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The Efficacy of Nanocurcumin Supplementation on Insulin Resistance, Lipids, Inflammatory Factors and Nesfatin among Obese Patients with Non-Alcoholic Fatty Liver Disease (NAFLD): A Trial Protocol

Running title: Nanocurcumin in NAFLD Patients: Trial Protocol

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Abstract

Objectives: Different studies have been conducted on curcumin roles in health with multiple properties including antioxidant and anti-inflammatory effects. Due to lack of studies regarding curcumin effects on obese NAFLD patients, our protocol is designed to assess the impact of nano-curcumin on blood sugar, lipids, inflammatory indexes, insulin resistance, and liver function, especially by nesfatin.

Setting: This trial will be conducted in a central hospital of Tehran, Iran. The level of care is primary.

Participants: 84 obese NAFLD patients diagnosed using ultrasonography will be employed according to the eligiblity criteria.

Interventions: The patients will be randomly divided into two equal groups with nanocurcumin or placebo supplements. Also, the lifestyle changes (low-calorie diet and physically active) will be advised. After the enrollment, intervention will begin for 3 months.

Primary and Seconary Outcome Measures: The general, 24-hr food-recall (at beginning-middle-end), and short-form international physical activity (IPAQ, at beginning and end) questionnaires will be completed. The blood pressure, anthropometrics, serum sugar (fasting blood sugar and insulin (FBS, FBI), glycosylated hemoglobin (HbA1c)), lipids (triglycerid (TG), total cholestrol (TC), low and high density lipoprotein-cholestrol (LDL-c, HDL-c)), and inflammatory profiles (interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α)), liver function (alanine and aspartate transaminase (ALT, AST)), insulin resistance and sensitivity indices, and nesfatin will be measured at the beginning and end of study.

Conclusions: This trial is the first that will determine efficacy of nanocurcumin on certain blood factors among obese patients with NAFLD. It is required to study the potential consequences of curcumin in various diseases especially NAFLD to be used clinically.

Trial registration: The Ethics Committee of Tehran University of Medical Sciences approved our protocol (IR.TUMS.REC.1395.2612). A written informed consent form will be dated by subjects in the beginning. The characteristics of trial registration at Iranian Registry of Clinical Trials (IRCT) are IRCT2016071915536N3 and 27/12/2015.

Article Summary

Strengths and limitations of this study:

- randomized double blinded design and protocol publication
- determining dietary intake and physical activity status and registering patient-reported possible problems
- selecting a single policlinic and slow recruiting of patients due to the eligibility criteria
- self-reporting of diet and physical activity status and lack of cooperation of some participants to complete the intervention

Keywords: *Nanocurcumin, Non-Alcoholic Fatty Liver Disease, Trial Protocol, Overweight, Obesity*

Introduction

Non-alcoholic fatty liver disease (NAFLD) occurs when triglyceride is deposited in liver cells (5% <) [Sherlock and Dooley, 2002]. Three degrees of NAFLD are mild (<33% fat accumulation), moderate (33-66% fat accumulation), and severe (>66% fat accumulation) [Sass et al., 2005]. The standard method of diagnosis is liver biopsy. Since biopsy is an invasive method, non-invasive diagnostic methods such as ultrasound, CT scan, and MRI are mostly used. However, exact differentiation between the disease stages by these techniques is difficult. Most of NAFLD patients are identified implicitly in medical examinations by elevated liver enzymes (Aminotransferases: ALT and AST about 1.5 to 2 times higher than normal level). According to recent studies, many patients with advanced non-alcoholic steatohepatitis (NASH) and even cirrhosis can have normal liver enzymes level [Angulo, 2002; Wei et al., 2008; Dowman et al., 2011; Harrison, 2012]. Thus, the prevalence of NAFLD is probably more than the reported one. The symptoms of NAFLD often include fatigue and discomfort in the right upper quarter of abdomen. The average of prevalence in adults is about 30 % (in obese (BMI≥25) and non-obese (BMI<25) patients nearly 65-85% and 15-20%, respectively) [Angulo, 2002; Amirkalali et al., 2014; Bagheri Lankarani et al., 2013; Karimi-Sari et al., 2015; Adibi et al., 2013]. NAFLD is more common in men. Disease pathology is a two-phase event including fat reposition in hepatocytes following hepatic steatosis and NASH. Insulin resistance has a key role in both phases, and oxidative stress and pro-inflammatory cytokines are major irritants [Sahebkar, 2011]. Common causes of macrovascular steatosis include insulin resistance, increasing blood insulin levels, central obesity, diabetes type 2, medications (glucocorticoid, estrogen, tamoxifen, amiodarone), nutrition status (starvation, protein deficiency, choline deficiency), liver diseases (Wilson's disease, chronic hepatitis C-III), Hindi child cirrhosis, and jejunum bypass [Harrison, 2012]. The liver fat content is directly related to insulin resistance. Activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) upregulates the production of pro-inflammatory cytokines that influence the insulin activity. So, inflammation, adipokines, oxidative stress, or lipid metabolites can change the insulin sensitivity, but intrahepatic fat content is not necessarily directly related to them [Fabbrini et al., 2010]. Age, family history, malnutrition, severe weight loss, gastrointestinal tract infection, certain medication, and some diseases (such as inflammatory bowel disease) are the other risk factors of NAFLD [Valenti et al., 2006; Ma et al., 2013; Lau et al., 2015]. In some studies, incidence of the disease has been

related to high intake of saturated fats or carbohydrates [Musso et al., 2003; Solga et al., 2004]. Some patients have normal weight although they may have abdominal obesity and insulin resistance [Assy et al., 2008; Yasutake et al., 2009].

Nesfatin as a secreted neuropeptide by the hypothalamus in mammals is involved in regulating appetite and body fat stores. Nesfatin gene is expressed in other locations such as brain, pancreas, stomach endocrine cells, and adipocytes. Nesfatin gene expression is activated by peroxisome proliferator-activated receptors (PPARs), especially PPARγ. Nesfatin plays an important role in glucose metabolism, phosphorylation of certain signaling proteins, and increasing insulin sensitivity in liver, particularly through AMPK (AMP-activated protein kinase) [Oh et al, 2006; Basar et al, 2012]. In a recent study, the serum nesfatin level of overweight/obese NAFLD patients (30-60 years) was found significantly lower than the healthy group [Basar et al., 2012].

The common treatment of NAFLD is lifestyle changing (gradual weight loss and increasing physical activity) that can improve the liver enzymes, fat reposition, inflammation, and fibrosis [Dixon et al., 2004; Luyckx et al., 1998; Palmer and Schaffner, 1990; Ueno et al., 1997; Shah et al., 2009]. It seems that changes of dietary ingredients can be presented as a therapy method for these patients [Zelber-Sagi et al., 2011] since losing weight and its maintenance for a long period of time is hard [Katan, 2009]. Accordingly, the assessment of relationship between NAFLD and certain nutrients or dietary ingredients is very important.

Different studies have been conducted on curcumin roles in health. Curcumin as turmeric spice (ginger family) in diet has multiple properties including antioxidant, anti-inflammatory, antimicrobial, and anti-carcinogenic effects [Chauhan et al., 2014]. Due to the importance of PPARs in metabolic pathways, numerous studies have been conducted to investigate the effects of curcumin on PPARs especially PPARγ gene expression. It increases both the activity and expression of PPARγ important in inhibiting inflammation and oxidative stress as the main factors of insulin resistance and NAFLD [Pescosolido et al., 2014].

The idea of this study is based on several properties of curcumin; it may modify the fatty liver disease. Curcumin has several molecular targets that can play important roles in pathophysiology and improvement of various diseases, especially NAFLD. Based on the relationship between progressive NAFLD and overweight/obesity and the efficacy of curcumin on blood profiles,

inflammation, liver function, serum nesfatin levels, and decreasing appetite in NAFLD patients, the proposed study is designed to evaluate the efficacy of curcumin in improving insulin resistance and other features of NAFLD, particularly via nesfatin. Despite multiple benefits of curcumin in health, its stability and bioavailability is very low, affecting its efficacy in therapy. Recently, many approaches are assessed to improve the stability and bioavailability of curcumin including the use of polymeric nanoparticles named nanocurcumin. For example, PLGA nanoparticles can increase the bioavailability of curcumin 22 times [Kamali et al., 2014]. In the proposed study, nanocurcumin will be used.

Methods

Study design

A double-blind randomized clinical trial will be conducted.

Objectives

1. Comparing the economic, occupational, marital status, and education levels of the subjects with nanocurcumin and placebo supplementation before the intervention
1. Comparing the mean of serum triglyceride, LDL-cholesterol, total cholesterol, HDL-cholesterol, fasting blood sugar (FBS), insulin (FBI), hemoglobin-A1c, insulin resistance, sensitivity indices (HOMA-IR, QUICKI), TNF-α, IL-6, hs-CRP, and nesfatin within each group and between the two groups before and after the intervention
2. Comparing the mean of weight, waist circumference, body mass index (BMI), body composition percentage, systolic and diastolic blood pressure within each group and between the two groups before and after the intervention
3. Comparing the mean of physical activity score and intake of energy, macronutrients, and micronutrients within each group and between the two groups before and after the intervention
4. Comparing the mean of age and height between the two groups before the intervention

Inclusion criteria

1. Age: 25-50 years

2. Overweight/obesity (25≤BMI<35)
3. Diagnosis of NAFLD by a radiologist based on the ultrasound test
4. The informed consent

Exclusion criteria

1. Alcohol consumption history during the last 12 months, based on person admission
2. Regular intake of nonsteroidal anti-inflammatory drugs, antibiotics, and corticosteroids during the last 6 months
3. Misuse of narcotics, psychotropic, and cigarettes over the last 6 months
4. Intake of anti-secretory drugs causing acholoridy, amiodarone, valproate, prednisone, tamoxifen, perhexiline, methotrexate, liver fat induced-drugs, hormone drugs, statins, antihypertensives, and ursodeoxycholic acid (UDCA) during the last 6 months
5. Intake of supplements such as probiotics, multivitamin-minerals, antioxidants, and omega-3 at least twice a week during the last 3 months
6. Diagnosis of pathological conditions affecting the liver such as viral hepatitis, acute or chronic liver failure, cholestasis, liver transplantation, acute systemic disease, cystic fibrosis disease, muscular dystrophy, previous surgery of gastrointestinal, neurological disorders, structural abnormalities of the gastrointestinal tract, diabetes, heart failure, thyroid disorders, kidney diseases, respiratory failure, psychological disorders, hereditary hemochromatosis, Wilson disease, alpha-1 antitrypsin deficiency, autoimmune diseases, celiac disease, and any malignancy
7. Rapid weight loss, total parenteral nutrition (TPN) history, and protein malnutrition over the last 6 months
8. Secondary causes of NAFLD such as drugs, surgical procedures, and environmental toxins
9. Conditions leading to the physical disability
10. Uncontrolled hypertension (>140/90 mmHg)
11. Breastfeeding, pregnancy, and or plan for pregnancy in the next 3 months
12. Professional athlete or regular exercise
13. Intake of multivitamin-mineral or antioxidant supplements at least twice a week during the study
14. Taking no more than 10% of prescription supplements

Subjects

Patients will be referred to a major executor, after diagnosis by a radiologist if meeting the eligibility criteria at the polyclinic of National Iranian Oil Company (NIOC) Central Hospital, Tehran, Iran. At the beginning, all study details will be clarified, and an informed consent form will be provided. Then, general, short-form IPAQ, and 24-hour food recall questionnaires will be fulfilled by interviewers. The necessary lifestyle changes including low-calorie diet (weight loss of 0.5-1 kg per a week during the trial according to BMI) and increase of the physical activity (aerobic, moderate intensity, at least three times a week about 30-45 min) will be prescribed. Anthropometrics including weight, height, and waist circumference will be measured by a digital scale, stadiometer, and non-elastic tape, respectively. Weight without shoes, minimum clothing, and with 100 gram accuracy, height in standing position without shoes with stocked heels to the wall, head and look frontwards and with 0.5 cm accuracy, and waist circumference in the middle of the last rib and the iliac crest, minimal clothing and with 0.5cm accuracy will be measured.

The 24-hour food recall questionnaire will be completed in the beginning, middle, and end of the study. The blood pressure will be measured after 10 min resting in a sitting position with a manometer (cuff in two-thirds of the upper right arm) at the beginning and the end of the study. At both the beginning and end of the intervention, 10 ml of blood will be taken from the brachial vein to measure the mentioned factors. Finally, these measurements will be presented to the patients privately.

The sample size

According to Chuengsamarn et al. [2012], the mean ± standard deviation of HOMA-IR index in the curcumin and the placebo groups were 3.22 ± 1.30 and 4.08 ± 1.35, respectively. The sample size was calculated as 42 patients in each group with 95% confidence interval, 80% power, and 15% loss. A total of 84 patients will be invited and divided into two equal groups using block randomization method as follows:

1. 42 overweight/obese NAFLD patients with nanocurcumin supplement and advices of lifestyle change (weight loss diet and increasing physical activity) for 3 months intervention
2. 42 overweight/obese NAFLD patients with placebo supplement and advices of lifestyle change (weight loss diet and increasing physical activity) for 3 months intervention

Intervention and randomization

The block randomization method is used to divide patients into two equal groups. The age and gender distribution will be controlled using stratified randomization. In this study, the ratio of supplementation groups is 1:1. An assistant will perform the block randomization, and the intervention allotment will be blinded to the investigator and patients. The subjects will be randomly allocated into two groups taking nanocurcumin and placebo supplements. The supplements as A and B packages will be blinded to the investigators and participants.

No side effects and toxicity, taking 210 mg, have been reported for nanocurcumin [Kanai et al., 2012]. The supplementation dose will be 80 mg/day Sinacurcumin (according to company order, two 40mg capsules per day: 1 capsule with breakfast and one with dinner). Sinacurcumin and placebo supplements will be prepared by Exir-nanosina pharmaceutical company. The placebo supplement will contain polysorbate 80, soy oil, purified water, sorbitol 70, methyl paraben, and propyl paraben with nanocurcumin particles.

Stability and bioavailability of curcumin is very low. Curcumin is hardly dissolved in water, metabolized rapidly, and absorbed very weakly in intestine, and its plasma levels are very low. Human studies have shown that consuming 12 grams of curcumin daily is safe. Less than 1% of taken curcumin enters the bloodstream and is mostly metabolized in liver. Today, new ways are investigated to enhance the bioavailability of curcumin, especially through polymeric nanoparticles as nanocurcumin. Poly-Lactic-co-Glycolic Acid (PLGA) as a nanoparticle can increase the bioavailability of curcumin 22 times in mice [Kamali et al., 2014].

The supplements will be distributed monthly, and possible complications will be recorded (number of ingested capsules and given back packets). Also, the progress of study will be followed by calling once a week.

Lifestyle changes

The low-calorie diet according to the BMI for weight loss of 0.5-1 kg/week and increase of the physical activity as lifestyle changes will be presented by a qualified dietician present in the polyclinic of National Iranian Oil Company (NIOC) Central Hospital, Tehran, Iran.

Assessments and measurements

The ultrasound test will be done by a radiologist after than 12h fasting. The measurements of blood lipids (TC, HDL, LDL, TG) and liver enzymes (ALT, AST) will be determined by special kits and Hitachi analyzer (or BT-3500) device after 12h fasting. Blood sugar (FBS) will be determined by the glucose oxidase method. Fasting insulin (FBI) and glycated hemoglobin (HbA1c) will be measured by electrochemiluminescence ((ECL) by *co base e411*® analyzer device) and immunoturbidimetric method. Insulin resistance and sensitivity indices (HOMA, QUICKI) will be calculated according to the following formula:

QUICKI = 1 / (log (FBI μU/mL) + log (FBS mg/dL))
HOMA1-IR = (FBI (mU/l) x FBS (mmol/l)) / 22.5

The serum inflammatory markers (IL-6, TNF-α, hs-CRP) and nesfatin will be determined by the ELISA method (sandwich ELISA format) and specific kits. The ELISA test will be done by Elisa washer (Combiwash Human®) and bioElisa reader devices (biokit® EL $_x$ 800).

The food intake status (at the beginning-middle-end) and physical activity (at the beginning and the end) will be investigated using the 24-hour food recall and the short-form IPAQ physical activity questionnaires. The dietary intakes will thus be examined and controlled. The body composition percentage including body fat and lean body mass will be determined by BIA (Bio-Impedance Analyzer) device (Tanita®).

At the beginning and the end of the study, the systolic and diastolic blood pressure will be determined using a mercury manometer. The reported values are in mmHg. The waist circumference, weight, and height will be measured using non-elastic tape, digital scale, and stadiometer, respectively. Weight with minimal clothing, without shoes and with 100 grams accuracy, height in standing position, without shoes, heels sticking to the wall, with flat and forward head and 0.5 centimeters accuracy, and waist circumference at the middle of the last rib and the iliac crest with minimal clothing will be measured at the beginning and the end. The blood taking, storage of blood samples, and the lab tests will be conducted at National Iranian Oil Company (NIOC) Central Hospital, Tehran, Iran.

The contents of enrolment, interventions, and assessments are presented in Figure 1. Furthermore, the SPIRIT checklist will be completed as an additional file. The progress of trial will be checked by an assistant regularly and independently.

Data analysis

The data entry, coding, security, and saving will be checked. The data normality will be examined using a Kolmogorov-Smirnov test. Chi-square/nonparametric, Wilcoxon, T-test, ANCOVA, and Pearson correlation coefficient statistical tests will be applied. Confidence interval (CI) of 95% will be considered in all tests. The significance value is considered less than 0.05. The SPSS statistical software will be applied to analyze data.

Data accessibility

The accessibility to the ultimate dataset is only limited to the major investigator. The results will be presented only via publication.

Discussion

The proposed study is novel regarding to the evaluation of the efficacy of nanocurcumin on various parameters such as blood sugar, lipids, inflammatory markers, insulin resistance, and nesfatin among overweight/obese patients with non-alcoholic fatty liver disease (NAFLD) for the first time. It is very relevant because of various clinical uses and lack of studies related to the advantages or disadvantages of curcumin in NAFLD patients. However, the clinical practice of curcumin for the treatment of some disorders needs to be investigated, taking into account the possible prospective of it in several diseases, especially NAFLD. Due to increasing the values of obesity and following NAFLD, significant alteration of some blood factors in these patients and few studies on efficacy of nano-curcumin, the proposed study has aimed to select these groups of patients as the most pertinent participants for intervention.

The strength points of the trial are: randomized double blinded design, protocol publication, determining dietary and physical activity status, and registering patient-reported possible problems.

The trial limitations are slow recruiting of patients due to the criteria, selecting a single policlinic, and lack of cooperation of some participants to complete the intervention.

Trial Status

The patient employment will continue at the time of protocol submission.

Abbreviations

NAFLD: non-alcoholic fatty liver disease, BMI: body mass index, NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells, TNF-α: tumor necrosis factor-alpha, PPAR: peroxisome proliferation activated receptor, HOMA-IR: homeostasis model assessment-insulin resistance, QUICKI: quantitative insulin sensitivity check index, IL-6: interleukin-6, hs-CRP: high-sensitivity C-reactive protein, TPN: total parenteral nutrition, CNS: central nervous system, IPAQ: international physical activity questionnaire, TC: total cholesterol, HDL-C: high density lipoprotein-cholesterol, LDL-C: low density lipoprotein-cholesterol, ALT: alanine transaminase, AST: aspartate transaminase, FBS: fasting blood sugar, FBI: fasting blood insulin, ELISA: enzyme-linked immunosorbent assay, ANCOVA: analysis of covariance

Declaration

Ethical Approval and Consent to participate

The ethical approval of this trial was conducted by the ethics committee of Tehran University of Medical Sciences (Ethical Code: IR.TUMS.REC.1395.2612). All participants will complete an informed consent form (in Farsi). Participation and continuing supplementation is free and voluntary for patients. In the trial, advises of lifestyle modification will be presented free to patients. The health care services of the hospital will be provided without inconsistency. Side effects of supplements have not been published previously. The personal information of patients will be kept confidential.

Consent for publication

Not applicable

Availability of supporting data

Not applicable

Competing interests

There is no potential conflict of interests with respect to the research, authorship, and publication.

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Authors' contributions

SAJT, MJHA, and SMR conceived and developed the idea for the study, and revised the manuscript. SM, SMA, and MDM contributed to data collection. MDM wrote numerous drafts of the study. MQ contributed to statistical interpretations. All authors read and approved the final manuscript.

Consent to publish

Not applicable

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Figure

The overall contents related to enrollment, interventions, and assessments are presented in Figure 1.

Figure 1: Content of enrollment, interventions, and assessments

Trial contents	STUDY PERIOD					
	Enrolment	Allocation	Post-allocation			Close-out
TIMEPOINT	$-t_1$	0	+1 Month	+1.5 Monthes	+2 Monthes	+3 Monthes
ENROLMENT:	X					
Eligibility screen	X					
Informed consent	X	X				
General questionnaire		X				
24hour food recall		X		X		X
SF-IPAQ questionnaire		X				X
Anthropometrics		X				X
Other questionnaires		X				X
Blood taking		X				X
Allocation		X				
INTERVENTIONS:						
[Intervention A]		X	X		X	
[Intervention B]		X	X		X	
ASSESSMENTS:						
Dietary status		X		X		X
Blood pressure		X				X
Inflammatory factors		X				X
Lipid profile		X				X
Blood sugar indices		X				X
Nesfatin		X				X
Physical activity status		X				X
Anthropometrics		X				X
Socioeconomic status		X				

Additional file 1

SPIRIP Checklist.doc; this checklist applies to protocols for all clinical trials and focuses on content rather than format. The checklist recommends a full description of what is planned; it does not prescribe how to design or conduct a trial. The SPIRIT recommendations aim to facilitate the drafting of high-quality protocols and enhance the transparency and completeness of trial protocols for the benefit of investigators, trial participants, patients, sponsors, funders, research ethics committees or institutional review boards, peer reviewers, journals, trial registries, policymakers, regulators, and other key stakeholders.

Click here for file:
[www.spirit-statement.org/wp-content/uploads/2013/08/SPIRIT-Checklist-download-8Jan13.doc]

Cover Letter

Dear editor,

Following is our manuscript entitled "**The efficacy of nanocurcumin supplementation on insulin resistance, lipids, inflammatory factors and nesfatin among overweight/obese patients with non-alcoholic fatty liver disease (NAFLD): A Trial Protocol**" for consideration for publication by the "*BMJ Open*". The authors would like to advise that all authors listed have contributed to the work and approved the content of the submitted manuscript. There is no conflict of interest to declare. All authors have agreed to submit the manuscript to the "*BMJ Open*". No part of the work has been published before. We believe that our protocol could be of interest to the readers of "*BMJ Open"*. The corresponding author will correspond with the journal. SAJT, MJHA, and SMR conceived and developed the idea for the study and revised the manuscript; SM, SMA, and MDM contributed to data collection. MDM wrote numerous drafts. MQ contributed to statistical interpretations.

Regards,

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Figure 1. Content of enrolment, interventions, and assessments.

Trial Contents	STUDY PERIOD					
	Enrolment	Allocation	Post-allocation			Close-out
TIMEPOINT	$-t_1$	0	+1 Month	+1.5 Monthes	+2 Monthes	+3 Monthes
ENROLMENT:	X					
Eligibility screen	X					
Informed consent	X	X				
General questionnaire		X				
24hour food recall		X		X		X
SF-IPAQ questionnaire		X				X
Anthropometrics		X				X
Other questionnaires		X				X
Blood taking		X				X
Allocation		X				
INTERVENTIONS:						
[Intervention A]		X	X		X	
[Intervention B]		X	X		X	
ASSESSMENTS:						
Dietary status		X		X		X
Blood pressure		X				X
Inflammatory factors		X				X
Lipid profile		X				X
Blood sugar indices		X				X
Nesfatin		X				X
Physical activity status		X				X
Anthropometrics		X				X
Socioeconomic status		X				

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	-
Protocol version	3	Date and version identifier	2
Funding	4	Sources and types of financial, material, and other support	14
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 14
	5b	Name and contact information for the trial sponsor	-
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	14
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	-
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3, 4, 5
	6b	Explanation for choice of comparators	-
Objectives	7	Specific objectives or hypotheses	6

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	2, 6, 9, 10
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8, 11
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9, 10
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	11
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	11
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	9, 10, 11
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	11
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	10, 11
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	10

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	11
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9, 10, 11
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	10
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	11
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	9
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	12
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	12
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	12
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	-
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	14
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	-

Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	12
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	13, 14
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	-
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7, 9
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	-
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	14
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	14
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	12
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	-
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	12
	31b	Authorship eligibility guidelines and any intended use of professional writers	-
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	-
Appendices			

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	- (In Farsi)
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	12

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated.

BMJ Open

The Efficacy of Nanocurcumin Supplementation on Insulin Resistance, Lipids, Inflammatory Factors, and Nesfatin among Obese Patients with Non-Alcoholic Fatty Liver Disease (NAFLD): A Trial Protocol

Journal:	*BMJ Open*
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Primary Subject Heading:	Nutrition and metabolism
Secondary Subject Heading:	Gastroenterology and hepatology, Complementary medicine
Keywords:	Nanocurcumin, Non-Alcoholic Fatty Liver Disease, Trial Protocol, Overweight, Obesity

The Efficacy of Nanocurcumin Supplementation on Insulin Resistance, Lipids, Inflammatory Factors, and Nesfatin among Obese Patients with Non-Alcoholic Fatty Liver Disease (NAFLD): A Trial Protocol

Running title: Curcumin Effects on Obese NAFLD Patients: A Trial Protocol

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Abstract

Objectives: Different studies have been conducted on the role of curcumin in health since having multiple properties, including antioxidant and anti-inflammatory effects. Due to the lack of studies regarding curcumin effects on obese NAFLD patients, our protocol was designed to assess nano-curcumin impacts on blood sugar, lipids, inflammatory indices, insulin resistance, and liver function, especially by nesfatin.

Setting: This trial will be conducted in the Oil Company central hospital of Tehran, Iran with a primary level of care.

Participants: 84 obese NAFLD patients diagnosed using ultrasonography will be employed according to the eligiblity criteria.

Interventions: The patients will be randomly divided into the 2 equal groups (nanocurcumin and placebo, two 40-mg capsules, per/day with meals for three months, follow-up monthly). Also, lifestyle changes (low-calorie diet and physical activity) will be advised.

Measures of the Primary and Secondary Outcomes: A general questionnaire, 24-hr food recall (at the beginning, middle, and end), and short-form International Physical Activity Questionnaire (IPAQ) will be completed. Blood pressure, anthropometrics, serum sugar indices (fasting blood sugar and insulin [FBS, FBI], insulin resistance and sensivity, and glycosylated hemoglobin [HbA1c]), lipids (triglycerid [TG], total cholestrol [TC], and low- and high-density lipoprotein-cholestrol [LDL-c, HDL-c]), inflammatory profiles (interleukin-6 [IL-6] and tumor necrosis factor-alpha [TNF-α]), liver function (alanine and aspartate transaminase [ALT and AST]), and nesfatin will be measured at the beginning and end of the study.

Conclusion: This trial would be the first experiment to determine nanocurcumin efficacy on certain blood factors among obese patients with NAFLD. Nevertheless, studying the potential consequences of curcumin in various diseases, especially NAFLD, is required for a clinical use.

Trial registration: Our protocol was approved by the Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.REC.1395.2612). The trial characteristics registered at the Iranian Registry of Clinical Trials (IRCT) were as follows: IRCT2016071915536N3 and 27/12/2015.

Keywords: *nanocurcumin, Non-Alcoholic Fatty Liver Disease (NAFLD), trial protocol, overweight, obesity*

A Summary on the Article

Strengths and limitations of this study:

- Providing a randomized double-blinded design and protocol publication
- Determining dietary intake and physical activity statuses and registering any possible patient-reported problems
- Selecting a single and slow policlinic recruitment of the patients to satisfy the eligibility criteria
- Self-reporting of the dietary intake and physical activity statuses and lack of cooperation of some participants to complete the intervention

Introduction

Non-Alcoholic Fatty Liver Disease (NAFLD) occurs when triglyceride (TG) is deposited in liver cells. Intrahepatic triglyceride (IHTG) content of more than 5% of liver weight or volume or visible intracellular triglyceride content of 5% of hepatocytes or more are chemically or histologically defined as excessive IHTG or steatosis, respectively [1]. The 3 degrees of NAFLD are mild (<33% of fat accumulation), moderate (33-66% of fat accumulation), and severe (>66% of fat accumulation) [2]. The standard method of diagnosis is liver biopsy. Since biopsy is an invasive method, non-invasive diagnostic approaches, such as ultrasound examination, CT-scan, and MRI are mostly employed. However, it is difficult to exactly differentiate between the disease stages by these techniques. Most NAFLD patients are implicitly identified via elevated liver enzymes (Aminotransferases: ALT and AST contents of about 1.5-2 times higher than normal levels) in medical examinations. According to the recent studies, many patients with advanced non-alcoholic steatohepatitis (NASH) and even cirrhosis can have normal levels of liver enzymes [3-6]. Thus, NAFLD prevalence is probably more than what has been reported. Its symptoms often include fatigue and discomfort in the right upper quarter of the abdomen. Its average prevalence in adults is about 30% (nearly 65-85% and 15-20% in obese (BMI≥25) and non-obese (BMI<25) patients, respectively) [7-11]. NAFLD is more common in men. The disease pathology is a two-phase event, including fat reposition in hepatocytes following hepatic steatosis and NASH. Insulin resistance has a key role in both phases, while oxidative stress and pro-inflammatory cytokines are the major irritants [12]. The common causes of macrovascular steatosis include insulin resistance, increasing blood insulin levels, central obesity, diabetes type 2, medications (e.g., glucocorticoid, estrogen, tamoxifen, and amiodarone), nutrition status (starvation, protein deficiency, and choline deficiency), liver diseases (Wilson's disease and chronic hepatitis C-III), Hindi child cirrhosis, and jejunum bypass [6]. Liver fat content is directly related to insulin resistance. Activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) upregulates the production of pro-inflammatory cytokines that influence on the insulin activity. Thus, inflammation, adipokines, oxidative stress, or lipid metabolites can change insulin sensitivity, but intrahepatic fat content is not necessarily directly related to any of them [1]. Age, family history, malnutrition, severe weight loss, gastrointestinal tract infection, certain medication, and some diseases, such as inflammatory bowel disease are the other risk factors of

NAFLD [13-15]. In some studies, the disease incidence has been related to the high intakes of saturated fats or carbohydrates [16,17]. Some patients have normal weights although they may have abdominal obesity and insulin resistance [18,19].

Nesfatin as a neuropeptide secreted by the hypothalamus in mammals is involved in the regulation of appetite and body fat stores. Nesfatin gene is expressed in other locations, such as brain, pancreas, endocrine cells of stomach, and adipocytes. Nesfatin gene expression is activated by Peroxisome Proliferator-Activated Receptors (PPARs), especially PPARγ. Nesfatin plays an important role in glucose metabolism, phosphorylation of certain signaling proteins, and increasing insulin sensitivity in the liver, particularly through AMP-activated protein kinase (AMPK) [20,21]. In a recent study, the serum levels of nesfatin in overweight/obese NAFLD patients with an age of 30-60 years were found to be significantly lower than those of the healthy group [21].

A common treatment for NAFLD is changing the lifestyle (gradual weight loss and increasing physical activity) that can improve liver enzymes, fat reposition, inflammation, and fibrosis [22-27]. It seems that changes in the dietary ingredients can be presented as a therapy method for these patients [28,29] since losing weight and its maintenance for a long period of time is a hard task [30]. Accordingly, assessment of the relationship between NAFLD and certain nutrients or dietary ingredients is very important.

Different studies have been conducted on the roles of curcumin in health. Curcumin as a turmeric spice of ginger family has multiple properties, including antioxidant, anti-inflammatory, antimicrobial, and anti-carcinogenic effects [31-38]. Due to the importance of PPARs in the metabolic pathways, numerous studies have been carried out to investigate curcumin effects on PPARs, especially on PPARγ gene expression. It increases both the activity and expression of PPARγ, which is important for inhibiting inflammation and oxidative stress as the main factors of insulin resistance and NAFLD [39-42].

NAFLD prevalence and implications are increasing. Due to the lack of any drugs for it and the role of nutrition (weight loss and changing food components associated with increased physical activity) as the key factor of treatment, assessment of the effects of some food components like curcumin as a polyphenol on NAFLD improvement can further help to find new

ways of treatment. Curcumin plays numerous metabolic roles in the improvement of insulin resistance through its antioxidant, anti-inflammatory, hypolipidemic, and antimicrobial effects. Despite the multiple benefits of curcumin for health, it has a very low stability and bioavailability that affect its efficacy in therapy. Recently, many approaches have been assessed to improve its stability and bioavailability by using polymeric nanoparticles named nanocurcumin. For example, PLGA nanoparticles can increase curcumin bioavailability up to 22 times [43]. Hence, in this study, nanocurcumin was applied. Fat accumulation in the liver, inflammation, and oxidative stress result in NAFLD onset and progression, which may be improved by curcumin. NAFLD exacerbates with overweight or obesity; yet, no human studies have been conducted on curcumin effects on them. Thus, this study aimed to assess the effects of curcumin on blood glucose, lipid, inflammatory profiles, liver function (fatty liver degree, ALT, and AST), and insulin resistance (HOMA-IR and QUICKI), especially through nesfatin in obese NAFLD patients.

Methods

Study design

In this research, a double-blind randomized clinical trial will be performed.

Objectives

1. Comparing the subjects' economic, occupational, and marital statuses, as well as education levels with nanocurcumin and placebo supplementations before the intervention
1. Comparing the means of serum triglyceride, LDL-cholesterol, total cholesterol, HDL-cholesterol, Fasting Blood Sugar (FBS), insulin (FBI), hemoglobin-A1c, insulin resistance, sensitivity indices (HOMA-IR, QUICKI), TNF-α, IL-6, hs-CRP, and nesfatin within each group and between the two groups before and after the intervention
2. Comparing the means of weight, waist circumference, Body Mass Index (BMI), body composition percentage, and systolic and diastolic blood pressures within each group and between the two groups before and after the intervention

3. Comparing the means of physical activity score and energy intakes, micronutrients, and macronutrients within each group and between the two groups before and after the intervention
4. Comparing the means of age and height between the two groups before the intervention

Inclusion criteria

1. Age: 25-50 years
2. Overweight/obesity (25≤BMI<35)
3. NAFLD diagnosis by a radiologist based on the ultrasound test
4. An informed consent

Exclusion criteria

1. A history of alcohol consumption during the last 12 months based on personal admission
2. Regular intakes of nonsteroidal anti-inflammatory drugs, antibiotics, and corticosteroids during the last 6 months
3. Misuses of narcotics, psychotropic medication, and cigarettes over the last 6 months
4. Intakes of anti-secretory drugs causing acholoridy, amiodarone, valproate, prednisone, tamoxifen, perhexiline, and methotrexate, liver fat-inducing drugs, hormone drugs, statins, antihypertensives, and ursodeoxycholic acid (UDCA) during the last 6 months
5. Intakes of supplements, such as probiotics, multivitamins/minerals, antioxidants, and omega-3 at least twice a week during the study or the last 3 months
6. Diagnosis of pathological conditions affecting the liver, such as viral hepatitis, acute or chronic liver failure, cholestasis, liver transplantation, acute systemic disease, cystic fibrosis disease, muscular dystrophy, previous gastrointestinal surgery, neurological disorders, structural abnormalities of the gastrointestinal tract, diabetes, heart failure, thyroid disorders, kidney diseases, respiratory failure, psychological disorders, hereditary hemochromatosis, Wilson's disease, alpha-1 antitrypsin deficiency, autoimmune diseases, celiac disease, and any types of malignancy
7. Rapid weight loss, Total Parenteral Nutrition (TPN), and protein malnutrition over the last 6 months
8. NAFLD secondary causes, such as drugs, surgical procedures, and environmental toxins

9. Conditions leading to physical disability
10. Uncontrolled hypertension (>140/90 mmHg)
11. Breastfeeding, pregnancy, or a plan for pregnancy in the next 3 months
12. Being a professional athlete or doing regular exercise
13. Taking no more than 10% of the prescription supplements

Subjects

The patients will be referred to a major executor after being diagnosed by a radiologist if meeting the eligibility criteria at the central hospital of the polyclinic of National Iranian Oil Company (NIOC), Tehran, Iran. At the beginning, all the study details will be clarified and an informed consent form will be provided. Then, a general questionnaire, the short form of IPAQ, and 24-hour food recall questionnaire will be filled by the interviewer. The necessary lifestyle changes, including a low-calorie diet (weight loss of 0.5-1 kg per week based on BMI during the trial) and increased physical activity (aerobic exercise of moderate intensity about 30-45 min at least 3 times a week) will be prescribed. Anthropometrics, including weight, height, and waist circumference will be measured using a digital scale, stadiometer, and non-elastic tape, respectively. Weight without shoes and minimum clothing with an accuracy of 100 g, height in a standing position without shoes with heels stuck to the wall and head looking frontwards with an accuracy of 0.5 cm, and waist circumference in the middle of the last rib and the iliac crest with minimal clothing with an accuracy of 0.5 cm will be measured.

The questionnaire of 24-hour food recall will be completed at the beginning, middle, and end of the study. Blood pressure will be measured with a manometer (cuff in two-thirds of the upper right arm) after 10 min of resting in a sitting position at the beginning and end of the study. At both the beginning and end of the intervention, 10 ml of blood will be taken from the brachial vein to measure the mentioned factors. Finally, these measurements will be privately presented to the patients.

Sample size

According to Chuengsamarn et al. [2012], the mean ± standard deviation of HOMA-IR index in the curcumin and placebo groups were 3.22 ± 1.30 and 4.08 ± 1.35, respectively [44].

The sample size was 42 patients in each group with a Confidence Interval (CI) of 95%, power of 80%, and loss of 15%. A total of 84 patients will be invited and divided into two equal groups by using the block randomization method as follows:

1. 42 overweight/obese NAFLD patients with nanocurcumin supplement and advice on lifestyle changes (a weight loss diet and increase of physical activity) for 3 months of intervention
2. 42 overweight/obese NAFLD patients with the placebo supplement and advice on lifestyle changes (a weight loss diet and increase of physical activity) for 3 months of intervention

Intervention and randomization

The block randomization method wasis used to divide the patients into two equal groups. Age and gender distributions will be controlled using a stratified randomization. The supplementation ratio is 1:1 for the groups in this study. An assistant performed the block randomization and the intervention allotment will be blinded to the investigator and patients. The subjects will be randomly allocated into the two groups of taking nanocurcumin and placebo supplements. The supplements offered in A and B packages will be blinded to the investigators and participants.

No side effects and toxicity caused by taking 210 mg of nanocurcumin have been reported [34]. The supplementation dose of Sinacurcumin is 80 mg/day (two 40-mg capsules per day according to company's order: 1 capsule with breakfast and one with dinner). Sinacurcumin and placebo supplements will be prepared by Exir-nanosina Pharmaceutical Company. The placebo supplement contained polysorbate 80, soy oil, purified water, sorbitol 70, methyl paraben, and propyl paraben associated with nanocurcumin particles.

Curcumin is of a very low stability and bioavailability. It is hardly dissolved in water, rapidly metabolized, and very weakly absorbed in the intestine so that it remains at a very low level in plasma. Human studies have shown that a daily consumption of 12 g of curcumin is safe. Less than 1% of curcumin taken enters the bloodstream to be mostly metabolized in the liver. Today, new ways are being investigated to enhance curcumin bioavailability, especially through polymeric nanoparticles called nanocurcumin. Poly-Lactic-co-Glycolic Acid (PLGA) as a nanoparticle can augment curcumin bioavailability in mice up to 22 times [43].

The supplements will be distributed on a monthly basis, while any possible complications regarding the numbers of ingested capsules and packets given back will be recorded. Also, the study progress will be pursued by calling the subjects once a week.

Lifestyle changes

A low-calorie diet for a weight loss of 0.5-1 kg/week based on the BMI and increased physical activity will be presented as the lifestyle changes by a qualified dietician present in the central hospital of the polyclinic of National Iranian Oil Company (NIOC), Tehran, Iran.

Assessments and measurements

The ultrasound test will be done by a radiologist after 12 h of fasting. The measurements of blood lipids (TC, HDL, LDL, and TG) and liver enzymes (ALT and AST) will be determined using special kits and Hitachi analyzer (or BT-3500) device after 12 h of fasting. Blood sugar (FBS) is determined using the glucose oxidase method. Fasting insulin (FBI) and glycated hemoglobin (HbA1c) will be measured via electrochemiluminescence (ECL) application using *co base e411*® analyzer device and immunoturbidimetric method. Insulin resistance and sensitivity indices (HOMA and QUICKI) are calculated according to the following formula:

QUICKI = 1 / (log (FBI μU/mL) + log (FBS mg/dL))
HOMA1-IR = (FBI (mU/l) x FBS (mmol/l)) / 22.5

The serum inflammatory markers (IL-6, TNF-α, and hs-CRP) and nesfatin will be determined using the ELISA method (sandwich ELISA format) and specific kits. The ELISA test will be done using Elisa washer (Combiwash Human®) and bioElisa reader devices (biokit® EL $_{x}$ 800).

Food intake status at the beginning, middle, and end of the study and physical activity at the beginning and end of the study will be investigated using the questionnaire of 24-hour food recall and the short form of IPAQ. The dietary intakes will thus be examined and controlled. The body composition percentage, including body fat and lean body mass will be determined using Bio-Impedance Analyzer (BIA) device (Tanita®).

At the beginning and end of the study, the systolic and diastolic blood pressures will be determined using a mercury manometer. The values are reported in mmHg. Waist circumference, weight, and height will be measured using a non-elastic tape, digital scale, and stadiometer, respectively. Weight with minimal clothing without shoes (100-g accuracy), height in a standing position without shoes with heels sticking to the wall and head keeping flat and looking forward (0.5-cm accuracy), and waist circumference at the middle of the last rib and the iliac crest with minimal clothing were measured at the beginning and end of the study. Blood taking, storage of blood samples, and performance of the lab tests will be conducted at the central hospital of the National Iranian Oil Company (NIOC), Tehran, Iran.

The details of enrolments, interventions, and assessments are presented in table 1. Furthermore, the SPIRIT checklist was completed in an additional file. The trial progress will be regularly and independently checked by an assistant.

Data analysis

The data entry, coding, security, and saving will be checked. The data normality will be examined using a Kolmogorov-Smirnov test. Chi-square/nonparametric, Wilcoxon, ANCOVA, and Pearson's correlation coefficient statistical tests, as well as the T-test will be applied. A CI (Confidence Interval) of 95% will be considered in all the tests. The significance value is considered to be less than 0.05. Finally, SPSS statistical software will be applied to analyze the data.

Data accessibility

Accessibility to the ultimate dataset is only limited to the major investigator. The results will be presented only via publication.

Discussion

This is a novel study proposed for the first time with regard to the evaluation of nanocurcumin efficacy on various parameters, such as blood sugar, lipids, inflammatory markers, insulin resistance, and nesfatin among overweight/obese patients with NAFLD. It is of high relevance due to the various clinical uses of curcumin and lack of any studies related to its

advantages or disadvantages in NAFLD patients. However, curcumin clinical practice for the treatment of some disorders needs to be investigated, while taking into account its possible prospective applications for several diseases, especially NAFLD. Due to the increasing values of obesity and NAFLD associated with significant alterations of some blood factors and the presence of few studies on nano-curcumin efficacy, the proposed research aimed to select these groups of patients as the most pertinent participants for intervention.

The strengths of the trial are using a randomized double-blind design and protocol publication, determining dietary and physical activity statuses, and registering any possible patient-reported problems.

The trial limitations are patients' slow recruitments and increase of the study period due to the multiple eligibility criteria, selection of a single policlinic center, participants' self-reporting on the drugs and supplement consumptions, dietary intakes, and physical activities, and lack of cooperation of some participants to complete the intervention, which would lead to a replacement with other patients if the loss percentage will be more than expected.

Trial Status

The patients' employments were continued at the time of the protocol submission.

Abbreviations

NAFLD: non-alcoholic fatty liver disease, BMI: body mass index, NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells, TNF-α: tumor necrosis factor-alpha, PPAR: peroxisome proliferation activated receptor, HOMA-IR: homeostasis model assessment-insulin resistance, QUICKI: quantitative insulin sensitivity check index, IL-6: interleukin-6, hs-CRP: high-sensitivity C-reactive protein, TPN: total parenteral nutrition, CNS: central nervous system, IPAQ: international physical activity questionnaire, TC: total cholesterol, HDL-C: high density lipoprotein-cholesterol, LDL-C: low density lipoprotein-cholesterol, ALT: alanine transaminase, AST: aspartate transaminase, FBS: fasting blood sugar, FBI: fasting blood insulin, ELISA: enzyme-linked immunosorbent assay, ANCOVA: analysis of covariance

Declaration

Ethical Approval and Consent

The ethical approval of this trial was conducted by the ethics committee of Tehran University of Medical Sciences (Ethical Code: IR.TUMS.REC.1395.2612). All the participants will be completed an informed consent form in Persian. Participation in and continuation of the supplementation is free and voluntary for the patients. In the trial, advice on the lifestyle modification will be presented to the patients free of charge. The health care services of the hospital will be provided without inconsistency. The side effects of the supplements had not been previously published. The patients' personal information will be kept confidential.

Consent for publication

Not applicable

Availability of supporting data

Not applicable

Competing interests

There is no potential conflict of interests with respect to the research, authorship, and publication.

Funding

The trial funding was supported by Tehran University of Medical Sciences.

Authors' contributions

SAJT, MJHA, and SMR conceived and developed the idea for the study and revised the manuscript. SM, SMA, and MDM contributed to the data collection. MDM wrote numerous drafts on the study. MQ contributed to the statistical interpretations. And ultimately, all the authors read and approved the final manuscript.

Consent to publish

Not applicable

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Table 1. Contents of enrolments, interventions, and assessments.

Trial Contents	STUDY PERIOD					
	Enrolment	Allocation	Post-allocation			Close-out
TIMEPOINT	$-t_1$	0	+1 Month	+1.5 Monthes	+2 Monthes	+3 Monthes
ENROLMENTS:						
Eligibility screen	X					
Informed consent	X	X				
General questionnaire		X				
24hour food recall		X		X		X
SF-IPAQ questionnaire		X				X
Anthropometrics		X				X
Other questionnaires		X				X
Blood taking		X				X
Allocation		X				
INTERVENTIONS:						
[Intervention A]		X	X		X	
[Intervention B]		X	X		X	
ASSESSMENTS:						
Dietary status		X		X		X
Blood pressure		X				X
Inflammatory factors		X				X
Lipid profile		X				X
Blood sugar indices		X				X
Nesfatin		X				X
Physical activity status		X				X
Anthropometrics		X				X
Socioeconomic status		X				

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	-
Protocol version	3	Date and version identifier	2
Funding	4	Sources and types of financial, material, and other support	14
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 14
	5b	Name and contact information for the trial sponsor	-
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	14
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	-
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3, 4, 5
	6b	Explanation for choice of comparators	-
Objectives	7	Specific objectives or hypotheses	6

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	2, 6, 9, 10
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8, 11
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9, 10
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	11
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	11
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	9, 10, 11
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	11
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	10, 11
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	10

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	11
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9, 10, 11
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	10
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	11
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	9
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	12
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	12
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	12
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	-
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	14
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	-

Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	12
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	13, 14
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	-
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7, 9
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	-
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	14
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	14
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	12
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	-
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	12
	31b	Authorship eligibility guidelines and any intended use of professional writers	-
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	-
Appendices			

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	- (In Farsi)
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	12

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated.