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Dietary supplement based on dihydromyricetin in metabolic dysfunction-associated steatotic liver disease: a double-blind, placebo-controlled, randomized clinical trial

Elisavet Michailidou[a], Stavros P. Papadakos[a], Eleni Koukoulioti[b], Sofia Paraskevopoulou[a], Maria Mela[c], Paraskevi Fytili[a], Panagiota Ioannidou[a], Evangelos Cholongitas[a], Konstantinos Triantafyllou[b], George V. Papatheodoridis[a]

Medical School, National and Kapodistrian University of Athens, General Hospital of Athens "Laiko"; Medical School, National and Kapodistrian University of Athens, University General Hospital of Athens "Attikon"; General Hospital of Athens "Evangelismos", Athens, Greece

Abstract

Background Despite its increasing prevalence, effective treatment options for metabolic dysfunction-associated steatotic liver disease (MASLD) are limited. We assessed the efficacy of a dietary supplement containing dihydromyricetin (DHM) in MASLD.

Methods Adult MASLD patients were randomized to receive a dietary supplement containing DHM (300 mg/day), vitamins C/E and choline (group A), or identical placebo (group B) for 12 months. Patients were assessed every ≤6 months for clinical and laboratory parameters and liver stiffness measurements (LSM).

Results Fifty-five patients were randomized to group A (n=28) or B (n=27), but 9 patients (group A/B=2/7) were withdrawn early for personal reasons. Median liver enzymes decreased at 6 or 12 months only in group A. Group A compared to B patients achieved higher 12-month rates of combined alanine aminotransferase (ALT)/γ-glutamyl transpeptidase (GGT) normalization (35% vs. 5%, P=0.028). Only in group A, glucose, glycated hemoglobin and total/low density lipoprotein mean levels had declined significantly at 6 and/or 12 months, whereas median liver stiffness measurements (LSM) were lower than baseline at both 6 and 12 months. In multivariate analysis, group A was the only factor associated with ALT/GGT normalization (P=0.038). Generalized estimating equation analysis revealed a significant treatment by time interaction for 12-month combined ALT/GGT normalization only in group A (P=0.021).

Conclusions The 6/12-month use of DHM supplement seems to result in improvements in liver enzymes and LSM, as well as in diabetes and lipid parameters in MASLD patients. Therefore, the use of such a supplement in MASLD needs further evaluation.

Keywords Metabolic dysfunction-associated steatotic liver disease, flavonoids, insulin resistance, elastography

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Correspondence to: Prof. George V. Papatheodoridis, MD, PhD, Director, 1st Department of Gastroenterology, Medical School of National and Kapodistrian University of Athens, General Hospital of Athens "Laiko", 17 Agiou Thoma Street, 11527 Athens, Greece, e-mail: gepapath@med.uoa.gr

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Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD), which is the new term for nonalcoholic fatty liver disease, represents the most common chronic liver disease globally, and is a major cause of morbidity and mortality in the developed world. In a recent meta-analysis, 38% of adults worldwide were reported to have MASLD, with the prevalence being highest in Latin America (44%) and lowest in Western Europe (25%) [1]. These rates are expected to increase, and the global prevalence of MASLD is estimated to reach 55% in adults by 2040 [2], in parallel with the increasing prevalence

rates of obesity and type 2 diabetes [3], although MASLD may also develop in individuals with normal weight (5%) (lean MASLD) [4].

MASLD is defined by the presence of steatosis in >5% of hepatocytes, diagnosed by imaging or histology, in combination with a metabolic risk factor, such as obesity/overweight, type 2 diabetes, hypertension, high triglycerides and low high-density lipoprotein (HDL) levels [5]. It covers a spectrum from simple steatosis to metabolic dysfunction-associated steatohepatitis (MASH) and cirrhosis, whereas hepatocellular carcinoma (HCC) may develop in MASLD patients with or even without cirrhosis [6].

The cornerstone of the management of MASLD/MASH has been weight loss and life-style modifications. In the presence of comorbidities, such as diabetes, specific agents, such as a glucagon-like peptide receptor agonist (GLP1), may be preferred, whereas a MASH-targeted agent, resmetirom (a thyroid hormone receptor-β agonist), has recently been approved by the Food and Drug Administration in the USA, but it is not available in most countries [7]. Given the difficulties inherent in weight loss and behavioral changes, and the limited therapeutic options, alternative approaches for the management of MASLD are eagerly needed.

Polyphenols are a group of naturally occurring compounds from plants, known for their antioxidant and anti-inflammatory properties, which have been evaluated for their potential health benefits in several chronic diseases, including cardiovascular disease, cancers and liver diseases such as MASLD [8]. Dihydromyricetin (DHM) is the most abundant flavonoid in *Ampelopsis grossedentata*, a plant native to China known for its traditional use in herbal medicine. In MASLD, DHM has been reported to reduce body weight, regulate cholesterol and triglyceride levels, improve insulin resistance, protect mitochondrial function by inhibiting oxidative stress [9]. In addition, DHM has been shown to have anti-inflammatory effects and to inhibit the activation of hepatic stellate cells, thus improving liver inflammation, steatosis and fibrosis [9].

Based on the existing data, we aimed to assess the efficacy of a dietary supplement enriched with natural explant titrated with DHM, choline, and vitamins C and E, in patients with MASLD.

[a]1st Department of Gastroenterology, Medical School, National and Kapodistrian University of Athens, General Hospital of Athens "Laiko" (Elisavet Michailidou, Stavros P. Papadakos, Sofia Paraskevopoulou, Paraskevi Fytili, Panagiota Ioannidou, Evangelos Cholongitas, George V. Papatheodoridis); [b]2nd Department of Gastroenterology, Medical School, National and Kapodistrian University of Athens, University General Hospital "Attikon" (Eleni Koukoulioti, Konstantinos Triantafyllou); [c]Gastroenterology Department, General Hospital of Athens "Evangelismos" (Maria Mela), Athens, Greece

Conflict of Interest: George V. Papatheodoridis has served as advisor for AbbVie, Astra Zeneca, Genesis, Gilead, GlaxoSmithKline, Janssen, Ipsen, Merck Sharp & Dohme, Novartis, Novo Nordisk, Roche, Takeda and Vir, as lecturer for AbbVie, Astra Zeneca, Gilead, GlaxoSmithKline, Janssen, Ipsen, Merck Sharp & Dohme, Novartis, Novo Nordisk and Roche and has received research grants from AbbVie, Gilead, Takeda, Vianex. The other authors declare no conflicts of interest

Patients and methods

Patient population

This study prospectively included adult patients (18-70 years old) with MASLD seen at the outpatient liver clinics of the 2 participating hospitals. Main inclusion criteria were alanine aminotransferase (ALT) and/or γ-glutamyl transpeptidase (GGT) levels greater than the upper limit of normal (ULN), presence of hepatic steatosis by either ultrasonography or histology, and absence of any other cause of hepatic steatosis or liver injury. In addition, patients had to have a body mass index (BMI) in the range 20-45 kg/m^2. Individuals with detectable hepatitis B virus (HBV) surface antigen (HBsAg) or antibodies against hepatitis C virus (anti-HCV) or against human immunodeficiency virus (anti-HIV), excessive alcohol consumption (>210 g per week in men or >140 g per week in women), use of any potentially hepatotoxic agent, or presence of systemic disease with potential hepatic involvement, were excluded. The study protocol was reviewed and approved by the Ethics Committees of the participating hospitals. All participants provided written informed consent prior to enrollment. The trial was registered at ClinicalTrials.gov (Identifier: NCT05052515) and conducted in accordance with the Declaration of Helsinki.

Study design

This was a double-blind, placebo controlled, randomized clinical trial that lasted 12 months. After enrollment, patients randomly received a 5-digit code assigning them to the DHM supplement or identical placebo. Both investigators and patients were blinded to the randomization and were unblinded after the end of the study.

The natural extract with DHM used in this study is a dietary supplement available on the market with the brand name Hepatrat® (Uni-Pharma, Athens, Greece). Every single tablet contains 80 mg of vitamin C, 12 mg of vitamin E, 82.5 mg of choline and 150 mg of DHM. The placebo tablets contained microcrystalline cellulose, sorbitol, pregelatinized starch, silicon dioxide colloidal and magnesium stearate; they were identical to the DHM tablets and identically packaged. All patients received 2 tablets b.i.d. for 12 months; thus, in the patients assigned to DHM supplement the total daily dose of DHM was 600 mg. Agent supplies for 6 months were given to the patients at the visits of 0 and 6 months.

Clinical and laboratory assessments

At the initial assessment, all patients underwent a complete clinical examination. Epidemiological characteristics, habits such as smoking and alcohol consumption, comorbidities and comedications were recorded in a predefined case report form. The alcohol consumption history was recorded by patients and friends and/or relatives where possible. The baseline

assessment of the participants also included measurements of their anthropometric characteristics (height, weight, waist circumference). The participants' BMI was calculated using the formula: weight in kilograms divided by (height in meters)^2. Clinical examination, assessment of habits, comorbidities and comedications use, and measurements of anthropometric characteristics were repeated at 6 and 12 months.

At the onset of the study, as well as at 6 and 12 months, all patients were screened with 8-hour fasting laboratory tests, including full blood count, prothrombin time, ALT and aspartate aminotransferase (AST), alkaline phosphatase, GGT, bilirubin (total, direct), total protein, albumin, cholesterol (total, HDL, low density lipoprotein [LDL]), triglycerides, glucose, insulin, glycated hemoglobin (HbA1c), urea, creatinine, uric acid and C-reactive protein (CRP). In addition, all patients were tested for HBV markers (HBsAg, anti-HBc, anti-HBs), anti-HCV, anti-HIV, thyroid-stimulating hormone, ferritin, transferrin saturation and α-fetoprotein at baseline.

Liver stiffness measurements (LSM)

At baseline, and at 6 and 12 months, all patients underwent LSM under fasting conditions, both by transient elastography (TE) including controlled attenuation parameter (CAP™) using a Fibroscan® 530 compact device equipped with M and XL probes (Echosens, Paris, France), and by 2D-shear-wave elastography (2D-SWE) using an Aixplorer® ultrasound system (Super Sonic Imagine S.A., Aix-en-Provence, France) with a SC6-1 convex probe; results were expressed in kPa. All examinations were performed by an experienced user who had previously completed >500 LSM by TE and 2D-SWE.

For TE, 10 valid LSM were carried out in each patient in the supine position via the intercostal approach, with the right arm in maximum abduction. Only successful LSM, defined by an interquartile range (IQR)/median value ratio <0.3, were recorded. For 2D-SWE, the examinations were conducted on the right lobe of the liver, through intercostal spaces, with the patient lying in the dorsal decubitus position and the right arm in maximal abduction. A liver portion ≥6-cm thick, free of large vascular structures, and at 15-mm depth below the liver capsule, was targeted using real-time B-mode ultrasonography. Only successful LSM were recorded, as defined by a region of interest of 15 mm diameter with complete and homogeneous filling, while the patient was in a state of complete apnea for 3 seconds. The median value of 10 valid measurements was selected only from results with IQR/median value ratio <0.3.

Endpoints

The primary endpoint was clinically beneficial improvement of ALT assessed at 12 months, and defined as either ALT normalization or ALT reduction >50% from the baseline values. Secondary endpoints included normalization of ALT at 6 or 12 months, normalization of GGT at 6 and 12 months, combined normalization of ALT and GGT levels at 6 or 12 months and improvement of liver stiffness at 6 or 12 months.

Sample size estimation

Based on the assumption that the primary endpoint might be achieved in 50% of patients randomized to the DHM group and 10% of patients randomized to the placebo group, it was estimated that 22 patients per treatment group would be required to demonstrate a statistically significant difference from the control group (with a 5% and 10% probability of type I and type II statistical errors, respectively). Additionally, allowing for a 10% loss to follow up, it was estimated that at least 25 patients should be randomized to each group.

Statistical analysis

All data were entered into a specifically designed SPSS database. Statistical analyses were performed using the SPSS software package (IBM® SPSS® Statistics 29.0, SPSS Inc, IBM, Chicago, IL, USA). Continuous variables were expressed as mean values ± standard deviation (SD) or median values (IQR) if they followed normal or non-normal distribution, respectively. The Student's *t*- or Mann-Whitney *U* test was used for comparisons of normally or non-normally distributed variables between groups, respectively. A paired *t*-test or Wilcoxon matched-paired signed test was used for the assessment of changes of continuous variables with normal or non-normal distribution over time, respectively. Categorical variables were presented as frequencies (percentages). The corrected chi-squared or 2-sided Fisher's exact test was used to test for association between 2 categorical variables. Intention-to-treat (ITT) and per protocol (PP) analyses of efficacy were performed. Univariate and multivariate logistic regression analyses were used to determine associations of independent variables with study endpoints, and their results were expressed as odds ratio (OR) and 95% confidence interval (CI). To appropriately handle repeated measurements (baseline, 6, 12 months), generalized estimating equations (GEE) with treatment-by-time interaction were used to assess the independent effect of DHM supplementation on biochemical and metabolic outcomes. A P-value of <0.05 was always considered to be statistically significant.

Results

Patient characteristics

A total of 55 participants were included in the study and randomly allocated to 2 groups: DHM supplement (Group A, n=28) or placebo (Group B, n=27). Of the 55 patients, 9 (2 from group A and 7 from group B) were lost to follow-up before the

completion of 12 months (all but 1 before the completion of 6 months).

Most baseline characteristics were balanced between the 2 groups, except for sex distribution, as females were more frequent in group A than B (71.4% vs. 40.7%, P=0.022). The mean age at baseline was 56 years in group A and 51 years in group B (P=0.142), whereas the mean BMI was 31 and 32 kg/m^2, respectively (P=0.522). The distribution of comorbidities, including hypertension, diabetes mellitus and hyperlipidemia, did not differ significantly between the 2 groups. The 2 groups also did not significantly differ in any baseline laboratory parameter or LSM (Table 1).

Tolerability and safety

The tolerability was excellent for both groups and no patient reported any drug-related adverse event.

Responses based on changes of the liver enzymes

The changes in the main liver enzymes at 6 and 12 months are presented in Table 2. The median ALT, AST and GGT values decreased significantly from baseline to 6 or 12 months in group A, but not in group B patients.

At 12 months, the rates of clinically beneficial improvement of ALT (ALT ≤ULN or reduction >50% from the baseline values) were only numerically, but not significantly higher in group A than group B at both ITT (13/28 or 46.4% vs. 8/27 or 29.6%, P=0.315) and PP analysis (13/26 or 50.0% vs. 8/20 or 40.0%, P=0.707). On the other hand, the most difficult to achieve endpoint, combined normalization of both ALT and GGT levels at 12 months, was achieved significantly more frequently in group A than in group B patients under both ITT (9/28 or 32.1% vs. 1/27 or 3.7%, P=0.012) and PP analysis (9/26 or 34.6% vs. 1/20 or 5.0%, P=0.028). The remaining ALT and GGT response rates at 6 or 12 months were numerically higher, but not significantly different in group A from group B (Table 3).

According to GGE analysis, ALT improvement was achieved in 46.4% of patients in the DHM group and 29.6% in the placebo group (OR 1.64, 95%CI 0.68-6.25; P=0.20; Fisher's exact P=0.27). Combined ALT and GGT normalization showed a significant treatment by time interaction (P=0.021), and DHM patients were more likely to normalize over time; rates were similar at 6 months (~15%) but diverged by 12 months (DHM: ~30% vs. placebo: ~4%). Sex was not a significant covariate (P=0.44).

For individual enzyme outcomes, the GEE analysis revealed no significant treatment-by-time interaction for ALT normalization (P=0.087) or GGT normalization (P=0.479), and sex did not significantly affect the outcome. Both enzymes tended to improve slightly in the DHM group compared with placebo, but these changes did not reach statistical significance (Table 4).

Table 1 Baseline characteristics of the study participants

Characteristics	Total (N=55)	DHM supplement (n=28)	Placebo (n=27)	P-value
Age, years	53.7±11.6	56.0±9.9	51.4±12.9	0.142
Female sex (%)	31 (56.4)	20 (71.4)	11 (40.7)	0.043
Hypertension, n (%)	23 (41.8)	14 (50.0)	9 (33.3)	0.210
Diabetes mellitus, n (%)	17 (30.9)	11 (39.3)	6 (22.2)	0.281
Body mass index, kg/m^2	31.6±5.0	31.4±5.0	32.3±5.5	0.522
ALT, IU/L	62 (52)	59 (49)	70 (46)	0.145
AST, IU/L	35 (29)	33.5 (18)	42 (34)	0.068
GGT, U/L	59 (136)	67.5 (149)	59 (120)	0.920
Platelets, ×10^3/mm^3	260±70	260±51	259±87	0.944
LSM by TE, kPa	6.2 (3.5)	6.25 (3.6)	6.0 (3.3)	0.946
LSM by 2D-SWE, kPa	6.3 (2.5)	5.7 (2.0)	6.6 (3.4)	0.192
CAP, Db/m	304±52	309±48	296±55	0.376

DHM, dihydromyricetin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transpeptidase; LSM, liver stiffness measurement; TE, transient elastography; 2D-SWE, 2D-shear wave elastography; CAP, controlled attenuation parameter

Changes in main metabolic parameters

The changes in the main metabolic parameters at 6 and 12 months are presented in Table 2. In group A patients, glucose and total cholesterol levels were significantly lower at 6 and 12 months, while HbA1c and LDL cholesterol levels had a trend for decrease at 6 months and were significantly lower at 12 months, compared to baseline values. In contrast, there were no significant changes in HDL cholesterol and triglyceride levels in group A patients. In addition, there was no significant change at 6 or 12 months in any metabolic parameter in group B patients.

Changes in body weight

The patients' body weight was numerically reduced at 12 months by a mean of 1.2 kg (P=0.103). The reduction in weight did not differ between patients of group A and B (P=0.571), but it was greater in patients who achieved combined normalization of ALT and GGT at 12 months compared to those who did not. In particular, the 10 patients who achieved both normal ALT and GGT at 12 months experienced a median weight loss of 5 kg, compared to only 1 kg of median weight loss in the 36 patients without such a response (P=0.035).

Changes in liver stiffness

LSM by both TE and 2D-SWE showed a significant decline in patients who received the DHM supplement, in contrast to

Table 2 Changes in main biochemical and metabolic parameters at 6 and 12 months

Parameters	Baseline	At 6 months	P-value[1]*	At 12 months	P-value[2]*
Dihydromyricetin supplement					
ALT, IU/L	59 (49)	50 (38)	0.042	42.5 (38)	0.010
AST, IU/L	33.5 (18)	30.5 (12)	0.067	28 (17)	0.033
GGT, U/L	67.5 (149)	54 (170)	0.045	61 (134)	0.022
Glucose, mg/dL	108±27	64±27	<0.001	100±17	0.015
HbA1c, %	6.3±1.3	6.1±1.1	0.083	6,0±1,2	0.046
Total cholesterol, mg/dL	200±31	191±35	0.049	191±30	0.036
LDL cholesterol, mg/dL	121±31	115±30	0.056	107±31	<0.001
HDL cholesterol, mg/dL	54±13	53±10	0.495	55±12	>0.99
Triglycerides, mg/dL	139±92	143±101	0.798	146±145	0.543
Placebo					
ALT, IU/L	70 (46)	51 (42)	0.192	58 (35)	0.117
AST, IU/L	42 (34)	31 (18)	0.095	33 (24)	0.121
GGT, U/L	59 (120)	58 (88)	0.082	61 (76)	0.376
Glucose, mg/dL	103±29	77±36	0.008	96±19	0.475
HbA1c, %	5.9±1.0	5.7±0.7	0.261	5.8±1.0	0.484
Total cholesterol, mg/dL	194±57	186±55	0.400	172±31	0.106
LDL cholesterol, mg/dL	117±49	109±40	0.316	102±26	0.242
HDL cholesterol, mg/dL	50±16	49±16	0.649	46±15	0.397
Triglycerides, mg/dL	156±87	136±53	0.178	139±64	0.357

*P values by paired t-test or Wilcoxon matched-paired signed test; [1]P-value for 0 vs. 6 months; [2]P-value for 0 vs 12 months. Quantitative variables are expressed as median values (interquartile range) or mean values ± standard deviation

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transpeptidase

Table 3 Response of main liver enzymes at 6 and 12 months

Response	Intention to treat analysis			Per protocol analysis		
Response at 6 months	DHM suppl.	Placebo	P-value	DHM suppl.	Placebo	P-value
	(n=28)	(n=27)		(n=26)	(n=21)	
ALT≤ULN, n (%)	11 (39.3)	8 (29.6)	0.639	11 (42.3)	8 (38.1)	>0.99
GGT≤ULN, n (%)	13 (46.4)	9 (33.3)	0.474	13 (50.0)	9 (42.9)	0.846
ALT & GGT≤ULN, n (%)	6 (21.4)	4 (14.8)	0.775	6 (23.1)	4 (19.0)	>0.99
Response at 12 months	(n=28)	(n=27)		(n=26)	(n=20)	
ALT response*, n (%)	13 (46.4)	8 (29.6)	0.315	13 (50.0)	8 (40.0)	0.707
ALT≤ULN, n (%)	13 (46.4)	7 (25.9)	0.194	13 (50.0)	7 (35.0)	0.309
GGT≤ULN, n (%)	12 (42.9)	8 (29.6)	0.460	12 (46.2)	8 (40.0)	0.676
ALT & GGT≤ULN, n (%)	9 (32.1)	1 (3.7)	0.012	9 (34.6)	1 (5.0)	0.028

*ALT response: ALT ≤ULN or reduction >50% compared to baseline values

ALT, alanine aminotransferase; GGT, γ-glutamyl transpeptidase; ULN, upper limit of normal

no significant LSM changes in patients who received placebo (Table 5). In particular, the median LSM by TE declined from 6.3 kPa at baseline to 5.4 kPa at 6 months (P=0.007), or 5.3 kPa at 12 months (P=0.001) of DHM supplement use. Similarly, the median LSM by 2D-SWE declined from 5.7 kPa at baseline to 5.5 kPa at 6 or 12 months (P=0.033) of DHM supplement use.

In line with these findings, GEE analysis confirmed that the probability of liver stiffness improvement was significantly

higher in the DHM group compared to placebo (Wald χ^2=6.17, P=0.013), while no significant effects of time or treatment-by-time interaction were detected. In contrast to LSM, median CAP values did not significantly change at 6 or 12 months in either group A or B. In particular, in patients who received the DHM supplement, there was only a trend for decline of median CAP values at 12 months compared to baseline (305 vs. 314 Db/m, P=0.092).

Table 4 Summary of generalized estimating equations (GEE) results

Outcome	Effect	Wald x^2	P-value
ALT normalization	Treatment×Time	2.921	0.087
	Sex	2.072	0.150
GGT normalization	Treatment×Time	0.501	0.479
	Sex	0.461	0.497
Combined ALT & GGT normalization	Treatment×Time	5.322	0.021
	Sex	0.535	0.465
LSM improvement	Treatment×Time	1.657	0.198
	Sex	0.006	0.940
	Treatment	6.171	0.013
ALT response* at 12 months (logistic regression)	Treatment	1.622	0.203

All analyses performed using GEE with binomial logit link and exchangeable correlation structure
*ALT response: ALT normal or reduction >50% compared to baseline values
ALT, alanine aminotransferase; GGT, γ-glutamyl transpeptidase; LSM, liver stiffness measurement

Factors associated with combined ALT and GGT normalization at 12 months

Since the rate of combined normalization of both ALT and GGT at 12 months was associated with the use of DHM supplement, the associations of such a response with patient and treatment characteristics were assessed. No baseline patient characteristic was associated with the probability of combined normalization of both ALT and GGT at 12 months (Table 6). In multivariate logistic regression analysis, including sex, body weight difference between baseline and 12 months, and study group, combined normalization of both ALT and GGT at 12 months was significantly associated only with the use of DHM supplement (OR 11.8, 95%CI 1.5-120.4; P=0.038) and had a trend for association with body weight reduction (OR per kg of decline 1.22, 95%CI 0.99-1.51; P=0.059). These findings remained consistent after adjustment for sex imbalance, using the GEE model to account for repeated measures and potential confounders.

Discussion

To the best of our knowledge, this is the first randomized study to assess the effect of a DHM supplement in patients with MASLD, and the first study to evaluate the efficacy and safety of such a supplement for a period of more than 3 months. In our double-blind, randomized, placebo-controlled study, the administration of DHM supplement for 12 months was found

Table 5 Elastographic changes at 6 and 12 months

Group	Liver stiffness by transient elastography, kPa				
	At baseline	At 6 months	P-value[1]*	At 12 months	P-value[2]*
DHM supplement (N=28)	6.25 (3.6) (n=28)	5.35 (1.4) (n=20)	0.007	5.3 (2.0) (n=25)	0.001
Placebo (N=27)	6.0 (3.3) (n=27)	7.0 (9.3) (n=17)	0.246	6.1 (5.1) (n=19)	0.931
	Liver stiffness by 2D-shear wave elastography, kPa				
	At baseline	At 6 months	P-value[1]*	At 12 months	P-value[2]*
DHM supplement (N=28)	5.7 (2.0) (n=28)	5.5 (2.0) (n=21)	0.033	5.5 (2.6) (n=25)	0.031
Placebo (N=27)	6.6 (3.4) (n=27)	6.6 (9.1) (n=17)	0.636	6.5 (2.4) (n=19)	0.573
	Controlled attenuation parameter, Db/m				
	At baseline	At 6 months	P-value[1]*	At 12 months	P-value[2]*
DHM supplement (N=28)	314 (96) (n=28)	296.5 (90) (n=20)	0.411	305 (62) (n=25)	0.092
Placebo (N=27)	310.5 (85) (n=26)	292 (107) (n=17)	0.796	320 (82) (n=19)	0.165

[1]P-value for 0 vs. 6 months; [2]P-value for 0 vs.12 months; *P-values by Wilcoxon matched-paired signed test

Table 6 Normalization of both alanine aminotransferase (ALT) and γ-glutamyl transpeptidase (GGT) at 12 months in association with baseline patient characteristics

Characteristics	ALT & GGT normal (n=10)	ALT and/or GGT elevated (n=36)	P-value
Age, years	57.0±11.1	52.2±12.4	0.271
Female sex (%)	8 (80.0)	18 (50.0)	0.090
Body mass index, kg/m²	31.0±5.2	31.8±5.5	0.704
Hypertension, n (%)	4 (10.0)	17 (47.2)	0.963
Diabetes mellitus, n (%)	4 (40.0)	11 (30.6)	0.573
LSM by TE, kPa	6.25 (4.6)	6.65 (3.5)	0.844
LSM by 2D-SWE, kPa	5.7 (2.0)	6.6 (2.6)	0.393
CAP, Db/m	300±58	307±49	0.722

LSM, liver stiffness measurement; TE, transient elastography; 2D-SWE, 2D-shear wave elastography; CAP, controlled attenuation parameter

to offer significant biochemical, metabolic and elastographic benefits in patients with MASLD, compared to substantially no change in patients with MASLD receiving placebo. Although the primary endpoint was not met, as the rate of clinically beneficial improvement of ALT at 12 months was only numerically but not significantly higher in patients receiving DHM supplement than placebo (46% vs. 30%), a more strict endpoint, namely the combined normalization of ALT and GGT at 12 months, was achieved significantly more frequently in the DHM supplement than the placebo group, in both ITT and PP analysis (35% vs. 5%, P=0.028). Using GEE models, we observed a significant time-dependent effect of DHM supplementation on combined ALT and GGT normalization over 12 months. Importantly, the GEE model adjusted for sex confirmed that the imbalance between groups did not influence outcomes.

Our findings support the potential hepatoprotective effect of DHM in MASLD, which has been previously suggested by *in vivo* preclinical and shorter clinical studies [10-12]. Firstly, a diet containing 0.1% ampelopsin, which represents the main constituent of *Ampelopsis grossedentata*, was reported to have hepatoprotective effects, expressed as marked suppression of the increase of aminotransferases and lactate dehydrogenase by preventing oxidative stress in D-galactosamine-induced liver injury in rats [13]. Subsequently, the effect of DHM, which is a flavonoid primarily derived from *Ampelopsis grossedentata*, was investigated in mice with thioacetamide-induced liver fibrosis. DHM administered as a nutritional supplement (20 or 40 mg/kg daily for 4 weeks) significantly reduced liver enzyme levels, alleviated oxidative stress and inhibited liver fibrosis progression by regulating apoptotic and inflammatory pathways, including phosphatidylinositol 3-kinase (PI3K)/Akt signaling and nuclear factor kappa B (NF-κB) [10]. More recently, DHM was reported to exhibit antioxidant and anti-inflammatory properties, and to protect mitochondrial function by inhibiting oxidative stress [14-16], as well as to have hepatoprotective effects by several mechanisms in rat models of liver injury [17]. In particular, DHM was shown to upregulate antioxidant defense mechanisms, such as nuclear factor erythroid 2 pathway, downregulate proinflammatory factors, such as NF-κB, interleukin 1b and tumor necrosis factor alpha, decrease the expression of pro-apoptotic proteins, such as cleaved caspase-3, and suppress lipid accumulation in hepatocytes. Additionally, DHM was shown to enhance mitochondrial function and inhibit endoplasmic reticulum stress, both of which contribute to improved liver health [17]. Based on the above preclinical data, DHM seems to have hepatoprotective effects by reducing inflammatory activity and also by having antifibrotic properties.

In clinical studies, DHM has been shown to have several metabolic effects that could be potentially beneficial in patients with MASLD. In particular, short-term use of DHM was shown to reduce insulin resistance, and to improve diabetes and renal function parameters. In 1 randomized placebo-controlled study that included 80 adult patients with type 2 diabetes mellitus, the authors evaluated the effects of 1-month administration of *Ampelopsis grossedentata* containing 970 mg of DHM on blood glucose metabolism, lipids and renal parameters. DHM supplementation, compared to placebo, resulted in significant reductions in fasting plasma glucose, glaciated albumin cystatin C and retinol binding protein-4, while the levels of other lipids and apolipoproteins did not substantially change [18]. Another double-blind, placebo-controlled study evaluated the effects of 3-month administration of DHM, given as 150 mg tablets b.i.d., on the glycemic and lipid metabolism of 60 patients with MASLD. The latter study showed that DHM achieved significant reductions in homeostatic model assessment (HOMA) index of insulin resistance, serum levels of glucose, liver enzymes and lipid parameters. Additionally, DHM decreased serum levels of liver enzymes, such as aminotransferases and GGT, and of several inflammatory biomarkers, such as tumor necrosis factor-α, cytokeratin 18 and fibroblast growth factor 21, while it also increased serum adiponectin levels [12].

The results from the above clinical studies are in agreement with the findings of several *in vitro* and animal studies, which also demonstrated that flavonoids, and especially DHM, can have beneficial effects on glucose and lipid homeostasis, mostly by improving insulin resistance, thereby exerting antidiabetic actions [19]. Moreover, according to the findings of these 2 placebo-controlled clinical studies [12,18], DHM appears to have a more rapid effect on glucose metabolism, which can be seen within the first month, while it may need to be given for longer to have an effect on lipid and perhaps liver parameters. It should be noted that DHM administration resulted in improvements of only glucose parameters when it was given for 1 month [18], improvements of glucose, liver enzymes and LDL cholesterol when it was given for 3 months [12], and improvements of glucose, liver enzymes and total and LDL cholesterol when it was given for ≥6 months in our study.

Our study demonstrated that 6- or 12-month administration of DHM at a daily dose of 600 mg combined with vitamins C/E and choline can lead to improvement of LSM, determined by either TE or 2D-SWE, despite the non-significant impact on liver steatosis as assessed by CAP. Although the median baseline

LSM values were relatively low in our study (F0-F1 range), the different pattern of changes of median LSM from baseline to 6 or 12 months in our DHM (significant reduction) and placebo (no significant change) group patients cannot be ignored, and are in agreement with previous reports suggesting potentially antifibrotic effects of DHM through inhibition of transforming growth factor-beta and hepatic stellate cell activation [9,11]. Although patients with higher baseline LSM might have shown greater changes, the small number of patients with high LSM made subgroup analyses meaningless Nevertheless, future studies should include patients with higher baseline stiffness (F2-F3) to better determine the clinical impact of this change.

Importantly, the supplement used in our trial contained DHM together with vitamins C/E and choline. Each of these components has independently demonstrated hepatoprotective or metabolic benefits [20-22]. Therefore, the observed effects cannot be solely attributed to DHM. However, given that the doses of vitamins C/E and choline were modest compared to those used therapeutically, it is plausible that DHM served as the principal active compound, while synergistic effects from the accompanying antioxidants enhanced the response.

Despite the promising results, some limitations must be acknowledged. Our study had a relatively small sample size, including only 55 patients in the ITT and 46 patients in the PP analysis. Thus, additional larger studies are required to confirm our findings. The multi-component nature of the supplement may not allow definitive attribution of the observed effects to DHM alone, although there are published data suggesting that liver diseases, including MASLD, can be favorably affected by DHM, but not by any other component of this supplement, including choline and low doses of vitamin C (80 mg per tablet) or vitamin E (12 mg per tablet). Furthermore, a small proportion of our patients had undergone liver biopsy before baseline and no patient underwent liver biopsy at the end of the study. Therefore, it was not possible for us to have adequate assessments of liver histological changes, which would have been crucial in order to determine possible changes of necroinflammatory activity, as well as to better understand the significance of improvements of median LSM after DHM use. Finally, most participants had normal baseline LSM, limiting the capacity to detect greater changes. Despite these limitations, the trial provides consistent evidence of biochemical, metabolic and elastographic improvement, with excellent safety and tolerability. The double-blind, placebo-controlled design and the 1-year duration strengthen the validity of the observations.

In conclusion, the up to 12-month use of a DHM supplement containing vitamins C and E and choline in our study was found to reduce the levels of liver enzymes and to achieve improvements of liver stiffness. These findings reinforce the hepatoprotective role of the DHM supplement in patients with MASLD. In addition, this DHM supplement achieved improvements in glucose and lipid parameters, which are usually therapeutic goals in patients with MASLD. Our results, in conjunction with previous reports [11,12,18] suggest that DHM may be a promising therapeutic agent for the treatment of MASLD. However, additional appropriate larger studies are warranted to confirm our findings and establish the long-term efficacy and safety profile.

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Summary Box

What is already known:

- Effective pharmacological treatments for metabolic dysfunction-associated steatotic liver disease (MASLD) remain limited, with lifestyle modification being the mainstay of management
- Polyphenols and flavonoids, such as dihydromyricetin (DHM), have shown antioxidant, anti-inflammatory and potential antifibrotic effects in preclinical liver disease models
- Prior clinical studies of DHM in MASLD have been short-term, and provided preliminary evidence of biochemical improvement

What the new findings are:

- A 12-month DHM supplement improved liver enzyme levels and reduced liver stiffness in MASLD patients compared to placebo
- Combined ALT and GGT normalization at 12 months were significantly more frequent with DHM than placebo
- DHM supplementation improved glucose and lipid profiles, suggesting additional metabolic benefits
- The supplement was safe and well-tolerated with no reported adverse events

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