Complementary Therapies in Medicine 51 (2020) 102428

Contents lists available at ScienceDirect

Complementary Therapies in Medicine

journal homepage: www.elsevier.com/locate/ctim

Effects of garlic powder supplementation on insulin resistance, oxidative stress, and body composition in patients with non-alcoholic fatty liver disease: A randomized controlled clinical trial

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ARTICLE INFO

Keywords:
Non-alcoholic fatty liver disease
Garlic
Insulin resistance
Oxidative stress
Body composition

ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide. Insulin resistance, oxidative stress, and obesity are major contributors to NAFLD pathogenesis. The effects of garlic powder supplementation on these risk factors in patients with NAFLD was investigated.
Methods: In this 12-wk, randomized controlled clinical trial, ninety patients with NAFLD were randomly assigned to two groups. The treatment group received four tablets of garlic (each coated tablet contained 400 mg garlic powder) daily and the control group received four tablets of placebo (each coated tablet contained 400 mg starch).
Results: A significant decrease was seen in the treatment group compared to the control group in waist circumference (P = 0.001), body fat percent (P < 0.001), serum concentration of fasting blood sugar (P = 0.01), insulin (P < 0.001), homeostatic model assessment for insulin resistance (P < 0.001), and malondialdehyde (P < 0.001), as well as significant increase in skeletal muscle mass (P = 0.002), serum concentration of superoxide dismutase (P < 0.001), and total antioxidant capacity (P < 0.001).
Conclusion: Garlic powder supplementation improved risk factors of NAFLD. Further studies are needed to determine the effects of garlic on hepatic features in patients with NAFLD. The study protocol was registered at Iranian clinical trials website under code IRCT20170206032417N4.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) refers to excessive fat accumulation in the liver (more than 5% of the liver weight) of non-alcoholic individuals and comprises a range from simple steatosis, which is a simple accumulation of fat without substantial inflammation or hepatocellular injury, to non-alcoholic steatohepatitis (NASH), which is an accumulation of fat along with inflammation and evidence of hepatocellular injury. Moreover, it can ultimately lead to fibrosis, cirrhosis, and hepatocellular carcinoma.[1] Currently, the global prevalence of NAFLD, as the most common chronic liver disease worldwide, is nearly 25 %[2]; however, its prevalence in Iran is reported to be as high as 33.9 %.[3] The global death rate of NAFLD population in 2015 was estimated to be 1.27 million.[4] Research findings suggest that obesity and insulin resistance are the most important risk factors associated with NAFLD pathogenesis and are contributed to the development of fat accumulation in the liver by disrupting the balance between lipid and glucose metabolism.[5,6] Furthermore, oxidative stress plays an important role in the pathogenesis of NAFLD by increasing the production of reactive oxygen species (ROS) and subsequently stimulating lipid peroxidation and producing malondialdehyde (MDA).[7] Also, adherence to a Western dietary pattern with a high-fat, high-protein, and high-fructose content can induce NAFLD by dysbiosis in the intestinal microbiota through increasing the proportion of Gram-negative to Gram-positive bacteria, overproducing lipopolysaccharide, and consequently increasing the hepatic inflammation.[8] Diet modification and weight loss are considered as important components of the main line of NAFLD treatment.[9,10]

For centuries, garlic (Allium sativum L.) has been known as a herbal medicine and is still used as a traditional medicine in various cultures.[11]

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https://doi.org/10.1016/j.ctim.2020.102428
Received 23 January 2020; Received in revised form 9 April 2020; Accepted 4 May 2020
Available online 11 May 2020
0965-2299/

The beneficial effects of garlic are attributed to its bioactive compounds including allicin, S-allylcysteine (SAC), ajoene, diallyl disulfide, SAC sulfoxide, and S-methylcysteine sulfoxide.[12,13] Strong scientific evidence indicate that garlic consumption could reduce blood pressure, prevent atherosclerosis, inhibit platelet aggregation, and increase the fibrinolytic activity. [14] As a result, the antioxidant effects of garlic have been confirmed.[14,15] Likewise, experimental studies have shown that garlic has beneficial effects on obesity and insulin resistance.[16–18] However, the effects of garlic on two major risk factors of NAFLD pathogenesis in the human population, that is, oxidative stress and insulin resistance, are still unclear. To the best of our knowledge, no clinical trial to date has investigated the effects of garlic consumption on oxidative stress and insulin resistance as major contributors to NAFLD pathogenesis. The trial conducted by Soleimani et al.[19] is the only clinical trial conducted to investigate the effect of garlic supplementation on obesity as another important factor in the pathogenesis of NAFLD. Accordingly, this clinical trial was designed to evaluate the effects of garlic powder supplementation on oxidative stress, insulin resistance, and body composition among patients with NAFLD.

2. Methods

2.1. Recruitment and eligibility screening

From August 2018 to November 2018, 110 patients with NAFLD were identified and screened at Imam Khomeini educational and medical center in Urmia, Iran. NAFLD was diagnosed by ultrasound on the basis of steatosis. The inclusion criteria included patients with grade 1–3 fatty liver and aged ≥18 y. The exclusion criteria, on the other hand, ruled out those individuals with viral hepatitis, liver cancer, other liver diseases, diabetes mellitus, untreated hypothyroidism, mental diseases, kidney diseases, pregnancy, lactation, low blood pressure, allergy to garlic, and those who were taking blood pressure lowering medications or were unwilling to participate in the study. Eventually, 90 patients above the age of 18 were included in the study.

2.2. Trial design

This 12-week, double-blind, randomized controlled clinical trial was conducted from December 2018 to March 2019. The risks and benefits of the study were explained to the participants and they were asked to sign a written consent approved by the ethics committee in Urmia University of Medical Sciences. Moreover, participants were told they were free to drop out at any time for any reason. The study protocol was registered at Iranian clinical trials website (http://www.irct.ir) under code IRCT20170206032417N4. At the beginning of the study, patients were randomly assigned into the two groups of treatment and control by applying a simple randomization sampling method. Randomization lists were computer-generated by a statistician. Next, the participants were assigned to the intervention or control group. The treatment group received 400 mg of garlic powder tablets (each coated tablet contained 1.5 mg allicin and approximately equaled two grams of fresh garlic) four times a day, and the control group received four placebos (coated tablets containing starch) on a daily basis. Medical treatment for all patients was the recommendation to lose weight without offering or following any certain method for this purpose. The patients, researcher, laboratory staff, and statistician were blinded to the intervention assignment until the end of the study.

2.3. Intervention

The participants were provided with garlic and placebo tablets once every three weeks. The dose of garlic powder was determined based on the effective dose mentioned in the study conducted by Lawson et al.[20] Because garlic bioavailability decreases after protein intake,[20] participants were asked to consume two tablets an hour before lunch and two tablets an hour before dinner. Garlic and placebo tablets were produced in Amin Pharmaceuticals Co., Isfahan, Iran. The appearance (shape, color, and odor) of the placebo tablets was similar to that of garlic powder tablets. The consumption of garlic powder and placebo tablets by each participant was checked once every three weeks, and when the consumed amount was estimated to be less than 80 % of the expected amount, the patient was excluded.

2.4. Dietary intake and physical activity assessment

A 3-day (one weekend day and two nonconsecutive weekdays) 24-h recall questionnaire was used to assess the average intake of food groups' servings including dairy, meats, grains, vegetables, fruits, fats, sugars. Moreover, the average consumption of garlic was specifically evaluated at weeks 0, 6, and 12. Furthermore, the patients' physical activity was assessed using the metabolic equivalent of task (MET) questionnaire at weeks 0, 6, and 12.

2.5. Laboratory evaluations

Laboratory tests were performed at weeks 0, and 12 to determine the serum total antioxidant capacity (TAC), MDA, superoxide dismutase (SOD), and insulin. Fasting blood sugar (FBS) concentration was measured at weeks 0, 6, and 12 as well. Blood was drawn after 12 h of fasting and was centrifuged for 10 min at a speed of 3600 rpm. Serum samples were evenly poured into the microtubes and were immediately frozen at -80 °C. Moreover, TAC, MDA, SOD, and insulin were measured employing the ELISA method (Stat Fax 4200; Microplate Reader, USA) and using commercial kits (Navand salamat, Urmia, Iran for TAC, MDA, and SOD; Q-1-DiaPlus, USA for Insulin). In addition, FBS was measured with routine enzymatic assays and with commercial kits (Pars Azmoon, Iran), using an auto-analyzer (AVIDA 1800 chemistry system; Siemens, United Kingdom). All laboratory assessments were performed in the laboratory of Nutrition Department applying the standard laboratory methods.

2.6. Glucose homeostasis

Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was employed to assess the insulin resistance using the following formula: HOMA-IR = [fasting insulin (mU/L) × fasting blood glucose (mg/dL)] / 405.

2.7. Anthropometric assessment

The height, weight, waist circumference (WC), body fat percentage (BFP), and skeletal muscle mass (SMM) were measured utilizing the stadiometer, scale, and bioelectrical impedance analyzer (BIA) (In Body 770, Korea) based on standard protocols at weeks 0, 6, and 12, with the patients wearing light clothes and no shoes. Then, the body mass index (BMI) of each patient was calculated using the following formula: weight (kg) / height (m) ^{2}.

2.8. Statistical analyses

With α = 0.05, power = 95 %, and based on a study conducted by Ashraf et al.,[21] the needed sample size was assessed to be 37 per study group.[27]. However, considering a drop-out rate of ∼20 %, the final needed sample size was estimated to be 45 per group. In order to eliminate the effects of confounding factors, differences in general characteristics and dietary intakes between the two groups at the baseline were compared conducting an independent *t*-test (for continuous variables), and chi-square (for categorical variables). To determine the effect of garlic powder and the placebo on serum concentration of FBS, and anthropometric variables, a general linear model of ANOVA for repeated measurements was applied. In these analyses,

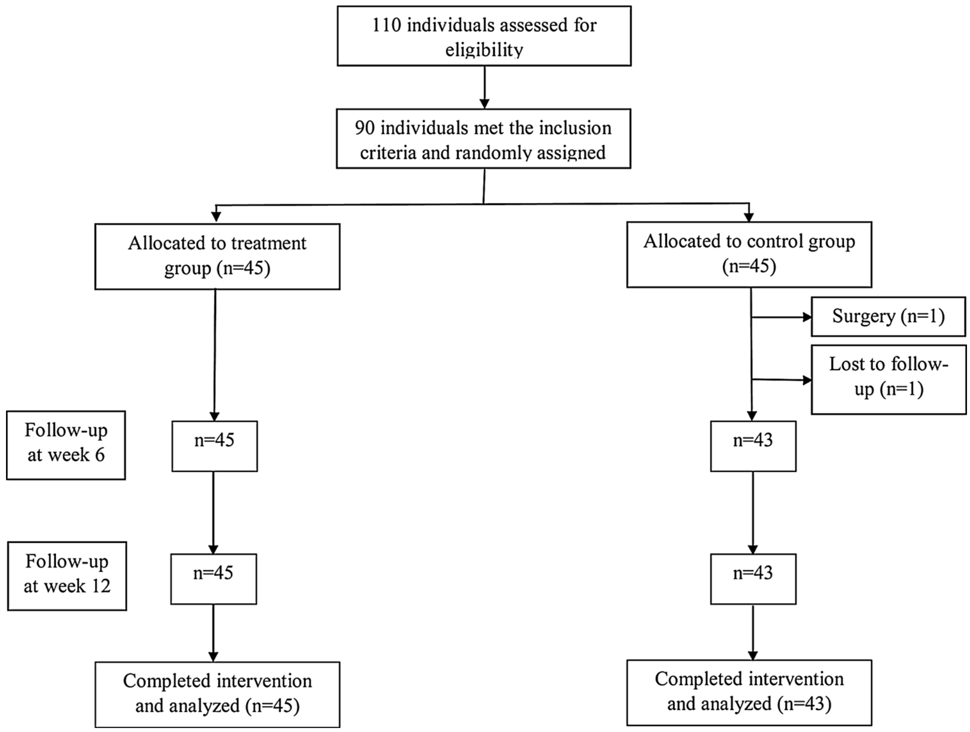

Fig. 1. Flow diagram of the study participants.

the groups were regarded as between-subject factors, and time was considered as a within-subject factor. Univariate ANCOVA was also carried out to compare the final values of HOMA-IR and serum concentrations of insulin and oxidative stress markers for controlling baseline values. Data were analyzed by running SPSS version 24 (SPSS, Inc.). For all analyses, $P < 0.05$ was considered as being significant, and all the data were shown as means ± SDs, if not indicated otherwise.

3. Results

3.1. Characteristics of the patients

A total of 90 participants were randomly assigned into the two groups. After randomization, two participants were excluded; due to surgery (n = 1), and lost to follow-up (n = 1). Finally, 88 participants completed the trial in the treatment (n = 45) and control groups (n = 43) (Fig. 1).

The results of the study showed that there were no significant differences between the two groups in terms of baseline demographic characteristics, except for the FBS. The control group had a higher FBS serum concentration than the treatment group (Table 1). During the study, there was no significant difference observed between the two groups in terms of physical activity. Moreover, based on the 3-day, 24-h food recalls, no significant differences in the mean intake of energy, food groups and garlic were seen between the two groups during this trial. Lastly, there was no significant difference observed between the two groups regarding the intake of drugs and supplements.

3.2. Outcomes

It was observed that WC (P = 0.001), BFP ($P < 0.001$), and SMM (P = 0.002) decreased significantly in the treatment group, in contrast to the control group (Table 2).

In addition, there was a significant decrease in FBS (P = 0.01), insulin ($P < 0.001$), MDA ($P < 0.001$), and HOMA-IR status ($P < 0.001$) as well as a significant increase in serum concentration of

Table 1
Demographic characteristics of patients with NAFLD.

	Treatment group (n = 45)	control group (n = 43)	*P*
Age, y	45.2 ± 12.4	44.2 ± 11.1	0.68
Sex, n (%)			0.08
Male	33 (73.3)	24 (55.8)	
Female	12 (26.7)	19 (44.2)	
Smoking status, n (%)			0.31
Current smoker	16 (35.6)	11 (25.6)	
Nonsmoker	29 (64.4)	32 (74.4)	
MET-h/d	28.8 ± 3.8	27.8 ± 3.6	0.23
Energy intake, kcal/d	2041 ± 123	2034 ± 136	0.80
ALT, IU/L	30.9 ± 15.7	24.9 ± 15.4	0.07
AST, IU/L	22.8 ± 11.1	21.2 ± 7.3	0.41
Glycemic indices			
FBS, mg/dL	90.1 ± 7.8	94.8 ± 11.3	0.02
insulin, mU/L	8.5 ± 2.7	8.3 ± 2.5	0.74
HOMA-IR	1.88 ± 0.6	1.94 ± 0.6	0.66
Serum OS markers			
TAC, nmol/mL	2.19 ± 0.44	2.26 ± 0.35	0.46
SOD, U/mL	521.83 ± 126.6	535.99 ± 156.1	0.64
MDA, nmol/mL	1.73 ± 0.38	1.71 ± 0.57	0.90
Anthropometric Indices			
Height, cm	172.2 ± 9.8	168.8 ± 11.0	0.13
Weight, kg	89.8 ± 11.9	90.0 ± 13.6	0.95
BMI, kg/m^2	30.2 ± 3.1	31.6 ± 3.8	0.08
WC, cm	105.6 ± 9.8	108.4 ± 10.4	0.19
BFP, %	33.6 ± 7.0	38.2 ± 8.1	0.006
SMM, kg	33.6 ± 6.1	31.2 ± 7.4	0.11

Data are expressed as mean ± standard deviation. P values were computed by independent *t*-test for continuous variables and by chi-square for categorical variables. MET-h: metabolic equivalent task hours; ALT: alanine transaminase; AST: aspartate transaminase; FBS: fasting blood sugar; HOMA-IR: homeostatic model assessment for insulin resistance; OS: oxidative stress; TAC: total antioxidant capacity; SOD: superoxide dismutase; MDA: malondialdehyde; BMI: body mass index; WC: waist circumference; BFP: body fat percent; SMM: skeletal muscle mass.

Table 2
Effects of garlic powder on anthropometric variables of patients with NAFLD*.

Anthropometric variables	Treatment group (n = 45)	control group (n = 43)	P_{Time}	P_{Group}	$P_{Time \times Group}$
Weight, kg			0.02	0.42	0.86
Week 0	89.8 ± 11.9	90.0 ± 13.6			
Week 6	89.5 ± 11.8	90.0 ± 13.6			
Week 12	89.2 ± 11.8	89.8 ± 13.4			
Mean difference	−0.6 ± 1.2	−0.1 ± 1.5			
BMI, kg/m^2			0.02	0.06	0.12
Week 0	30.2 ± 3.1	31.6 ± 3.8			
Week 6	30.1 ± 3.1	31.6 ± 3.8			
Week 12	30.0 ± 3.1	31.5 ± 3.8			
Mean difference	−0.19 ± 0.4	−0.03 ± 0.5			
WC, cm			0.001	0.008	0.001
Week 0	105.6 ± 9.8	108.4 ± 10.4			
Week 6	104.9 ± 9.7	108.9 ± 10.9			
Week 12	104.0 ± 9.7	108.4 ± 10.8			
Mean difference	−1.58 ± 2.1	−0.03 ± 1.9			
BFP, %			< 0.001	0.002	< 0.001
Week 0	33.6 ± 7.0	38.2 ± 8.1			
Week 6	33.3 ± 7.1	38.4 ± 8.0			
Week 12	32.5 ± 7.3	38.1 ± 7.9			
Mean difference	−1.08 ± 1.1	−0.10 ± 1.1			
SMM, kg			0.08	0.05	0.002
Week 0	33.6 ± 6.1	31.2 ± 7.4			
Week 6	33.7 ± 6.2	30.8 ± 7.2			
Week 12	34.1 ± 6.3	31.0 ± 7.2			
Mean difference	+ 0.5 ± 1.1	−0.2 ± 1.0			

*Data are expressed as mean ± standard deviation (SD).
P: P values were computed by general linear model ANOVA for repeated measurements.
NAFLD: non-alcoholic fatty liver disease; BMI: body mass index; WC: waist circumference; BFP: body fat percent; SMM: skeletal muscle mass.

TAC ($P < 0.001$), and SOD ($P < 0.001$) in the treatment group compared to the control group (Table 3), and finally, there was no significant difference found between the two groups in their weight and BMI at the end of the study.

4. Discussion

To the best of our knowledge, this study was the first clinical trial to investigate the effects of garlic powder supplementation on insulin resistance and oxidative stress in patients with NAFLD. Garlic is a part of the Mediterranean diet, and adherence to the this diet which is based on the regular consumption of olive oil, plant foods, the moderate consumption of fish, seafood, and dairy, and low alcohol (mostly red wine) intake, balanced by a comparatively limited use of red meat and other meat products, has beneficial effects in the management of NAFLD.[22] We demonstrated that supplementation with garlic powder for 12 weeks led to improved insulin resistance, oxidative stress, and body composition indices. Insulin resistance, as an important risk factor, becomes involved in the pathogenesis of NAFLD through mechanisms such as altering the production of adipokines, increasing lipolysis and subsequent delivery of free fatty acids to the liver.[23] A possible mechanism through which garlic can improve insulin resistance could be the regulation of lipogenesis by reducing the activation of enzymes involved in the formation of adipose in the liver and increasing the adiponectin levels.[24,25] Consistent with our findings, Liu et al.[25] showed improvement of HOMA-IR in diabetic rats consuming garlic oil and diallyl trisulfide. In addition, in line with our study, Choudhary et al.[26] as well as Ashraf et al.[27] observed that garlic consumption led to the improvement of FBS among individuals with metabolic syndrome and those with diabetes, respectively.

Reducing mitochondrial dysfunction, decreasing kupffer cells activation, decreasing gene expression of oxidative stress indices, and increasing gene expression of antioxidant indices are potential mechanisms through which garlic can improve oxidative stress.[28,29] Consistent with the results of the present study, Koseoglu et al.[30] showed an improvement in TAC during garlic powder supplementation in healthy subjects. Moreover, Ziamajidi et al.[15] observed a significant decrease in MDA and total oxidant status after treatment of diabetic rats with garlic.

Obesity is one of the most important factors involved in the NAFLD pathogenesis. We found that garlic powder supplementation reduced WC, BFP, and increased SMM without affecting the weight. It should be noted that reducing WC without losing weight can be an indication of the improvement in central obesity, which is considered an important risk factor for NAFLD pathogenesis.[31] Possible mechanisms of garlic affecting obesity are reduction of intestinal absorption of triglycerides, inhibition of PPAR gamma gene expression, and consequently suppression of human preadipocyte differentiation and lipid accumulation in differentiated preadipocyte, as well as the thermogenic effects of garlic.[16,32,33] Very few clinical trials, however, have examined the effect of garlic powder supplementation on body composition. In a 15-week study by Soleimani et al.,[19] garlic powder supplementation improved obesity indices such as weight and fat mass in patients with NAFLD; nonetheless, no significant weight loss was observed in our study. A logical reason for this contrast may be the difference in intervention duration; as in our 12-week study we observed a trend in weight loss; however, it was not significant. Consistent with our results including a significant decrease in the BFP, Joo et al.[16] found that garlic supplementation in rats fed a high-fat diet reduced adipose tissue mass; demonstrating the beneficial effects of garlic on body fat mass. It should be noted that no serious adverse effects were reported throughout the study.

Our study has strengths such as having a high compliance rate (more than 97 %) and checking dietary garlic consumption.

One of the limitations of the study is the use of liver ultrasonography due to its low cost, noninvasiveness, and availability, and this is despite the fact that biopsy is known as the gold standard in liver steatosis assessment. So it was possible that some patients with NAFLD are missed in sampling of NAFLD patients (false negative diagnosis), although possible of false positive diagnosis was very low.[34] Another limitation of the study is using BIA, which is not the gold standard for body composition measurement. Finally, euglycemic clamp technique

Table 3
Effects of garlic powder on serum levels of glycemic and oxidative stress indices*.

	Treatment group (n = 45)	control group (n = 43)	$P^{\dagger}$	$P^{\dagger\dagger}$
glycemic indices				
FBS, mg/dL			0.01[a]	0.02[a]
Week 0	90.1 ± 7.8	94.8 ± 11.3		
Week 6	89.2 ± 7.7	97.8 ± 9.7		
Week 12	86.9 ± 8.2	95.7 ± 10.5		
Mean difference	−3.1 ± 6.8	−0.8 ± 7.9		
Insulin, mU/L			< 0.001	0.001
Week 0	8.5 ± 2.7	8.3 ± 2.5		
Week 12	5.6 ± 2.5	7.1 ± 2.8		
Mean difference	−2.8 ± 2.5	−1.1 ± 1.5		
HOMA-IR			< 0.001	< 0.001
Week 0	1.88 ± 0.6	1.94 ± 0.6		
Week 12	1.21 ± 0.5	1.71 ± 0.7		
Mean difference	−0.6 ± 0.5	−0.2 ± 0.4		
Oxidative stress indices				
TAC, nmol/mL			< 0.001	< 0.001
Week 0	2.19 ± 0.44	2.26 ± 0.35		
Week 12	2.25 ± 0.39	1.99 ± 0.29		
Mean difference	+ 0.05 ± 0.2	−2.26 ± 0.2		
SOD, U/mL			< 0.001	< 0.001
Week 0	521.83 ± 126.6	535.99 ± 156.1		
Week 12	554.99 ± 118.0	506.77 ± 123.5		
Mean difference	+ 33.1 ± 36.5	−29.2 ± 79.4		
MDA, nmol/mL			< 0.001	< 0.001
Week 0	1.73 ± 0.38	1.71 ± 0.57		
Week 12	1.42 ± 0.37	1.72 ± 0.63		
Mean difference	−0.3 ± 0.2	0.0 ± 0.4		

*Data are expressed as mean ± standard deviation (SD).
†P values were computed by general linear model ANOVA for repeated measurements in FBS and by univariate ANCOVA in the rest of variables after controlling baseline values.
†† Adjusted based on mean changes of WC and baseline values of parameters.
[a] *P* value represent the effect of time × group interaction.
NAFLD: non-alcoholic fatty liver disease; FBS: fasting blood sugar; HOMA-IR: homeostatic model assessment for insulin resistance; TAC: total antioxidant capacity; SOD: superoxide dismutase; MDA: malondialdehyde; WC: waist circumferences.

or intravenous glucose tolerance testing could measure insulin sensitivity more accurately.[35]

This 12-week clinical trial, demonstrated that garlic powder supplementation led to improve insulin resistance, oxidative stress, and body composition. According to these results, further studies are needed to determine the effect of garlic powder supplementation on hepatic features in patients with NAFLD.

Funding

This trial supported by the Urmia University of Medical Sciences.

Author statement

The authors' responsibilities were as follows—Abbas Ali Sangouni and Mohammad Alizadeh: conceived and designed the study and analyzed the data; Mohammad Reza Mohammad Hosseini Azar: provided material and technical support, Abbas Ali Sangouni: wrote the manuscript; Mohammad Alizadeh: critically revised the manuscript for important intellectual content; Mohammad Alizadeh: had primary responsibility; and all authors: read and approved the final manuscript.

Declaration of Competing Interest

The authors have declared no conflict of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ctim.2020.102428.

References

1. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: Practice guideline by the american association for the study of liver diseases, american college of gastroenterology, and the American Gastroenterological Association. *Hepatology.* 2012;55(6):2005–2023.
2. Araújo AR, Rosso N, Bedogni G, Tiribelli C, Bellentani S. Global epidemiology of non-alcoholic fatty liver disease/non-alcoholic steatohepatitis: what we need in the future. *Liver Int.* 2018;38(Suppl 1):47–51.
3. Moghaddasifar I, Lankarani KB, Moosazadeh M, et al. Prevalence of non-alcoholic fatty liver disease and its related factors in Iran. *Int J Organ Transplant Med.* 2016;7(3):149–160.
4. Estes C, Razavi H, Loomba R, Younossi Z, Sanyal AJ. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. *Hepatology.* 2018;67(1):123–133.
5. Pagano G, Pacini G, Musso G, et al. Nonalcoholic steatohepatitis, insulin resistance, and metabolic syndrome: Further evidence for an etiologic association. *Hepatology.* 2002;35(2):367–372.
6. Polyzos SA, Kountouras J, Mantzoros CS. Adipose tissue, obesity and non-alcoholic fatty liver disease. *Minerva Endocrinol.* 2017;42(2):92–108.
7. Rolo AP, Teodoro JS, Palmeira CM. Role of oxidative stress in the pathogenesis of nonalcoholic steatohepatitis. *Free Radic Biol Med.* 2012;52(1):59–69.
8. Abenavoli L, Scarpellini E, Rouabhia S, Balsano C, Luzza F. Probiotics in non-alcoholic fatty liver disease: Which and when. *Ann Hepatol.* 2013;12(3):357–363.
9. Zelber-Sagi S, Godos J, Salomone F. Lifestyle changes for the treatment of nonalcoholic fatty liver disease: A review of observational studies and intervention trials. *Therap Adv Gastroenterol.* 2016;9(3):392–407.
10. Clark JM. Weight loss as a treatment for nonalcoholic fatty liver disease. *J J Clin Gastroenterol.* 2006;40(Suppl 1):S39–43.
11. Bayan L, Koulivand PH, Gorji A. Garlic: a review of potential therapeutic effects. *Avicenna J Phytomed.* 2014;4(1):1–14.
12. Iciek M, Kwiecień I, Włodek L. Biological properties of garlic and garlic-derived organosulfur compounds. *Environ Mol Mutagen.* 2009;50(3):247–265.
13. El-Bayoumy K, Sinha R, Pinto JT, Rivlin RS. Cancer chemoprevention by garlic and garlic-containing sulfur and selenium compounds. *J Nutr.* 2006;136(3 Suppl):864S–869S.
14. Chan JYY, Yuen ACY, Chan RYK, Chan SW. A review of the cardiovascular benefits and antioxidant properties of allicin. *Phytother Res.* 2013;27(5):637–646.
15. Ziamajidi N, Nasiri A, Abbasalipourkabir R, Moheb SS. Effects of garlic extract on TNF-α expression and oxidative stress status in the kidneys of rats with STZ + nicotinamide-induced diabetes. *Pharm Biol.* 2017;55(1):526–531.
16. Joo H, Kim CT, Kim IH, Kim Y. Anti-obesity effects of hot water extract and high hydrostatic pressure extract of garlic in rats fed a high-fat diet. *Food Chem Toxicol.* 2013;55:100–105.
17. Lee M-S-S, Kim I-H-H, Kim I-H-T, Kim Y. Reduction of body weight by dietary garlic is associated with an increase in uncoupling protein mRNA expression and activation of AMP-Activated protein kinase in diet-induced obese mice. *J Nutr.* 2011;141(11):1947–1953.
18. Padiya R, Khatua TN, Bagul PK, Kuncha M, Banerjee SK. Garlic improves insulin sensitivity and associated metabolic syndromes in fructose fed rats. *Nutr Metab.* 2011;8:53.
19. Soleimani D, Paknahad Z, Askari G, Iraj B, Feizi A. Effect of garlic powder consumption on body composition in patients with nonalcoholic fatty liver disease: A randomized, double-blind, placebo-controlled trial. *Adv Biomed Res.* 2016;5:2.
20. Lawson LD, Hunsaker SM. Allicin bioavailability and bioequivalence from garlic supplements and garlic foods. *Nutrients.* 2018;10(7).
21. Ashraf R, Aamir K, Shaikh AR, Ahmed T. Effects of garlic on dyslipidemia in patients with type 2 diabetes mellitus. *J Ayub Med Coll Abbottabad.* 2005;17(3):60–64.
22. Abenavoli L, Di Renzo L, Boccuto L, Alwardat N, Gratteri S, De Lorenzo A. Health benefits of Mediterranean diet in nonalcoholic fatty liver disease. *Expert Rev Gastroenterol Hepatol.* 2018;12:873–881.
23. Polyzos S, Kountouras J, Zavos C. Nonalcoholic fatty liver disease: The pathogenetic roles of insulin resistance and adipocytokines. *Curr Mol Med.* 2009;9(3):299–314.
24. Maeda T, Miki S, Morihara N, Kagawa Y. Aged garlic extract ameliorates fatty liver and insulin resistance and improves the gut microbiota profile in a mouse model of insulin resistance. *Exp Ther Med.* 2019;18(1):857–866.
25. Liu CT, Hse H, Lii CK, Chen PS, Sheen LY. Effects of garlic oil and diallyl trisulfide on glycemic control in diabetic rats. *Eur J Pharmacol.* 2005;516(2):165–173.
26. Choudhary PR, Jani RD, Sharma MS. Effect of raw crushed garlic (Allium sativum L.) on components of metabolic syndrome. *J Diet Suppl.* 2018;15(4):499–506.
27. Ashraf R, Khan RA, Ashraf I. Garlic (Allium sativum) supplementation with standard antidiabetic agent provides better diabetic control in type 2 diabetes patients. *Pak J Pharm Sci.* 2011;24(4):565–570.

28. Trio PZ, You S, He X, He J, Sakao K, Hou DX. Chemopreventive functions and molecular mechanisms of garlic organosulfur compounds. *Food Funct.* 2014;5(5):833–844.
29. Agarwal KC. Therapeutic actions of garlic constituents. *Med Res Rev.* 1996;16(1):111–124.
30. Koseoglu M, Isleten F, Atay A, Kaplan YC. Effects of acute and subacute garlic supplement administration on serum total antioxidant capacity and lipid parameters in healthy volunteers. *Phyther Res.* 2010;24(3):374–378.
31. Pang Q, Zhang JY, Song SD, et al. Central obesity and nonalcoholic fatty liver disease risk after adjusting for body mass index. *World J Gastroenterol.* 2015;21(5):1650–1662.
32. Keophiphath M, Priem F, Jacquemond-Collet I, Clément K, Lacasa D. 1,2-vinyldithiin from garlic inhibits differentiation and inflammation of human preadipocytes. *J Nutr.* 2009;139(11):2055–2060.
33. Ince DI, Sönmez GT, Ince ML. Effects garlic on aerobic performance. *Turk J Med Sci.* 2000;30(6):557–561.
34. Saleh HA, Abu-Rashed AH. Liver biopsy remains the gold standard for evaluation of chronic hepatitis and fibrosis. *J Gastrointestin Liver Dis.* 2007;16(4):425–426.
35. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: A method for quantifying insulin secretion and resistance. *Am J Physiol Metab.* 1979;237(3):E214–23.