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Original Article

Triple Therapy with Garlic, Silymarin and Curcumin in Non-Alcoholic Fatty Liver Disease: A Randomized, Placebo-Controlled Clinical Trial

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Abstract

Background and Aim: Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in the world. We aimed to evaluate the effect of a mixture of garlic, silymarin, and curcumin on hepatic parameters and overall improvement of NAFLD.

Materials and Methods: Individuals between 18 and 70 years of age with altered liver enzymes and confirmed pattern of fatty liver in their hepatic ultrasound were entered into this randomized, triple-blind, placebo-controlled trial and assigned to two groups. They received either capsules containing curcumin, silymarin and garlic or an identical placebo. After 3 months, the patients were re-evaluated for laboratory tests, clinical evaluation, and liver fibroscan. Data were analyzed in consistent with the intention-to-treat approach.

Results: After randomization and blinding, 50 individuals entered this study. The mean age of the participants was 42.51 ± 11.13 (mean± SD) years of old, and 80% of the participants were men. After triple therapy, no significant differences were observed in laboratory tests between the two groups, except for the triglyceride level (188.86 ± 90.66 vs. 146.23 ± 70.38, p-value=0.04). However, hepatic fibroscans were noticeably ameliorated in the treatment group compared to the control (p-value <0.001).

Conclusion: We observed no significant amelioration in the majority of biochemical indices of the patients, but patterns of NAFLD in fibroscans were considerably improved. Although we cannot entirely attribute this finding to our therapy with silymarin, curcumin, and garlic, a healthy lifestyle combined with these supplements can help improve the state of NAFLD. Further studies with larger sample size and different doses are recommended.

Keywords: NAFLD, Garlic, Silymarin, Curcumin, Liver

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Introduction

The first description of non-alcoholic fatty liver disease (NAFLD) was presented in the 1950s when fatty liver was diagnosed in a group of obese patients. In 1980, Mr. Ludwig et al. examined 20 obese, diabetic, and non-alcoholic patients at the Mayo Clinic and found a similarity between the histopathology of liver biopsy specimens in these patients and that of patients with alcoholic liver disease, and the term "non-alcoholic fatty liver disease" was coined (1). NAFLD includes a range of liver diseases, including: 1) Simple fatty liver; 2) non-alcoholic steato-hepatitis (NASH); and 3) Fibrosis and cirrhosis caused by NAFLD (2).

The precise prevalence of NAFLD has remained unknown, but it is estimated that 14% to 20% of the general population have NAFLD and 3% have NASH. However, its prevalence varies in distinct regions of the world and the highest prevalence reported among non-alcoholic individuals has been 76% (3). It is most common in middle-aged people (40s to 60s) and is equally common in men and women, but men are more likely to develop more advanced forms such as NASH (1, 3). NAFLD is the most common liver disease in the West and is thought to have a prevalence between 5% and 30% in Asia (4). Studies have shown that NAFLD alone is the most common cause of elevated liver enzymes and also the most common cause of cryptogenic cirrhosis. Its prevalence is increasing with the prevalence of obesity and, by its nature, insulin resistance, so that 75% of people with body mass index (BMI) above 30 and about 90% of people with BMI> 35 suffer from chronic disease (3, 4). There is a strong correlation between type II diabetes (with or without obesity) and NAFLD. Type II diabetes, hyperglycemia, and glucose intolerance occur in adults with NASH. Presence of NAFLD in diabetic patients may significantly increase the risk of cardiovascular disease (2).

Most patients with NAFLD are diagnosed following the accidental detection of elevated liver enzymes (ALT and AST), because majority of them have no symptoms and only hepatomegaly can be found in their physical examination. The combination of history, physical examination, blood tests, and radiological findings are useful to rule out other liver diseases. The role of liver biopsy in NAFLD diagnosis is still debated, because histologically, NAFLD and alcoholic liver disease are indistinguishable from each other. Therefore, standard diagnostic criteria are not known to date (5, 6).

Despite the high prevalence of this disease and the possibility of its progression to complications such as chronic liver failure and hepatocellular carcinoma, no standard treatment has been approved so far (7). However, multifactorial nature of NAFLD requires the use of different herbal and pharmaceuticals to be under study. Thus, the most used materials, either alone or in combination regimens, are silymarin, vitamin E, vitamin D, polyunsaturated fatty acids of the omega-3 series, astaxanthin, coenzyme Q10, berberine, curcumin, resveratrol, extracts of Salvia milthiorriza, and probiotics (2, 8, 9). Promising impacts of garlic in the treatment of liver inflammation and fatty liver (10) have also been investigated. It has been indicated that different doses of garlic have positive impacts on hyperlipidemia and lipid profile in both humans and animals studies (11, 12).

In this study, we aimed to investigate the effect of a mixture of distinct herbal medicines, i.e. garlic, silymarin, and curcumin on hepatic parameters and overall improvement of NAFLD.

Materials and Methods

Study design

This study was a randomized, triple-blind, placebo-controlled trial on two parallel groups with IRCT registration number of IRCT20190602043787N1 in the registry of clinical trials (http://www.irct.ir). It was conducted based on the guidelines of the Declaration of Helsinki and its study method was approved by the Ethical Committee of Mashhad University of Medical Sciences (code: IRMUMS.MEDICAL.REC.1398.152). All the participants signed a written informed consent

approved in advance by the Ethical Committee.

Participants

Exclusion criteria included diabetes, hypothyroidism, any use of medication/drugs, severe heart and lung

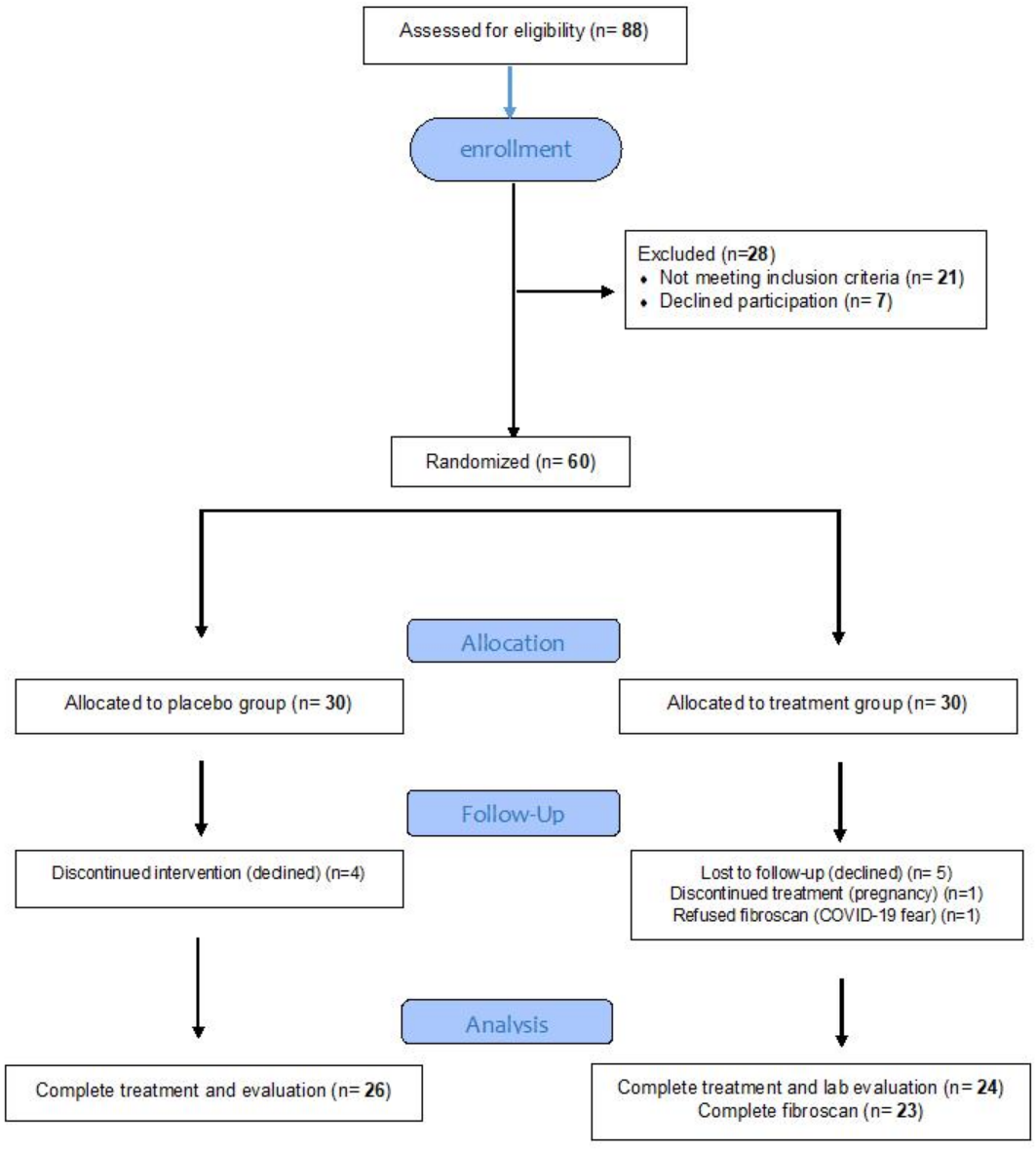

Figure 1. CONSORT flow chart.

We randomly divided all the subjects into two groups of treatment and control using a balanced block randomization technique. Individuals between 18 and 70 years of age, who had altered liver enzymes (aspartate aminotransferase: AST, alanine aminotransferase: ALT) and confirmed pattern of fatty liver in their hepatic ultrasound were entered into the study.

diseases, history of underlying liver disease, cirrhosis and cancer, breastfeeding or pregnant women or those planning for imminent pregnancy, individuals with altered tests of HbC Ab, HCV Ab, ANA, ASMA, SI, Ferritin, TIBC, Anti TTG IgA HBs Ag, and ceruloplasmin; and declining to sign the informed consent.

Interventions

The research plan and its objectives were explained to the patients, and informed written consent was obtained. A checklist was completed under the supervision of the main researcher (appendix 1), and then each patient received a drug package specified with a code by a partnered physician. Only the main researcher was aware of the packages content and their codes. The patients were treated and then followed up only according to the relevant codes in a group for a 3-month duration.

The treatment packages contained three herbs in the form of soft gel capsules each containing curcumin (380 mg), silymarin (70 mg) and garlic (200 mg) to be ingested on daily basis for 12 weeks (2, 12, 13). Similar capsules containing the formulation excipients were used daily by the control group. All of the capsules were prepared by the pharmaceutical laboratory of the School of Pharmacy, Mashhad University of Medical Sciences. Along with the treatment capsules, both groups were put on a designed diet to restrict fat and carbohydrate intake.

Semi purified curcumin (50% purified) had been used in this product.

The herbarium code for the plants under study is as follows:

Silybum marianum: IBRC P1006710, Allium *sativum* UPS BOT V-173002, *Curcuma Longa:* NHMUK BOT BM000948011

For the garlic the total dried powder of the bulb had been used, and total dried seeds had been used for silybum.

Silymarin and allicin, as the active components of Silybum marianum and garlic respectively, were determined.

Placebo capsule was filled by Lactulose.

At the end of the three-month intervention, the patients were re-evaluated for laboratory tests (ALT-AST-ALP-Bili-FBS-TG-CHOL-LDL-HDL), clinical evaluation, and a blind liver fibroscan. Hepatic fibroscan was performed using 3.5-or 5-MHz convex probe (Essence®, Germany). The patients had to be on 8–12 h fasting duration prior to examination.

Clinical Evaluations

In the beginning of the study, pharmacological, anthropometric and clinical parameters were measured for each study subject. Familial history for heart disease, diabetes (two blood sugars >126 mg/dL), hypercholesterolemia (serum cholesterol ≥200 mg/dL), hypertriglyceridemia (serum triglycerides ≥200 mg/dL), smoking and alcohol consumption were also documented in study subjects. Waist (as umbilical) circumferences and body mass index (BMI) as kilograms per square meter were measured. Liver function tests, including ALT, AST, alkaline phosphatase, bilirubin and NAFLD steatosis score were calculated to assess the state of hepatic steatosis in each individual once at the beginning of the intervention and afterwards at the end of the study. According to the results of hepatic fibroscan, the percentages of liver fat were graded as follows: 0-9% normal, 10-33% grade 1, 34-68% grade 2, and 69-100% as grade 3 fatty liver.

Response to treatment was assessed by two factors, i.e. an increase of more than 5 units in the difference between ALT before and after receiving either the combination drug or placebo, and changes in liver scans.

Sample Size and Outcome

The frequency of patients whose ALT decreased by 5 units (U) was considered as the main outcome of the study. Given that there was no similar study, the researchers' assumptions were used to determine the sample size. Considering $\alpha = 0.05$ and $\beta = 0.2$, and calculating a 10% drop, the final sample size was estimated 30 patients in each group using PASS software.

Randomization and Blinding

Following an initial screening process, nutrition plan and lifestyle were explained to each participant face-to-face. All the subjects were randomly assigned to either the treatment group (curcumin 380 mg, silymarin 70 mg, and garlic 200 mg per day) or the placebo group (control condition) by a fixed randomization scheme based on computerized random numbers. All the individuals were informed that two treatment regimens were being assessed (figure 1).

Statistical Analysis

Data were analyzed in consistent with the intention-to-treat approach using SPSS software (version 16; SPSS Inc., Chicago, IL). We used "last value carried forward" to analyze the lost data **.**For data with normal distribution, the independent t-test was used to compare quantitative variables between the two groups, otherwise Mann-Whitney test was applied.

The qualitative variables between the two groups were compared by Chi-square and Fisher's exact. Paired T test was used to compare quantitative variables before and after the intervention, otherwise Wilcoxon test was used. To compare the qualitative variables, we used Mcnemar's test. A two-sided p-value < 0.05 was considered statistically significant. The characteristics of the study subjects were presented by descriptive statistical methods, including central indices, dispersion and frequency distribution in tables and graphs.

Results and Discussion

Trial participants

A total of 50 individuals who met all our inclusion criteria and were willing to participate, entered this study. The participants who had been randomly assigned to two groups of treatment (n = 24) and control (n = 26) were evaluated during the study, but of 50 persons completed a 3-month follow-up visit and preclinical tests, only 23 in the treatment group accepted to go through hepatic fibroscan. One patient refused to show-up at our radiology clinic because of fear of contracting COVID-19 (figure 1). The mean age of participants was 42.51 ± 11.13 (mean± SD) years of old, and 80% of the participants were men. Comparing the individual characteristics of the participants, none of the demographical variables were statistically different between the two groups. Descriptions and comparisons of participants' demographic data have been depicted in Table 1. All baseline characteristics, including demographics, history of illnesses, anthropometrics and body composition such as waist to hip ratio were similar between the two groups. Furthermore, no difference was observed in baseline dietary intake between the two groups (Table 1).

Laboratory Parameters

We measured laboratory tests once before the interventions and afterwards at the end of triple therapy (three months later). A comparison of laboratory tests before and after the combination therapy in the treatment group showed that there were statistical differences in mean AST (39.79 ± 15.44 vs 34.04 ± 15.01, p value =0.02), total bilirubin (1.01 ± 0.63 vs 0.74 ± 0.44, p value =0.008), and direct bilirubin (0.22 ± 0.08 vs 0.18 ± 0.09, p value =0.03). In the control group; however, mean ALT (46.42 ± 28.11 vs. 39.07 ± 21.78, p value =0.01), triglyceride (173.16 ± 79.35 vs. 146.23 ± 70.38, p value =0.04), HDL (39.37 ± 4.9 vs 43.03 ± 6.91, p value =0.01), total bilirubin (0.82 ± 0.38 vs 0.62 ± 0.19, p-value =0.007) and direct bilirubin (0.23 ± 0.14 vs 0.17 ± 0.06, p-value =0.02) were statistically significant before and after dietary regulation.

In contrast to our expectations, after triple therapy there were no remarkable distinctions in laboratory tests between the two groups, except for the triglyceride level (188.86 ± 90.66 vs. 146.23 ± 70.38, p-value=0.04). Detailed test results have been shown in Table 2.

Outcome Assessment

Response to treatment was assessed by two factors, i.e. a decrease of more than 5 units in the difference between ALT before and after receiving either the combination drug or placebo, and hepatic fibroscan changes. The number of patients with 5 units of reduction in their ALT was 15% and 50% in the control and in the treatment groups, respectively.

Hepatic Fibroscan Assessments

As shown in Table 3, liver fibroscan before and after receiving either the combination therapy or placebo was significantly different in both groups. Although in the treatment group the number of people with more than 5 unite decrease in ALT was higher than that in the placebo group, this difference was not statistically significant between the two groups (Figure 2, Table 3). Totally, 23 individuals in the treatment group and 26 in the control group underwent liver scans after extensive follow-up (Table 3). Despite several follow-ups, one of the participants in the treatment group did not show up for a second scan due to fear of contracting the SARS-CoV-2 virus.

Before the treatment, the majority of participants (77.3% and 82.6% in the treatment and control group, respectively) who underwent liver fibroscan for fatty liver were staged as grade 3. In the second fibroscan, the number of individuals with grade 2 and 3 fatty liver patterns reduced in both groups, but the rate of reduction in grade 3 was higher in the treatment group in comparison with placebo. No significant difference in hepatic fibroscan was observed when both groups were compared (Table 3). However, after receiving either the combination therapy or placebo, hepatic

fibroscan results were significantly ameliorated in the treatment group in comparison with the control group

Table 1: Baseline characteristics of study participants, including demographic data, history, and nutritional state. Data have been shown as mean±SD and number (percent) for each variable.

Characteristics	Category	Treatment Group N=24	Control Group N=26	P Value
Sex number (percent)	Female	5 (20.8)	5 (19.2)	0.88**
	Male	19 (79.2)	12 (80.8)	
Age (year) IQR		41 (34-49)	40 (33-48)	0.79***
Weight (kg) IQR		85.5 (76.25-96.5)	86 (79.25-98.5)	0.75***
Height (meter) IQR		175 (164.75-180)	174 (165.25-180.75)	0.75***
Body mass index (kg/m^2) mean ± standard deviation		28.46 ± 5.03	29.07 ± 3.69	0.62*
Waist circumference mean ± standard deviation		100.75 ± 11.55	104.95 ± 8.59	0.16*
Pelvis circumference IQR		104.5 (100.5-109.5)	105 (99-108)	0.83***
Waist to hip ratio IQR		0.97 (0.9-0.99)	1 (0.95-1)	0.06***
Education number (percent)	Elementary	2 (8.3)	5 (19.2)	0.19**
	Middle school	1 (4.2)	5 (19.2)	
	High school	3 (12.5)	3 (11.5)	
	Higher education	18 (75)	13 (50)	
Occupation number (percent)	Unemployed	1 (4.2)	2 (8.3)	0.14**
	Employed	16 (66.7)	7 (29.2)	
	Worker	2 (8.3)	4 (16.7)	
	Retired	1 (4.2)	3 (12.5)	
	Housewife	3 (12.5)	3 (12.5)	
	Self-employed	1 (4.2)	5 (20.8)	
Previous illness of the father number (percent)	Positive	5 (21.7)	4 (15.4)	0.56**
Previous illness of the mother number (percent)	Positive	6 (25)	2 (7.7)	0.09**
History of diabetes	Positive	3 (130)	3 (11.5)	0.87**

number (percent)				
History of smoking number (percent)	Positive	4 (16.7)	3 (11.5)	0.6**
History of hyperlipidemia number (percent)	Positive	9 (37.5)	8 (30.8)	0.61**
History of hypertriglyceridemia number (percent)	Positive	10 (41.7)	7 (26.9)	0.27**
History of hypercholesterolemia number (percent)	Positive	2 (8.3)	3 (11.5)	0.7**
History of fatty liver number (percent)	Positive	1 (4.2)	5 (19.2)	0.1**
History of alcohol consumption number (percent)	Positive	4 (16.7)	3 (11.5)	0.6**
Family history number (percent)	Cardiovascular disease	4 (26.7)	5 (35.7)	0.8**
	Hypertension	6 (40)	4 (26.8)	
	Diabetes	3 (20)	4 (28.6)	

Dietary characteristics of study participants

Type of nutrition	**Type or amount**	**Treatment Group** N=24	**Control Group** N=26	**P Value**
Type of oil consumed number (percent)	)PUFA,W6(	16 (66.7)	14 (53.8)	0.65**
	)SFA(solid oil	0	2 (7.7)	
	)SFA(animal oil	8 (33.3)	8 (30.8)	
	)MUFA (sesame	0	2 (7.7)	
Amount of oil consumed number (percent)	No oil	0	2 (7.7)	0.15**
	Low	22 (91.66)	20 (76.9)	
	Fatty	2 (8.34)	4 (15.4)	
Type of milk consumed number (percent)	Low fat	18 (75)	13 (50)	0.14**
	High fat	3 (12.5)	4 (15.4)	
	None	3 (12.5)	9 (34.6)	

Tea sugar content number (percent)	No sugar	2 (8.3)	6 (23.1)	0.11**
	Low sugar	11 (45.8)	10 (38.5)	
	Medium sugar	11 (45.8)	7 (26.9)	
	High sugar	0	3 (11.5)	
Sauce consumption number (percent)	No sauce	6 (26.1)	13 (50)	0.22**
	Low sauce	14 (60.9)	11 (42.3)	
	High sauce	3 (13)	2 (7.7)	
Type of Sauce consumed number (percent)	Mayonnaise	6 (25)	10 (38.5)	0.1**
	Ketchup	2 (8.3)	5 (19.2)	
	Mayonnaise and ketchup	10 (41.7)	3 (11.5)	
	None	5 (20.8)	8 (30.8)	
Breakfast intake number (percent)	Yes	17 (70.8)	22 (88)	0.13**
	No	7 (29.2)	3 (12)	
Consumption of sweets in the last month number (percent)	1-3 times	17 (73.9)	20 (87)	0.05**
	4-8 times	0	2 (8.7)	
	8-1 times	6 (26.1)	1 (4.3)	
Consumption of fast food in the last month number (percent)	None	10 (43.5)	12 (48)	0.45**
	Once a week	10 (43.5)	8 (32)	
	Twice a week	1 (4.3)	0	
	Once a month	2 (8.7)	5 (20)	
Type of bread consumed number (percent)	whole wheat (sangak)	11 (45.8)	10 (38.5)	0.36**
	white bread (lavash)	6 (25)	7 (26.9)	
	whole wheat (barbari)	5 (20.8)	9 (34.6)	
	whole wheat (baguette)	2 (8.3)	0	
Amount of bread per day Number (percent)	90 g	3 (13)	4 (16)	0.72**
	180 g	8 (34.8)	5 (20)	
	270 g	6 (26.1)	8 (32)	
	More than 270 g	6 (26.1)	8 (32)	

IQR:inter quartile range - *: independent t sample test, **: chi square test, ***: manWhitney u test
SFA - Saturated fatty acids; MUFA - Monounsaturated Fatty Acids; PUFA - W6 Polyunsaturated fatty acids

(p-value <0.001) (Table 3).

A s far as we have searched, this is the first triple-blind randomized clinical trial to evaluate the effect of a three-drug regimen consisting of curcumin, silymarin, and garlic on nonalcoholic fatty liver disease.

Nonalcoholic fatty liver disease is currently one of the most common chronic liver disorders worldwide that strongly correlates with lifestyle, including dietary pattern and physical activity (14-16). Since lipids, glucose, proteins, and other nutrients are catabolized in the liver, several metabolic disorders can easily arise when disproportionate dietary intake overloads the liver. Hence, access to effective yet safe and affordable drugs for treatment of NAFLD is a necessity (17). To date, various research studies have suggested several chemical and herbal treatments for this purpose, including silymarin, vitamin E, vitamin D, polyunsaturated fatty acids of the omega-3 series, astaxanthin, coenzyme Q10, berberine, curcumin, resveratrol, extracts of Salvia milthiorriza, probioticsm, and garlic (2, 8-10). Among all, silymarin

has been by far one of the most promising substances to improve liver function in NAFLD. However, it has less significant effects on triglycerides and cholesterol

Table 2: Laboratory tests of the study participants before and after the treatment. For every test, normal range and P-values have been shown.

Characteristics	**Normal range (**lab unit**)**	**Treatment group (Mean ± SD)**	**Control group (Mean ± SD)**	**P value***
Cr before	0.7-1.4 (mg/dL)	1.012 ± 0.16	0.98 ± 0.16	0.39
Cr after		1.01 ± 0.17	0.84 ± 0.22	0.23
P value**		0.35	0.15	
FBS before	75-110 (mg/dL)	97.16 ± 8.4	101.33 ± 14.94	0.24
FBS after		96.43 ± 12.11	99.42 ± 12.45	0.29
P value		0.91	0.67	
AST before	Up to 40 (U/L)	39.79 ± 15.44	31.13 ± 13.45	0.03
AST after		34.04 ± 15.01	27.57 ± 13.91	0.08
P value		0.02	0.13	
ALT before	Up to 40 (U/L)	54.75 ± 35.52	46.42 ± 28.11	0.44
ALT after		42.56 ± 27.92	39.07 ± 21.78	0.8
P value		0.05	0.01	
ALP before	64-306 (U/L)	145.17 ± 51.13	183.58 ± 65.99	0.03
ALP after		154.22 ± 37.39	173.16 ± 52.23	0.16
P value		0.42	0.08	
TG before	<200 (mg/dL)	185.08 ± 70.01	173.16 ± 79.35	0.58
TG after		188.86 ± 90.66	146.23 ± 70.38	0.04
P value		0.99	0.04	
Cholesterol before	<200 (mg/dL)	176.87 ± 37.17	186.83 ± 31.84	0.32
Cholesterol after		175.68 ± 42.94	179.76 ± 33.08	0.71
P value		0.55	0.32	
LDL before	Up to 150 (mg/dL)	112.29 ± 36.14	109.08 ± 24.62	0.72
LDL after		108.9 ± 41.98	116.12 ± 29.33	0.49
P value		0.37	0.2	
HDL before	> 35(mg/dL)	39.58 ± 11.75	39.37 ± 4.9	0.41
HDL after		40.45 ± 11.81	43.03 ± 6.91	0.07

P value		0.29	0.02	
Bilirubin direct before	0-0.3 (mg/dL)	0.22 ± 0.08	0.23 ± 0.14	0.99
Bilirubin direct after		0.18 ± 0.09	0.17 ± 0.06	0.63
P value		0.03	0.01	
Bilirubin total before	0.1-1.5 (mg/dL)	1.01 ± 0.63	0.82 ± 0.38	0.42
Bilirubin total after		0.74 ± 0.44	0.62 ± 0.19	0.99
P value		0.008	0.007	

Cr: creatinine, IU: International Unite. FBS: fasting blood sugar, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, TG: triglyceride, LDL: low-density lipoprotein, HDL: high-density lipoprotein, TSH: thyroid stimulating hormone

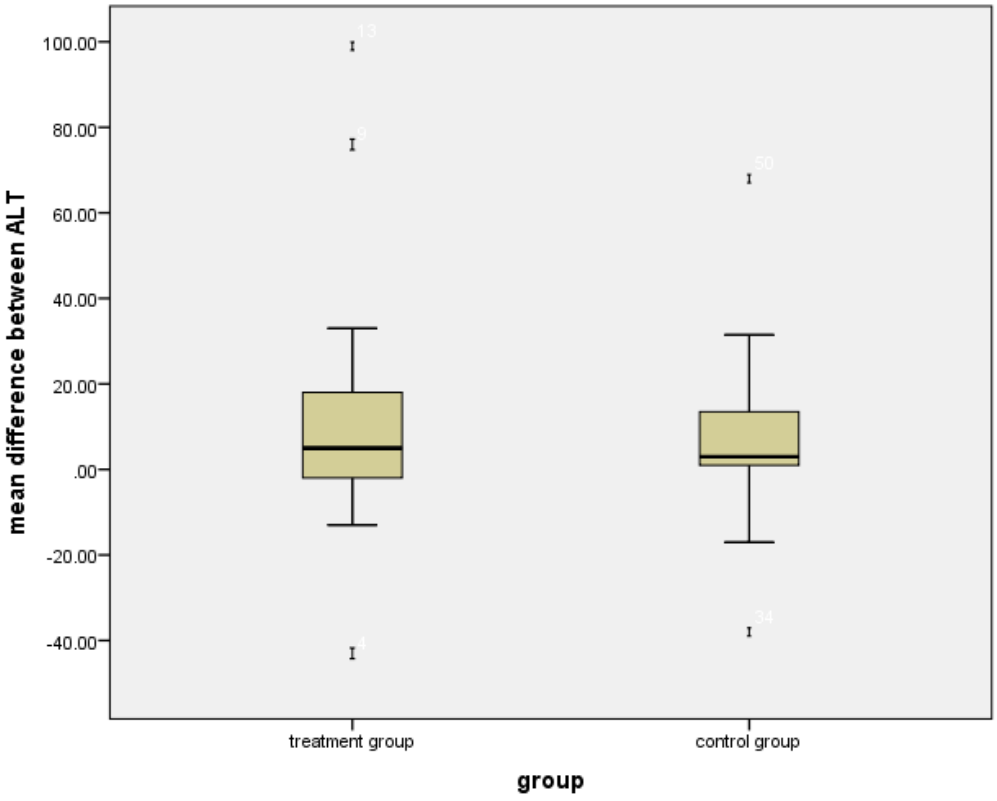

Figure 2. Mean difference of serum ALT (alanine aminotransferase) decrease between the two groups after the treatment (mean ± SD: 11.43 ± 28.79 vs. 7.34 ± 18.38 in the treatment and control groups, respectively).

*: independent sample t test - **: paired sample t test

Table 2: Response to treatment between the two groups. Hepatic fibroscan results were compared once before the treatment and then after triple therapy.

Groups	Grading	Treatment group number (percent) n=23	Control group number (percent) n=26	P value*
Hepatic fibroscan Before treatment	0-9% (Normal)	0	0	0.58
	10-33% (Grade 1)	1 (4.2)	3 (11.5)	
	34-68% (Grade 2)	4 (16.7)	3 (11.5)	
	69-100% (Grade 3)	19 (79.2)	20 (76.9)	
Hepatic fibroscan After treatment	0-9% (Normal)	1 (4.3)	1(3.8)	0.8
	10-33% (Grade 1)	1 (4.3)	2 (7.7)	
	34-68% (Grade 2)	9 (39.1)	7 (26.9)	
	69-100% (Grade 3)	12 (52.2)	16 (61.5)	
P value		<0.001	0.01	
ALT Difference	5≥	13 (56.5)	12 (46.2)	0.53
	<5	11 (43.5)	14 (53.8)	

ALT: alanine aminotransferase; *: chi square test

restoration (17), which is partly consistent with the findings of our trial. We observed significant amelioration of triglycerides when a mixture of silymarin, garlic and curcumin was prescribed to NAFLD individuals. Effects of garlic alone on triglycerides and cholesterol levels have been previously studied, but no significant results have been observed (18). Thus, such a finding in our patients' TG level may well be attributed to curcumin, as it has been previously documented that curcumin has the potential to decrease cholesterol and triglycerides in NAFLD (13).

Using biochemical tests and fibroscan investigations, we observed significant improvements in the pattern of NAFLD in patients who received our combination therapy with curcumin, garlic and silymarin. While ALT levels were not considerably changed in the treatment group in comparison with that of the control, improvement in fibroscans for fatty liver assessment was noticeable in the former group. Comparable to our findings, some of the previous clinical trials have revealed promising alterations in liver function of NAFLD patients following a mixture of silymarin, phosphatidylcholine and vitamin E (19).

Our patients had higher ALT levels before the intervention. Following the end of the treatment period, both serum ALT and AST levels proved to have remarkably reduced in the treatment group, whereas only ALT decreased in control group. Since we set alanine aminotransferase difference as our response-to-treatment standard, we attributed such a finding to improvement in the individuals' lifestyle rather than our treatment cocktail. Healthy diet could surprisingly decrease fat accumulation in the liver, thus lowering ALT levels. On the other hand, ALT per se cannot be used as an appropriate alternative to assess histopathologic reactions following herbal treatments. Additionally, efficacy of silymarin can be under the influence of confounding variables such as diet and physical activity (20); the fact that we also observed in this trial.

In a case control study in which either silymarin (210 mg for 8 weeks) or placebo were blindly prescribed to non-alcoholic steato-hepatitis patients silymarin reduced ALT and AST more significantly than the placebo (21), which is in agreement with our findings. In our research, no significant differences were observed in further laboratory tests except for bilirubin between the two groups at the end of the treatment. We observed a statistically significant reduction in bilirubin (total and direct) in both groups when

compared before and after the intervention. The fact that such differences exists in both groups suggest that a change in lifestyle might play a role here. Furthermore, recent studies (22) have revealed that bilirubin and the enzyme that produces it (biliverdin reductase A) have the potential to protect the liver against lipid accumulation and hepatic steatosis partly due to its antioxidant and cytoprotective properties (23). A cross-sectional study on more than 17000 patients on routine health check-ups evaluated the correlation between bilirubin levels and NAFLD (24). The authors reported that NAFLD prevalence had an inverse dose-dependent relationship with serum bilirubin level. However, there was no differentiation made between unconjugated and conjugated bilirubin in this research. In our trial, we observed a decrease in both total and direct bilirubin that when interpreted alongside the fibroscan results can largely confirm the above-mentioned findings. Various radiological techniques can diagnose NAFLD, but ultrasonography is a safe and noninvasive method that can be employed for screening purposes. Although hepatic fibroscan became a challenge in our study due to the onset of the COVID-19 pandemic and patients fear to partake, majority of the treatment group (23 out of 26) underwent this evaluation, and the results were remarkable after triple therapy. We observed a significant amelioration in stages 2 and 3 of NAFLD in both groups. Both lifestyle adjustment and treatment cocktail can be credited for this outcome. It has been shown that curcumin has the ability to reduce aminotransferases levels in addition to improve hepatic steatosis and inflammation (25). Moreover, although silymarin can alleviate hepatic injuries because of its antioxidant and cytoprotective properties, some trials have reported no definite effect of silymarin on liver diseases (20), which is partially in accordance with our findings.

Positive effects of garlic intake on NAFLD in animal models have been indicated in some studies (26-28). However, until recently no human studies were available on whether garlic has shown similar results on NAFLD in humans. A clinical trial reported that 15-week supplementation with garlic could decrease fat bulk in NAFLD patients (29), which is partially in line with our findings. Another randomized trial revealed that after a 12-week intervention with garlic extract, hepatic dysfunction was improved in adults aged between 20 and 75 (30). In a recent study by Zhang *et al*. in which Chinese men and women were evaluated for the effect of raw garlic on NAFLD, an inverse relationship between daily garlic intake and NAFLD was reported in men only (31). We also observed amelioration in ultrasonography and some biochemical tests, but no sex differences existed. Sangouni *et al*. studied the effect of a 12-week diet modification with 1600 mg/day garlic on NAFLD patients (11). Their results showed significant decreases in the serum ALT, AST, and cholesterol. The research group stated that garlic could improve hepatic parameters and lipid profile, which is relatively similar to our findings. We also observed a decrease in similar tests that may well be attributed to garlic.

Utilization a regimen comprised of three recognized herbal medicines with the highest rate of clinical studies is the strength of this trial. Although it has been proved that hepatic fibroscan is of high sensitivity and specificity to assess stages of NAFLD, liver biopsy is the gold standard method for diagnosis because microscopic histological evaluations can raise accuracy (32). However, considering the invasive nature of liver biopsy, ethical limitations in human studies, and because histological patterns of NAFLD and alcoholic liver disease are hardly distinguishable, this procedure was not employed in our study. Moreover, further paraclinical evaluations for NAFLD such as ferritin and serum gammaglobulin levels were not included in this trial.

Another major limitation to our study was facing the unpredictable COVID-19 pandemic in the middle of our observations, which greatly affected patients' follow-up. Some participants refused to continue treatment and replaced other supplements in the hope of preventing viral infection. A few of them declined showing up for laboratory tests and liver scan, although the research team managed to offer at-home sampling for their convenience. One individual, declined fibroscan to avoid radiology room and fear of contracting the virus.

Conclusion

The cornerstone of NAFLD prevention and treatment

is lifestyle amendment by adopting optimal physical activity along with a healthy diet. In this study, all the participants were put on a healthy lifestyle combined with triple therapy with silymarin, curcumin, and garlic supplementation, which could enhance the NAFLD state. Although this improvement cannot completely be attributed to our treatment intervention, some biochemical indices as well as fibroscan patterns were improved to a large extent. Further studies with larger sample size and different doses are recommended.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Appendix 1- Checklist of study participants entering the study

Form code: ---- study group: treatment□ placebo□

First and Last name: ----

Address: ------

Phone number: ------

Emergency phone number: --------

Demographics:

Date of birth: ---

Gender: Male□ female□

Education: Primary education□ Middle school education□ diploma□ academic education □

Marital status: Married□ Single□

Occupation: jobless □ Clerk□ Retired □ housewife□

Body weight (kg): ---- Height: ------ Body Mass Index: -----

waist circumference: ------ WHR (waist-to-hip ratio): ------

Drug history: ---------

Family history: -------

- History of heart disease and death in father? Yes□ No □

- History of heart disease and death in mother? Yes □ No □

- History of diabetes No□ Yes □ , if yes, how many years: ---

- History of smoking No □ quit smoking□ currently smoking□

- History of alcohol consumption No□ Yes□, if yes, how many years: ---

- Underlying liver disease (Cirrhosis, liver cancer, hepatitis) Yes □ No□

- History of hyperlipidemia Yes □ No□