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Original Research Article

The Effects of Liv.52 DS Tablets on Various Liver Parameters in Non-Alcoholic Fatty Liver Disease: Preliminary Trend Identified from a Cumulative Efficacy Analysis

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Conflict of interest: Nil

Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) is a clinical condition where excess fat accumulates within the liver and progresses from hepatic steatosis to hepatic inflammatory conditions, fibrosis, and cirrhosis. It culminates in liver failure, hepatocellular carcinoma, or liver transplantation.

Objectives: We conducted a cumulative efficacy analysis on four clinical studies evaluating individuals with NAFLD post-Liv.52 DS administration.

Methods: Two of the 4 studies were controlled/comparative studies [placebo/ ursodeoxycholic acid (UDCA)], and the other two were single-arm studies. In total, 159 subjects only from Liv.52 DS arm were considered for cumulative analyses. A dose of 2 tablets twice daily was recommended across the 4 studies. Treatment duration in 3 studies was 3 months, and in 1 study it was 2 months. Improvements in clinical signs and symptoms, SGPT, SGOT, hepatomegaly via ultrasonography, NAFLD fibrosis scores (NFS), AST to Platelet Ratio Index (APRI), and fibrosis-4 (FIB-4) scores were considered for analyses. When compared with baseline values, changes in parameters at end of study (EOS) were statistically analysed using chi-square or paired t-tests. Statistical analyses were performed using Graph Pad Prism software (Version 6.07).

Results: Significant trends in symptomatic improvements, including reduced hepatomegaly, SGPT, and SGOT levels, were observed. NFS, APRI, and FIB-4 scores confirmed a fibrosis-free liver state. No serious or non-serious adverse events were reported or observed across studies.

Conclusions: Our cumulative efficacy analysis of Liv.52 DS tablet administration for NAFLD confirmed that recommended dose was well tolerated and showed beneficial improvement trends in individuals with NAFLD.

Keywords: hepatomegaly, liver fibrosis, non-alcoholic fatty liver disease, cumulative efficacy analysis

Abbreviations: Non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT).

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a clinical condition characterized by the accumulation of fat in hepatocytes without excessive alcohol consumption.[1] NAFLD ranges from non-alcoholic fatty liver (NAFL) to non-alcoholic steatohepatitis (NASH) at the more severe end of the spectrum. In NAFL, hepatic steatosis is present but without inflammation, whereas in NASH, hepatic steatosis is associated with lobular inflammation and apoptosis, potentially leading to fibrosis and cirrhosis.[2-4]

The most common major risk factors for NAFLD include central obesity, type 2 diabetes mellitus, dyslipidemia, and metabolic syndrome.[5] Disease prevalence is growing exponentially in Western and general Indian populations, with higher incidence rates among obese and diabetic individuals.[6-8]

The global prevalence of NAFLD is estimated at approximately 25%, making it the most common chronic and progressive liver disease in industrialized countries. Recent research models predicted an adult NAFLD prevalence rate of more than 30% of the total population, with approximately 20% diagnosed with NASH.[9]

Significant weight loss and lifestyle moderation are now considered effective NAFLD preventative measures. While some individuals have successfully benefited from long-term weight reduction, a need exists to develop novel pharmacological interventions for individuals with NAFLD. Although several clinical trials showed promising results by therapeutically targeting NAFLD and NASH, no approved pharmacological treatments are available.

Considering the asymptomatic nature of NAFLD (most cases) before NASH transition, associated clinical complications, and limited therapeutic options for the effective management of these conditions, Liv.52 DS tablets have been developed to treat the disease. Comprising a polyherbal formulation consisting of *Capparis spinosa*, *Cichorium intybus*, Mandura bhasma, *Solanum nigrum*, *Terminalia arjuna*, *Cassia occidentalis*, *Achillea millefolium*, *and Tamarix gallica* extracts, Liv.52 DS tablets have been subjected to repeated safety and efficacy evaluations for NAFLD management compared with ursodeoxycholic acid (UDCA)/placebo and individually. Considering this therapeutic indication's importance, the cumulative analysis investigated four clinical studies involving Liv.52 DS tablets. However, to specifically evaluate the overall effectiveness of Liv.52 DS tablets for NAFLD, only Liv.52 DS arm data were considered.

Study Aim

To assess the cumulative efficacy of Liv.52 DS tablets for NAFLD.

Materials and Methods

Four clinical studies were considered for analyses to assess the safety and efficacy of Liv.52 DS tablets **(Table 1)** in NAFLD. A twice-daily dose regimen was common in all four studies. However, three studies were conducted over 3 months, and one study over 2 months. Two studies were

comparative/controlled in nature (Liv.52 DS vs. UDCA/Liv.52 DS vs. placebo), and the remaining two were single-arm studies comprising Liv.52 DS alone. Only clinical data specifically related to the Liv.52 DS arm of studies were considered for cumulative analyses.

In total, 155 subjects received two Liv.52 DS tablets twice daily for 2–3 months were considered for cumulative efficacy analyses. Basic study details **(Table 2)**.

Predefined study outcomes were: improvement in NAFLD clinical signs and symptoms, hepatomegaly assessed by ultrasonography (USG), SGOT and SGPT levels, NAFLD fibrosis scores (NFS), aspartate aminotransferase (AST) to Platelet Ratio Index (APRI), and FIB-4 scores EOS compared with baseline. Also, we enumerated adverse events and compliance with the intervention at EOS when compared with baseline data for safety evaluations. Data were presented as the mean ± standard deviation for quantitative variables and proportions and percentages for categorical variables. Comparisons of continuous variables within groups were performed using paired t-tests. Comparisons of different categorical variables were performed using the chi-square/Fisher exact test. Statistical analyses were conducted in GraphPad Prism, Version 6.07 for Windows (GraphPad Software, San Diego, CA, USA). Significance was accepted at $p < 0.05$ for all efficacy measures.

Results

In total, 155 subjects were considered for cumulative analyses from four different clinical studies **(Table 1, Table 2).**

Table 1: **Clinical Study Information for Cumulative Analyses**

Study No.	Study investigator and site details	Year	Study Design	Duration	N
1	Dr. S.G. Maity Kothari Medical College Kolkata, India.	2009	Randomized, comparative clinical study [50]	12 weeks	N = 35 Liv.52 DS N = 19 UDCA N = 16
2	Dr. Shantanu Ghosh, Jawaharlal Nehru Medical College, Bhagalpur, Bihar, India.	2013	Open-label clinical study [51]	3 months	N = 50
3	Dr. Gontar Siregar University of Sumatera Utara, Medan, Indonesia.	2017	Open-label clinical study [52]	2 months	N = 60
4	Dr. Sharad C. Shah Breach Candy Hospital, Mumbai, India.	2018	Placebo-controlled clinical study	3 months	N = 60 Liv.52 DS N = 26 Placebo N = 26

Table 2: Basic Study Details

Number of clinical trials	4
Number of subjects	155
Age range	18–75
Gender ratio: male/female	78/77

Dose regimen	Two tablets, orally, twice daily
Treatment duration	2–3 months

Effect of Liv.52 DS tablets on SGOT and SGPT levels:

Cumulative analyses of SGPT and SGOT levels in 153 subjects revealed a significant reduction in SGPT ($p < 0.0001$) and SGOT ($p < 0.0001$) levels at EOS when compared with baseline **(Table 3a, 3b).** This is illustrated in Fig 1**.**

Table 3a: Cumulative Analysis of SGOT (AST) Levels

N	Baseline	EOS	p value
	Mean ± SD	Mean ± SD	
50	32.08 ± 4.62	29.25 ± 4.32	
19	56.74 ± 31.77	39 ± 11.47	
60	60.33 ± 13.04	54.12 ± 17.26	
24	32.88 ± 17.06	28.18 ± 10.19	
N = 153	**45.51 ± 16.62**	**37.64 ± 10.81**	p < 0.0001
Paired t test			

Table 3b. Cumulative Analysis of SGPT (ALT) Levels

N	Baseline	EOS	p value
	Mean ± SD	Mean ± SD	
50	61.12 ± 10.51	41.08 ± 7.69	
19	68.89 ± 21.25	45.56 ± 19.87	
60	70.68 ± 18.49	61.9 ± 22.7	
24	47.54 ± 24.61	38.72 ± 17.58	
N = 153	**62.06 ± 18.72**	**46.8216.96**	p < 0.0001
Paired t test			

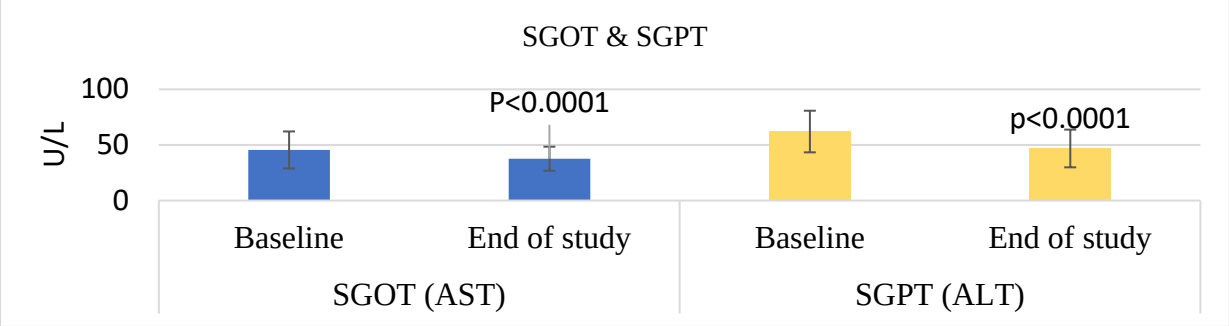

Figure 1: Demonstrates the effect of Liv.52 DS tablets on SGOT and SGPT levels. Blue bar elicits the significant reduction of SGOT levels from baseline to end of study ($p<0.0001$). Beige bar elicits the significant reduction of SGPT levels from baseline to end of the study($p<0.0001$).

Effect of Liv.52 DS tablets on Fatty Liver Grading Using Ultrasonography:

USG data for fatty liver changes were available for 90 subjects at baseline. At study entry, out of 90 subjects, 50 (55.56%) subjects had grade I, 36 (40%) had grade II, and four (0.44%) had grade III fatty liver.

Cumulative analyses of USG findings (N = 90) at EOS revealed that 34 (38%) subjects had grade 0, 54 (60%) had grade I, two (2%) had grade II, and no subjects had grade III fatty livers. These results

indicated an improving trend in grading fatty liver via USG, post-Liv. 52 DS administration **(Table 4).** These findings are depicted in Fig 2**.**

Table 4: Effect of Liv.52 DS Tablets on Fatty Liver Grading (Ultrasonography Findings)

Grade	At entry (N = 90)	At EOS (N = 90)
0	0 (0%)	34 (38%)
I	50 (55.56%)	54 (60%)
II	36 (40%)	2 (2%)
III	4 (4.44%)	0 (0%)

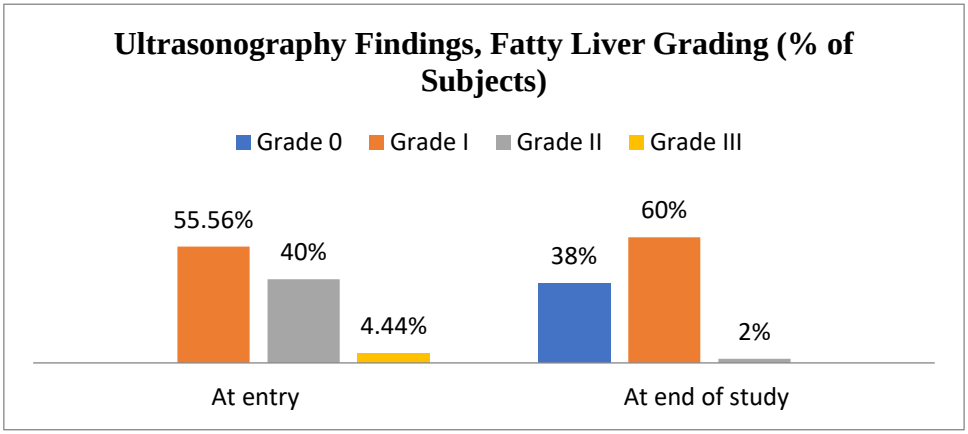

Figure 2: Demonstrates the effect of Liv.52 DS tablets on fatty liver through ultrasonography. Significant shift in the gradings of fatty liver was observed at the end of the study as compared to the baseline. Percentage of Subjects with grade III was completely nil at the end of the study as compared to the baseline 4.44%, while percentage of subjects with grade II was reduced to 2% at the end of the study as compared to 40% at the baseline. Percentage of subjects with Grade I increased from 55.56% to 60% at the end of the study. Total 38% of subjects achieved Grade 0 at the end of the study.

Effect of Liv.52 DS tablet on Hepatomegaly (USG):

At entry (N=150), cumulative analyses showed that 135 (90%) subjects had hepatomegaly (>16cm) and 15 (10%) had no hepatomegaly (<16cm)**.** After treatment with Liv.52 DS, 58 (38.66%) subjects had no hepatomegaly (<16cm) ($p < 0.0001$).The number of subjects with hepatomegaly (>16cms) reduced from 135 (90%) to 92 (61.33%) at EOS **(Table 5).** This is illustrated in Fig 3**.**

Table 5: Effect of Liv.52 DS Tablets on Hepatomegaly, N (%)

	Baseline (N = 150)	EOS (N = 150)	
	No. of subjects (%)	No. of subjects (%)	p value
No hepatomegaly	15 (10%)	58 (38.66%)	$p < 0.0001$
Hepatomegaly	135 (90%)	92 (61.33%)	

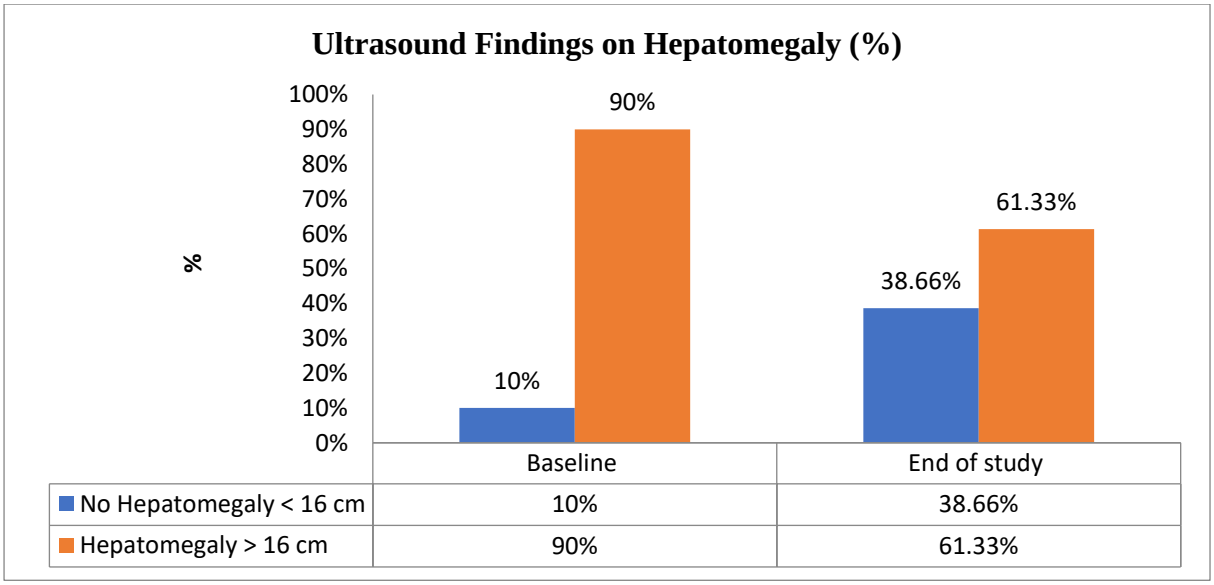

Figure 3: Demonstrates the effect of Liv.52 DS tablets on hepatomegaly based on ultrasonography findings. At baseline, 90% of the subjects had hepatomegaly and remaining 10% did not have hepatomegaly at the baseline. However, at the end of the study, there were 38.66% of subjects with no hepatomegaly and remaining 61.33% of subjects did had hepatomegaly.

Effect of Liv.52 DS tablets on Symptomatic Assessments:

Abdominal discomfort due to hepatomegaly

At screening, 76 subjects had abdominal discomfort due to hepatomegaly. At EOS, 29 (38%) subjects had these symptoms, while the remaining 47 (62%) did not (p<0.0001) **(Table 6).**

Table 6: Effect of Liv.52 DS Tablets on Symptomatic Assessments

Abdominal discomfort due to hepatomegaly	**Screening** (N = 76)	**EOS** (N = 76)	**p value**
Absent	0 (0%)	47 (62%)	p < 0.0001
Present	76 (100%)	29 (38%)	
Fatigue	**Screening** (N = 53)	**EOS** (N = 53)	**p Value**
Absent	0 (0%)	40 (75%)	p < 0.0001
Present	53 (100%)	13 (25%)	
Weakness	**Screening** (N = 54)	**EOS** (N = 54)	**p Value**
Absent	0 (0%)	34 (63%)	p < 0.0001
Present	54 (100%)	20 (37%)	
Fisher's exact test. Significance < 0.05			

Fatigue

At screening, as per cumulative analyses, fatigue was present in 53 subjects, while at EOS, 13 (25%) subjects had fatigue, while the remaining 40 (75%) did not (p<0.0001) **(Table 6).**

Weakness

At screening, "weakness" was identified in 54 subjects, while at EOS, 20 (37%) subjects had this symptom, and the remaining 34 (63%) did not (p<0.0001) **(Table 6).**

Effect of Liv.52 DS on Liver Fibrosis-NAFLD Fibrosis Score (NFS)

As per cumulative data, NAFLD fibrosis scores were available for 136 subjects. At baseline, 77 subjects had a NAFLD score which indicated the absence of fibrosis (F0–F2). However, 59 subjects with indeterminate NAFLD scores suggested no advanced fibrosis (F3–F4) at study entry. At EOS, the NAFLD score in 77 subjects remained unchanged compared with baseline NAFLD scores, suggesting no progression to fibrosis. However, 59 subjects with indeterminate NAFLD scores at baseline had slight improvements toward normal. No subjects had NAFLD scores indicating advanced fibrotic liver changes at EOS. **(Table 7)**

Table 7: Effect of Liv.52 DS on Liver Fibrosis - NAFLD Fibrosis Scores (NFS) (N=136)

Scale		**Baseline (N = 77)**	**EOS (N = 77)**
NAFLD Fibrosis score < −1.455 = F0–F2	Mean	−2.938	−2.9055
	SD	1.1675	1.1919
		Baseline (N = 59)	**EOS (N = 59)**
NAFLD Fibrosis score −1.455–0.675 = indeterminate score	Mean	−0.815	−1.0345
	SD	0.437	0.5385
NAFLD Fibrosis score > 0.675 = F3–F4		0	0
Paired t test, values are the mean ± SD			
NAFLD Fibrosis Score (NFS): −1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m^2) + 1.13 × IFG/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio − 0.013 × platelet (×10^9/l) − 0.66 × albumin (g/dl).			

Effect of Liv.52 DS on APRI and FIB-4 scores

Data related to APRI scores were available for 134 subjects. An APRI score < 0.7 indicated a fibrosis-free liver state. At baseline, this score was 0.38, and at EOS, it was 0.34. The FIB-4 score at baseline was 0.94, and at EOS, it was 0.95. These scores indicated no fibrosis progression in the liver. **(Table 8)**

Table 8: AST to Platelet Ratio Index (APRI) and Fibrosis-4 (FIB-4) Scores (N = 134)

Score	**Baseline**	**EOS**
APRI score	0.38*	0.34*
FIB-4 Index	0.94$^{\#}$	0.95$^{\#}$
*scores < 0.7 were not sensitive or specific enough to determine fibrosis or cirrhosis levels (Paul *et al.* 2020)		
$^{\#}$score <1.45, subjects do not have advanced fibrosis, but might have approximate fibrosis stage: 0-1, based on Ishak fibrosis staging (Sterling *et al* 2006). FIB-4 = Age × AST (U/L)/Platelet count (10^9/L) × square root of ALT (U/L)		

Discussion and Conclusions

Understanding NAFLD/NASH pathogenesis is essential for the establishment of proper and correct therapeutic disease interventions. However, disease development is highly complex and has been designated “multiple-hit theory”. [10]

Steatogenesis is represented by excessive triacylglycerol (TAG) accumulation and the disrupted conversion of TAG into Very Low-Density Lipoprotein, ultimately leading to hepatosteatosis.[11] Approximately 60% of fatty acids leading to TAG formation originate from white adipose tissue [12]; thus, adipocyte dysfunction may lead to fatty acid overflow and NAFLD/NASH progression. While TAG accumulation in hepatocytes does not cause hepatocellular damage, TAG precursors and intermediates such as palmitate, diacylglycerol, and ceramide are likely detrimental to these cells, causing hepatocellular damage. Palmitate increases oxidative and endoplasmic reticulum stress leading to c-Jun N-terminal kinase activation and lipoapoptosis. [13-15] Increased free cholesterol and persistent exposure to organic pollutants also causes mitochondrial dysfunction, metabolic disorders, altered hepatic lipogenesis and inflammasome activation. [16] Cytotoxic states mediated by these specific lipids (i.e., lipotoxicity) are another major cause of hepatocellular injury, facilitating NAFLD progression to NASH.

Damaged hepatocytes release several pro-inflammatory mediators, including damage-associated molecular patterns and pathogen-associated molecular patterns to recruit immune cells and activate Kupffer cells. Activated immune cells release bioactive molecules that further damage hepatocytes or render them more sensitive to various substances such as microbiome-derived lipopolysaccharides, secondary bile acids, and gut-derived food contaminants, thus amplifying cell death and inflammation [17-18]. Damaged hepatocytes and activated immune cells also promote hepatic stellate cell (HSC) activation lobules [19]. Although HSC activation is a key event during liver fibrogenesis regardless of etiology, fibrogenesis occurring mainly in the perisinusoidal space is relatively specific to steatohepatitis [20-22].

Although therapeutic options for NAFLD include weight reduction in the obese, good glucose control in diabetics, and more exercise in general, preventing fibrosis should be the core objective for NAFLD treatment as it may culminate in cirrhosis and portal hypertension if untreated. Several large clinical trials using a variety of agents are currently underway and should provide additional treatment options for those with NASH [23-24].

In terms of NAFLD pathogenesis and therapeutic limitations, the polyherbal Liv.52 DS tablet formulation was designed to manage hepatic disorders, with a wide spectrum of therapeutic applications to correct hepatic dysfunction. Eight active medicinal herbs included *C. spinosa [25-28], C. intybus[29-33], S. nigrum[34-36], T. arjuna [37-42],C. occidentalis [43-44], A. millefolium[45], T. gallica [46],* and Mandura bhasma[47-48], with carefully selected hepato-protective pharmacological properties during product development.

Based on this cumulative efficacy analysis of Liv.52 DS tablets in NAFLD, the oral administration of these tablets at recommended doses provided significant symptomatic improvements and SGPT and SGOT reductions. This may have been due to the suppressed oxidative degradation of DNA in tissue debris, enhanced phagocytic activity, natural killer cell activity, cell proliferation, and/or free radical scavenging effects.

A significant reduction in patients with hepatomegaly also occurred, which may have been due to enhanced antioxidant activity by inhibiting nitric oxide (NO) production and decreasing inducible NO synthase levels in lipopolysaccharide-stimulated peritoneal macrophages.

Few non-invasive methods (NFS, APRI, and FIB-4 scores assessing fibrotic changes in the liver) revealed a liver fibrosis-free state at EOS. This may have

been due to enhanced hepatocellular repair and reduced hepatocellular damage via potent antioxidant and hepato-protective herb activities. The patient group had predominantly grade I–II fatty livers; biopsies could not be performed, so fibrosis scoring systems were used.

We observed no clinically significant adverse events, neither reported nor observed, in all studies.

Improved hepatic laboratory parameters and symptomatic improvements were very encouraging. However, study duration (2–3 months) was too brief to substantiate the long-term hepato-protective activities of Liv.52 DS for NAFLD. Additionally, as per recent AASLD guidelines [49], histologically proven NASH for diagnosis and further assessment were lacking in these studies. However, preliminary improvement trends in hepatic parameters were encouraging. In the absence of approved therapeutic interventions, the preliminary trends observed here provide invaluable insights for NAFLD treatment. Similarly, the absence of any safety concern during 3 months of intervention was also encouraging sign. In the future, the investigation of Liv.52 DS in randomized, placebo-controlled, long-term prospective studies in large number of subjects, as per prevailing guidelines, is warranted.

Based on our clinical evidence, the oral administration of Liv.52 DS tablets at the recommended dose may elicit beneficial improvements in individuals with NAFLD.

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Statement of Ethics:

All the studies considered in these cumulative analyses were conducted ethically in accordance with the World Medical Association Declaration of Helsinki. Among the 4 clinical studies considered for this study, three of the studies are published and only one is not published. This unpublished study was apprised to ethics committee of Breach Candy Medical Research Centre, Mumbai. Informed consent was obtained from all the study participants and was documented as appropriate.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Authors Contribution:

Prof. (Dr). Sharad C. Shah, Dr. Gontar Siregar are the principal investigators for respective clinical studies and responsible for review of cumulative analyses. Dr. Prasanna Shah and Dr. Nusly P J Pocha being subject matter expert contributed in the review of manuscript.

Dr. Srikrishna H A is responsible for preparation/drafting of the manuscript. Dr. Rajesh Kumawat contributed in the review and finalization of manuscript.

Data Availability Statement:

All the published articles of the three studies are available on the corresponding journal Homepage under Archival section. The data that support the findings of these studies are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

References:

1. Aijaz Ahmed, Robert.J.Wong, Stephen A. Harrison. Non-alcoholic Fatty Liver Disease Review: Diagnosis, Treatment, and Outcomes. *Clin Gastroenterol Hepatol* 2015; 13:2062.
2. Mariana Verdelho Machado, Anna Mae Diehl. Pathogenesis of Non-alcoholic Steatohepatitis. *Gastroenterology* 2016; 150:1769.
3. Patrik Nasr, Simone Ignatova, Stergios Kechagias, Mattias Ekstedt. Natural history of non-alcoholic fatty liver disease: A prospective follow-up study with serial biopsies. *Hepatol Commun* 2018; 2:199.
4. Zobair Younossi, Quentin M Anste, Milena Marietti, Timothy Hardy, Linda Henry, Mohammed Eslam, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 2018; 15:11
5. Zobair M Younossi, Maria Stepanova, Mariam Afendy, Yun fang, Youssef Younossi, Hesham Mir, Manirath shishord. Changes in the prevalence of the most common causes of chronic liver diseases in the United States from 1988 to 2008. *Clin Gastroenterol Hepatol* 2011; 9:524.
6. Christopher D Williams, Joel Stengel, Michael I. Asike, Dawn M. Torres, Janet Shaw, Maricela Contreras, Cristy L. Landt, and Stephen A. Harrison. Prevalence of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology* 2011; 140:124-131.
7. Vernon G, A. Baranova, Z.M. Younossi, Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther* 2011; 34:274.
8. Mariana Lazo, Ruben Hernaez, Mark S Eberhardt, Susanne bonekmp, Ihab Kamel, Eliseo Guallar, *et al*. Prevalence of non-alcoholic fatty liver disease in the United States: the Third National Health and Nutrition Examination Survey, 1988-1994. *Am J Epidemiol* 2013; 178:38.
9. Elke Roeb, Non-alcoholic fatty liver diseases: current challenges and future directions, *Ann Transl Med* 2021;9(8):726.
10. Naoki Tanaka, Toshifumi Aoyoma, Shioko Kimura, Frank J Gonzalez. Targeting nuclear receptors for the treatment of fatty liver disease. Pharmacol Ther 2017; 179: 142-157.
11. Kerry L Donnelly, Coleman I Smith, Sarah J Schwarzenberg, Jose Jessurun, Mark D Boldt, Elizabeth J Parks. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with non-alcoholic fatty liver disease. *J Clin Invest* 2005; 115: 1343-1351
12. Naoki Tanaka, Shogo Takahashi, Yuan Zhang, Kristopher W Krausz, Philip B Smith, Andrew D Patterson *et al*. Role of Fibroblast growth factor 21 in the early stage of NASH induced by methionine- and choline deficient diet. *Biochim Biophys Acta* 2015; 1852: 1242-1252
13. Naoki Tanaka, Shogo Takahashi, Xiao hu, Yu Lu, Naoyuki Fujimori, Srujana Golla *et al*. Growth arrest and DNA damage-inducible 45α protects against non-alcoholic steatohepatitis induced by methionine- and choline-deficient diet. *Biochim Biophys Acta Mol Basis Dis* 2017; 1863: 3170-3182
14. Yuko Akazawa, Kazuhiko Nakao. To die or not to die: death signaling in non-alcoholic fatty liver disease. *J Gastroenterol* 2018; 53: 893-906

15. Lay T Gan, Derrick M Van Rooyen, Mark E Koina, Robert S McCuskey, Narcissus C Teoh, Geoffrey C Farrell. Hepatocyte free cholesterol lipotoxicity results from JNK1-mediated mitochondrial injury and is HMGB1 and TLR4-dependent. *J Hepatol* 2014; 61: 1376-1384

16. Limin Zhang, Robert G Nichols, Jared Correll, Iain A Murray, Naoki Tanaka, Philip B Smith *et al.* Persistent Organic Pollutants Modify Gut Microbiota-Host Metabolic Homeostasis in Mice Through Aryl Hydrocarbon Receptor Activation. *Environ Health Perspect* 2015; 123: 679-688

17. Huikuan Chu, Yi Duan, Ling Yang, Bernd Schnabl. Small metabolites, possible big changes: a microbiota centered view of non- alcoholic fatty liver disease. *Gut* 2019 Feb;68(2):359-370.

18. Youngmin A Lee, Michael C Wallace, Scott L Friedman. Pathobiology of liver fibrosis: a translational success story. *Gut* 2015; 64: 830- 841

19. Carlo Saitta, Gianluca Tripodi, Adalberto Barbera, Antonio Bertuccio, Antonina Smedile, Alessia Ciancio, *et al*. Hepatitis B virus (HBV) DNA integration in patients with occult HBV infection and hepatocellular carcinoma. *Liver Int* 2015; 35: 2311-2317

20. Kohichiroh Yasui, Etsuko Hashimoto, Yasuji Komorizono, Kazuhiko Koike, Shigeki Arii, Yasuharu Imai *et al*, Japan NASH Study Group, Ministry of Health, Labour, and Welfare of Japan NASH Study Group, Ministry of Health, Labour, and Welfare of Japan. Characteristics of patients with non-alcoholic steatohepatitis who develop hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 2011; 9: 428-433; quiz e50

21. Carrie R Wong, Mindie H Nguyen, Joseph K Lim, Hepatocellular carcinoma in patients with non-alcoholic fatty liver disease. *World J Gastroenterol* 2016; 22: 8294-8303

22. Ramy Younes, Elisabetta Bugianesi, Should we undertake surveillance for HCC in patients with NAFLD? *J Hepatol* 2018; 68: 326-334

23. Das K, P Kar. Non-Alcoholic Steatohepatitis; JAPI; 2005:53: 195-199.

24. Kerkar Nanda, Non-alcoholic steatohepatitis in children. *Pediatr Transplantation* 2004; 8: 613-618.

25. Gadgoli C, S H Mishra *et al*, Antihepatotoxic activity of p-methoxy benzoic acid from *Capparis spinosa*. *J Ethnopharmacol* 1999; 66(2): 187–192.

26. Mona M Mantawy, Manal A Hamed, Elham M Sammour, Mahmoud Sanad. Influence of *Capparis spinosa* and *Acacia arabica* on certain biochemical haemolymph parameters of Biomphalaria alexandrina. *J Egyptian Society Parasitol* 2004; 34 (2): 659–677

27. H Fallah Huseini, S M Alavian, R Heshmat, M R Heydari, K Abolmaali. The efficacy of Liv.52 on liver cirrhotic patients: A randomized, double- blind, placebo-controlled first approach. *Phytomedicine* 2005;12 (9): 619–624

28. Maria Paola Germanò, Rita De Pasquale, Valeria D'Angelo, Stefania Catania, Virginia Silvari, Chiara Costa. Evaluation of extracts and isolated fraction from *Capparis spinosa* L. buds as an antioxidant source. *J Agri Food Chem* 2002; 50(5): 1168–1171

29. Z Amirghofran, M Azadbakht, M H Karimi. Evaluation of the immunomodulatory effects of five herbal plants. *J Ethnopharmacol* 2000; 72(1–2): 167–172.

30. Joung-Hoon Kim, Yeun-Ja Mun, Won-Hong Woo, Kyung-Soo Jeon,

Nyeon-Hyoung An, Joung-Suk Park, Effects of the ethanol extract of *Cichorium intybus* on the immunotoxicity by ethanol in mice. *Intl Immunol* 2002; 2(6): 733–744.

31. G Gazzani, M Daglia, A Papetti, C Gregotti. *In vitro* and *ex vivo* anti- and prooxidant components of *Cichorium intybus*. *J Pharmaceut Biomed Analysis* 2000; 23(1): 127-133.

32. Rafael Llorach, Francisco A Tomás-Barberán, Federico Ferreres. Lettuce and chicory by products as a source of antioxidant phenolic extracts. *J Agri Food Chem*. 2004; 52(16): 5109–5116.

33. Sedef Nehir El, Sibel Karakaya. Radical scavenging and iron-chelating activities of some greens used as traditional dishes in Mediterranean diet. *Intl J Food Sci Nutrition* 2004; 55(1): 67–74.

34. Y-O Son, J Kim, J-C Lim, Y Chung, G-H Chung, J-C Lee. Ripe fruit of *Solanum nigrum* L. inhibits cell growth and induces apoptosis in MCF-7 cells. *Food Chem Toxicol* 2003; 41(10): 1421–1428

35. Kyung-Sun Heo, Kye-Taek Lim. Antioxidative effects of glycoprotein isolated from *Solanum nigrum* L. *J Med Food2004*; 7(3): 349–357.

36. V Prashanth Kumar, S Shashidhara, M M Kumar, B Y Sridhara. Cytoprotective role of *Solanum nigrum* against gentamicin-induced kidney cell (Vero cells) damage *in vitro*. Fitoterapia2001; 72(5): 481–486.

37. Ram A, P Lauria, R Gupta, P Kumar, V N Sharma. Hypocholesterolaemic effects of *Terminalia arjuna* tree bark. *J Ethnopharmacol* 1997; 55(3): 165–169.

38. Prasenjit Manna, Mahua Sinha, Parames C Sil. Aqueous extract of *Terminalia arjuna* prevents carbon tetrachloride induced hepatic and renal disorders. *BMC Complement Alternative Med* 2006;6: 33.

39. T C J Munasinghe, C K Seneviratne, M I Thabrew, A M Abeysekera. Antiradical and antilipoperoxidative effects of some plant extracts used by Sri Lankan traditional medical practitioners for cardioprotection. *Phytotherapy Res* 2001; 15(6): 519–523

40. Ali A, G Kaur, K Hayat, M Ali, M Ather. A novel naphthanol glycoside from *Terminalia arjuna* with antioxidant and nitric oxide inhibitory activities. *Pharmazie* 2003; 58(12): 932–934.

41. Hua-Yew Cheng, Chun-Ching Lin, Ta-Chen Lin, Antiherpes simplex virus type 2 activity of casuarinin from the bark of *Terminalia arjuna* Linn. *Antiviral Res* 2002; 55(3): 447–455.

42. R Perumal Samy, S Ignacimuthu, A Sen. Screening of 34 Indian medicinal plants for antibacterial properties. *J Ethnopharmacol* 1998; 62(2): 173–182.

43. B Bin-Hafeez, I Ahmad, R Haque, S Raisuddin. Protective effect of *Cassia occidentalis* L. on cyclophosphamide-induced suppression of humoral immunity in mice. *J Ethnopharmacol* 2001; 75(1): 13–18.

44. M A Jafri, M Jalis Subhani, K Javed, S Singh, Hepatoprotective activity of leaves of *Cassia occidentalis* against paracetamol and ethyl alcohol intoxication in rats. *J Ethnopharmacol* 1999; 66(3): 355–361.

45. Sheikh Yaeesh, Qamar Jamal, Arif-Ullah Khan, Anwarul Hassan Gilani. Studies on hepatoprotective, antispasmodic, and calcium antagonist activities of the aqueous-methanol extract of *Achillea millefolium*. *Phytotherapy Res* 2006; 20(7): 546–551.

46. Anuradha Sehrawat, Sarwat Sultana, *Tamarix gallica* ameliorates thioacetamide-induced hepatic oxidative stress and hyperproliferative response in Wistar rats. *J Enzyme Inhibition Med Chem* 2006; 21(2): 215–223.

47. A Kanase, S Patil, B Thorat. Curative effects of Mandura Bhasma on liver and kidney of albino rats after induction of acute hepatitis by CCl4. *Indian J ExpBiol* 1997; 35(7): 754–764.

48. Mandura bhasma: The Ayurvedic Formulary of India, Volume-I, Ministry of Health and Family Welfare, Department of ISM & H, Government of India: 613-615

49. Naga Chalasani, Zobair Younossi, Joel E Lavine, Michael Charlton, Kenneth Cusi, Mary Rinella *et al*. The Diagnosis and Management of Non-alcoholic Fatty Liver Disease: Practice Guidance from American Association for the Study of Liver Diseases. Hepatology, Volume. 67, NO. 1, 2018.

50. Maity SG and MandalAK. A Clinical Comparative Study to Evaluate the Efficacy and Safety of Liv.52 DS Tablets in Non Alcoholic Steatohepatitis (NASH)11.World Journal of Pharmaceutical Research. 2015, Volume 4, Issue 7, 398-414.

51. Shantanu Ghosh, Singh TB, Mandal KD, Suresh Kumar. Evaluation of safety and efficacy of a Polyherbal formulation Liv.52 DS in the Management of Non-Alcoholic Steatohepatitis (NASH): An open clinical study. International Journal Current Research and Academic Review 2014; 2(9):305-316.

52. Goantar Siregar, Rangesh Paramesh, Rajesh Kumawat, Palaniyamma D, Srikrishna H A. A prospective interventional clinical study to evaluate the safety and efficacy of Liv.52 DS in the management of non-alcoholic fatty liver disease. Eur J Clin Exp Med 2021; 19(2):129-136