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In-Vitro and *In-Vivo* Assessment of Effects of Nano-Curcumin on Liver Enzymes, Inflammatory Cytokines, Degree of Steatosis, and Related Genes in NAFLD

Tahavori A[1,2,3], Shateri K[1,2], Abbasi N[1,2] and Pashaei MR[1,2]*

[1]Department of Internal Medicine, Urmia University of Medical Sciences, Iran

[2]Department of Gastroenterology, Urmia University of Medical Sciences, Iran

[3]Department of Psychiatric, Urmia University of Medical Sciences, Iran

***Corresponding author:** Mohammad Reza Pashaei, Department of Gastroenterology , School of Medicine, Urmia University of Medical Sciences, Urmia, Iran, Email: dr.pashaei@yahoo.com

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is characterized by elevated triglycerides, liver enzymes, NAFLD-promoting genes, inflammatory biomarkers, and the degree of hepatic steatosis. This study aimed to evaluate the effect of nano-curcumin on the liver enzymes, expression of PNPLA3 and TM6SF2, pro-and anti-inflammatory cytokines, and hepatic steatosis and fibrosis in patients with NAFLD. In this study, 66 patients with NAFLD received one capsule containing 500 mg nano-curcumin. Then, FBS, liver enzymes, cholesterol, TG, BMI, LDL, and HDL levels were measured. Besides, the amount of pro-and anti-inflammatory cytokines in sera. In the in-vitro step, the expression of tissue-specific factors including PNPLA3 and TM6SF2 along with cytokines were determined by Real-time PCR and Western blotting in Hep G2 cells, respectively. The results revealed a significant reduction in LDL, BMI, TG, ALT, AST, and FBS levels after consumption of nano-curcumin. In this regard, TNF-α and IL-6 had a decreasing concentration compared to placebo-received patients, while IL-4 was elevated.

Furthermore, the addition of nano-curcumin to Hep G2 cells showed a significant reduction of PNPLA3 as NAFLD-promoting gene along with TNF-α and IL-6 at both mRNA and protein levels. At the same time, IL-4 and TM6SF2 were upregulated as reducers of lipid accumulation. In conclusion, regarding the scarcity of effective treatments for NAFLD and since the nano-curcumin was safe and well-tolerated in this study and affected diverse contributing factors in NFALD, it could be nominated as a potential therapeutic target for patients with NFLAD.

Keywords: NAFLD; Nano-Curcumin; Liver Enzyme; Inflammation; Expression

Introduction

A sedentary lifestyle has become widespread in many industrialized and even underdeveloped countries, leading to hypertension, diabetes, cardiovascular diseases, and obesity [1]. Body fat increases with aging, especially in women; simultaneously, lean body mass decreases [2]. Several factors such as genetic background, alcohol consumption, drugs and hormones, and inactivity can lead to obesity [3].

Nonalcoholic fatty liver disease is one of the most common chronic liver disorders globally, affecting most obese, inactive, and type 2 diabetics [4]. The condition is characterized by elevated triglycerides, liver enzymes, some inflammatory biomarkers, and the degree of hepatic steatosis [5]. Nonalcoholic fatty liver disease comprises a wide range of liver disorders, including simple steatosis, fibrosis, and cirrhosis of the liver; if left untreated, it can eventually lead to hepatocellular carcinoma and death [6]. Nonalcoholic fatty liver disease usually occurs due to metabolic syndrome disorders such as obesity, insulin resistance, hypertension, dyslipidemia, and impaired adipose metabolism. Also, it increases the risk of death from cardiovascular diseases [5,7]. The prevalence of nonalcoholic fatty liver is 24.5% in normal people, 67% in overweight people and 94% in obese people [8].

Nonalcoholic fatty liver disease pathogenesis is closely associated with obesity and insulin resistance. Obesity and insulin resistance increase lipolysis in adipose tissue and the flow of free fatty acids to the liver, paving the way for increased inflammation in the liver [9]. Another part of the disease's pathogenesis is impaired mitochondrial function, which is followed by increased oxidative stress, cytokine levels, and pro-inflammatory factors that provide the basis for liver tissue damage [10]. Although no definitive treatment has yet been found for this disease, researchers have suggested combining an appropriate diet with physical activity to prevent and treat it [11]. Recent studies have shown that diets containing antioxidants and anti-inflammatory agents such as bioactive ingredients in some medicinal plants can be effective in treating nonalcoholic fatty liver disease [12,13].

Patatin-like phospholipase domain-containing protein 3 (PNPLA3) is an essential regulator of fat in hepatocytes which hydrolyzes TG and catalyzes the transfer of unsaturated fatty acids (PUFAs) from di and triacylglycerols to phosphocholine. The elevated expression of PNPLA3 is involved in the progression of NFLAD and insulin resistance [14,15]. In this regard, it has been revealed that the down-regulation of PNPLA3 led to a reduction in hepatic fat and improved insulin sensitivity. Therefore, PNPLA3 has been known as an attractive target for treating NAFLD [16-18]. On the other hand, TM6SF2 (Transmembrane 6 Superfamily Member 2) has been known as a regulator of fat metabolism and lipid droplet accumulation in the liver. Overexpression of TM6SF2 is involved in reduced liver cell steatosis. Since lipid droplet accumulation in the hepatocytes is the hallmark of NAFLD, inhibition of TM6SF2 can be a potential therapeutic approach in NAFLD [19-21].

Cytokines play an important role in regulating inflammatory processes throughout the body, some of which can handle various functions, including inflammation, immune responses, and metabolic processes, such as insulin resistance [22,23]. Pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin 6 (IL-6) have been shown to be elevated in patients with NAFLD, while IL-4 as an anti-inflammatory cytokine has the opposite effect [22,24-26].

Curcumin (Turmeric) is an active and main ingredient of turmeric plants (flowering plants of the ginger family). It is known by the scientific name Curcuma longa, which has medicinal properties that provide many health benefits. It looks like ginger root with a more intense yellow to golden color. Of course, in addition to curcumin, other compounds such as desmethoxycurcumin and bisdemethoxycurcumin are also present in turmeric [27,28]. Curcumin contains the most important compounds of plant origin, which is an old medicine for treating many diseases. Curcumin is used in traditional medicine in some countries such as India and Iran to treat respiratory, liver, gastrointestinal disorders, sinusitis, wound drying, and pain relief [29,30]. Numerous pharmacological effects of curcumin have been reported in clinical trials, including anti-inflammatory, antimicrobial, and treating diabetic, rheumatoid arthritis, psoriasis, Alzheimer's, and cancer diseases [31,32]. Curcumin has an anti-inflammatory effect and interferes with many molecules that are effective in inflammatory responses, including decreased cyclooxygenase II, lipoxygenase, citric oxide synthase, inhibition of inflammatory cytokines such as tumor necrosis factor-alpha and interleukins, as well as reduction of cellular kinases pathway [33,34]. The antioxidant activity and scavenging free radicals by curcumin are due to its chemical structure. Curcumin has a design and conjugated double bonds that trap and eliminate free hydroxyl radicals. In addition to the direct removal of free radicals, curcumin can increase the activity of intracellular enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, which have antioxidant roles [35-37]. A systematic study showed that curcumin/turmeric is a relatively low-cost and safe natural treatment that has a beneficial effect on NAFLD at higher doses. As adjunctive therapy, it reduces side effects from conventional drug treatment [38].

Curcumin has long been available in various forms such as powders, capsules, and tablets. Today, to enhance the solubility and strength of curcumin, nanotechnology has been used to produce curcumin. Nano-curcumin, the same as curcumin derived from the turmeric plant, is embedded in nano-lipid structures for further absorption. Curcumin is insoluble in water and acidic pH and soluble in alkaline PH. Curcumin is rapidly eliminated from the body due to water insolubility and hydrophobic properties. The nano-method curcumin formulation has solved the adsorption problem,

The basis of this formulation is that the curcumin capsule is bonded with polymer particles with a hydrophobic core and a hydrophilic shell. Nanoparticles are fabricated by Emulsification Solvent Evaporation technique with different drug to polymer ratios. In this technique, nano-micelles and nanoparticles are used, which can significantly increase the gastrointestinal absorption of the drug. This study used nano-curcumin, which can have both lipophilic and hydrophilic effects [39].

Material and Methods

In-Vivo Assessments

Study Design and Participants

Adult patients, both male and female, referring to the liver and gastrointestinal clinic of Imam Khomeini Hospital with symptoms of metabolic syndrome including waist circumference ≥ 102 cm (male) or≥ 88cm (female), blood pressure ≥ 130/ 85mmHg, triglycerides ≥ 1.7 mmol/L, high-density lipoprotein cholesterol (HDL-C) <1.03 mmol/L (males) or<1.29 mmol/L (females), and fasting blood glucose ≥ 6.1 mmol/L were screened for eligibility. Inclusion criteria were patients aged 18 years or older and evidence of NAFLD in Fibroscan (CAP > 263 dB/m). Exclusion criteria were as follow: patients with chronic liver diseases such as various types of hepatitis, chronic biliary disorders, diabetes mellitus, cancer, hereditary disorders affecting the condition of the liver (iron and copper storage diseases), untreated hypothyroidism, autoimmune diseases, taking hepatotoxic pills, such as phenytoin, tamoxifen, and lithium, taking effective medications for blood sugar and lipid levels, taking multivitamin and mineral and vitamin E supplements, undergoing weight loss surgery in the past year or strict weight-loss diets in the past three months, not being pregnant or breastfeeding, and with history of alcohol consumption. Also, being pregnant during the intervention, weight loss of more than 10% of the baseline weight, using more than 10% of the prescribed supplements at each follow-up, or unwillingness to continue cooperation led to exclusion from the study.

Sixty-six patients participated in this double-blind clinical trial study after meeting the inclusion criteria. They were divided equally into intervention and control groups using the block randomization method. Intervention allocation blinding was performed for both participants and investigators before the beginning, kept during the intervention, and opened after the data analysis by a field worker.

Intervention

At the beginning of the study (week 0), a general information questionnaire was completed for all patients. Patients in the intervention group received 80 mg of nano-curcumin (one capsule), and the other group received one capsule of placebo (starch) per day for 12 weeks. Curcumin capsules were filled with BCM-95 (BIO-CURCUMIN®), a proprietary combination of 95% curcuminoids and essential oil of turmeric-ar-turmerone. Placebo capsules were similar to the turmeric supplement in size, color, shape. Both curcumin and placebo capsules were produced by Arjuna Natural Extract, India, and labeled as A or B, so both investigators and participants were unaware of the capsules' contents. Patients were interviewed every four weeks. A 4-week supply of tablets was given to the patients at the beginning of the study and the end of the fourth and eighth weeks.

Lifestyle Modifications

Patients in both groups were recommended to follow a modified diet and physical activity according to guidelines published for overweight and obese individuals by the NIH and the North American Association for the Study of Obesity. According to this recommendation, the nutrients were distributed in such a way that less than 30% of the energy was provided from fats, 10% from saturated fatty acids (SFAs), 15% from fatty acids with a double bond (MUFAs), and 5% from polyunsaturated fatty acids (PUFAs), 15-18 percent energy from proteins, and 52-55 percent energy from carbohydrates. Also, a dietary cholesterol intake of less than 300 mg and an increase in 20 to 30 g / daily fiber intake were recommended. All patients were advised to exercise three times a week for at least 30 minutes each time.

Clinical, Para-Clinical Assessment

Weight was measured for all patients at the beginning of the study using a Squeal Scale to an accuracy of 100 g. Height was measured using a Seca scale without shoes and a precision of 0.5 m. BMI was calculated by dividing weight in kilograms to height in squared meters. Every four weeks, anthropometric measurements were performed.

Blood samples were taken from all patients with 10 to 12 hours of incontinence at week 0 and week 12 (beginning and end of the study) to perform biochemical tests. All biochemical tests were measured in a laboratory. Concentrations of liver enzymes, including ALT, AST, and GGT, were determined by colorimetry. The rate of steatosis in patients was measured using ultrasonic elastography at the beginning and end of the study. The fiber scanner's CAP test (Controlled Attenuation Parameter) was used to evaluate steatosis, and the results were reported in decibels per meter (dB / m). A nutritionist was consulted to control the effect of the turmeric diet.

Elisa Assay

For determining the effect of nano-curcumin on concentrations of IL6, TNF-alpha, and IL-4 in patients, ELISA kits were obtained from Takarabio (Kusatsu, Japan). They were performed in respect of the manufacturer protocol. The detection threshold was one pg/mg.

In-Vitro Assessments

Cell Culture and Treatment

Hepatoma cell lines are used as in vitro alternatives to primary human hepatocytes. HepG2 is a human hepatoma most commonly used in drug metabolism and hepatotoxicity studies in NAFLD in-vitro. This cell line is non-tumorigenic cells with high-proliferative activity and an epithelial-like morphology. In this regard, Hep G2 cell line was obtained from Iran Pasteur Institute (Tehran, Iran). After thawing the cells, they were grown in cell culture flasks containing RPMI culture media with 10% Fetal Bovine Serum (FBS) + 10 pen-strep (100 IU / ml penicillin and 100 mg/ml streptomycin) (Takara, Japan) and preserved inside a humid incubator containing 5% CO2. After treating Hep G2 cells with nano-curcumin, the cells were cultured for two days, and subsequent experiments then started.

RNA Extraction

According to the manufacturer's protocol, a RiboEx RNA extraction kit (Takarabio, Japan) was carried out to extract total RNA from Hep G2 cells after nano-curcumin addition. A conventional PCR was then performed to synthesize cDNAs according to the manufacturer's protocol (Exiqon, Denmark). Untreated cells were considered as the negative control.

Real-Time PCR

The real-time polymerase chain reaction was performed using a light cycler 96 (Roche, Germany) using SYBR® Premix Ex Taq™ (Takarabio, Japan) according to the manufacturer protocol to assess the expression levels at target genes in Hep G2 cells. All primer sequences shown in Table 1 were purchased from Bio fact Pharma Ltd (Korea). The mRNA level of Beta-Tubulin was used as an internal control.

Name	Sequences	
PNPLA3	Forward	5' CCTGTGGAATCTGCCATTGCGA3'
	Reverse	5' GAGCAGACACATCAGCACTCGA3'
TM6SF2	Forward	5' GGTATTTGCTGGAGCCATTGGC3'
	Reverse	5' CCAGTGCCAATAGCAGGTTGCT3'
TNF-α	Forward	5' CTCTTCTGCCTGCTGCACTTTG 3'
	Reverse	5' ATGGGCTACAGGCTTGTCACTC 3'
IL-6	Forward	5' AGACAGCCACTCACCTCTTCAG 3'
	Reverse	5' TTCTGCCAGTGCCTCTTTGCTG 3'
IL-4	Forward	5' CCGTAACAGACATCTTTGCTGCC 3'
	Reverse	5' GAGTGTCCTTCTCATGGTGGCT 3'

Table 1: Primer pairs and their related nucleotide sequences.

Western Blot

Whole proteins were extracted from Hep G2 cells using a radioimmunoprecipitation assay (RIPA) protein extraction kit (Santa Crus Biotechnology, Santa Cruz, CA, USA). In the next step, 50 μg of the extracted protein was loaded on sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to PVDF membranes using a semi-dry staining system (Bio-Rad, Inc.). The membrane was incubated with 3% BSA and 0.5% Tween-20 in PBS for 2 hours to block the membrane. The next step was to use anti-goat monoclonal antibodies to detect the target genes. Next, the membrane was incubated with a secondary rabbit anti-goat conjugated to HRP (1:5000; diluted in PBS) and kept at room temperature for 1 hour.

Finally, we used an electro-chemiluminescence (ECL) method to visualize protein strips through a Western blot imaging device. Protein concentrations in each group were measured using ImageJ software and normalized to the corresponding reference gene.

Data Analysis

The normality of the data distribution was checked through the Kolmogorov-Smirnov test. Between-group comparisons were performed using an independent sample t-test (if not every day, its nonparametric Mann-Whitney test was used). Within-group comparisons were performed using paired-samples t-test (for normally distributed data), or Wilcoxon signed ranks test (for non-normally distributed information). In the *in-vitro* assessments, all experiments were carried out in triplicate and repeated three times. Data have been expressed as the mean ± SD. P-value < 0.05 was considered statistically significant. Statistical analysis of data was performed using SPSS software version 17.

Results

Nano-Curcumin Improves Fat Metabolism and Liver Function by Affecting the Amount of TG and Cholesterol as Well as Liver Enzymes

The demographic and baseline characteristics of the study groups are illustrated in Table 2. The mean ages of the curcumin and placebo groups were 37.73±10.24 and 37.33±9.96, respectively. No significant difference was observed between the two groups in BMI, gender, LDL, TG, ALT, FBS, Fibroscan except HDL and AST at baseline. There was a significant difference between the two groups in HDL (p=0.01) and AST (p=0.01) (Table 2).

Variables	Curcumin		P-value -	Placebo		P-value -
	Before	After		Before	After	
BMI	30.15±3.23	28.06±2.6	<0.001	30.2±3.23	29.27±2.85	< 0.001
LDL	108.91±16.45	101.00±12.85	<0.001	111.88±14.06	108.15±11.56	< 0.001
HDL	35.27±3.95	35.94±3.64	NS	337.55±3.74	37.73±4.17	NS
Triglycerides	176.45±40.25	171.76±37.87	<0.001	173.85±38.8	167.73±34.78	< 0.001
ALT	40.33±9.81	32.91±7.89	<0.001	38.85±8.75	34.94±7.01	< 0.001
AST	29.55±6.73	25.33±5.09	<0.001	29.67±6.89	27.91±5.56	< 0.001
FBS	100.76±20.08	97.12±15.48	<0.001	101.30±22.14	98.67±19.32	< 0.001
Fibroscan	6.44±1.23	6.16±1.12	NS	6.02±1.60	5.81±1.45	NS

Table 2: Comparison of baseline characteristics between curcumin and placebo groups.

Within both groups, a significant decrease has been demonstrated in LDL, BMI, TG, ALT, AST, and FBS (p<0.001) after 12 weeks of intervention; however, these reductions were statistically significant ($p > 0.05$). HDL and Fibroscan were without changes within both groups (Table 3).

Between-group comparisons of curcumin and placebo groups (Table 4) revealed a significant reduction in BMI (p<0.001) and LDL level (p=0.002), ASL level (p<0.001), and AST level (p=0.001) after 12 weeks of intervention. No significant difference was observed in changes of TG level (p=0.334), HDL level (0.355), FSB level (p=0.546), and fibroscan (p=0.316).

Variables	Curcumin	Placebo	P-value
Age	37.73±10.24	37.33±9.96	0.87
BMI	30.15±3.23	30.2 ± 3.23	0.97
Gender (Male/Female)	M: 45.5	M: 42.4	1
	F: 54.5	F: 57.6	1
LDL	108.91±16.46	111.88±14.06	0.43
HDL	35.27±3.95	37.55±3.74	0.01
Triglycerides	176.45±40.25	173.85±38.28	0.79
ALT	40.33±9.82	32.91±7.89	0.51
AST	29.55±6.74	29.67±6.89	0.01
FBS	100.76±20.09	101.30±22.14	0.91
Fibroscan	6.44±1.24	6.02±1.60	0.23

Table 3: Within-group comparison of biochemical parameters between curcumin and placebo groups.

Nano-Curcumin Could Modify the Production of Pro-And Anti-Inflammatory Cytokines in Patients

The results of ELISA showed that the use of nano-curcumin reduced the production of TNF-α and IL-6 (**** and ***) as pro-inflammatory cytokines. In contrast, the production of IL-4 had an increasing pattern. (Figure 1; * P <0.05, *** P <0.001, **** P <0.0001)

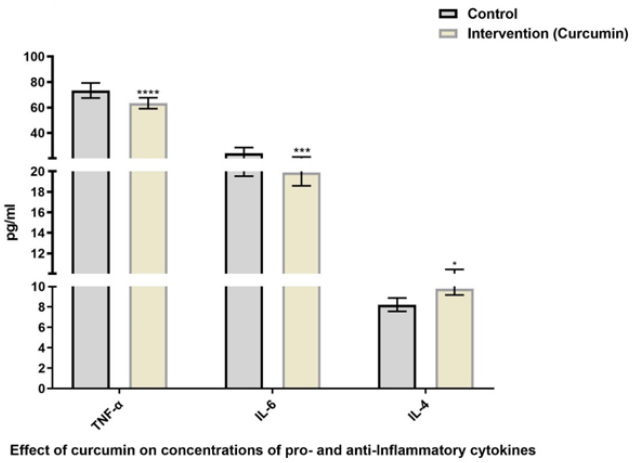

Figure 1: Effects of Nano-Curcumin on pro-and Anti-Inflammatory Cytokines Production.

It can be seen from this figure that nano-curcumin reduces the concentration of IL-6 and TNF-α in comparison with placebo-received patients while IL-4 has an increasing pattern. *P<0.05, ***P<0.001, and ****P<0.0001.

Expression of PNPLA3 and TM6SF2 Along with pro- and Anti-Inflammatory Cytokines were Regulated by Nano-Curcumin in Hep G2 Cells

Real-time PCR and Western blotting results showed that adding nano-curcumin to Hep G2 cells reduced PNPLA3, TNF-α, and IL-6 at both mRNA and protein levels as NFLAD-promoting agents. At the same time, TM6SF2 and IL-4 were upregulated as a reducer for lipid droplets accumulation and initiating of NFLAD. (Figures 2 and 3; * P <0.05, ** P <0.01, *** P <0.001, **** P <0.0001).

Figure 2 and 3: Effects of Nano-Curcumin on Aimed Targets Expression. These figures show that nano-curcumin reduces mRNA and protein expression of PNPLA3, TNF-α, and IL-6 compared to untreated cells. At the same time, it caused an augmentation of TM6SF2 and IL-4 expression at both mRNA and protein expression levels. *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001.

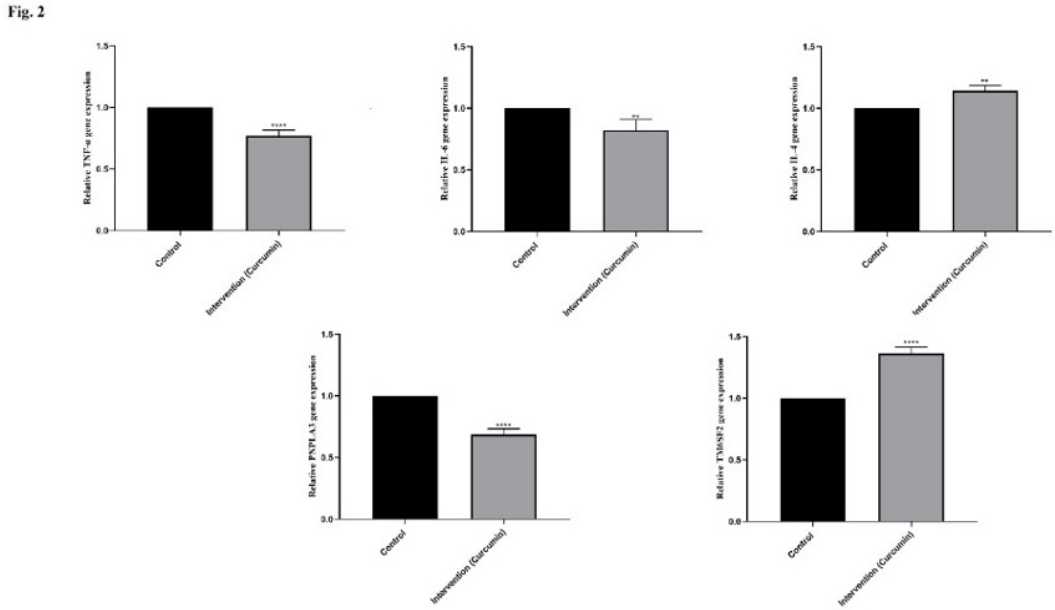

Figure 2: Effects of nano-curcumin on aimed targets expression.

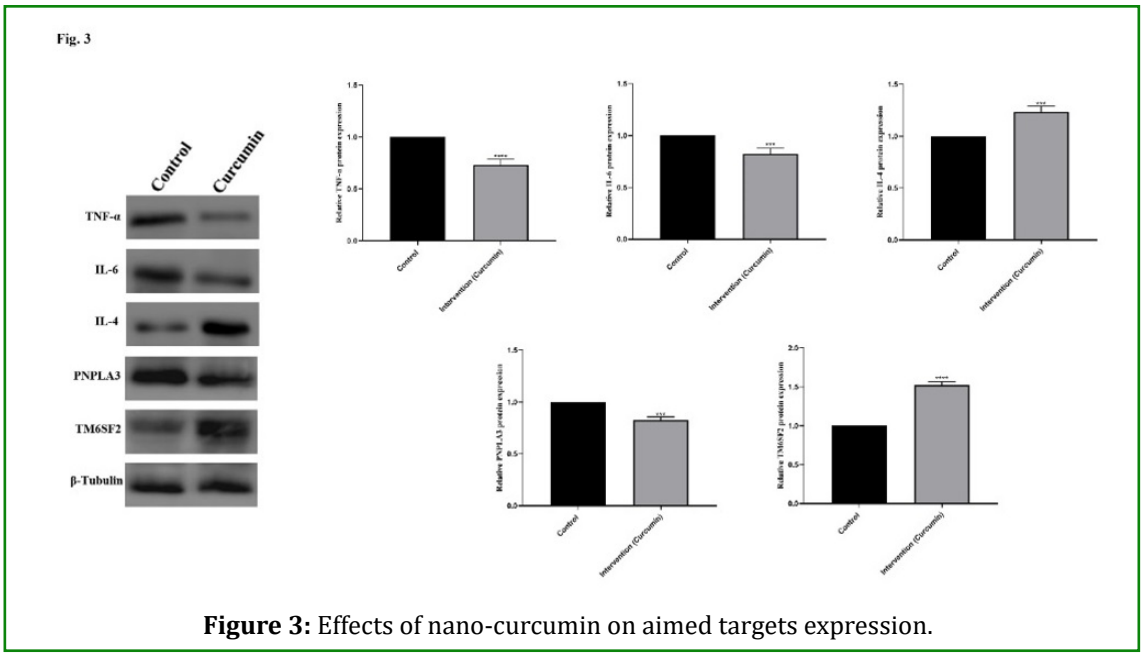

Figure 3: Effects of nano-curcumin on aimed targets expression.

Discussion

The present study's findings suggested that supplementation with nano-curcumin significantly affected BMI, LDL level, AST level, and ALT level in patients with NAFLD. In contrast, it did not impact triglyceride levels, HDL levels, FBS levels, and hepatic fibroscan outcomes in these patients. To the authors' best knowledge, this is the first randomized, double-blind, placebo-controlled clinical trial assessing the effects of nano-curcumin supplementation in NAFLD patients using Fibroscan.

These findings agree with a study indicating that curcumin regulates diet-induced hepatic steatosis by activating AMP-activated protein kinase, which causes the prevention of gradual gathering of hepatic lipid [40]. Another study, conducted by Panahi et al., investigated the efficacy and safety of phagosomal curcumin in patients with NAFLD. They presented short-term curcumin supplementation improved sonographic findings of NAFLD and hepatic transaminase levels. The result showed a significant difference between the curcumin and placebo groups in BMI reduction, LDL level, ASL level, and ALT level, which is entirely consistent with the present study [41]. In the same line with our research, in a randomized placebo-controlled trial, patients with NAFLD were randomly divided into two groups treated with curcumin and placebo for eight days based on ultrasound evidence. They confirmed the efficacy of curcumin supplementation in improving lipid and glycemic profile and reducing the liver fat content of subjects with NAFLD [42].

Furthermore, in another similar study conducted by Saadati et al., the effects of curcumin supplementation on liver enzymes, lipid profile, glucose homeostasis, hepatic steatosis, and fibrosis were investigated in patients with NAFLD. According to the study results, anthropometric indices in both groups decreased significantly, without any significant difference between the two groups, which is also entirely consistent with our results [43]. IN addition, Navekar et al. conducted a study to present turmeric supplementation effects on serum glucose indices and leptin levels in patients with NAFLD. They showed that turmeric supplementation was associated with a significant decrease in the weight and BMI of subjects compared to baseline values; however, the differences were not substantial [43]. Another study revealed that the difference between the curcumin group and placebo group was significant in triglyceride levels. A significant difference in hypoglycemia between curcumin and placebo groups was reported, demonstrating alteration in FBS levels [42].

Additional to NAFLD, curcumin has been presented in several experimental studies to increase the liver activity of Acyl-CoA oxidase and blunt lipid peroxidation through enhancing hepatic glutathione levels and glutathione reductase activities [44].

Several studies have debated curcumin's antioxidant and anti-inflammatory properties [45-47]. According to these studies, curcumin decreases the concentration of malondialdehyde (MDA) in serum, C-reactive protein, and lipid peroxidation products. Curcumin was also revealed to be involved in increasing the total antioxidant capacity (TAC) [48,49]. In line with our study, it was shown that the production and expression of IL6 and TNF-alpha have been attenuated by curcumin [50,51] while IL-4 has been increased [52,53].

Since it has been revealed that increased PNPLA3 is involved in the development of NFLAD and insulin resistance, its inhibition has been shown to be effective in this regard [54]. Therefore, in conjugation with our study, curcumin is involved in improved glucose Intolerance and NAFLD through regulating PNPLA3 by approximately 56% reduction of its expression [55]. TM6SF2, on the other hand, governs liver fat metabolism and hepatic lipid droplet, which is decreased in NFLAD, so its augmentation should be effective in this regard [20,56]. There is no study revealing the effect of curcumin on the expression of TM6SF2, indicating it is the first time that this axis has been identified.

The therapeutic ability of curcumin for treating hepatic disorders was the subject of several studies. According to the results of these studies, the molecular mechanism of the hepatoprotective action of curcumin is due to its antioxidant features and hindering activity against various pro-inflammatory and profibrotic cytokines [38,57,58].

Conclusion

In conclusion, the findings of this study confirmed the effect of nano-curcumin supplementation in improving BMI and liver enzyme levels in patients with NAFLD. This study showed that low-dose turmeric in the form of nano (which can be the strength of this study) can effectively improve the condition of patients with fatty liver and can be used in the long term as a supplement to exercise and diet modification. It was also revealed that the production and expression of cytokines are modified by nano-curcumin. Interestingly, the expression of two central genes (PNPLA3 and TM6SF2) contributing to the pathogenesis of NFLAD was altered by nano-curcumin. Besides, curcumin was safe and well-tolerated in this trial. Numerous studies confirmed the safety and tolerability of curcumin [41]. Doses between 4000 mg/day are tolerated without any serious adverse event [59]. As a final remark, due to the several pleiotropic actions, the safety of curcumin, and the scarcity of effective treatments for NAFLD, its application in patients with NAFLD may be a potential therapeutic approach.

Ethics Approval and Consent to Participate

The ethics committee of Urmia University of Medical Sciences approved this double-blind, randomized placebo-controlled clinical trial as IR. UMSU.REC.1398.389. No additional costs were imposed on patients, and their identities were not disclosed. Written informed consent was obtained from patients before participation in the study.

Consent for Publication

All authors and institutions have confirmed this manuscript for publication.

Availability of Data and Materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Competing Interests

None.

Funding Information

None.

Credit Author's Contributions

Mohammad Reza Pashaei: Conceptualization, Writing - original draft preparation.
Nilufar Abbasi and Kamran Shateri: Methodology, Visualization, Investigation.
Amir Tahavori: Conceptualization, Formal Analysis, Writing - review & editing, Supervision.

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