3	17.50000	<0.001*
		V4 (end of the study)	31.93333	<0.001*
AST/SGOT (IU/l)	Visit 1 (baseline)	Visit 3	29.13333	<0.001*
		V4 (end of the study)	65.23333	<0.001*
Bilirubin (mg/dl)	Visit 1 (baseline)	Visit 3	0.15167	<0.001*
		V4 (end of the study)	0.24833	<0.001*

Continued.

Variable	Baseline	Subsequent visits	Mean difference (visit 1 – subsequent visit)	P value difference
ALP (IU/l)	Visit 1 (baseline)	Visit 3	14.43333	<0.001*
		V4 (end of the study)	25.03333	<0.001*
Group B - placebo				
ALT/SGPT (IU/l)	Visit 1 (baseline)	Visit 3	10.56782	<0.001*
		V4 (end of the study)	14.91264	<0.001*
AST/SGOT (IU/l)	Visit 1 (Baseline)	Visit 3	17.22299	<0.001*
		V4 (end of the study)	29.01609	<0.001*
Bilirubin (mg/dl)	Visit 1 (baseline)	Visit 3	0.08933	0.001*
		V4 (end of the study)	0.15092	<0.001*
ALP (IU/l)	Visit 1 (baseline)	Visit 3	8.02414	0.006*
		V4 (end of the study)	11.85172	<0.001*

*refers to p value <0.05

Table 3: Intergroup comparison of primary outcomes (group A (KaraLiv) versus group B (placebo)).

Tests	Group	Mean	Standard deviation	Mean difference (group A – group B)	P value
Visit 1 (baseline)					
ALT/SGPT visit 1 (baseline) (IU/l)	Group A	79.1333	7.51887	2.60000	0.155
	Group B	76.5333	6.43125		
AST/SGOT visit 1 (baseline) (IU/l)	Group A	122.4000	11.77256	2.86667	0.307
	Group B	119.5333	9.66948		
Bilirubin visit 1 (baseline) (mg/dl)	Group A	1.2183	0.13269	0.01500	0.650
	Group B	1.2033	0.12215		
Alkaline phosphatase visit (baseline) (IU/l)	Group A	119.7333	15.24950	2.43333	0.504
	Group B	117.3000	12.62769		
Visit 3 (day 28)					
ALT/SGPT visit 3 (IU/l)	Group A	61.6333	7.21819	-4.33218	0.011*
	Group B	65.9655	5.29476		
AST/SGOT visit 3 (IU/l)	Group A	93.2667	9.26221	-9.04368	<0.001*
	Group B	102.3103	8.56114		
Bilirubin visit 3 (mg/dl)	Group A	1.0667	0.09911	-0.04733	0.050*
	Group B	1.1140	0.08115		
Alkaline phosphatase visit 3 (IU/l)	Group A	105.3000	9.34455	-3.97586	0.102
	Group B	109.2759	9.02733		
Visit 4 (day 56)					
ALT/SGPT V4 (end of the study) (IU/l)	Group A	47.2000	5.08141	-14.42069	<0.001*
	Group B	61.6207	4.27963		
AST/SGOT V4 (end of the study) (IU/l)	Group A	57.1667	8.51807	-33.35057	<0.001*
	Group B	90.5172	8.65044		
Bilirubin V4 (end of the study) (mg/dl)	Group A	0.9700	0.07506	-0.08241	<0.001*
	Group B	1.0524	0.05604		
Alkaline phosphatase V4 (end of the study) (IU/l)	Group A	94.7000	8.07785	-10.74828	<0.001*
	Group B	105.4483	6.02724		

*Refers to p value <0.05

Table 4: Intergroup comparison of primary outcomes between group A (KaraLiv™) and group B (placebo).

Measures	Group A			Group B		
	Visit 1	Visit 3 (day 28)	Visit 4 (day 56)	Visit 1	Visit 3 (day 28)	Visit 4 (day 56)
ALT/SGPT (IU/l)	79.13 (7.52)	61.63 (7.22)	47.20 (5.08)	76.53 (6.43)	65.97 (5.29)	61.62 (4.28)
AST/SGOT (IU/l)	122.40 (11.77)	93.27 (9.26)	57.17 (8.52)	119.53 (9.67)	102.31 (8.56)	90.52 (8.65)
Bilirubin (mg/dl)	1.22 (0.13)	1.07 (0.1)	0.97 (0.08)	1.20 (0.12)	1.11 (0.08)	1.05 (0.06)
ALP (IU/l)	119.73 (15.25)	105.30 (9.34)	94.70 (8.08)	117.30 (12.63)	109.28 (9.03)	105.45 (6.03)

*Refers to p value <0.05

Table 5: Comparison of blood markers between baseline and end of study in group A and group B.

Variables	Group A			Group B		
	Visit 1 Mean (SD)	Visit 4 Mean (SD)	P value (visit 1 – visit 4)	Visit 1 Mean (SD)	Visit 4 Mean (SD)	P value (visit 1 – visit 4)
Haemoglobin (gm/dl)	12.32 (1.72)	12.44 (1.67)	0.011*	12.72 (1.77)	12.86 (1.74)	0.775
RBC (millions/ cubmm)	4.17 (0.43)	4.23 (0.45)	0.030*	4.33 (0.38)	4.39 (0.38)	0.595
Total leukocyte count (cells/ cubmm)	7560.67 (1201.11)	7570.67 (1030.33)	0.921	7332.76 (948.74)	7244.14 (898.04)	0.686
Platelets (lakhs/ cubmm)	2.58 (0.42)	2.70 (0.36)	0.003*	2.73 (0.43)	2.76 (0.44)	0.846
Neutrophils (% relative value)	63.07 (1.95)	62.53 (2.03)	0.181	62.24 (2.25)	63.07 (1.62)	0.126
Lymphocytes (% relative value)	26.27 (1.89)	26.87 (1.87)	0.182	27.31 (2.11)	27.07 (2.48)	0.712
Eosinophil (% relative value)	6.83 (1.23)	6.33 (0.96)	0.011*	6.41 (0.87)	5.97 (0.78)	0.021*
Basophils (% relative value)	0.10 (0.31)	0.07 (0.25)	0.573	0.07 (0.26)	0.17 (0.38)	0.184
Monocytes (% relative value)	3.73 (0.78)	4.20 (1.1)	0.055	3.97 (0.94)	4.07 (1.19)	0.721
Serum creatinine (mg/dl)	1.06 (0.11)	1.06 (0.11)	0.751	1.06 (0.10)	1.06 (0.11)	0.956
BUN (mg/dl)	12.80 (2.55)	11.63 (1.94)	0.001*	12.86 (2.29)	11.59 (2.06)	0.156
Urea (mg/dl)	34.43 (5.88)	32.13 (5.09)	0.000*	34.62 (5.12)	32.93 (4.46)	0.036*

*Refers to p value <0.05

Table 6: Summary of biomarkers in group A (KaraLiv™ group).

Test	Mean	Standard deviation	Mean difference (visit 1 – visit 4)	P value
MDA (µmol/l)				
Visit 1 (baseline)	3.437	0.433	0.925	<0.001*
Visit 4 (end of the study)	2.512	0.367		
SOD (U/ml)				
Visit 1 (baseline)	179.967	13.718	-36.167	<0.001*
Visit 4 (end of the study)	216.133	20.844		
GGT (U/l)				
Visit 1 (baseline)	69.033	11.637	14.600	<0.001*
Visit 4 (end of the study)	54.433	10.592		
SHBG (µmol/ml)				
Visit 1 (baseline)	140.100	37.890	11.100	<0.001*
Visit 4 (end of the study)	129.000	36.100		

*Refers to p value <0.05

Table 7: Summary of biomarkers in group B (placebo group).

Test	Mean	Standard deviation	Mean difference (visit 1 – visit 4)	P value
MDA (µmol/l)				
Visit 1 (baseline)	3.331	0.578	0.019	0.715
Visit 4 (end of the study)	3.312	0.498		
SOD (U/ml)				
V1 visit 1 (baseline)	205.759	26.235	-1.758	0.253
Visit 4 (end of the study)	207.517	27.865		
GGT (U/l)				
Visit 1 (baseline)	66.517	11.642	3.034	0.007*

Continued.

Test	Mean	Standard deviation	Mean difference (visit 1 – visit 4)	P value
Visit 4 (end of the study)	63.483	13.325		
SHBG (µmol/ml)				
Visit 1 (baseline)	115.103	41.694	3.586	0.080
Visit 4 (end of the study)	111.517	44.103		

*Refers to p value <0.05

Table 8: Intergroup comparison between the groups using independent t-test at visit 1 and visit 4.

Test	Mean	Standard deviation	Mean difference (group A – group B)	P value
MDA visit 1 (baseline) (µmol/l)				
Group A	3.4370	0.43338	0.10631	0.084
Group B	3.3307	0.57775		
MDA visit 4 (end of the study) (µmol/l)				
Group A	2.5123	0.36705	-0.80008	0.026*
Group B	3.3124	0.49809		
SOD visit 1 (baseline) (U/ml)				
Group A	179.9667	13.71755	-25.79195	0.000*
Group B	205.7586	26.23473		
SOD visit 4 (end of the study) (U/ml)				
Group A	216.1333	20.84381	8.61609	0.072
Group B	207.5172	27.86526		
GGT visit 1 (baseline) (U/l)				
Group A	69.0333	11.63669	2.51609	0.887
Group B	66.5172	11.64235		
GGT visit 4 (end of the study) (U/l)				
Group A	54.4333	10.59174	-9.04943	0.313
Group B	63.4828	13.32458		
SHBG visit 1 (baseline) (µmol/ml)				
Group A	140.1000	37.89036	24.99655	0.206
Group B	115.1034	41.69391		
SHBG visit 4 (end of the study) (µmol/ml)				
Group A	129.0000	36.10043	17.48276	0.113
Group B	111.5172	44.10266		

*Refers to p value <0.05

Table 9: Quality of life parameters assessed using SF36 at V1 and V4 for both group A and group B.

Parameters	Visit 1			Visit 4		
	Mean	Standard deviation	P value (group A – group B)	Mean	Standard deviation	P value (group A – group B)
Physical functioning						
Group A	26.72	31.42	0.167	69.83	26.78	<0.001*
Group B	25	27.67		35.67	26.56	
Role limitation due to physical health						
Group A	17.50	38.16	0.435	62.93	48.51	<0.001*
Group B	14.17	35.02		13.79	34.63	
Role limitations due to emotional problems						
Group A	30	46.08	0.567	100	0.00	<0.001*
Group B	33.33	47.40		32.18	46.99	
Energy/fatigue						
Group A	27.33	22.89	0.632	42.50	26.21	<0.001*
Group B	28.50	21.17		27.93	20.91	

Continued.

Parameters	Visit 1			Visit 4		
	Mean	Standard deviation	P value (group A – group B)	Mean	Standard deviation	P value (group A – group B)
Emotional well-being						
Group A	32.13	21.97	0.931	52.41	21.35	<0.001*
Group B	31.93	23.56		32.00	22.41	
Social functioning						
Group A	30.83	19.73	0.689	55.43	21.51	<0.001*
Group B	29.58	23.69		27.16	24.00	
Pain						
Group A	22	15.82	0.053	33.97	21.21	<0.001*
Group B	17.25	13.23		18.19	15.12	
General health						
Group A	29.17	23.43	0.821	60.69	22.39	<0.001*
Group B	28.66	22.31		26.72	21.98	

*Refers to p value <0.05

DISCUSSION

The study found that the herbal supplement KaraLiv™, compared . the placebo, resulted in statistically significant reductions in ALT/ SGPT, AST/SGOT, bilirubin, and ALP levels. These results indicate improvement in liver function in the KaraLiv™ treated group.

In the KaraLiv™ group, blood ALT reduced by 40% at the end of the study. Thus, the KaraLiv™ group experienced their ALT levels return much closer to the normal range after treatment. ALT is an enzyme mainly in the liver and assists the liver in metabolizing proteins, removing toxins, storing important nutrients, and making bile (a key fluid for digestion).[18] The liver also uses ALT to produce glycogen, an energy reserve, which is stored mainly in the liver; when the liver is functioning improperly, ALT is released into the blood.[18] When elevated blood ALT levels begin to lower, the liver condition is improving, and the key bodily functions begin returning to normal.

ALT is frequently measured along with AST to measure how well the liver is functioning. In the KaraLiv™ group, mean blood AST reduced by 53%. Thus, the KaraLiv™ group experienced their AST levels return much closer to the normal range after treatment. AST is an enzyme found in many different tissues and organs in the body, such as the liver, kidneys, brain, and heart. AST helps with metabolizing amino acids, removing toxins, and producing glucose.[19] When there is damage to the organs, AST gets released into the blood. When elevated levels of AST begin to decrease, the organ damage that initially resulted in AST release is beginning to resolve. Thus, decreasing levels of AST in patients treated with KaraLiv™ likely indicates that liver damage is beginning to resolve.

ALP, another key liver enzyme, assists many key bodily functions. In the liver, ALP helps in the transport of enzymes and nutrients.[20] ALP levels in the blood increase when there is a blockage or damage to the liver.[20] Subsequent reduction in ALP levels after elevation can indicate that the damage to the liver has reduced or resolved. In the KaraLiv™ group, serum ALP reduced by 21% at the end of the study.

One of the key roles of the liver is to clear bilirubin from the blood. Bilirubin is produced during the normal breakdown of red blood cells. Elevated serum bilirubin indicates an increased level of red blood cell breakdown or that the liver isn't filtering bilirubin from the blood effectively.[21] Reductions in elevated serum bilirubin can indicate that liver function is improving, and waste is being removed from the blood more effectively. In the KaraLiv™ group, blood bilirubin levels reduced by 20% at the end of the study. Therefore, the KaraLiv™ treated group experienced a reduction of bilirubin levels into the normal range after treatment.[14]

Measurement of the secondary parameters may lead to determining a possible mechanism of action for KaraLiv™. MDA levels are used as a measure of oxidative stress that can occur when the liver malfunctions.[22] A reduction in the MDA levels can indicate that the liver damage is reduced. In the KaraLiv™ group, MDA showed a 27% reduction. One way this reduction in oxidative stress could be occurring is through the increase of SOD levels (an enzyme that reduces oxidative stress).[23] The KaraLiv™ group exhibited increased SOD levels by 20% by the end of the study. Additionally, GGT (an enzyme which participates in the metabolism of glutathione, an important antioxidant in the body) could also be responsible for the reduction in oxidative stress.[24] GGT is mainly found in the liver, but when the liver is impaired, GGT leaks into the blood.[25] A reduction in elevated GGT levels in the blood can indicate that the liver's condition is improving and, thus, the GGT in the liver may be able assist in glutathione metabolism. In the KaraLiv™ group, mean GGT levels in the blood decreased by 21%. These data may indicate that KaraLiv™ improves liver function by reducing oxidative stress in the liver.

The KaraLiv™ group also experienced a statistically significant outperformance compared to the placebo in the secondary outcomes relating to QOL and psychological health. The SF 36 questionnaire showed a significant improvement in all parameters from V1 to V4 in the KaraLiv™ group compared to the placebo group. The KaraLiv™ group also experienced significant improvements in their emotional well-being and social functioning.

The safety parameters were assessed by both clinical laboratory tests and by assessing vital signs. The blood tests and vital signs showed no significant variation from the normal range after or during treatment. These data indicate that KaraLiv™ is a safe product to consume in the doses tested.

The study has limitations. The trial was conducted on 60 patients for two months. While the results were promising, a large study with more patients and a longer duration must be conducted to draw broader conclusions about the long-term effectiveness of the product.

CONCLUSION

The study demonstrated that KaraLiv™ decreased the ALT, AST, bilirubin, and ALP levels in the treatment group, indicating its effectiveness in improving liver function. KaraLiv™ patients also experienced a decrease in MDA, GGT, and SHBG and an increase in SOD levels, highlighting KaraLiv™'s hepato-protective functionality. KaraLiv™ did not significantly alter vital signs or blood parameters, indicating that it is a safe treatment option. There were no serious adverse side-effects observed for patients taking KaraLiv™, further indicating that it is a safe product. More long-term studies are needed to further understand the hepato-protective capability of KaraLiv™, but results of this study support its use in improving liver function.

Funding: The study was funded by Green Chem, Karnataka, India
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Ward FM, Daly MJ. Hepatic disease. Walker R, Edwards C, editors. Clinical Pharmacy and Therapeutics. New York: Churchill Livingstone. 1999;195-212.
2. Dash S, Pattanaik S, Rout SS, Bose A. Evaluation of hepatoprotective activity of the aerial parts of phyllanthus reticulates against paracetamol induced hepatic damage in rats. Asian J Pharm Clin Res. 2015;8(4):221-4.
3. Cornelius CE. Liver function. Clinical biochemistry of domestic animals. Academic Press. 1980;201-57.
4. Maher JJ. Exploring alcohol's effects on liver function. Alcohol Health Res World 1997;21(1):5.
5. Karan M, Vasisht K, Handa SS. Antihepatotoxic activity of Swertia chirata on carbon tetrachloride induced hepatotoxicity in rats. Phytother Res. 1999;13(1):24-30.
6. Chaterrjee TK. Medicinal Plants with Hepatoprotective Properties, Herbal Options. Calcutta: Books and Applied Allied (P) Ltd. 2000;143.
7. Thyagarajan SP, Jayaram S, Gopalakrishnan V, Hari R, Jeyakumar P, Sripathi MS. Herbal medicines for liver diseases in India. J Gastroenterol Hepatol. 2002;17:370-6.
8. Mardani S, Khodadadi S, Ahmadi A, Kazemi E, Rafieian-kopaei M. The effects of Momordica charantia on liver function and histological structure. Ann Res Antioxid. 2016;1(1):12.
9. Al Zarzour RH, Ahmad M, Asmawi MZ, Kaur G, Saeed M, Al-Mansoub, et al. Phyllanthus Niruri Standardized Extract Alleviates the Progression of Non-Alcoholic Fatty Liver Disease and Decreases Atherosclerotic Risk in Sprague-Dawley Rats. Nutrients. 2017;9(7):766.
10. Chua LS. Review on liver inflammation and antiinflammatory activity of Andrographis paniculata for hepatoprotection. Phytother Res. 2014;28(11):1589-98.
11. Fu R, Zhang Y, Guo Y, Peng T, Chen F. Hepatoprotection using Brassica rapa var. rapa L. seeds and its bioactive compound, sinapine thiocyanate, for CCl4-induced liver injury. J Funct Foods. 2016;22:73-81.
12. Acharya SR, Acharya NS, Bhangale JO, Shah SK, Pandya SS. Antioxidant and hepatoprotective action of Asparagus racemosus wild root extracts. Indian J Exp Biol. 2012;50(11):795-801.
13. Rafie R, Hosseini SA, Hajiani E, Malehi AS, Mard SA. Effect of Ginger Powder Supplementation in Patients with Non-Alcoholic Fatty Liver Disease: A Randomized Clinical Trial. Clin Exp Gastroenterol. 2020;13:35-45.
14. Lala V, Goyal A, Bansal P, Minter DA. Liver function tests. StatPearls. 2020.
15. Zelber-Sagi S, Ivancovsky-Wajcman D, Fliss-Isakov N, Hahn M, Webb M, Shibolet O, et al. Serum malondialdehyde is associated with non-alcoholic fatty liver and related liver damage differentially in men and women. Antioxidants. 2020;9(7):578.
16. Younus H. Therapeutic potentials of superoxide dismutase. Int J Health Sci (Qassim). 2018;12(3):88-93.
17. Whitfield JB. Gamma Glutamyl Transferase. Criti Rev Clin Lab Sci. 2001;38(4):263-355.
18. Kim WR, Flamm SL, Di Bisceglie AM, Bodenheimer HC. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. Hepatology. 2008;47(4):1363-70.
19. Panteghini M. Aspartate aminotransferase isoenzymes. Clinical biochemistry. 1990;23(4):311-9.
20. Sharma U, Pal D, Prasad R. Alkaline phosphatase: an overview. Indian J Clin Biochem. 2014;29(3):269-78.
21. Hamoud AR, Weaver L, Stec DE, Hinds TD. Bilirubin in the liver–gut signaling axis. Trends Endocrinol Metabol. 2018;29(3):140-50.
22. Vuppalanchi R, Juluri R, Bell LN, Ghabril M, Kamendulis L, Klaunig JE, et al. Oxidative stress in chronic liver disease: relationship between peripheral and hepatic measurements. Am J Med Sci. 2011;342(4):314-7.
23. Di Naso FC, Simões Dias A, Porawski M, Marroni NA. Exogenous superoxidedismutase: action on liver

oxidative stress in animals with streptozotocin-induced diabetes. Exp Diabetes Res. 2011;754132.
24. Courtay C, Oster T, Michelet F, Visvikis A, Diederich M, Wellman M et al. γ-Glutamyltransferase: Nucleotide sequence of the human pancreatic cDNA. Biochem Pharmacol. 1992;43(12):2527-33.

Cite this article as: Rajendran K, Ramaswamy R, Venkateshwarlu K, Rajendran R, Naik KS. Assessment of safety and efficacy of a dietary supplement KaraLiv™ in supporting liver health: a double-blind, parallel, placebo-controlled randomized clinical trial. Int J Basic Clin Pharmacol 2021;10:1170-81.