CURCUMIN AS ADJUVANT TREATMENT IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD): A SYSTEMATIC REVIEW AND META-ANALYSIS.

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Abbreviation

ALT: alanine aminotransferase

AST: aspartate aminotransferase

BMI: Body mass index

LDL: low-density lipoprotein

HDL: high-density lipoprotein

NAFLD: Non-alcoholic fatty liver disease

NASH: Non-alcoholic steatohepatitis

QUICKI: Quantitative insulin check index

TC: total cholesterol

TG: total glycerides

ABSTRAK

Objektif: Matlamat kajian ini adalah untuk menentukan kesan curcumin pada morfologi ultrasonografi hati, dan keberkesanan curcumin sebagai rawatan adjuvan untuk NAFLD.

Kaedah: Perpustakaan Cohcrane dan PubMed telah dicari secara sistematik untuk mengenal pasti kajian rawak terkawal dari tahun 2000 hingga Januari 2021. Hasil utama adalah keterukan NAFLD, resolusi steatosis hati, parut hati, enzim hati, dan profil lipid. 16 RCT dengan sejumlah 1028 peserta telah dimasukkan ke dalam meta-analisis.

Keputusan: Curcumin meningkatkan pemulihan NAFLD (RR: 3.52, 95% CI 1.27 hingga 9.72; P = 0.02) dan meningkatkan resolusi steatosis hati (RR 3.96, 95% CI 1.54 hingga 10.17; P = 0.004) berdasarkan penemuan ultrasonografi hati. Suplemen curcumin mengurangkan aspartat aminotransferase (MD -4.00, 95% CI -5.72 hingga -2.28; P <0.001), alanine aminotransferase (MD -7.02, 95% CI -9.83 hingga -4.20; P <0.001), jumlah kolesterol (MD - 11.86, 95% CI -19.25 hingga -4.46; P = 0.002) dan BMI (MD: -0.41, 95% CI -0.75 hingga -0.07; P = 0.02).

Kesimpulan: Suplemen curcumin mempunyai kesan yang baik terhadap penemuan ultrasonografi hati, mengurangkan enzim hati serum, jumlah kolesterol, dan BMI dalam pesakit yang ada NAFLD. Oleh itu, mempromosikan curcumin sebagai rawatan adjuvan pada pesakit NAFLD mungkin wajar.

ABSTRACT

Objective: The aim of this review is to determine the effect of curcumin on the liver ultrasonographic morphology, and the effectiveness of curcumin as adjuvant treatment for NAFLD.

Methods: The Cohcrane library and PubMed were searched systematically to identify randomized controlled trials from 2000 to January 2021. The primary outcomes were NAFLD severity, liver steatosis resolution, liver scarring, liver enzymes, also lipid profiles. 16 RCTs with a total of 1028 participants were included in the meta-analysis.

Results: Curcumin improved NAFLD severity (RR: 3.52, 95% CI 1.27 to 9.72; P = 0.02) and increased the liver steatosis resolution (RR 3.96, 95% CI 1.54 to 10.17; P = 0.004) based on the liver ultrasonographic finding. Curcumin supplementation reduced aspartate aminotransferase (MD -4.00, 95% CI -5.72 to -2.28; P < 0.001), alanine aminotransferase (MD -7.02, 95% CI -9.83 to -4.20; P < 0.001), total cholesterol (MD -11.86, 95% CI -19.25 to -4.46; P = 0.002) and BMI (MD: -0.41, 95% CI -0.75 to -0.07; P = 0.02).

Conclusion: Curcumin supplementation has a favourable effect on liver ultrasonographic findings, reduced serum liver enzymes, total cholesterol, and BMI in participants with NAFLD. Therefore, promoting curcumin as adjuvant treatment on NAFLD patients might be justified.

Keyword: curcumin, non-alcoholic fatty liver disease, liver ultrasound, lipids profiles, liver enzymes, systematic review and meta-analysis

1. CHAPTER 1: INTRODUCTION

1.1. Introduction

NAFLD occurs in developed and developing countries and is present across a full range of ethnicities, such that it is now considered to be the most common liver condition in the world. The prevalence of NAFLD is up to 30% in developed countries and nearly 10% in developing nations, making NAFLD the most common liver condition in the world (1). The onset of NAFLD may occur in childhood, with children as young as two years being reported with the condition. The prevalence increases with age, rising from 0.7% in children aged 2-4 years, to 17% in late adolescence in the US (2).

Non-alcoholic fatty liver disease (NAFLD) is defined by macrovesicular steatosis in ≥5% hepatocytes, in the absence of a secondary cause such as alcohol or drugs. It encompasses a spectrum of disease from non-alcoholic fatty liver (NAFL) through to non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis (3).

The pathogenesis of NAFLD and NASH has classically been thought of as a "two hit" process; the first resulting in hepatic triglyceride accumulation and the second being responsible for hepatocyte death and scar formation. Hepatic steatosis results from an imbalance in lipid flux (4). Accumulation of free fatty acid and their metabolites causes hepatocellular apoptosis, hepatic damage, and steatosis, resulting in disease progression to liver cirrhosis and increase risk of becoming hepatocellular carcinoma (5).

Several risk factors for NAFLD have been identified: male, obesity, type 2 diabetes, dyslipidemia, metabolic syndrome and polycystic ovarian syndrome (6).

The majority of patients with NAFLD are asymptomatic and often come to medical attention following incidental abnormalities in serum hepatic transaminases or abdominal imaging.

Some patients may report abdominal discomfort or fullness presumably due to stretching of the hepatic capsule and hepatomegaly may be present on clinical examination (1).

To diagnosed NAFLD, there must be (1) evidence of hepatic steatosis (HS), either by imaging or histology, and (2) lack of secondary causes of hepatic fat accumulation such as significant alcohol consumption, long term use of a steatogenic medication, or monogenic hereditary disorders (6).

Traditionally, ultrasound has been the diagnostic modality of choice for identifying steatosis and has the advantage of low cost and high accessibility (3). However, ultrasound cannot differentiate between non-alcoholic fatty liver or non-alcoholic steatohepatitis. Therefore, the gold standard for diagnosing NASH remains a liver biopsy. Given that liver biopsy is not feasible in studies of the general population , limited by cost, sampling error, and procedure-related morbidity and mortality, clinical decision aids (e.g., NAFLD fibrosis score, FIB-4 index) are being developed for identifying NAFLD patients with higher likelihood of having bridging fibrosis (stage 3) or cirrhosis (stage 4) (7).

The assessment and management of blood pressure, lipids, weight, smoking status and diabetes control are the cornerstone of managing NAFLD. This will mainly be done in primary care. There is good evidence that weight loss not only reduces liver fat content, but histological resolution of NASH can occur with as little as 5% weight loss, and almost half of patients with 10% weight loss will have fibrosis regression (3)

Pharmacological treatments aimed primarily at improving liver disease should generally be limited to those with biopsy-proven NASH and fibrosis. Pioglitazone improves liver histology in patients with and without T2DM with biopsy-proven NASH (6).

Turmeric (Curcuma Longa) has active constituents in its rhizome called curcuminoids with the most prominent curcuminoid called curcumin. Curcumin is acknowledged as powerful antioxidant and anti-inflammatory agent. Curcumin can decrease major NAFLD risk factors such as hyperuricemia, dyslipidaemia, and insulin resistance through the reduction of serum lipids and uric acid concentration (8).

Curcumin is given through oral capsule and taken daily. A review reported that consumption of curcumin significantly reduced liver enzymes level, decreased serum LDL and total cholesterol (9).

Curcumin has been shown to reduce liver enzymes and cholesterol level in patients with NAFLD (9, 10). It can also decrease hepatic steatosis through lipid homeostasis and subsequently control hepatic inflammation (11).

Why it is important to do this review

Lifestyle modification consisting of diet, exercise, and weight loss has been advocated to treat patients with NAFLD. However, all these lifestyle modifications took considerable time to put into action and quite hard to achieve and difficult to sustain. Currently, there is no recommended pharmacotherapy for NAFLD patients without advanced liver fibrosis(6). There are currently no reviews on the effect of curcumin on ultrasound morphological characteristic of liver among patients with NAFLD. If curcumin can be shown to improved liver enzymes and liver steatosis in the liver, curcumin supplementation might serve as valuable adjunct therapy for the prevention and treatment of NAFLD.

CHAPTER 2: OBJECTIVES AND OUTCOMES OF THE STUDY

2.1 Objectives

The effectiveness of curcumin on NAFLD severity, liver fibrosis, liver steatosis resolution, liver enzymes and lipid profile on patient with non-alcoholic fatty liver disease

2.2 Outcomes of the study

Primary outcomes

1) NAFLD severity based on ultrasound morphology.
2) Resolution of liver fat based on ultrasound morphology.
3) Liver scarring/fibrosis based on Fibro-scan
4) Serum liver enzymes
5) Serum lipid profiles

Secondary outcomes

1) Insulin sensitivity (Quantitative insulin sensitivity check index)
2) Body mass index
3) Adverse effect (e.g nausea, stomach-ache)

CHAPTER 3: MANUSCRIPT

3.1. Title Page

Curcumin as adjuvant treatment in patients with non-alcoholic fatty liver (NAFLD) disease: a systematic review and meta-analysis.

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3.2. Abstract

Objective: The aim of this review is to determine the effect of curcumin on the liver ultrasonographic morphology, and the effectiveness of curcumin as adjuvant treatment for NAFLD.

Methods: The Cohcrane library and PubMed were searched systematically to identify randomized controlled trials from 2000 to January 2021. The primary outcomes were NAFLD severity, liver steatosis resolution, liver scarring, liver enzymes, also lipid profiles. 16 RCTs with a total of 1028 participants were included in the meta-analysis.

Results: Curcumin improved NAFLD severity (RR: 3.52, 95% CI 1.27 to 9.72; P = 0.02) and increased the liver steatosis resolution (RR 3.96, 95% CI 1.54 to 10.17; P = 0.004) based on the liver ultrasonographic finding. Curcumin supplementation reduced aspartate aminotransferase (MD -4.00, 95% CI -5.72 to -2.28; P < 0.001), alanine aminotransferase (MD -7.02, 95% CI -9.83 to -4.20; P < 0.001), total cholesterol (MD -11.86, 95% CI -19.25 to -4.46; P = 0.002) and BMI (MD: -0.41, 95% CI -0.75 to -0.07; P = 0.02).

Conclusion: Curcumin supplementation has a favourable effect on liver ultrasonographic findings, reduced serum liver enzymes, total cholesterol, and BMI in participants with NAFLD. Therefore, promoting curcumin as adjuvant treatment on NAFLD patients might be justified.

Keyword: curcumin, non-alcoholic fatty liver disease, liver ultrasound, lipids profiles, liver enzymes, systematic review and meta-analysis

3.3 Background

The prevalence of the NAFLD is up to 30% in developed countries and nearly 10% in developing nations (1). The majority of patients with NAFLD are asymptomatic and often come to medical attention following incidental abnormalities in serum hepatic transaminases or abdominal imaging (1).To diagnose NAFLD, there must be: evidence of hepatic steatosis, either by imaging or histology, and lack of secondary causes of hepatic fat accumulation (6). The assessment and management of blood pressure, lipids, weight, smoking status, and diabetes control are the cornerstone of managing NAFLD. There is good evidence that 5% weight loss reduces liver fat content and histological resolution of NAFLD (3). However, a study showed that 80% of patients failed to lose at least 5% body weight, indicating that weight loss is largely unsuccessful in real-world clinical settings among patients with NAFLD (12).

Pharmacological treatments aimed primarily at improving liver disease should generally be limited to those with biopsy-proven non-alcoholic steatohepatitis (NASH) and fibrosis. However, liver biopsy is invasive, carries complications (pain, bleeding, infection), and requires operator and liver pathologists (13).

The limitations, risks, and cost of liver biopsy before pharmacological treatment have led us to look for an alternative treatment. The ideal treatment would be low cost, effective and require no liver biopsy-proven non-alcoholic steatohepatitis when initiating the treatment.

Turmeric (Curcuma Longa) has active constituents in its rhizome called curcuminoids, with the most prominent curcuminoid called curcumin. Curcumin is acknowledged as a powerful antioxidant and anti-inflammatory agent (8).

Curcumin is given through an oral capsule and taken daily. A review reported that the consumption of curcumin significantly reduced liver enzymes, decreased low-density lipoprotein and total cholesterol (9) among NAFLD patients. Another review found curcumin showed significant effects on fasting blood sugar, insulin level, and homeostasis model of assessment insulin resistance (14) among women with polycystic ovary syndrome.

For years, medicinal plants have been used in different health schemes and used as traditional medicines for treating diseases. Curcumin is anticipated to function as antiviral drugs to treat the current COVID-19 virus based on in vitro and in vivo studies as it showed high inhibitory activity towards the virus (15). Computer simulation and molecular docking showed the good ability of this monomer to bind to the COVID virus and host target so that they could block the virus-host binding sites (16).

There are currently no reviews on the effect of curcumin on ultrasound morphological characteristics of the liver among patients with NAFLD. If curcumin can be shown to improve liver ultrasound morphological characteristics such as NAFLD severity and increase liver steatosis resolution, then curcumin supplementation might serve as a valuable adjunct therapy for the treatment of mild to moderate NAFLD.

3.4. Material and methodology

3.4.1. Protocol and registration

The systematic review was registered in PROSPERO, International prospective register of systematic reviews (Registration number: CRD42021229160). We followed the Preferred Reporting Item for Systematic Reviews and Meta-Analysis Statement (PRISMA) guidelines (17).

3.4.2. Literature search

We systematically searched the Cochrane Central Register of Controlled Trials (CENTRAL 2021, Issue 3 of 12) and PubMed (2000 to 2020). The keyword applied were (curcuma OR curcuma longa OR curcumin OR turmeric) AND (fatty liver OR non-alcoholic fatty liver disease OR metabolic associated fatty liver disease OR non-alcoholic steatohepatitis OR non-alcoholic fatty liver). We restricted the publications to only the ones using English Language.

We searched for completed and ongoing trials through the World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP) http://www.who.int/ictrp/en/ and www.clinicaltrials.gov (searched 2 January 2021).

3.4.3. Types of study, participants, and intervention

RCTs comparing turmeric/curcumin with placebo, or no treatment was included. We included blinded and open-labelled studies with participants diagnosed with NAFLD through liver ultrasonography. Inclusion criteria are:

1) Fibroscan: Evidence of NAFLD in fibroscan
2) Ultrasound: Evidence of liver steatosis by ultrasound
3) Treatment of NAFLD with turmeric/curcumin extract by oral route

Exclusion Criteria:

1) NAFLD secondary to alcohol consumption
2) Presence of Hepatitis B or C

3.4.4. Types of outcome measures

The primary outcomes were the number of participants with an improvement of NAFLD severity (based on liver ultrasound, fibroscan), number of participants with liver steatosis resolution, liver scarring, serum liver enzymes, and lipid profiles. Secondary outcomes include QUICKI, BMI, and adverse effect (gastrointestinal symptoms). The follow-up period for the outcomes was at least eight weeks after the intervention.

3.4.5. Study selection and data extraction

Two reviewers screened and collected the literature independently. The result of the reviews was discussed, and any discrepancies were resolved. Three reviewers reviewed the data extracted from the trials. A standardized form was used to extract data from the included studies for the assessment of study quality and evidence synthesis. The data extracted were as follows: study setting, participant characteristics methodology, dosage, study duration, method of diagnosing NAFLD, patient's ultrasonographic morphologic characteristics, patient's weight and height, liver function test, lipid profiles, QUICKI, and the occurrence of related adverse events.

3.4.6. Assessment risk of bias and GRADE method

Two reviewers performed the risk of bias assessment based on the Cochrane Handbook for Systemic Review of Interventions (18). We assessed the risk of bias based on random

sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessors, completeness of outcome data, selectivity of outcome reporting, and other biases (18). Two reviewers assessed the quality of evidence for primary and secondary outcomes according to the GRADE methodology (19).

3.4.7. Data synthesis and statistical analysis

We measured the treatment effect for dichotomous outcomes using risk ratios (RRs) and absolute risk reduction, and for continuous outcomes, we used mean differences (MDs); both with 95% confidence intervals (CIs). We reported the results of the random-effects model. We adjusted results from the trials, showing the unit of analysis errors based on the mean cluster size and intra-cluster correlation coefficient (18). If the mean changes for standard deviation were not reported in the study, we calculate the standard deviations by using Review Manager Calculator 5.4 software (20) or correlation coefficient (18). We contacted the original trial authors to request missing or inadequately reported data (21, 22).

We conducted meta-analyses using Review Manager 5.4 software (20) and used a random-effects model to pool data. We assessed the presence of heterogeneity in two steps. Firstly, we assessed obvious heterogeneity at face value by comparing the populations, settings, interventions, and outcomes. Secondly, we assessed statistical heterogeneity utilizing the I^2 statistic (18).

We performed sensitivity analysis on the primary outcomes and subgroup analysis on serum liver enzymes and lipid profiles on curcumin dosage and intervention duration. We were unable to perform the subgroup analysis on liver steatosis and NAFLD severity due to insufficient data.

Funnel plots were performed to detect publication bias for outcomes with more than ten trials.

3.5. RESULT

3.5.1. Study selection

We retrieved 2059 records from the electronic search and one record from the other sources (Figure 1). The publication year of the trials ranged from 2012 to 2020. After the duplicates were removed, we screened a total of 2026 records. We reviewed full manuscript of 24 studies. We identified 16 articles as meeting the review inclusion criteria and eight were not eligible for inclusion (two are non-english articles, five did not report any of the outcome of interest, one did not use ultrasound as diagnostic criteria.)

3.5.2. Study characteristics

We included 16 trials with a total of 1028 participants (21-36). All studies had a randomized parallel design. Nine out of 16 trials declared funding from the university (22, 23, 25, 27, 29, 30, 33, 35, 36). Seven trials did not state their source of funding (21, 24, 26, 28, 31, 32, 34).

Only one out of 16 trials was conducted in Thailand (24), with the rest of the trials being conducted in Iran . Despite 15 were conducted in Iran, they were conducted by different researcher teams. Two trials have equal sex distribution between groups (26, 34). Two trials did not mention the sex of the participants (21, 36). Participants with NAFLD were the target population of all included trials. Three out of 16 trials used fibroscan to diagnose NAFLD (21, 22, 35), and 13 trials used liver ultrasound to diagnose NAFLD (23-34, 36). The ultrasound was performed by a trained operator and NAFLD severity was graded based on liver steatosis : absent (score 0), mild (score 1), moderate (score 2), severe (score 3) (37).

Participants in the trials were randomized into the intervention and control groups. Four trials used phospholipid curcumin 50 mg/day as intervention (23, 25, 28, 29), while three

trials received curcumin 1500 mg/day (21, 22, 35). Two trials used curcumin 1000 mg/day phytosomal formulation, equivalent to curcumin 200 mg/day (31, 32), and two trials' participants received 500 mg curcumin and 5mg piperine/day as intervention (33, 36). Turmeric 500 mg/day (30) and 2000 mg/day (26) were received by each of the participants in the intervention group. One trial received curcumin 80 mg/day (27), and one trial received curcumin 70 mg/day (34). The intervention group in one trial did not mention any curcumin dosage (24). All the included studies did not independently test the curcumin product for purity and potency.

For nine trials, the study duration was eight weeks (23, 25, 26, 28, 29, 31, 32, 34, 36). The duration of study for six trials was 12 weeks (21, 22, 27, 30, 33, 35) and one trial was for 24 weeks(24).

Intervention and control group capsules were similar in appearance in all trials. Three of 16 trials used maltodextrin as a placebo (21, 22, 35). Starch as a placebo was used in two trials (25, 30). One trial used lactose capsules as control (31),and another trial used wheat flour capsules as control (26). Nine trials did not specify the content of the capsule in the control group (23, 24, 27-29, 32-34, 36).

Three trials reported that they had measured the number of participants with the improvement of the ultrasonographic morphologic of NAFLD severity (32-34). Six trials reported the number of participants with liver steatosis resolution (26, 27, 32-34, 36); another three trials measured liver scarring(fibrosis) as outcomes (21, 22, 35).

The comparison for liver enzymes was reported in 14 trials (21-30, 32-34, 36). Ten trials measured lipid profiles as outcomes (21, 23, 24, 26-28, 31, 33, 34, 36).

For secondary outcomes, 11 trials reported that they had measured BMI (23, 25-30, 32, 34-36). The QUICKI was reported in three trials (21, 27, 31). Three trials reported the number of participants with gastrointestinal adverse effects (27, 34, 36).

We excluded eight trials. Two of these were written in the Persian language; hence they were excluded (38, 39). One study does not meet the inclusion criteria (40), as the participants recruited were not diagnosed with NAFLD and therefore, were excluded. Five trials were excluded because they did not report any outcomes of interest (41-45).

3.5.3. Risk of bias in included study

The assessment of the risk of bias is shown in Figure 2. Figure 2(A) shows the proportion of studies assessed as low, high, or unclear risk of bias for each risk of bias indicator. Figure 2(B) shows the risk of bias indicator for individual studies. The details of these trials are found in the table of characteristics of the studies concerned (Table 1).

Ten trials described the method of randomization used. Two trials randomized the participants using the computer-generated number (22, 30), and four trials used a random number table (21, 23, 35, 36). Four trials used block randomization (25, 27-29). The methods of randomization were not reported in the other six trials (24, 26, 31-34); thus we judged them as unclear risk. Allocation concealment was not clear in three trials (24, 26, 31).

Four trials did not specify the blinding method, and thus we judge the blinding of participants and personnel as unclear risk of bias (24, 26, 31, 35). Eleven trials used placebo control that is identical in size, shape and colour to the intervention group (21-23, 25, 27, 29, 30, 32-34, 36). One trial has a high risk of performance bias as the researcher knew the content of the packages and the types of drugs (28).

The blinding of outcome assessors was not described in five trials (26, 30, 31, 33, 35), and thus we judge it as unclear risk for detection bias.

Two trials have high missing data (defined as more than 20% missing data), and thus we judge it as a high risk of bias for attrition bias (23, 24). Three trials did not specify the reason for patient withdrawal from the trials (23, 26, 34), and thus we judge them as unclear risk.

Five trials were prospectively registered in the Iranian Registry of Clinical Trials (27, 28, 32, 34, 36). The outcome listed in the registered protocol were those reported.

We encountered moderate to severe heterogeneity in our primary outcome.

3.5.4. Comparison of Curcumin vs Placebo

3.5.4.1. Effect of curcumin on NAFLD severity, liver steatosis resolution, liver scarring.

For the primary outcomes, curcumin improved NAFLD severity (32-34) (RR: 3.52, 95% CI 1.27 to 9.72; I^2 statistic=83%; P = 0.02; three trials, 234 participants) (Figure 3, Table 2) and increased the number of participants with liver steatosis resolution (26, 27, 32-34, 36) (RR 3.96, 95% CI 1.54 to 10.17; I^2 statistic = 34%; P = 0.004; six trials, 437 participants) (Figure 3, Table 2) compared to the placebo group based on ultrasonographic findings.

However, there is no difference in liver scarring (21, 22, 35) (MD -0.30, 95% CI -0.66 to 0.05; I^2 statistic = 0%; P = 0.09; three trials, 144 participants) (Figure 3, Table 2) between the two group.

Sensitivity analysis showed no impact of each study on liver steatosis resolution and liver scarring.

However, the effect estimate changed with Panahi 2019 (33) (unclear risk for random sequence generation) (RR 6.2, 95% CI 0.89 to 43.10; I^2 statistic = 86%; P = 0.07 ; two trials, 164 participants) or Rahmani 2016 (unclear risk for random sequence generation) (34) (RR 4.82, 95% CI 0.35 to 66.70; I^2 statistic = 92%; P = 0.24 ; two trials, 157 participants) to non-significant for NAFLD severity.

3.5.4.2. Effect of curcumin on liver enzymes

Curcumin reduced aspartate aminotransferase (21-30, 32-34, 36) (MD -4.00, 95% CI -5.72 to -2.28; I^2 statistic = 82%; P < 0.001; 14 trials, 792 participants) (Figure 4, Table 2) and alanine aminotransferase (21-29, 32-34, 36) (MD -7.02, 95% CI -9.83 to -4.20; I^2 statistic =83%; P < 0.001; 13 trials, 750 participants) (Figure 4, Table 2) compared to the placebo group

Subgroup analysis revealed no change in the significance of heterogeneity and showed curcumin dosage of less than 500 mg/day reduced serum liver enzymes (Supplemental Figure 1) compared to the placebo group.

3.5.4.3. Effect of curcumin on lipid profiles

Curcumin reduced the total cholesterol (21, 23, 24, 26-28, 31, 33, 34, 36) (MD -11.86, 95% CI -19.25 to -4.46; I^2 statistic =86%; P = 0.002; ; ten trials, 613 participants) (Figure 4, Table 2) compared to the placebo group.

However, there is no difference in low-density lipoprotein (21, 23, 24, 26-28, 31, 33, 34, 36) (MD -8.78, 95% CI -18.74 to 1.18; I^2 statistic: 97%; P = 0.08; ten trials, 613 participants) (Figure 5, Table 2); high-density lipoprotein (21, 23, 26-28, 31, 33, 34, 36) (MD 0.43, CI 95% -3.88 to 4.74; I^2 statistic: 98%; P = 0.85; nine trials, 592 participants) (Figure 5, Table

2); triglycerides (21, 23, 24, 26-28, 31, 33, 34, 36) (MD -13.00, 95% CI -27.94 to 1.94; I^2 statistic: 96%; P= 0.09; ten trials, 613 participants) (Figure 5, Table 2) between the two groups.

Subgroup analysis showed heterogeneity reduced in curcumin dosage of more than ≥ 500 mg/day for low-density lipoprotein (21, 33, 36) (MD 8.81, 95% CI 2.12 to 15.51; I^2 statistic = 38%; P = 0.09; three trials, 175 participants) (Supplemental Figure 2), high-density lipoprotein(21, 33, 36) (MD -1.97, 95% CI -3.38 to -0.56; I^2 statistic = 22%; P = 0.006; three trials, 175 participants) (Supplemental Figure 2), and triglycerrides (21, 33, 36) (MD -5.63, 95% CI -16.62 to 5.36; I^2 statistic = 39%; P = 0.32; three trials, 175 participants) (Supplemental Figure 3), and total cholesterol (21, 33, 36) (MD -4.16, 95% CI -8.55 to -0.66; I^2 statistic = 0%; P = 0.02; three trials, 175 participants) (Supplemental Figure 3). Heterogeneneity also reduced in duration of intervention > 8 weeks for total cholesterol (21, 24, 27, 33) (MD -6.29, 95% CI -9.65 to 2.93; I^2 statistic = 0%; P < 0.001; four trials, 224 participants) (Supplemental Figure 4).

The subgroup analysis of curcumin dosage showed curcumin dosage less than 500 mg/day reduced low-density lipoprotein (23, 27, 28, 31, 34) (MD -19.25, 95% CI -32.52 to -5.98; I^2 statistic = 97%; P = 0.004; five trials, 354 participants) (Supplemental Figure 2) compared to the placebo group. Curcumin dosage equal or more than 500 mg/day reduced high density lipoprotein (21, 33, 36) (MD -1.97, 95% CI -3.38 to -0.56; I^2 statistic = 22%; P = 0.006; three trials, 175 participants) (Supplemental Figure 2) compared to the placebo group.

The subgroup analysis showed that study duration of more than eight weeks reduced triglycerides (21, 24, 27, 33) (MD -24.97, CI -42.23 to – 6.72; I^2 statistic: 94%; P = 0.007; four trials, 224 participants) (Supplemental Figure 4) compared to the placebo group.

Sensitivity analysis showed a change in the effect estimates with curcumin reducing the low-density lipoprotein compared to placebo with the exclusion of Panahi 2019 (33) (unclear risk for random sequence generation) (MD -11.41, 95% CI -21.02 to -1.81; I^2 statistic: 95%; P = 0.02; nine trials, 543 participants). No change was observed in the overall effect estimates for total cholesterol, high-density lipoprotein, and triglycerides.

3.5.4.4. Effects of curcumin on BMI, QUICKI score and adverse effect

For secondary outcomes, curcumin reduced BMI (23, 25-30, 32, 34-36) (MD: -0.41, 95% CI -0.75 to -0.07; I^2 statistic: 79%; P = 0.02; eleven trials, 650 participants) (Figure 6, Table 2) compared to the placebo group. There is no difference in QUICKI (21, 27, 31) (MD 0.01, 95% CI -0.00 to 0.02; I^2 statistic: 94%; P: 0.30; three trials, 221 participants) (Figure 6, Table 2) and adverse effect (27, 34, 36) (RR: 2.09, 95% CI 0.35 to 12.56; I^2 statistic: 2%; P = 0.42; three trials, 214 participants) (Figure 6, Table 2) between the two groups. The adverse effect reported were nausea, vomiting and abdominal pain.

3.6. Discussion

3.6.1. Summary of main results and limitations

Our findings showed that curcumin improved NAFLD severity and increased liver steatosis resolution based on the liver ultrasonographic findings and reduced serum liver enzymes.

Curcumin suppressed nuclear factor-Kβ and reduced oxidative stress and inflammation, making it anti-oxidant and anti-inflammatory (46). This finding raises the possibility that curcumin can help to protect the liver by lowering oxidative stress, as oxidative stress has been implicated in the pathogenesis of NAFLD (47). This hypothesis has been emphasised when a study showed that curcumin improved the antioxidant defense system and lower lipid peroxidation level (48), which improved NAFLD severity.

In addition, curcumin reduced total cholesterol with its lipid-lowering property by interacting with the expression of multiple genes, such as peroxisome proliferator-activated receptor alpha (PPAR-α), cholesteryl ester transfer protein, and lipoprotein lipase (49). Curcumin supplementation thus played a role in decreasing plasma triglyceride and cholesterol levels by inhibiting the expression of lipogenic genes. This decreased the severity of NALFD by lowering the fat level of the liver (50).

Curcumin reduced body fat mass by inhibiting adipocyte differentiation by suppressing PPAR- γ and increasing adenosine monophosphate-activated protein kinase, resulting in increased lipolysis (51).

There is no major side effect (diarrhoea, vomiting, epigastric pain) reported in participants receiving curcumin.

The subgroup analysis showed that only curcumin with a dosage less than 500 mg/day reduced serum liver enzymes and low-density lipoprotein compared to the placebo group. Curcumin dosage equal or more than 500 mg/day reduced high-density lipoprotein, increase low-density lipoprotein in participants with NAFLD compared to the placebo. Lower doses appear to be sufficient to affect the status of patients with NAFLD.

Curcumin reduces serum liver enzymes, and total cholesterol in trials with a duration of intervention of eight weeks and more than eight weeks compared to the placebo group. The supplementation of curcumin for more than eight weeks reduced triglycerides.

We have included 16 trials, but the finding of this review may not apply to NAFLD in adolescents. We included two trials with turmeric as the comparison group, as curcumin is an active ingredient of turmeric. There are three trials with unspecified curcumin dosage (including two trials using turmeric). However, they were excluded in the meta-analysis in the subgroup analysis for curcumin dosage due to unspecified curcumin dosage.

Turmeric powders are used as spices and seasoning in food preparation and are easily available commercially. However, due to their low concentration of curcumin component, averaging 3.14% by weight (52), commercial turmeric powders are not suitable to be used or replaced with oral curcumin tablets.

There are several limitations presents in this meta-analysis. The formulation of curcumin might differ due to the different bioavailability of the curcumin used in the trials. This might cause a difference in absorption and metabolism despite the similar dosage. Some studies used unformulated curcumin. Besides, different dosages and sources of curcumin were used for intervention in the studies that were included.

A high degree of heterogeneity was detected between studies, with subgroup analysis performed to explore them. Some confounding factors, such as nutritional consumption and physical activity were not included in most of the studies, and we were unable to include them in this analysis.

Most of the included studies had a small number of participants, ranging from 47 participants to 102 participants, affecting the precision of the outcomes. This downgrades the quality of evidence for the primary outcomes of liver scarring, liver steatosis resolution, and NAFLD severity. Unlike the biochemical result, the ultrasound is operator- dependent, and all the trials used trained operators to perform ultrasound scan.

Most of the studies included are from Iran and this limits the generalizability of results to other regions or populations.

The overall level of evidence contributing to this review using the GRADE approach is of low to moderate quality. There was no evidence of selective reporting, or bias although we cannot exclude this because only five trials had published the protocol.

Funnel plots were constructed for the primary outcomes of serum liver enzymes and lipid profiles . The visual inspection of the funnel plot (Supplemental Figure 5) for changes in high-density lipoprotein revealed potential publication bias. This downgrades the quality of evidence for the outcomes of high-density lipoprotein. Ultrasound assessment is heavily operator-dependent, which may affect the precision of the results.

3.6.2. Agreement and disagreement with other studies and reviews

Five systematic reviews have examined the effect of curcumin on NAFLD (9, 10, 53-55). Our reviews included all their trials except Ghaffari 2017 (42) (not related to our prespecified outcomes), and Jarahzadeh (2017) (thesis, not published).

Goodarzi 2019 (10) and Jalali 2020 (9) found that curcumin reduced liver enzymes in NAFLD compared to the placebo group, which is similar to our findings. Mansour-Ghanaei 2019 (53) and Wei 2019 (54) showed that curcumin reduced aspartate aminotransferase; however, there is no difference in alanine aminotransferase between the two groups.

Wei 2019 (54) found that there is no difference in total cholesterol between the two groups. Our finding is similar to Jalali 2020 (9) that curcumin reduced total cholesterol in participants compared to the placebo group. Wei 2019 (54) and Jalali 2020 (9) showed that curcumin reduced the low-density lipoprotein compared to the placebo group. Wei 2019 (54) also showed that curcumin reduces triglycerides compared to the placebo group. Our review showed that there is no difference in low-density lipoprotein and triglycerides between the two groups. Wei 2019 (54), Jalali 2020 (9) and our review showed no difference in high-density lipoprotein between the two groups.

For secondary outcomes, Baziar 2020 (55) and our review showed that curcumin reduced BMI in participants with the NAFLD compared to the placebo group; however, Jalali 2020 (9) found no difference in BMI between the two groups.

We found no other systematic reviews that report on liver ultrasonographic findings in NAFLD with curcumin supplementation.

3.7. Quality assessment using GRADE

The assessment of quality using GRADE is shown in Table 2. It is evident that the differences between curcumin and placebo for NAFLD severity, high-density lipoprotein, and QUICKI were low. The evidence for liver steatosis resolution, liver scarring, serum liver enzymes, total cholesterol, low-density lipoprotein, triglycerides, BMI, and adverse effects was moderate.

3.8. Implication for for the practice and research the practice

3.8.1. Implication for the practice

Curcumin has been shown to improve NAFLD's severity and increase liver steatosis resolution compared to the placebo groups. Besides improving the liver ultrasonographic findings, curcumin also reduced serum liver enzymes, total cholesterol, and BMI. There is no major side effect, and our findings showed no difference in the adverse effect between the two groups. Curcumin was also found to reduce triglycerides if taken more than eight weeks' duration.

3.8.2. Implication for research

Further studies should include other liver enzymes such as gamma-glutamyl transpeptidase and albumin as outcomes. RCTs are needed with larger sample sizes and adequate duration to confirm the efficacy of curcumin on NAFLD.

3.9. Author's conclusion

Our meta-analysis shows that curcumin has favourable effect on liver ultrasonographic findings, reduced serum liver enzymes, total cholesterol, and BMI in participants with NAFLD. Therefore, promoting curcumin as an adjuvant treatment on NAFLD patients might be justified.

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Conflict of interest

The authors declared that there are no competing interests