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Effects of garlic and its major bioactive components on non-alcoholic fatty liver disease: A systematic review and meta-analysis of animal studies

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ABSTRACT

This systematic review and meta-analysis study was conducted to summarize the effects of garlic or its major components on hepatic triglyceride and cholesterol content, serum alanine transaminase (ALT) and aspartate transaminase (AST) levels, and liver weight. We searched PubMed, Embase, Scopus, and Web of Knowledge databases. Data were pooled, and standardized mean difference (SMD) and 95% confidence intervals (CI) were estimated using the random-effect model. Out of 958 reports, 28 articles were eligible, of which 22 studies were included in the meta-analysis. Most of the included studies demonstrated the beneficial effects of garlic on hepatic histopathological features. The pooled results showed that garlic significantly decreased hepatic triglyceride and cholesterol, ALT, AST, and liver weight. The certainty of the estimates was very low to low according to GRADE. In conclusion, our study demonstrated amelioration of hepatic histopathological characteristics, hepatic lipid content, serum liver enzymes, and liver weight following garlic administration.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD), the most common chronic liver disease, is characterized by hepatic triglyceride (TG) accumulation > 5 % of liver weight or volume and persistent abnormalities in liver enzymes (Fabbrini et al., 2010). An interplay between genetic background, epigenetic, diet, gut microbiota, oxidative stress, and environmental, metabolic, and hormonal factors can play a crucial role in the NAFLD development and progression (Shao et al., 2020). The global prevalence of NAFLD among adults was 25.24 % in 2016 (Younossi et al., 2016). Adherence to a healthy diet, reducing caloric intake, weight loss, and physical activity remain the cornerstone of NAFLD management (Beaton, 2012). The growing incidence and lack of definitive treatment encourage many researchers to seek agents, especially nutraceuticals, for NAFLD prevention and suppression.

Garlic (*Allium sativum L.*) is a traditional herbal food used all over the world, which its therapeutic efficacy has origins in ancient medical texts (Rivlin, 2001). Garlic contains various bioactive ingredients, including organic sulfides, saponins, phenolic compounds, and polysaccharides. Organosulfur compounds such as allicin, diallyl sulfide, alliin, ajoene, diallyl disulfide (DADS), diallyl trisulfide, S-allyl cysteine (SAC), and S-allylmercaptocysteine (SAMC) are major active components of garlic (De Greef et al., 2021; Shang et al., 2019). The amount of these compounds could be affected by different processing methods; as black garlic (fermented garlic prepared under high temperature and humidity) is reported to have much more functional compounds such as SAC and DADS than fresh garlic (Kimura et al., 2017). Numerous recent meta-analyses confirmed the health-promoting effects of garlic on obesity (Mofrad et al., 2019), blood pressure (Schwingshackl et al., 2016), lipid profile (Shabani et al., 2019), glucose parameters (Shabani et al., 2019), inflammation (Darooghegi Mofrad et al., 2019), and oxidative stress (Moosavian et al., 2020). In another meta-analysis of randomized controlled trials, garlic supplementation reduced serum aspartate transaminase (AST), but not alanine transaminase (ALT) levels, in patients with different health conditions (Panjeshahin et al., 2020). There are also extensive and emerging reports based on experimental research

Abbreviations: AGEs, advanced glycation end products; ALT, alanine transaminase; AMPK, AMP-activated protein kinase; AST, aspartate transaminase; CI, confidence intervals; DADS, diallyl disulfide; GRADE, Grading of Recommendations Assessment, Development, and Evaluation; H&E, hematoxylin-eosin; HFD, high-fat diet; NAFLD, Non-alcoholic fatty liver disease; NAS, NAFLD activity score; NF-κB, Nuclear factor-kappa B; MCD, methionine-choline deficient diet; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analysis; PROSPERO, International Prospective Register of Systematic Reviews; SAC, S-allyl cysteine; SAMC, S-allylmercaptocysteine; SD, standard deviations; SMD, standardized mean difference; TG, triglyceride.

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with conflicting findings (Lai et al., 2014; Lee et al., 2017; Lin et al., 2008; Hamed et al., 2010; Yang et al., 2019; Yu et al., 2021) on the effects of garlic or its constituents on NAFLD. Nonetheless, an evaluation of preventive and suppressive attributes of garlic on NAFLD has not been conducted in a systematic review or meta-analysis. The purpose of this study was providing a comprehensive review and meta-analysis of animal studies on the effects of garlic or its products and major bioactive components on hepatic histopathological parameters, hepatic lipid content, liver enzymes, and liver weight with a focus on mechanisms of action.

2. Methods

2.1. Research design and methods

The present systematic review was reported according to the PRISMA (Preferred Reporting Items for Systematic Reviews and meta-Analysis) guideline (Liberati et al., 2009). Moreover, the abstract was written according to the PRISMA 2020 extension checklist (Beller et al., 2013). The protocol of the present study has been registered in the International Prospective Register of Systematic Reviews (PROSPERO) with the identification number CRD42021274804.

2.2. Data source and search strategy

A systematic search was performed for the relevant articles published from 1 January 1970 to 14 June 2021 on the PubMed, Embase, Scopus, and Thomson Reuters Web of Knowledge. Searches were conducted using the following MeSH terms and relevant keywords: intervention (alliin OR isoalliin OR allium OR garlic OR carisano OR "s-allyl cysteine" OR "s-allylcysteine" OR "allyl cysteine" OR "s-2-propenyl-l-cysteine" OR allimin OR allicin OR ajoene OR "allylthiosulphinic acid allyl ester" OR "diallyl disulfide-oxide" OR allylthiosulfinate OR "diallyl disulfide" OR "allyll disulfide" OR garlicin OR allitin OR diallylsulfide OR "diallyl sulfide" OR "allyl sulfide" OR "diallyl trisulfide" OR "diallyl disulphide" OR "allyl disulfide" OR allitridum OR allitridin OR "allyl trisulfide" OR dasuansu OR allitridi OR "s-(2-propenyl)cysteine sulfoxide" OR "s-allylcysteine sulfoxide" OR s-allylmercaptocysteine) AND outcome (nafl OR "fatty liver" OR "nonalcoholic steatohepatitis" OR steato* OR "nonalcoholic fatty liver disease" OR "non-alcoholic steatohepatitis" OR "non-alcoholic fatty liver" OR "non alcoholic fatty liver" OR nafld OR nash). The PubMed search strategy is shown in Supplementary Box 1. No language restriction was included in the search. Moreover, forward and backward citation tracking was manually done for all of the included studies to ensure the inclusion of all eligible studies. In addition, Phytotherapy Research, Evidence Based Complementary and Alternative Medicine, and Frontiers in Pharmacology were selected as the key journals and underwent hand searching. The literature search was performed by two independent authors (S.S.Z. and A.R.S).

2.3. Eligibility criteria and screening methods

All searched records were exported to the EndNote software, version X8.0, and duplicates were deleted. Two independent reviewers screened titles and abstracts and then the full text of some selected studies based on the inclusion and exclusion criteria (S.S.Z. and A.R.S.). For any discrepancies between reviewers, the consensus was achieved through discussion. Study inclusion and exclusion criteria were structured using the PICOS (Population, Intervention, Comparison, Outcome, and Study Design) format (Table 1). Investigations were included in the current systematic review if they: (a) used rodent models of diet (high-fat diet (HFD), high fructose-diet, and methionine-choline deficient diet (MCD))-induced NAFLD (proven by hepatic histopathological analysis by hematoxylin-eosin (H&E) or hepatic TG content) (Kleiner et al., 2005), (b) assessed the effects of garlic or its major active components either orally or by injection on the NAFLD prevention or suppression, (c) had negative and positive control groups, and (d) examined hepatic fat content (TG and cholesterol), liver enzymes (ALT and AST), and liver weight. Previous reviews were used to find the main compounds of garlic (Amagase, 2006; Shang et al., 2019). Studies with the animal models of AFLD, fibrosis, cirrhosis or other liver diseases, genetic models of obesity, diabetes, or NAFLD, drug or toxin-induced fatty liver, and animals with alloxan or streptozotocin-induced diabetes were ineligible. In-vitro and human studies and investigations on the analogs and synthetic forms of garlic's ingredients, components related to parts of garlic other than cloves, and concurrent administration of garlic or its compounds with other agents were excluded.

Table 1
PICOS (Population, Intervention, Comparison, Outcome, and Study Design) criteria.

Parameter	Inclusion criteria	Exclusion criteria
Population	Rodents, all strains and species, all ages, and both sexes with high-fat diet, high fructose-diet, and methionine-choline deficient diet-induced non-alcoholic fatty liver disease	Animals other than rodents; animals with other diseases; human; cell lines
Intervention	Garlic; alliin; S-allylcysteine; S-allylmercaptocysteine; allicin; diallyl trisulfide; allyl sulfide; ajoene; diallyl disulfide (injection or oral)	Concurrent administration of interested interventions with other agents; analogs and synthetic forms of garlic's ingredients; not major components of garlic; components related to parts of garlic other than cloves
Comparison	Positive control group with no (or a placebo) treatment	No negative or positive control groups
Outcomes	Hepatic triglyceride and cholesterol content, serum alanine transaminase and aspartate transaminase levels, and liver weight	
Study design	Animal studies Published up to June 2021	*In vitro* and *ex vivo* studies; human studies; reviews; newspapers; magazines; conference abstracts; book chapters; notes; short surveys; letters; editorials; case report studies; clinical trial registration

2.4. Data extraction

Data extraction form was developed and pilot-tested by two independent authors (S.S.Z. and A.K.) and summarized in Table 2. Moreover, the means and standard deviations (SDs) of the hepatic TG and cholesterol, serum ALT and AST, and liver weight of animals in both treatment (animals with NAFLD under treatment with garlic or its major components) and positive control groups (reference group, animals with NAFLD with no or placebo treatment) were extracted. For studies reporting median and standard errors for considered outcomes, mean and SDs were calculated based on the sample sizes in each group. If a study did not report the required data, three emails were sent to the authors to obtain further information. According to the Cochrane criteria, when several doses of one treatment were evaluated in the same study, we combined groups to generate a single pair-wise comparison.

But, if outcomes were estimated by varying types of considered interventions in the same study, we split the shared positive control group into two or more groups with smaller sample sizes to include two or more independent comparisons and prevent multiplicity (Higgins & Green, 2011).

2.5. Risk of bias assessment

Two reviewers (S.S.Z. and M.R.F.) conducted risk of bias assessment independently with SYRCLE's risk of bias tool (Hooijmans et al., 2014a), and any discrepancies between them were resolved by discussion. This tool is an adapted version of the Cochran risk of bias tool (Higgins & Green, 2011) for animal studies and consists of 10 items or six domains of bias, including sequence generation (selection bias), baseline characteristics (selection bias), allocation concealment (selection bias), random housing (performance bias), blinding of caregivers and investigators (performance bias), random outcome assessment (detection bias), blinding of outcome assessors (detection bias), incomplete outcome data (attrition bias), selective outcome reporting (reporting bias), other bias. The risk of bias was judged as low, unclear, or high in each item. Then, for quality analysis, randomization, attrition bias, and reporting bias, which had the most differences between studies, were selected as the key domains based on the discussion among the reviewers. Studies with a high risk of bias in one or more key domains were considered high-risk (Higgins & Green, 2011).

2.6. Statistical analysis

The effects of garlic or its major constituent on the hepatic histopathological features were assessed by qualitative synthesis. Moreover, the treatment effect sizes on the hepatic TG and cholesterol, serum ALT and AST, and liver weight were evaluated by the random-effect model and the DerSimonian-Laird method (DerSimonian & Laird, 1986) due to methodological heterogeneity. The pooled effect sizes were considered as standardized mean differences (SMD, Glass's Δ) and a 95 % confidence interval (CI). The heterogeneity was assessed using Cochran's Q and I^2 statistics. The I^2 values of 0–40 %, 30–60 %, 50–90 %, and 75–100 % were considered as "perhaps not important", "moderate heterogeneity", "substantial heterogeneity", and "considerable heterogeneity", respectively (Higgins & Green., 2011). Subgroup analyses were implemented only if more than two studies were available for each category to identify possible sources of heterogeneity. This analysis was performed based on the types of animals (rats, rabbits, and mice) and interventions (garlic and its components), treatment duration ($\leq$6 and $>$ 6 weeks), NAFLD induction method (HFD and MCD), study model (prevention and suppression), and risk of bias (high and low). Publication bias was evaluated by visual assessment of funnel plot asymmetry and Egger's test. Then, if publication bias was detected, the trim-and-fill analysis was used to impute the missing studies. Sensitivity analysis also was conducted by the leave-one-out method to examine the impact of each study on the pooled SMD. All tests were performed using Stata MP Version 16 (StataCorp, College Station, TX, USA), and p-values < 0.05 were considered statistically significant.

2.7. Certainty of the evidence

The certainty of each outcome was evaluated using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach. Outcomes were rated as high-quality evidence and were downgraded based on the risk of bias, inconsistency, indirectness, imprecision, and publication bias criteria. Upgrading also was performed if the effects were consistent across different species (Hooijmans et al., 2018).

3. Results

3.1. Search results

The PRISMA flow diagram of the literature search is depicted in Fig. 1. The systematic search yielded 958 references, and two other studies also were found by the forward and backward citation tracking. After removing duplicates (n = 259) and title and abstract screening of the remaining articles (n = 701), 649 studies were excluded according to our predefined criteria. Then, the full-text of the other studies were reviewed for eligibility (n = 52), although one article had no full-text, and our efforts to communicate with the authors were unsuccessful (Zhang B. et al., 2002). Finally, 28 studies were included (Supplementary Table 1). Among them, six articles were only included in the qualitative analysis because they had examined the effects of garlic or its components solely on histopathological characteristics (Qamar et al., 2016; Sangi, 2016; Xiao et al., 2013a; Zalejska-Fiolka et al., 2015), had not mentioned the number of animals in each group (Chen et al., 2014), or had reported incomplete data (Xiao et al., 2013b). The other articles were included both in the quantitative and qualitative analysis.

3.2. Study characteristics

The main characteristics of the included studies are summarized in Table 2. These studies were published from 2008 to 2021 in Asia (Arifah et al., 2020; Chen et al., 2014; Ha et al., 2015; Han et al., 2011; Irfan et al., 2019; Jannah et al., 2021; Jiang et al., 2021; Jung et al., 2011; Kim & Kim, 2011; Lai et al., 2014; Lee et al., 2017; Li et al., 2010; Lin et al., 2008; Qamar et al., 2016; Sangi, 2016; Shin et al., 2014; Xiao et al., 2013a; Xiao et al., 2013b; Yang et al., 2018; Yang et al., 2019; Yu et al., 2021; Zhang N. et al., 2019; Zhang Y. et al., 2019; Zhongming et al., 2019), Africa (Egypt) (Amal & Sanaa, 2012; Seif el-Din et al., 2014; Hamed et al., 2010), and Europe (Zalejska-Fiolka et al., 2015). One study was in Chinese (Zhongming et al., 2019) and the others were in English. Of the 28 reviewed articles (extracted from 26 independent investigations), 18 eligible examinations were on the preventive (Amal & Sanaa, 2012; Chen et al., 2014; Ha et al., 2015; Jannah et al., 2021; Jiang et al., 2021; Lai et al., 2014; Lee et al., 2017; Lin et al., 2008; Qamar et al., 2016; Hamed et al., 2010; Sangi, 2016; Xiao et al., 2013a; Xiao et al., 2013b; Yang et al., 2018; Yang et al., 2019; Zalejska-Fiolka et al., 2015; Zhang N. et al., 2019; Zhongming et al., 2019) and 10 on the suppressive effects (Arifah et al., 2020; Seif el-Din et al., 2014; Han et al., 2011; Irfan et al., 2019; Jung et al., 2011; Kim & Kim, 2011; Li et al., 2010; Shin et al., 2014; Yu et al., 2021; Zhang Y. et al., 2019) of garlic or its major constituent on NAFLD. Thirteen studies utilized rats (Chen et al., 2014; Seif el-Din et al., 2014; Ha et al., 2015; Irfan et al., 2019; Li et al., 2010; Qamar et al., 2016; Hamed et al., 2010; Sangi, 2016; Xiao et al., 2013a; Xiao et al., 2013b; Yang et al., 2018; Zhang Y. et al., 2019; Zhongming et al., 2019), 12 were on mice (Arifah et al., 2020; Han et al., 2011; Jannah et al., 2021; Jiang et al., 2021; Jung et al., 2011; Kim & Kim, 2011; Lai et al., 2014; Lee et al., 2017; Lin et al., 2008; Shin et al., 2014; Yang et al., 2019; Zhang N. et al., 2019), two used rabbits (Amal & Sanaa, 2012; Zalejska-Fiolka et al., 2015), and the other study applied both rats and mice (Yu et al., 2021). Twenty-three studies were performed on male animals (Amal & Sanaa, 2012; Arifah et al., 2020; Chen et al., 2014; Seif el-Din et al., 2014; Ha et al., 2015; Han et al., 2011; Irfan et al., 2019; Jannah et al., 2021; Jiang et al., 2021; Kim & Kim, 2011; Lai et al., 2014; Lee et al., 2017; Li et al., 2010; Lin et al., 2008; Hamed et al., 2010; Sangi, 2016; Shin et al., 2014; Yang et al., 2018; Yang et al., 2019; Zalejska-Fiolka et al., 2015; Zhang N. et al., 2019; Zhang Y. et al., 2019; Zhongming et al., 2019); three were on females (Jung et al., 2011; Xiao et al., 2013a; Xiao et al., 2013b), and one was conducted in both sexes (Yu et al., 2021). The other study did not report the sex of the animals used in the examination (Qamar et al., 2016). The number of rodents in each group ranged from 4 (Arifah et al., 2020; Jannah et al., 2021) to 12 (Li et al., 2010; Lin et al., 2008),

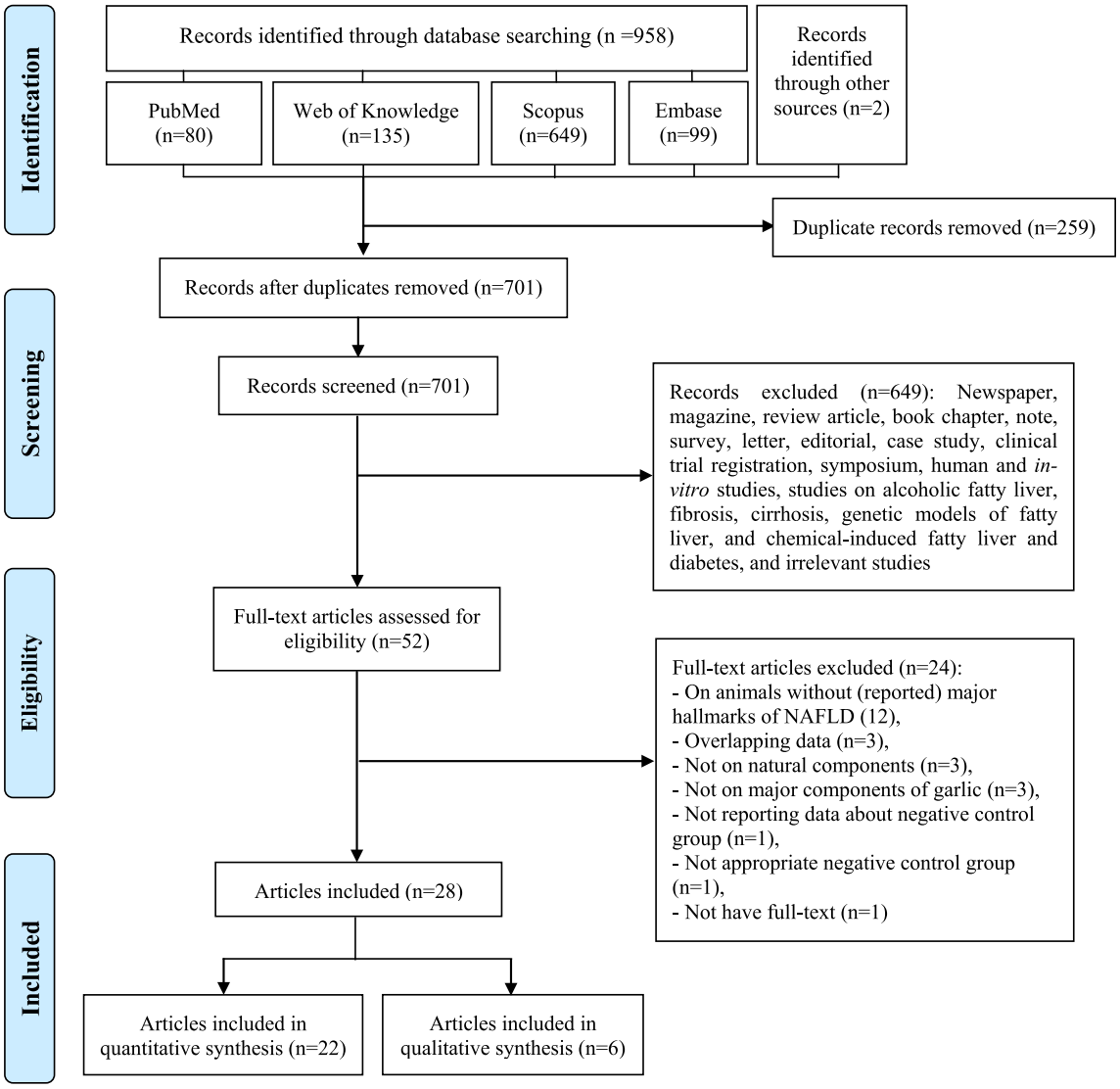

Fig. 1. PRISMA flow diagram of the literature search and study selection process.

although it was not specified exactly in three studies (Chen et al., 2014; Hamed et al., 2010; Yu et al., 2021), and only Hamed *et al.* (Hamed et al., 2010) replied to our email request in regards to missing information. In the included studies, NAFLD was induced in the animals by HFD (Amal & Sanaa, 2012; Arifah et al., 2020; Chen et al., 2014; Seif el-Din et al., 2014; Ha et al., 2015; Han et al., 2011; Irfan et al., 2019; Jannah et al., 2021; Jiang et al., 2021; Jung et al., 2011; Kim & Kim, 2011; Lai et al., 2014; Lee et al., 2017; Li et al., 2010; Qamar et al., 2016; Hamed et al., 2010; Sangi, 2016; Shin et al., 2014; Xiao et al., 2013a; Xiao et al., 2013b; Yang et al., 2018; Yang et al., 2019; Yu et al., 2021; Zalejska-Fiolka et al., 2015; Zhang N. et al., 2019; Zhang Y. et al., 2019; Zhongming et al., 2019) or MCD (Lin et al., 2008; Yu et al., 2021; Zhang N. et al., 2019). The animals in the eligible studies were treated with regular garlic (Amal & Sanaa, 2012; Seif el-Din et al., 2014; Jannah et al., 2021; Kim & Kim, 2011; Qamar et al., 2016; Hamed et al., 2010; Sangi, 2016; Zalejska-Fiolka et al., 2015), fermented garlic (fermentation by yeast (Jung et al., 2011), bacteria (Irfan et al., 2019; Lee et al., 2017), or aging process (Chen et al., 2014; Ha et al., 2015; Irfan et al., 2019; Jung et al., 2011; Lee et al., 2017; Shin et al., 2014)), garlic oil (Arifah et al., 2020; Lai et al., 2014; Yang et al., 2018; Zhang Y. et al., 2019), SAC (Lin et al., 2008), allicin (Li et al., 2010; Zhongming et al., 2019), ajoene (Han et al., 2011), SAMC (Xiao et al., 2013a; Xiao et al., 2013b; Yu et al., 2021), and DADS (Lai et al., 2014; Yang et al., 2019; Zhang N. et al., 2019). Garlic or its components were given to animals by oral administration (Amal & Sanaa, 2012; Arifah et al., 2020; Chen et al., 2014; Seif el-Din et al., 2014; Ha et al., 2015; Han et al., 2011; Irfan et al., 2019; Jannah et al., 2021; Jiang et al., 2021; Jung et al., 2011; Kim & Kim, 2011; Lai et al., 2014; Lee et al., 2017; Li et al., 2010; Lin et al., 2008; Qamar et al., 2016; Hamed et al., 2010; Sangi, 2016; Shin et al., 2014; Yang et al., 2018; Yang et al., 2019; Yu et al., 2021; Zalejska-Fiolka et al., 2015; Zhang N. et al., 2019; Zhang Y. et al., 2019; Zhongming et al., 2019), intraperitoneal injection (Xiao et al., 2013a; Xiao et al., 2013b), or Shenque acupoint (Zhang Y. et al., 2019). The duration of intervention administration ranged from 30 days (Jannah et al., 2021) to 20 weeks (Zhang N. et al., 2019).

Table 2
Data extraction of animal studies focusing on the effects of garlic on non-alcoholic fatty liver disease (NAFLD).

First author, year	Animals	n/ treatment group	Steatosis induction method	Type of treatment, rout of administration, dosage	Treatment duration	Comparator	Main effects	Risk of bias
Jiang G, 2021	Male C57BL/6 mice	10	HFD for 6 weeks	Aged black garlic extract, oral gavage, 100 mg/kg, everyday	6 weeks, along with HFD	HFD	↓ Hepatic steatosis and serum ALT ↔ AST	Low
Jannah FN, 2021	Male *Mus musculus* Balb/C mice, 34 g	4	HFD for 30 days	Single clove garlic extract, oral, 100 (a), 200 (b), and 400 (c) mg/kg, everyday	30 days, along with HFD	HFD	(ab) ↓ Liver weight and hepatic steatosis (c) ↔ Liver weight and steatosis	High
Arifah SN, 2020	Male Balb/C mice, 10 weeks old, 25 g	4	HFD for 80 days	Single clove garlic oil, oral gavage, 12.5, 25, and 50 mg/kg, everyday	35 days from 46th to 80th days	HFD	↓ Liver weight and steatosis	High
Yu Q, 2020	(a) Female SD rats, 8–10 weeks old, 180–200 g; (b) male C57BL/6N Mice, 18–24 g	(a) 6–9; (b) 6–7	(a) HFD for 12 weeks; (b) MCD diet for 6 weeks	SAMC, oral gavage, 200 mg/kg, three times/week	(a) 4 weeks from 9th to 12th weeks; (b) 3 weeks from 4th to 6th weeks	HFD (a), MCD (b)	(ab) ↓ Steatosis, NAS score, liver TG, and ALT	High
Zhang N, 2019	Male C57Bl/6J mice, 6–7 weeks old, 22 g	5	(a) MCD diet for 4 weeks, (b) HFD for 20 weeks	DADS, oral gavage, 20, 50 and 100 mg/kg, everyday	4 (a) or 20 (b) weeks, along with MCD or HFD	MCD + vehicle (corn oil) (a), HFD + vehicle (corn oil) (b)	(ab) ↓ Hepatic steatosis and inflammation, (ab) ↓ serum ALT and AST, hepatic cholesterol and TG and weight in a dose-dependent manner	High
Zhang Y, 2019	Male Wistar rats, 180–200 g	7	HFD for 13 weeks	Garlic oil: Shenque, 25 (a), 50 (b), and 100 (c) mg/kg/day; Oral, 50 mg/kg/day (d)	6 weeks, from 8th to 13th weeks	HFD	(a-d) ↓ Liver weight, ↓ Steatosis, especially in the c group	Low
Yang Y, 2019	Male C57BL/6 mice, 5 weeks old, 19.92 g	10	HFD for 8 weeks	DADS, oral gavage, 10 and 20 mg/kg, everyday	8 weeks, along with HFD	HFD	↑ Hepatic steatosis, ↔ Hepatic TG and cholesterol	Low
Irfan M, 2019	Male SD rats, 180–200 g	5	HFD for 5 weeks	Non-fermented (a) or fermented (b) garlic extract, oral gavage, 300 mg/kg, everyday	4 weeks, from 2th to 5th weeks	HFD + vehicle	↓ Hepatic steatosis, especially by fermented garlic, (ab): ↔ ALT and AST, (b): ↓ Liver weight	High
Zhongming Y, 2019	Male SD rats, 346 g	10	HFD for 7 weeks	Allicin, oral gavage, 30 mg/kg/day	7 weeks, along with HFD	HFD + water	↓ Hepatic steatosis, liver weight, ALT and AST	Low
Yang C, 2018	Male SD rats, 120–150 g	12	HFD for 60 days	Garlic oil, oral gavage, 11.6 (a), 46.3 (b), and 92.6 (c) mg/kg, everyday	60 days, along with HFD	HFD + vehicle (Tween 80)	(bc) ↑ Liver/body weight and ↓ steatosis	Low
Lee HS, 2017	Male C57/BL6 J mice, 20 g	6	HFD for 8 weeks	Non-fermented (NFGE) or fermented (FGE) garlic extract, oral gavage, 500 mg/kg, everyday	8 weeks, along with HFD	HFD + saline	↓ Steatosis, ALT and hepatic weight and TG, especially by FGE, ↓ AST and hepatic cholesterol by FGF	Low
Sangi SMA, 2016	Male rats, 90–120 days old, 200–240 g	6	HFD for 8 weeks	Diet containing 6 % fresh crushed garlic	8 weeks, along with HFD	HFD	↓ Hepatic steatosis	High
Qamar A, 2016	Albino rats, 90–120 days old, 200–240 g	5	HFD for 4 (a) or 8 (b) weeks	Diet containing 6 % fresh crushed garlic	4 (a) or 8 (b) weeks, along with HFD	HFD	(ab) ↓ Liver weight and hepatic fatty changes	High
Zalejska-Fiolka J, 2015	Male Chinchilla rabbits, 2800 g	6	High oxidized olive oil (a) or high oxidized rapeseed oil (b), for 12 weeks	Diet containing 4 mg/kg garlic extract	12 weeks, along with HFD	HFD	(ab) ↓ Hepatic steatosis	High
Ha AW, 2015	Male SD rats, 4 weeks old	8	HFD for 5 weeks	Diet containing 0.5 % (a) or 1.5 % (b) black garlic extract	5 weeks, along with HFD	HFD	(ab) ↓ ALT, ↔ Liver weight and cholesterol and TG content, (b) ↓ Total hepatic lipid content and AST	Low
Shin JH, 2014	Male C57BL/6 mice, 7 weeks old	8	HFD for 4 weeks	Aged black garlic extract, oral, 100 (a) and 200 (b) mg/kg, 5 days/week	4 weeks following steatosis induction	HFD + vehicle	b: ↓ Steatosis and liver weight; ab: ↓ Plasma ALT and AST, ↔ Hepatic TG and plasma ALP	High
Chen YC, 2014	Male Wistar rats, 8 weeks old	ns	HFD for 8 weeks	Diet containing 0.1 % (a), 0.3 % (b), and 0.6 %	8 weeks, along with HFD	HFD	(abc) ↔ Liver weight, ALT, and AST, ↓	High

(*continued on next page*)

Table 2 (*continued*)

First author, year	Animals	n/ treatment group	Steatosis induction method	Type of treatment, rout of administration, dosage	Treatment duration	Comparator	Main effects	Risk of bias
				(c) methanol extract of black garlic			Steatosis, hepatic TG and cholesterol	
Lai YS, 2014	Male C57BL/6J mice, 6 weeks old	10	HFD for 12 weeks	(a) Garlic essential oil, 25, 50, and 100 mg/kg, (b) DADS, 10 and 20 mg/kg, Oral gavage, everyday	12 weeks, along with HFD	HFD + vehicle (olive oil)	(ab) ↓ Fatty liver score, hepatic TG and cholesterol, and serum ALT and AST, dose dependently	Low
Seif el-Din SH, 2014	Male SD rats, 120 g	10	HFD for 20 weeks (a), HFD for 12 weeks (b)	Garlic, oral gavage, 500 mg/kg	(ab) 8 weeks from 13th to 20th weeks	HFD	(ab) ↓ Steatosis and liver injury, liver weight index, and serum ALT, AST, and ALP	Low
Xiao J, 2013 (a)	Female SD rats, 8 weeks old, 180–200 g	7	HFD for 8 weeks	SAMC, i.p. injection, 200 mg/kg, three times/week *	8 weeks, along with HFD	HFD	↓ Hepatic fatty droplets, inflammatory foci numbers, and NAS score	High
Xiao J, 2013 (b)	Female SD rats, 8 weeks old, 180–200 g	7	HFD for 8 weeks	SAMC, i.p. injection, 200 mg/kg, three times/week *	8 weeks, along with HFD	HFD	↓ Steatosis, liver injury, fibrosis, and serum ALT	High
Amal AF, 2012	Male white New Zealand rabbits, 1000–1200 g	7	HFD for 4 weeks	Aqueous garlic extract, oral, 500 mg/kg, everyday	4 weeks, along with HFD	HFD	↓ Steatosis, ↓ ALT, AST, and ALP	High
Han, 2011	Male C57BL/6 mice, 7 weeks old	8	HFD for 8 weeks	Ajoene, oral gavage, 10 (a) or 30 (b) mg/kg, 5 days/week	4 weeks from 5th to 8th weeks	HFD + vehicle (polyethyleneglycol)	(ab) ↓ Hepatic TG accumulation, steatosis and injury, especially by the high dose,(b) ↓ ALT and liver weight	High
Jung YM, 2011	Female ICR mice, 5.5 weeks old	5	HFD for 91 days	400 mg/kg non-fermented black garlic extract (a), or 100 (b), 200 (c), and 400 (d) mg/kg fermented black garlic extract, oral gavage, everyday	63 days from 28th to 91th days	HFD	(a-d): ↓ ALT, AST, and steatohepatitis	High
Kim M, 2011	Male C57BL/6 mice, 4 weeks old	10	HFD for 9 weeks	Diet containing 2 % (a) or 4 % (b) garlic	4 weeks from 6th to 9th weeks	HFD	(ab): ↓ Liver weight, ALT, and steatosis, ↓ AST especially by high dose	Low
Hamed MR, 2010	Male SD rats, 225 g	10	HFD for 8 weeks	Fresh garlic homogenate, oral gavage, 250 mg/kg, everyday	8 weeks, along with HFD	HFD + water	↓ Hepatic steatosis, ↓ ALT and AST	High
Li W, 2010	Male Wistar rats, 4 months old, 220–250 g	12	HFD for 12 weeks	Allicin was added to HFD, 60 mg/kg/day	8 weeks from 5th to 12th weeks	HFD	↓ Fatty degeneration, ↓ ALT and AST	Low
Lin CC, 2008	Male C57BL/6 mice, 3–4 weeks old	12	MCD diet for 7 weeks	SAC 1 g/L in drinking water	7 weeks, along with MCD	MCD	↓ Hepatic TG, ↓ ALT and AST, Improvement of liver weight,↔ Hepatic cholesterol	High

ALP: alkaline phosphatase, ALT: alanine aminotransferase, AST: aspartate aminotransferase, DADS: diallyl disulfide, ELISA: enzyme-linked immunosorbent assay, HFD: high-fat diet, i.p.: intraperitoneal, MCD: methionine-choline deficient diet, NAS: NAFLD activity scoring, ns: not stated, SAC: S-allyl cysteine, SAMC: S-allyl-mercaptocysteine, SD: Sprague–Dawley, TG: triglyceride.

3.3. Risk of bias assessment

The risk of bias evaluation of the included studies is presented in Fig. 2 and Supplementary Fig. 1. Accordingly, 12 of the articles (42.8 %) did not use randomization, and the others were randomized but did not report the method of the allocation sequence generation. The baseline characteristics of the animals (sex, age, or weight) were not stated to be similar in two of the reviewed studies (7.1 %). None of the studies described the allocation concealed, random housing of animals, blinding of caregivers and investigators, and random outcome assessment. The primary outcome assessment was not blinded in 26 studies (92.8 %). The risk of attrition bias was high in four of the included studies (14.3 %), low in 10 of the articles (35.7 %), and unclear in the others (50 %). The risk of reporting bias was also high in 9 reviewed studies (32.1 %) and low in the others. Finally, 17 (60.7 %) studies were considered high-risk, and the others low-risk of bias.

3.4. Main outcomes

3.4.1. Histopathology

Twenty-six eligible studies (92.8 %) assessed the effects of garlic or its components on H&E histopathological analysis. Among them, three investigations examined the intervention effects on the hepatic steatosis severity by NAFLD activity score (NAS) and reported the efficacy of SAMC and allicin on amelioration of total NAS score (Xiao et al., 2013a; Yu et al., 2021; Zhongming et al., 2019). NAS is a validated histological feature scoring system that comprises the full spectrum of the NAFLD lesions, including steatosis, hepatocellular ballooning, lobular inflammation, and fibrosis (Kleiner et al., 2005). Most of the other studies also demonstrated beneficial effects of the interventions on steatosis (Amal & Sanaa, 2012; Arifah et al., 2020; Chen et al., 2014; Seif el-Din et al., 2014; Han et al., 2011; Irfan et al., 2019; Jannah et al., 2021; Jiang et al., 2021; Kim & Kim, 2011; Lee et al., 2017; Li et al., 2010; Qamar et al.,

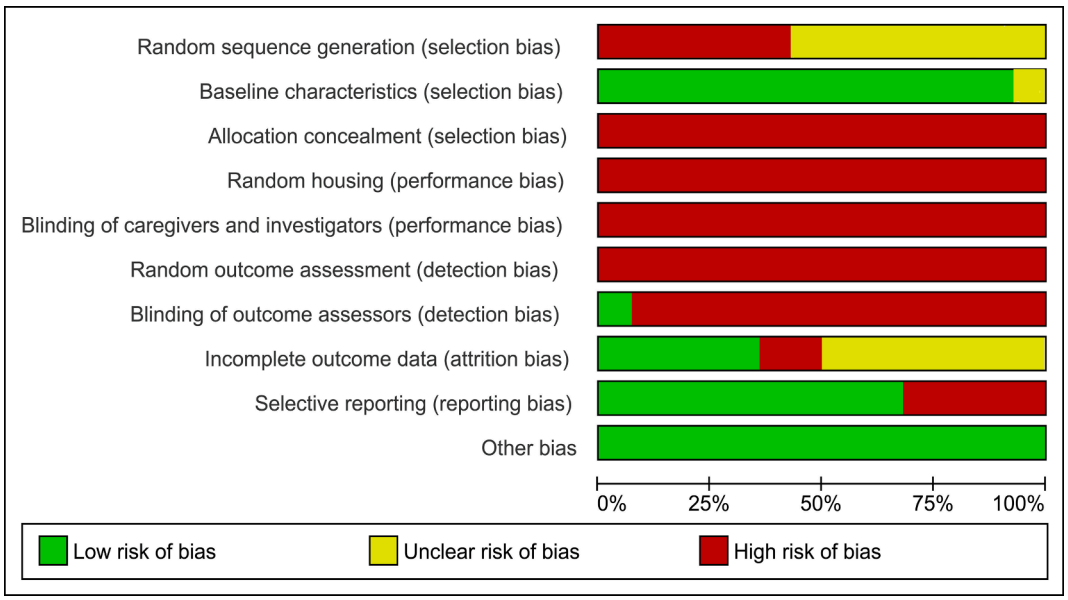

Fig. 2. Risk of bias graph.

2016; Hamed et al., 2010; Sangi, 2016; Xiao et al., 2013a; Xiao et al., 2013b; Yang et al., 2018; Zalejska-Fiolka et al., 2015; Zhang N. et al., 2019; Zhang Y. et al., 2019), inflammation (Jung et al., 2011; Zhang N. et al., 2019), ballooning (Jannah et al., 2021), hepatic injury, and fibrosis (Seif el-Din et al., 2014; Han et al., 2011; Xiao et al., 2013b). Nonetheless, in the study by Jannah *et al.*, the consumption of 400 mg/kg garlic extract, contrary to the lower doses of 100 and 20 mg/kg, could not prevent steatosis induction in male *Mus musculus* Balb/C mice (Jannah et al., 2021). In contrast, in another study, oral gavage of 10 and 20 mg/kg DADS for eight weeks caused fat deposition in the liver of C57BL/6 mice fed normal-fat-diet and HFD (Yang et al., 2019) (Table 2).

3.4.2. Hepatic lipid content

Hepatic TG was assessed in 10 (35.7 %) and hepatic cholesterol in seven (25 %) eligible studies. Although, two of the studies were not included in the quantitative synthesis due to not reporting sample size and incomplete outcome data (Chen et al., 2014; Zhang N. et al., 2019). Chen *et al.* and Zhang *et al.* assessed the effects of oral administration of black garlic extract and DADS, respectively, and reported decreased hepatic TG and cholesterol content (Table 2).

The meta-analyses were performed on 11 and seven effect sizes for hepatic TG and cholesterol, respectively. The pooled SMDs for the effects of garlic or its bioactive constituent on hepatic TG and cholesterol in the intervention compared to comparison groups were −1.72 (95 % CI: −2.60 to −0.85, p-value < 0.001, Fig. 3a) and −1.11 (95 % CI: −2.17 to −0.06, p-value = 0.04, Fig. 3b), respectively. Substantial between-study heterogeneity was observed in both analyses (I^2 = 71.28 % and p-value for Q test < 0.001 for hepatic TG and I^2 = 69.76 % and p-value for Q test < 0.001 for hepatic cholesterol). For hepatic TG, there were no significant differences in the subgroup analyses stratified by types of animals (p-value = 0.91) and interventions (p-value = 0.39), treatment duration (p-value = 0.59), NAFLD induction method (p-value = 0.06), study model (p-value = 0.87), and risk of bias (p-value = 0.21). Analyses of subgroups stratified by intervention type (p-value for Q test = 0.78), treatment duration (p-value for Q test = 0.46), and study model (p-value for Q test = 0.42) reduced the heterogeneity to zero. When hepatic cholesterol data were stratified by intervention type, the pooled effect sizes of garlic (SMD = -0.73, 95 % CI: −1.56 to 0.09) and its compounds (SMD = -1.62, 95 % CI: −3.53 to 0.30) were statistically similar (p-value = 0.41), and the heterogeneity was significantly reduced (I^2 = 0.00 %, p-value for Q test = 0.39) (Table 3).

3.4.3. Serum liver enzymes

Nineteen of the included studies (67.8 %) examined the effects of garlic or its ingredients on serum ALT, and 16 studies (57.1 %) assessed serum AST. In the study by Chen *et al.* (Chen et al., 2014), black garlic

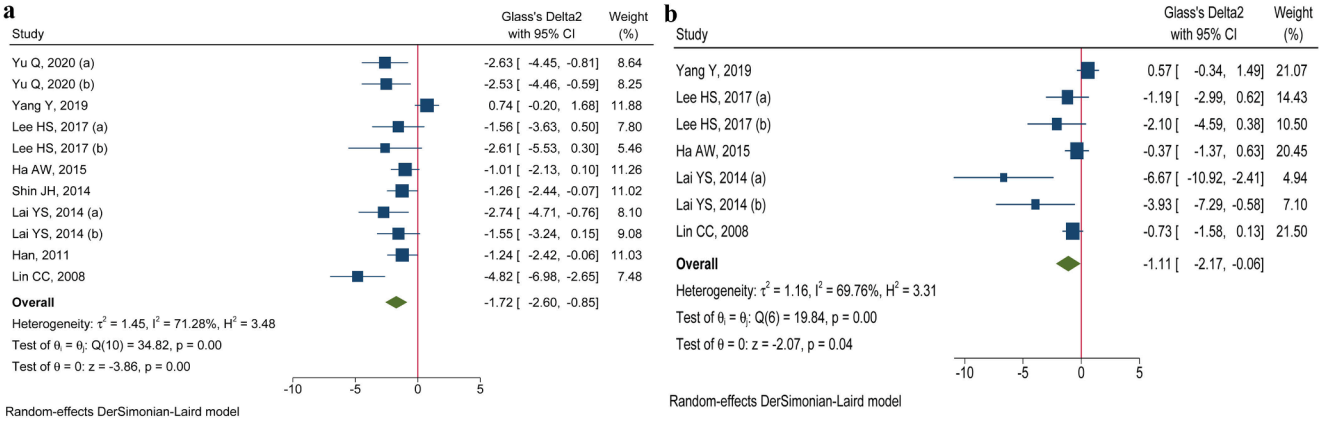

Fig. 3. Forest plot depicting the effects of garlic or its components on hepatic triglyceride (a) and cholesterol (b) content in animal models of non-alcoholic fatty liver disease.

Table 3
Stratified meta-analysis of the effects of garlic or its major components on hepatic lipid content, liver enzymes, and liver weight.

Variable		Subgroups	Number of effect sizes	Pooled SMD (95 % CI)	P-for-difference	P-for-heterogeneity	I^2 (%)
Hepatic TG	All		11	−1.72 (−2.60 to −0.85)	<0.001	<0.001	71.28
	Type of animals	*Mice*	9	−1.77 (−2.84 to −0.69)	0.91	< 0.001	75.23
		Rat	2	−1.65 (−3.21 to −0.10)		0.14	54.76
	Interventions	*Garlic*	4	−1.27 (−2.00 to −0.54)	0.39	0.78	0.00
		Compounds	7	−1.97 (−3.36 to −0.57)		< 0.001	82.18
	Treatment duration	*≤6*	5	−1.45 (−2.05 to −0.85)	0.59	0.46	0.00
		>6 weeks	6	−1.96 (−3.76 to −0.17)		< 0.001	83.22
	NAFLD induction method	*HFD*	9	−1.31 (−2.12 to −0.50)	0.06	0.01	62.51
		MCD	2	−3.62 (−5.86 to −1.38)		0.12	58.19
	Study model	*Prevention*	7	−1.76 (−3.14 to −0.37)	0.87	< 0.001	79.88
		Suppression	4	−1.63 (−2.33 to −0.92)		0.42	0.00
	Risk of bias	*High*	5	−2.26 (−3.39 to −1.12)	0.21	0.04	61.31
		Low	6	−1.22 (−2.40 to −0.03)		0.01	69.91
Hepatic cholesterol	All		7	−1.11 (−2.17 to −0.06)	0.04	< 0.001	69.76
	Interventions	*Garlic*	3	−0.73 (−1.56 to 0.09)	0.41	0.39	0.00
		Compounds	4	−1.62 (−3.53 to 0.30)		< 0.001	82.84
Serum ALT	All		23	−1.99 (−2.62 to −1.35)	< 0.001	< 0.001	64.89
	Type of animals	*Mice*	14	−2.33 (−3.15 to −1.51)	0.23	< 0.001	56.73
		Rat/Rabbit	9	−1.54 (−2.54 to −0.53)		< 0.001	72.56
	Interventions	*Garlic*	13	−1.66 (−2.30 to −1.03)	0.17	0.06	40.49
		Compounds	10	−2.65 (−3.91 to −1.40)		< 0.001	78.79
	Treatment duration	*≤6*	11	−1.57 (−2.26 to −0.88)	0.08	0.02	52.24
		>6 weeks	12	−2.79 (−3.97 to −1.61)		< 0.001	73.54
	NAFLD induction method	*HFD*	20	−1.79 (−2.38 to −1.20)	0.17	< 0.001	56.06
		MCD	3	−4.95 (−9.46 to −0.45)		< 0.001	88.89
	Study model	*Prevention*	12	−1.76 (−2.53 to −1.00)	0.48	< 0.001	61.98
		Suppression	11	−2.25 (−3.37 to −1.14)		< 0.001	68.72
	Risk of bias	*High*	13	−1.72 (−2.60 to −0.84)	0.33	< 0.001	65.70
		Low	10	−2.37 (−3.35 to −1.39)		< 0.001	66.86
Serum AST	All		20	−1.53 (−2.29 to −0.78)	< 0.001	< 0.001	74.22
	Type of animals	*Mice*	12	−1.34 (−2.25 to −0.43)	0.60	< 0.001	72.22
		Rat/Rabbit	8	−1.79 (−3.17 to −0.40)		< 0.001	76.58
	Interventions	*Garlic*	13	−1.23 (−2.15 to −0.30)	0.24	< 0.001	71.32
		Compounds	7	−2.25 (−3.69 to −0.82)		< 0.001	80.73
	Treatment duration	*≤6*	8	−0.85 (−2.05 to 0.36)	0.14	< 0.001	76.51
		>6 weeks	12	−2.02 (−3.01 to −1.03)		< 0.001	71.89
	NAFLD induction method	*HFD*	18	−1.29 (−2.00 to −0.59)	0.31	< 0.001	69.24
		MCD	2	−6.30 (−16.03 to 3.43)		< 0.001	92.97
	Study model	*Prevention*	12	−1.39 (−2.06 to −0.71)	0.74	< 0.001	63.08
		Suppression	8	−1.80 (−4.10 to 0.50)		< 0.001	83.81
	Risk of bias	*High*	10	−1.88 (−3.24 to −0.53)	0.47	< 0.001	69.85
		Low	10	−1.29 (−2.19 to −0.39)		< 0.001	76.25
Liver weight	All		18	−1.38 (−1.94 to −0.81)	< 0.001	< 0.001	54.04
	Type of animals	*Mice*	10	−2.25 (−3.22 to −1.28)	**< 0.001**	0.02	54.19
		Rat	8	−0.59 (−1.05 to −0.14)		0.35	10.53
	Interventions	*Garlic*	9	−1.32 (−2.11 to −0.53)	0.73	0.04	50.66
		Compounds	9	−1.53 (−2.42 to −0.63)		0.01	61.38
	Treatment duration	*≤6*	9	−1.34 (−2.14 to −0.53)	0.78	0.03	54.06
		>6 weeks	9	−1.51 (−2.39 to −0.63)		0.01	58.70
	Study model	*Prevention*	9	−1.23 (−2.07 to −0.39)	0.59	0.01	57.85
		Suppression	9	−1.54 (−2.31 to −0.77)		0.06	47.06
	Risk of bias	*High*	7	−1.19 (−1.77 to −0.61)	0.34	0.92	0.00
		Low	11	−1.73 (−2.66 to −0.80)		< 0.001	70.60

ALT: alanine aminotransferase, AST: aspartate aminotransferase, HFD: high fat diet, MCD: methionine-choline deficient diet, NAFLD: non-alcoholic fatty liver disease, SMD: standardized mean differences, TG: triglyceride.

prevented the HFD-induced increment of the ALT and AST levels in male rats. Xiao *et al.* (Xiao et al., 2013b) also reported prevention of the ALT elevation by SAMC administration. These studies did not report the required data to be included in the meta-analysis (Table 2).

The meta-analyses were done on 23 and 20 independent effect sizes for serum ALT and AST, respectively. The combined SMDs for the impact of garlic or its constituent on serum ALT (SMD = -1.99, 95 % CI: −2.62 to −1.35, p-value < 0.001, Fig. 4) and AST (SMD = -1.53, 95 % CI: −2.29 to −0.78, p-value < 0.001, Fig. 5) were statistically significant with substantial between-study heterogeneities (I^2 = 64.89 % and p-value for Q test < 0.001 for serum ALT and I^2 = 74.22 % and p-value for Q test < 0.001 for serum AST). No significant differences were observed in the subgroups of ALT and AST levels. However, the heterogeneity was significantly reduced following subgroup analysis by type of intervention (I^2 = 40.49 % and p-value for Q test = 0.06) (Table 3).

3.4.4. Liver weight

Liver weight was investigated in 16 of the eligible studies (57.1 %) that two of them were not included in the quantitative synthesis due to the following reasons. (a) One study was on the MCD-induced NAFLD, and contrast to the HFD-induced model, reported a reduced liver weight after MCD administration (Lin et al., 2008). In this study, SAC was effective on improvement of MCD-induced changes on liver weight. (b) Another study did not report the sample size in each group (Chen et al., 2014), and observed no effect of different doses of black garlic extract on liver weight (Table 2).

Fourteen studies (18 independent effect sizes) were included in the quantitative synthesis for liver weight. The meta-analysis suggested a significant effect of garlic or its components on liver weight (SMD = −1.38, 95 % CI: −1.94 to −0.81, p-value < 0.001) with substantial heterogeneity (I^2 = 54.04 % and p-value for Q test < 0.001) (Fig. 6). This

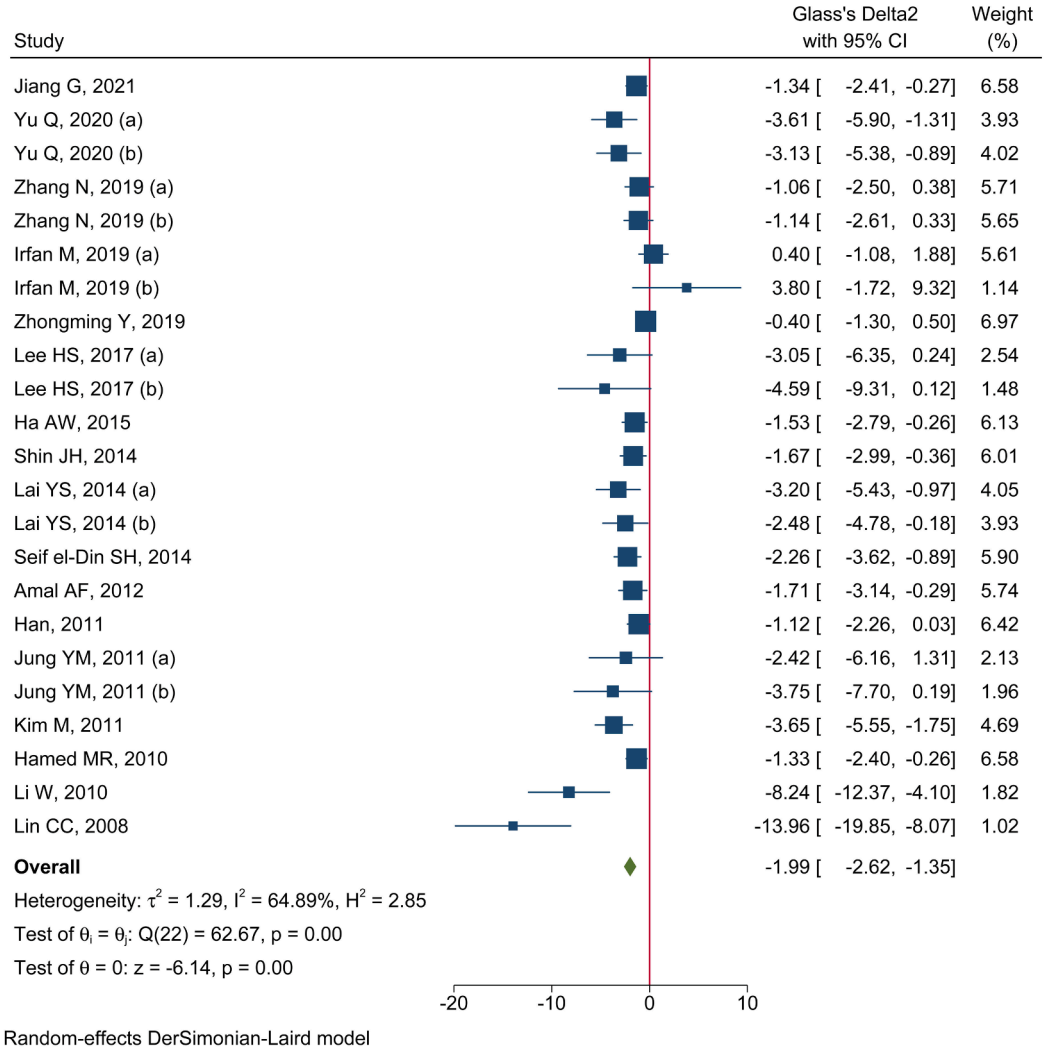

Fig. 4. Forest plot depicting the effects of garlic or its components on serum alanine transaminase levels in animal models of non-alcoholic fatty liver disease.

impact was significantly stronger in the mice (SMD = −2.25, 95 % CI: −3.22 to −1.28) compared to the rats (SMD = −0.59, 95 % CI: −1.05 to −0.14, p-value < 0.001). The heterogeneity was significantly reduced in subgroup analyses based on the type of animals (I^2 = 10.53 %, p-value for Q test = 0.35), study model (I^2 = 47.06 %, p-value for Q test = 0.06), and risk of bias (I^2 = 0.00 %, p-value for Q test = 0.92) (Table 3).

3.5. Sensitivity analysis and publication bias

No particular study had a significant impact on the summary effect in the sensitivity analysis for all outcomes. The pooled SMD ranged from −1.42 to −1.91 for hepatic TG, −0.69 to −1.51 for hepatic cholesterol, −1.79 to −2.11 for serum ALT, −1.30 to −1.68 for AST, and −1.12 to −1.52 for liver weight (Supplementary Fig. 2).

Visual inspection of funnel plot asymmetry (Fig. 7) and also Egger's test suggested potential publication bias for the effects of garlic or its compounds on hepatic TG (p-value = 0.003), hepatic cholesterol (p-value = 0.015), serum ALT (p-value = 0.001), serum AST (p-value = 0.035), and liver weight (p-value < 0.001). According to the trim-and-fill analysis, four, three, seven, and eight potential missing studies were required on the right side of the funnel plots related to hepatic TG, hepatic cholesterol, serum ALT, and liver weight, respectively, to make it symmetric (Supplementary Fig. 3). The correction caused a slight change in the pooled SMD of hepatic TG (SMD = −1.08, 95 % CI: −1.98 to −0.18) and serum ALT (SMD = -1.34, 95 % CI: −2.31 to −0.37). Although, the pooled effects of garlic on hepatic cholesterol (SMD = −0.36, 95 % CI: −2.10 to 1.39) and liver weight (SMD = −0.70, 95 % CI: −1.54 to 0.13) became non-significant after the trim-and-fill correction for publication bias.

3.6. Exploratory outcomes

In a separate analysis, we compared the effects of fermented or raw garlic on the NAFLD-related markers. No significant differences were detected in the effects of fermented in comparison with raw garlic on serum ALT (SMD = −1.59, 95 % CI: −2.35 to −0.84 *vs* SMD = −1.74, 95 % CI: −2.79 to −0.68, p-value = 0.83), AST (SMD = −1.91, 95 % CI: −3.43 to −0.39 *vs* SMD = −0.73, 95 % CI: −2.02 to 0.56, p-value = 0.25), and liver weight (SMD = −0.71, 95 % CI: −1.60 to 0.17 *vs* SMD = −2.20, 95 % CI: −3.90 to −0.49, p-value = 0.13) (Supplementary Fig. 4).

3.7. Certainty of the evidence

The certainty of the estimates was rated low for hepatic TG and serum ALT and was graded very low for hepatic cholesterol, serum AST, and liver weight. One of the reasons for downgrading the evidence was the risk of bias since almost all included studies were not blinded,

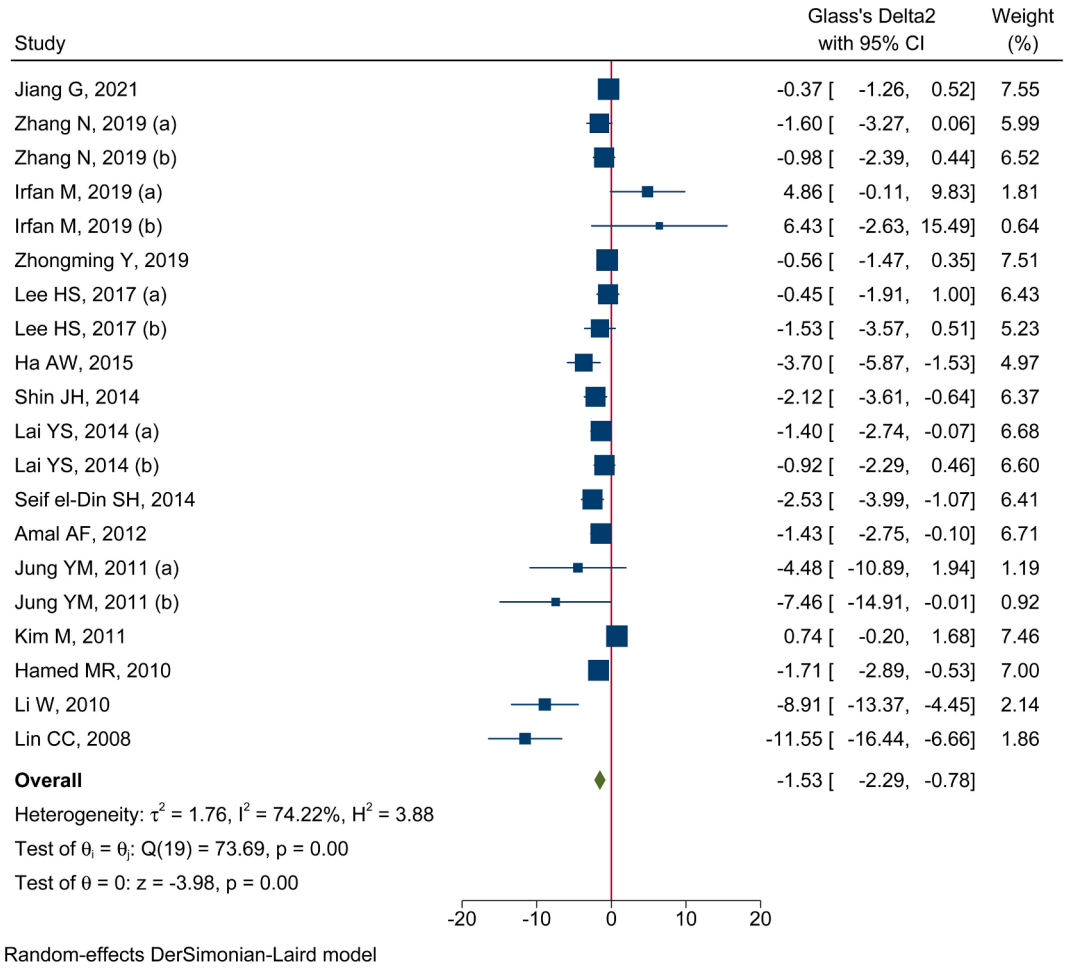

Fig. 5. Forest plot depicting the effects of garlic or its components on serum aspartate transaminase levels in animal models of non-alcoholic fatty liver disease.

although most of them were randomized. Moreover, all outcomes were graded down due to indirectness and publication bias. The evidence was also downgraded one level for inconsistency for serum AST because of significant unexplained between-study heterogeneity. Hepatic cholesterol and serum AST also were rated as imprecise because of the wide confidence interval. The GRADE assessment and the explanations related to each judgment are reported in Supplementary Table 3.

4. Discussion

This systematic review and meta-analysis study assessed the effects of garlic on histopathological parameters, hepatic lipid content, serum liver enzymes, and liver weight. Most of the included studies demonstrated the efficacy of garlic or its ingredients on hepatic steatosis severity, inflammation, ballooning, hepatic injury, and fibrosis. The meta-analysis also showed that these interventions significantly reduced hepatic lipid content, serum liver enzymes, and liver weight. The efficacy of interventions on liver weight was greater among investigations performed on mice. While, the effects of garlic on hepatic lipid content and serum liver enzymes were similar among different subgroups. Type of animals, interventions, treatment duration, study model, and risk of bias were suggested as potential sources of heterogeneity for some studied outcomes. Due to the nature of animal research, statistical and methodological between-study heterogeneity, the quality weaknesses of the included studies regarding performance and detection bias, low/very low certainty of evidence, and the observed publication bias in the present meta-analysis, interpretation of the findings should proceed with caution.

The results of the present study are in line with the limited available human studies. In a recent clinical trial by Sangouni *et al.*, 12-week garlic powder supplementation at the dose of 1600 mg/day resulted in hepatic steatosis amelioration and liver enzyme levels reduction (Sangouni et al., 2020). In another clinical trial study, 110 NAFLD patients (26 participants also had diabetes) were treated with 800 mg/day garlic powder for 15 weeks, and their hepatic steatosis and liver enzymes were improved (Soleimani et al., 2020). Zhang *et al.* also performed a cross-sectional study on 24,106 Chinese subjects of both sexes and demonstrated a significant inverse association between raw garlic consumption and NAFLD, only in men (Zhang S. et al., 2019). In another case-control investigation, higher consumption of allium vegetables (raw garlic and onions) was associated with a lower risk of NAFLD (Emamat et al., 2020). However, in a previous meta-analysis of randomized controlled trials, garlic supplementation reduced AST levels, but not ALT, in a population consisting of patients with elevated serum gamma-glutamyl transferase, hypertension, arsenical palmer keratosis, hypercholesterolemia, coronary artery disease, and peritoneal dialysis (Panjeshahin et al., 2020).

Our findings are also close to those obtained from other animal models. Takemura *et al.* evaluated the effects of SAC (0.45 % diet) on Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a type 2 diabetes model, and suggested therapeutic potential of this sulfur-containing amino acid in diabetes and NAFLD (Takemura et al., 2013). In another study, aged garlic extract ameliorated glucose intolerance and hepatic TG accumulation in ddY–H mice, a mouse model of spontaneous insulin-resistance and NAFLD (Maeda et al., 2019). Some other studies also reported the beneficial effects of garlic products on acute liver damages

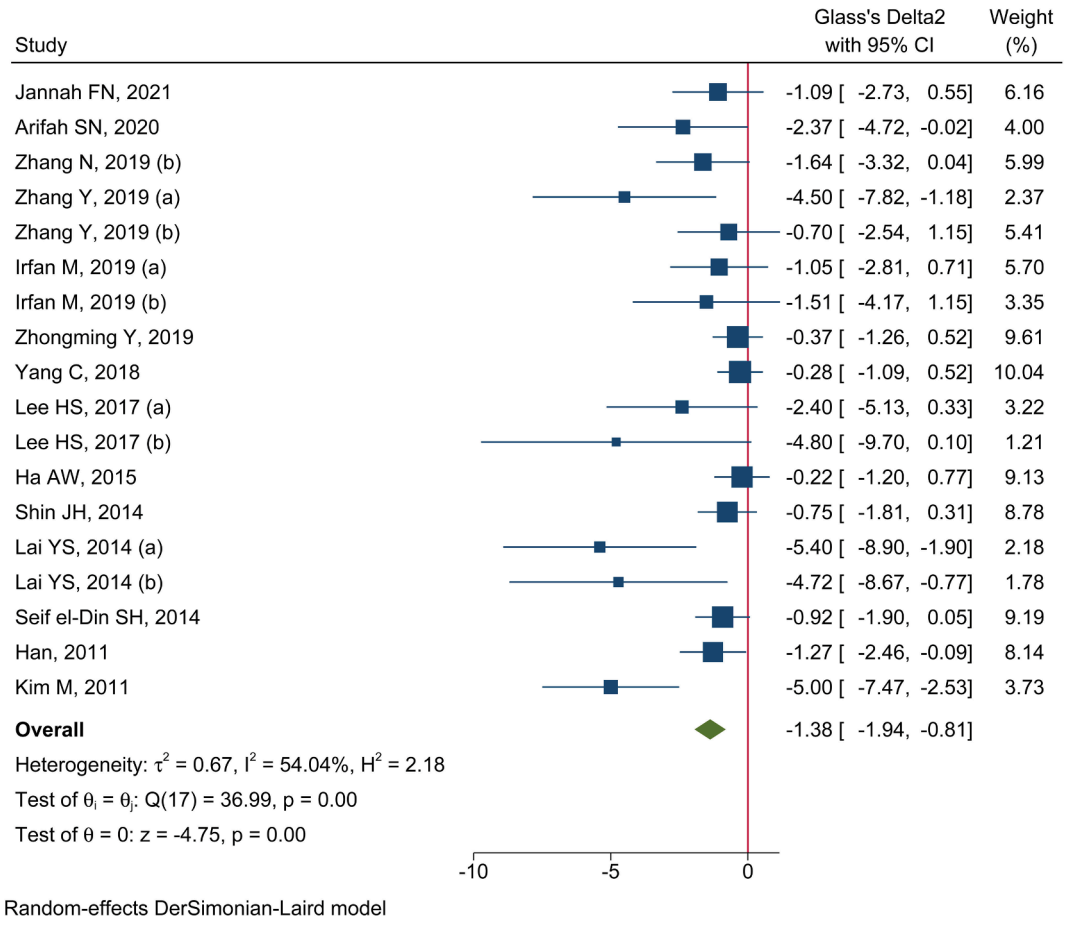

Fig. 6. Forest plot depicting the effects of garlic or its components on liver weight in animals with high-fat diet-induced non-alcoholic fatty liver disease.

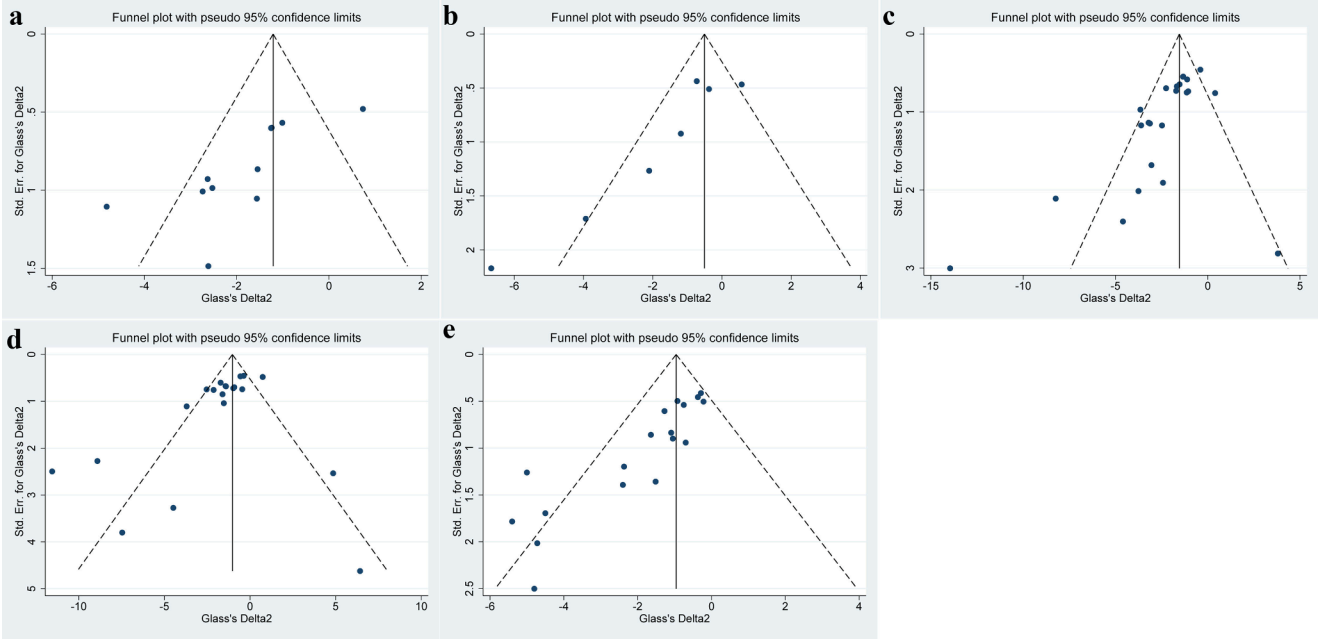

Fig. 7. Publication bias for studies that investigated hepatic triglyceride (a), hepatic cholesterol (b), alanine transaminase (c), aspartate transaminase (d), and liver weight (e) following garlic administration.

induced by lipopolysaccharide/d-galactosamine (Rousta et al., 2020) or carbon tetrachloride (Almatroodi et al., 2020). Garlic also is thought to have preventive and therapeutic efficacy against acute and chronic ethanol-induced liver injury (Guan et al., 2018).

In the present study, no differences between fermented and raw garlic effects on the serum liver enzymes and weight were observed.

Nonetheless, the literature suggests a higher nutritional profile, mineral content, and anti-glycation, anti-hypertensive, and antioxidant properties of fermented compared to fresh garlic (Elosta et al., 2017; Harauma & Moriguchi, 2006; Munday et al., 1999; Tahir et al., 2022). Moreover, Seo *et al.* demonstrated the reduction of blood lipid and insulin resistance after consumption of aged black, but not fresh, garlic in db/db mice (Seo et al., 2009). More studies are warranted to elucidate the health-promoting effects of black garlic than fresh ones.

NAFLD is characterized by an imbalance between TG acquisition from lipid uptake (dietary fats and fatty acids generated from adipose lipolysis) and de-novo lipogenesis on one hand and lipid utilization by TG export (via very low-density lipoprotein) and fatty acid β-oxidation in the liver on the other hand (Cano et al., 2021). Multiple parallel factors are implicated synergistically in the development and progression of NAFLD. There is a bidirectional relationship between NAFLD and metabolic syndrome parameters, including insulin resistance (Birjandi et al., 2016; Wainwright & Byrne, 2016). Insulin resistance is caused by numerous factors, including secretion of cytokines and chemokines (tumor necrosis factor-α, interleukin-6, and CC-chemokine ligand-2) from the adipose tissue in obesity. Insulin resistance occurs in various tissues such as the liver, adipose tissue, and muscle and leads to increased hepatic gluconeogenesis, an enhanced release of free fatty acids and inflammatory cytokines from adipose tissues, impaired muscle glucose uptake, and subsequently, initiation and progression of hepatic steatosis. Moreover, increases in hepatocyte apoptosis in response to cellular damages, including hypoxia, DNA damage, and TG accumulation, are involved in the NAFLD progression (Alkhouri et al., 2011). Genetic background, lipotoxicity, oxidative stress, endoplasmic reticulum stress, mitochondrial dysfunction, gut microbiota, and dietary factors are other common pathogenic mechanisms of NAFLD (Tilg et al., 2021; J. Yu et al., 2016). Moreover, increases in hepatocyte apoptosis in response to cellular damages, including hypoxia, DNA damage, and TG accumulation, are involved in the NAFLD progression (Alkhouri et al., 2011). Therefore, identifying preventive or therapeutic agents affecting these targets may help NAFLD management.

Garlic products may improve the imbalance between hepatic lipid influx and efflux by decreasing serum levels of free fatty acids (Chen et al., 2014; Xiao et al., 2013b; Yu et al., 2021), increasing fecal lipid excretion (Chen et al., 2014; Maeda et al., 2019), alleviating hepatic lipogenesis (downregulation of fatty acid synthesis, sterol regulatory element-binding protein-1c, β-Hydroxy β-methylglutaryl-CoA reductase, acetyl-CoA carboxylase, and stearoyl-coenzyme A desaturase 1) (Chen et al., 2014; Ha et al., 2015; Han et al., 2011; Lee et al., 2017; Zhang N. et al., 2019), and increasing hepatic lipid β-oxidation (upregulating AMP-activated protein kinase (AMPK), sirtuin 1, peroxisome proliferator-activated receptor alpha, and carnitine palmitoyltransferase I) (Chen et al., 2014; Lai et al., 2014; Lee et al., 2017; Zhang N. et al., 2019). Garlic may improve insulin resistance by suppressing adipose tissue growth and preadipocytes differentiation, increasing insulin secretion, upregulating adiponectin, and downregulating plasminogen activator inhibitor 1, resistin, tumor necrosis factor-alpha, and glucose transporter type 4 (Chen et al., 2014; Lembede et al., 2018; Zhang Y. et al., 2019). Amelioration of inflammation and oxidative stress could also contribute to the hepatoprotective action of garlic (Arifah et al., 2020; Jiang et al., 2021; Zalejska-Fiolka et al., 2015). Garlic compounds reduced Nuclear factor-kappa B (NF-κB)/Inhibitor of NF-κB (IκB) and NOD-like receptor protein 3/6 inflammatory pathways and upregulated the nuclear factor erythroid 2-related factor 2 (NRF2) expression (Xiao et al., 2013b; Yu et al., 2021; Zhang N. et al., 2019). NRF2 is a regulator of detoxifying and antioxidant defense gene expression in the liver that could be a potential target to attenuate oxidative damage (Bocci & Valacchi, 2015). Garlic is also reported to inhibit formation of advanced glycation end products (AGEs), which are formed when reducing sugars nonenzymatically react with proteins or lipids, and glycation-derived free radicals (Ahmad & Ahmed, 2006). AGEs have been implicated in the steatosis progression to nonalcoholic steatohepatitis and liver fibrosis (Fernando et al., 2019). Furthermore, SAMC is reported to downregulate the intrinsic and extrinsic apoptotic mechanisms by targeting liver kinase B1/AMPK and phosphoinositide 3-kinase/protein kinase B pathways (Xiao et al., 2013a).

Evidence also suggested that the beneficial effects of garlic or its constituents are through their influence on gut microbiota composition (Maeda et al., 2019). Wu *et al.* reported glucose tolerance improvement, inhibition of lipopolysaccharide formation, and induction of short-chain fatty acids production by increasing the number of *Bacteroidaceae*, *Lactobacillaceae*, and *Akkermansiaceae* in the gut (J. Wu et al., 2020). Ingestion of *Lactobacillus* attenuated NAFLD progression by lowering cholesterol (Lee et al., 2021). Moreover, allicin prevented trimethylamine *N*-oxide (TMAO) formation after carnitine intake by modulating gut microbiota (W.-K. Wu et al., 2015). TMAO is a gut microbiota-liver metabolite that may aggravate steatosis by modulating bile acid metabolism (Tan et al., 2019).

Unexpectedly, and despite the mentioned studies, oral administration of a low dose of DADS caused NAFLD development in the animals fed a normal-fat diet by regulating the expression of lipid metabolism-associated genes and gut microbiota similar to the HFD-induced condition (Yang et al., 2019). Due to the contradictory evidence, more studies on the effects of garlic and its components on NAFLD are warranted.

The present systematic review and meta-analysis study is the first report on the effects of garlic on NAFLD prevention and suppression with consideration of the underlying mechanisms. Preclinical animal studies provide valuable opportunities to understand the impact of an agent and its route of action and better design new clinical trials. When human evidence is lacking, performing a meta-analysis of animal studies could comprehensively assess the efficacy of an intervention and the sources of heterogeneity, reduce the unnecessary duplication of animal studies, and lead to a more robust result than a single animal study (Hooijmans et al., 2014b). Nevertheless, our research contains several limitations; the most important one is obtaining results from animal models that may not be translated to humans. High heterogeneity and publication bias are two hallmarks of meta-analysis of laboratory animal research (Hooijmans et al., 2014b; ter Riet et al., 2012), as are detected in the current analysis. Another limitation of the present study is the lack of dose–response analysis due to the diversities in the type of garlic product or ingredient and also route of administration. Furthermore, most of the included studies had high risk of bias. Finally, the certainty of evidence is low and very low, which suggests results should be interpreted cautiously.

5. Conclusions and future research directions

Garlic and its components had beneficial effects on NAFLD prevention and suppression in animal models, which were documented by attenuation of hepatic steatosis and injuries, liver TG and cholesterol content, serum ALT and AST levels, and liver weight. These results were almost similar in the subgroups of types and duration of interventions, disease and study models, and risk of bias. However, the reduction of liver weight was higher in mice than in rats. Future high-quality animal studies on the efficacy of fresh and black garlic on NAFLD-related biomarkers and its underlying mechanisms, especially regarding microbiota, AGEs, and signaling pathways, are needed because of the low/very low certainty of present evidence.

Data Sharing: Data described in the manuscript are available from the Corresponding author [A.R.S.], upon reasonable request.

Ethical approval: The present study was approved by the Ethics Committee of Shiraz University of medical sciences, Shiraz, Iran.

CRediT authorship contribution statement

Sara Shojaei-Zarghani: Conceptualization, Methodology, Data curation, Formal analysis, Software, Writing – original draft.

Mohammad Reza Fattahi: Conceptualization, Methodology, Supervision, Writing – review & editing. **Asma Kazemi:** Conceptualization, Methodology, Data curation, Formal analysis, Software, Writing – review & editing. **Ali Reza Safarpour:** Conceptualization, Methodology, Data curation, Formal analysis, Software, Project administration, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2022.105206.

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