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Does flavonoid supplementation alleviate non-alcoholic fatty liver disease? A systematic review and meta-analysis of randomized controlled trials

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Abbreviations: non-alcoholic fatty liver disease, NAFLD; randomized clinical trials, RCTs; low-density lipoprotein cholesterol, LDL-C; aspartate aminotransferase, AST; alanine aminotransferase, ALT; nuclear factor-κB, NF-κB; high-sensitivity C-reactive protein, hs-CRP; tumor necrosis factor-α, TNF-α; body mass index, BMI; γ-glutamyl transpeptidase, GGT; fasting blood sugar, FBS; homeostatic model assessment of insulin resistance, HOMA-IR; high-density lipoprotein cholesterol, HDL-C; total cholesterol, TC; triglycerides, TG; interleukin-6, IL-6.

Keywords: Flavonoid; non-alcoholic fatty liver disease; liver function; lipid profile; inflammation

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ABSTRACT

Higher flavonoid intake is associated with reduced risk of non-alcoholic fatty liver disease (NAFLD). However, there is a large discrepancy in the effects of flavonoid supplementation on makers of NAFLD. To fill such knowledge gap, we systematically reviewed randomized clinical trials (RCTs) to critically assess flavonoid supplementation effect on liver function, lipid profile, inflammation, and insulin resistance in adults with NAFLD. A systematic search was conducted from 4 databases from inception until May 2023. Twelve RCTs were included in the final analysis demonstrating beneficial effects of flavonoid supplementation on ALT (SMD = -3.59, p = 0.034), AST (SMD = -4.47, p = 0.001), GGT (SMD = -8.70, p = 0.000), CK-18M30 (SMD = -0.35, p = 0.042), TG (SMD = -0.37, p = 0.001), LDL-C (SMD = -0.38, p = 0.039), TC (MD = -0.25 mmol/l, p = 0.017), steatosis score (MD = -18.97, p = 0.30), TNF-α (MD = -0.88, p = 0.000), and NF-κB (MD = -1.62, p = 0.001). This meta-analysis suggests that flavonoid alleviates NAFLD through exerting favourable effects on liver function, lipid profile, and inflammation, indicating flavonoid supplementation presents a promising drug regimen for the management of NAFLD and its associated complications.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) represents a broad spectrum of liver disorders associated with excessive deposition of fatty acids within hepatocytes, ranging from steatosis to hepatocellular carcinoma. The pathogenesis of NAFLD is complex and multifactorial, comprising fat accumulation in the liver, inflammation and insulin resistance [1]. Non-alcoholic steatohepatitis (NASH) is an active form of NAFLD, characterized by liver necrotizing inflammation and faster fibrosis progression [2]. The prevalence of NAFLD is on the rise, affecting one-third of the global population [3]. Specifically, the overall prevalence of NAFLD is 38% in a large middle-aged US cohort [4]. Notably, individuals suffering from NAFLD are at higher risk of developing coronavirus disease 2019 (COVID-19) [5, 6]. Meanwhile, the enhanced physical inactivity, overeating and depression associated with COVID-19 exert adverse effects on NAFLD, ultimately contributing to elevated liver-related mortality [7].

In recent years, increasing efforts have been given towards management of fatty liver diseases using nutraceuticals, over the last decade with obvious increase in research papers from 16 records in 2008 to reach 218 in 2022 as retrieved by searching SCOPUS database for literature using keywords of cereal polyphenols (Appendix A1 Fig. 1). As shown in Fig. 1, drawn for the co-occurrences on fatty liver and flavonoids as analyzed by the VOS viewer bibliometric visualization software, circles in different colors represent the keywords related to different topics, and the links between circles demonstrate their relations. Four main clusters in literature could be visualized using VOS viewer as such: with the major cluster 1 of 358 items related to natural products chemistry and health effects and a second cluster on biochemical markers associated with fatty liver including term flavonoids appearing (552 times) and fatty liver (312 times) in a total of 997 items all highlighting their close connection. The result indicates huge intersections

between flavonoids and NAFLD, presenting great research potential. In contrast, only one cluster made up of 1 item belonged to anthocyanins revealing that few literature is made towards assessment of anthocyanins in fatty liver management compared to flavonoids. Furthermore, papers grouped by subject areas revealed that largest reports of is in pharmacology, medicine and biochemistry amounting for 60.5% of all fields as depicted in Appendix A1 Fig. 2.

Hence, it is imperative to find remedies to counteract or alleviate NAFLD. Lifestyle modifications including increased physical activity and healthy diet constitute the cornerstone of treatment for NAFLD [8, 9]. Importantly, increasing evidence has revealed a protective role of flavonoids in mitigating NAFLD [10, 11]. Specifically, flavonoids exert a hepatoprotective effect by regulating the activity of Cytochrome P450 2E1 (CYP2E1) [12]. Flavonoids from *Chimonanthus nitens* Oliv. leaves alleviated NAFLD in mice by decreasing liver fat deposition, oxidative stress, and inflammatory response as well as regulating the gut–liver axis [13]. Furthermore, dihydromyricetin alleviated streptozotocin-induced liver impairment and inflammation in diabetic rats through the regulation of NF-κB and AMPK signaling pathway [14].

Flavonoids comprise secondary metabolites that are found ubiquitous in plants, categorised into subclasses encompassing flavanones, flavonols, flavanols, flavones, anthocyanidins and isoflavones [15]. They are well characterized for multiple health effects, including anti-inflammatory [16], antioxidant [17] and anti-hypercholesterolemic activities [18], as well as counteracting insulin resistance [19]. Therefore, flavonoids are considered as promising candidates for the prevention and treatment of NAFLD [20]. Indeed, higher flavonoid intake is associated with a lower risk of NAFLD progression in the elderly overweight/obese population [21]. However, results of randomized clinical trials (RCTs) evaluating the effects of flavonoids on NAFLD appear to be controversial in literature [22,

[23], obscuring the mechanism by which flavonoids regulate NAFLD and limiting future clinical application of flavonoids. Hence, given that no comprehensive reviews regarding the effects of flavonoids on liver function, lipid profile, inflammatory markers and insulin resistance have been conducted, the aim of the present study was to systematically review the evidence and quantify the impact of flavonoids on NAFLD risk factors.

2. Methods

2.1. Protocol and Registration

The study was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement checklist [24]. The study was registered on https://inplasy.com/ with the registration number of INPLASY202260057, and the DOI number of 10.37766/inplasy2022.6.0057.

2.2. Search Strategy and Selection Criteria

Two reviewers (K.J. and F.D.) carried out literature searching, screening, quality evaluation, and data extraction, with a third reviewer (L.L.) as arbitrator. Systematic literature search was conducted in Cochrane Central Register of Controlled Trials (CENTRAL), PubMed, ScienceDirect and Web of Science from the inception of the database until May 2023.

The following terms were included in the literature search: “flavonoid” as well as names of individual compounds, liver function (“alanine aminotransferase” or “aspartate aminotransferase” or “γ-glutamyl transpeptidase” or “cytokeratin 18-M30” or “fibrosis score”), lipid profile (“triglycerides” or “low-density lipoprotein cholesterol” or “high-density lipoprotein cholesterol” or “total cholesterol” or “steatosis score”), inflammation (“tumor necrosis factor-α” or “high-sensitivity C-reactive protein” or “nuclear factor-κB”), insulin resistance (“homeostasis model assessment” or “insulin” or “fasting blood sugar”), “nonalcoholic fatty liver disease”, “randomized controlled trial”, “intervention”

(more information is shown in Appendix A2). Bibliographic search was conducted by reviewing the reference lists of the included studies or key texts. Moreover, manual search and lists of references from additional studies were included, and other similar systematic reviews were checked to identify potential studies that might meet the inclusion criteria.

Inclusion criteria were as follows: (1) Randomized controlled trials, (2) evaluated the effect of consuming flavonoids on changes in at least one of these outcomes (liver function, lipid profile, inflammation, insulin resistance) in adults with NAFLD, (3) provided sufficient data regarding the baseline and final values within groups or presented the data regarding the average change from baseline, (4) articles published in English. The studies meeting any of the following criteria were excluded: (1) having non-randomized or semi-randomized design, (2) absence of a control group, (3) having a duration of less than 8 weeks.

2.3. Study Selection and Quality Evaluation

2.3.1. Data Collection Process

For each study included in this systematic review, the following information was extracted: (1) study (author's last name and year of publication); (2) study design; (3) country; (4) population (number, age, BMI); (5) intervention group; (6) control group; (7) duration; (8) main results.

2.3.2. Quality Evaluation

The bias risk of RCTs included in this systematic review and meta-analysis was assessed using the Risk of Bias2 (RoB2) tool from the Cochrane Collaboration, with a domain-based evaluation that classifies five domains of each RCT into "low risk of bias", "some concerns" or "high risk of bias" [25]. The five domains include bias arising from the randomization, deviations from intended intervention, missing data, measurement of the outcome, and selective outcome reporting. Moreover, Nutri Grade (Grading of

Recommendations Assessment, Development, and Evaluation) scoring system was used to assess the quality according to the following seven aspects: (1) risk of bias, study quality, and study limitations, (2) precision, (3) heterogeneity, (4) directness, (5) publication bias, (6) funding bias, (7) study design [26].

2.3.3. Statistical Analysis

The statistical analysis was conducted using the Stata version 14.0 (College Station, TX77845, USA) and the Review Manager 5.4 software (The Cochrane Collaboration, London, UK, 2020). Mean difference (MD) or the standardized mean difference (SMD) was used to determine the effect size, using a random-effects model (DerSimonian-Laird approach (Kelley and Kelley 2012)). Outcomes were presented as mean changes from baseline. The standard deviation (SD) of the change was calculated if the data were not stated in the RCTs, using the following equation: $[SD_{pre}^2+SD_{post}^2-2\times Corr\ (pre, post) \times SD_{pre}\times SD_{post}]^{0.5}$. Specifically, SD_{pre} was the SD before the intervention, SD_{post} was the SD after the intervention, and Corr (pre, post) was within-participant correlation. If the correlation was not stated, the within-participant correlation was set as 0.5. If not directly reported, the SD was calculated from standard error or confidence interval. A subgroup analysis based on dose (≥500 mg/d or <500 mg/d), and flavonoid type were conducted to detect potential sources of heterogeneity. Heterogeneity across studies was assessed by I^2 statistics. Heterogeneity was classified as "small", "moderate", or "substantial" if I^2 was <25%, 25–75%, and >75%, respectively [27].

The individual influence of each study on the overall result was evaluated by removing them individually. Egger's test and visual inspection of funnel plots were performed to evaluate publication bias. All statistical results with p value <0.05 were considered statistically significant.

3. Results

3.1. Study Selection

The process of study selection is shown as a PRISMA flow diagram (Fig. 2). A total of 1048 publications were identified by searching the databases and other sources. Following the removal of duplication, 583 publications remained. The titles and abstracts of the included studies were screened, and 568 were excluded after this preliminary filter. Subsequently, full text screening was carried out and 12 publications were included in the final meta-analysis.

3.2. Study Characteristics

The characteristics of the included fourteen studies are shown in Table 1, involving a total of 831 patients with NAFLD, with 418 participants in intervention group and 413 participants in control group. All studies included men and women. Specifically, one study did not specify the ratio of men to women. The sample size of the studies ranged from 36 to 108. The duration of the studies ranged from 8 to 48 weeks, with 12 weeks being the most common duration (n = 8). The intervention group included hesperidin, silybum, anthocyanin, genistein, dihydromyricetin at doses ranging from 94 to 2100 mg/d. The study design of all 12 RCTs was parallel.

3.3. Quality Evaluation

The RoB2 tool summary and risk of bias graphs are shown in Appendix A3. Among all studies, 12 studies (100%) had no domain as “high risk”, and 10 studies (83.3%) had five domains as “low risk”. Evaluation of the quality of the present meta-analysis based on the Nutri Grade scoring system demonstrated a score of 9.4, indicating high meta-evidence.

3.4. Outcomes

The effects of flavonoid consumption on adults with NAFLD were evaluated in the present study. The outcome assessed included liver function (alanine aminotransferase

(ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (GGT), cytokeratin 18-M30 (CK-18M30), fibrosis score, steatosis score), lipid profile (triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC)), inflammation (tumor necrosis factor-α (TNF-α), high-sensitivity C-reactive protein (hs-CRP), nuclear factor-κB (NF-κB)) and insulin resistance (homeostatic model assessment of insulin resistance (HOMA-IR), insulin, fasting blood sugar (FBS)), each described separately in the next subsections.

3.4.1. Liver function

The pooled results demonstrated that there was a significant decrease in ALT concentrations in intervention group, compared with control group (SMD = -3.59, 95% CI [-6.90, -0.28], p = 0.034), with a small heterogeneity (I^2 = 16.4%, p = 0.296) (Fig. 3A). Subgroup analysis showed that dihydromyricetin significantly reduced ALT concentrations (Appendix A4).

Similarly, the pooled results showed that AST significantly decreased in intervention group, compared with control group (SMD = -4.47, 95% CI [-7.08, -1.86], p = 0.001) with a small heterogeneity (I^2 = 0.0%, p = 0.507) (Fig. 3B). Subgroup analysis demonstrated that flavonoid supplementation significantly reduced AST concentrations with the dose of ≥500 mg/d. Regarding flavonoid type, subgroup analysis showed that silymarin significantly reduced AST concentrations (Appendix A4).

The pooled results showed that there was a significant decrease in GGT in intervention group, compared with control group (SMD = -8.70, 95% CI [-12.86, -4.54], p = 0.000), with a small heterogeneity (I^2 = 0.0%, p = 0.960) (Fig. 3C). Subgroup analysis showed that flavonoid supplementation significantly reduced GGT concentrations with the dose of ≥500 mg/d. Regarding flavonoid type, subgroup analysis showed that hesperidin significantly reduced GGT concentrations (Appendix A4).

Likewise, there was a significant reduction in CK-18M30 in intervention group, compared with control group (SMD = -0.35, 95% CI [-0.70, -0.01], p = 0.042), with a small heterogeneity (I^2 = 0.0%, p = 0.487) (Fig. 3D).

In contrast, fibrosis score did not differ between intervention group and control group, with a MD of 0.11 (95% CI [-0.44, 0.66], p = 0.696) and a small heterogeneity (I^2 = 0.0%, p = 0.849) (Fig. 3E).

Similarly, steatosis score significantly decreased in intervention group, compared with control group (MD = -18.97, 95% CI [-36.15, -1.80], p = 0.030), with a small heterogeneity (I^2 = 0.0%, p = 0.904) (Fig. 3F).

3.4.2. Lipid profile

The pooled analysis demonstrated that flavonoid supplementation significantly decreased TG levels (MD = -0.37 mg/dl, 95% CI [-0.59, -0.15], p = 0.001) compared to placebo with a small heterogeneity (I^2 = 0.0%, p = 0.649) (Fig. 4A). Subgroup analysis demonstrated that flavonoid supplementation significantly reduced TG concentrations with the dose of ≥500 mg/d and <500 mg/d. Regarding flavonoid type, subgroup analysis showed that genistein significantly reduced TG concentrations (Appendix A4).

The results showed that there was likewise a significant difference in LDL-C level between the intervention group and the control group (SMD = -0.38, 95% CI [-0.74, -0.02], p = 0.039), with a small heterogeneity (I^2 = 20.9%, p = 0.270) (Fig. 4B). Regarding flavonoid type, subgroup analysis showed that hesperidin and dihydromyricetin significantly reduced LDL-C concentrations (Appendix A4).

In contrast, results demonstrated that no significant difference was observed in HDL-C levels between the intervention group and the control group (SMD = 0.05, 95% CI [-0.06, 0.16], p = 0.344), with a small heterogeneity (I^2 = 0.0%, p = 0.927) (Fig. 4C).

The results demonstrated that TC levels in the intervention group decreased significantly compared with placebo (MD = -0.25 mmol/l, 95% CI [-0.45, -0.04], p = 0.017), and the heterogeneity was small (I^2 = 0.0%, p = 0.937) (Fig. 4D). Subgroup analysis showed that flavonoid supplementation significantly reduced TC concentrations with the dose of ≥500 mg/d (Appendix A4).

3.4.3. Inflammation

The pooled analysis showed that TNF-α levels in the intervention group decreased significantly compared with placebo (MD = -0.88 pg/ml, 95% CI [-1.15, -0.62], p = 0.000), and the heterogeneity was small (I^2 = 0.0%, p = 0.392) (Fig. 5A). Subgroup analysis showed that flavonoid supplementation significantly reduced TNF-α concentrations with the dose of ≥500 mg/d. Regarding flavonoid type, subgroup analysis showed that hesperidin, genistein and dihydromyricetin significantly reduced TNF-α concentrations (Appendix A4).

The results demonstrated that there was no significant difference in hs-CRP between the intervention group and the control group (MD = -0.26 ng/dl, 95% CI [-0.66, 0.14], p = 0.200), with a small heterogeneity (I^2 = 0.0%, p = 0.637) (Fig. 5B).

The pooled analysis showed that there was a significant decrease in NF-κB in intervention group compared to control group (MD = -1.62 ng/mg protein, 95% CI [-2.56, - 0.69], p = 0.001), with a small heterogeneity (I^2 = 0.0%, p = 0.811) (Fig. 5C).

3.4.4. Insulin resistance

Comprehensive analysis showed that the HOMA-IR level in the intervention group did not decrease significantly compared with the placebo group (MD = -0.05, 95% CI [-0.25, 0.15], p = 0.642), with a moderate heterogeneity (I^2 = 0.0%, p = 0.842) (Fig. 6A).

The results demonstrated that there was no significant difference in insulin concentrations between the control group and the intervention group (MD = -0.07 mU/L,

95% CI [-0.40, 0.25], p = 0.659), with a moderate heterogeneity (I^2 = 35.9%, p = 0.197) (Fig. 6B).

No significant difference was observed in FBS levels between the control group and the intervention group (MD = -0.23 mg/dl, 95% CI [-0.49, 0.02], p = 0.075), with a small heterogeneity (I^2 = 0.0%, p = 0.866) (Fig. 6C).

Sensitivity analysis of markers of liver function, lipid profile, inflammatory markers and insulin resistance revealed that the overall effect did not change and visual interpretation of the funnel plots demonstrated no evidence of publication bias (Appendix A5-A8, respectively). The individual influence of each study on the overall result was evaluated by removing them individually (Appendix A9). Forest plots of subgroup analysis are shown in Appendix A10.

4. Discussion

This meta-analysis demonstrated that long-term consumption of flavonoids significantly reduced ALT, AST, GGT, and CK-18M30, compared with the control group. Given that ALT, AST, GGT, and CK-18M30 levels represent structural and cellular liver damage, with elevated levels indicating increased severity of liver injury [28], the present study showed that flavonoids alleviated the severity of NAFLD. Consistent with a previous meta-analysis investigating the effect of silymarin on patients with NAFLD [29], ALT and AST were also significantly reduced following silymarin administration. Similarly, consumption of silymarin for 24 weeks significantly reduced the levels of AST and ALT in patients with serum hepatitis C virus [30]. In support of these findings, intake of flavonoid extract of *P. curatellifolia* seeds for 14 days dose dependently prevented acetaminophen-induced increase in serum activities of ALT, AST, and GGT in rats [31].

Dyslipidemia is a common feature of NAFLD [32]. The present meta-analysis showed that flavonoids significantly reduced TG, TC and LDL-C in patients with

NAFLD, albeit not HDL-C. Similarly, a previous meta-analysis demonstrated that flavonoid consumption led to a significant reduction in TG, TC and LDL-C in participants with type 2 diabetes [33]. In support of these findings, flavonoids from mulberry leaves inhibited lipid accumulation and alleviated hepatic injury in NAFLD rat model [34]. Specifically, quercetin significantly decreased lipid accumulation in HepG2 cells, through the inhibition of mRNA and protein expression level of SREBP2 and HMGCR, as well as the upregulation of SR-BI mRNA expression, providing evidence for a protective role of quercetin in the NAFLD treatment [34]. Furthermore, a recent systematic review and meta-analysis suggested that flavonoid-containing artichoke leaf extract resulted in a significant reduction in ALT, AST, TC, TG and LDL-C in NAFLD patients [35].

Inflammation is an important hallmark in the development of NAFLD [36]. The present meta-analysis showed that TNF-α and NF-κB in patients with NAFLD significantly decreased following flavonoid supplementation likely attributed to flavonoids potential anti-inflammatory actions [37]. In support of those findings, administration of dihydromyricetin (100–400 mg/kg/day) for 6 weeks inhibited TNF-α and IL-1β levels in diabetic rats by the regulation of NF-κB signaling pathway [38]. Consistent with those findings, flavonoids have been reported to inhibit enzymes or transcription factors important in inflammation, such as TNF-α, IL-6 and NF-κB [16].

Given that insulin resistance in both the adipose tissue and the liver contributes to an accumulation of free fatty acids in hepatocytes, insulin resistance has been suggested as an independent risk factor for the progression of NAFLD [39]. However, this meta-analysis demonstrated that flavonoid consumption led to no significant changes in insulin, HOMA-IR and FBS levels in patients with NAFLD. In contrast, a meta-analysis on patients with diabetes reported that HOMA-IR and FBS were significantly reduced

following flavonoid intake, whereas insulin was unaffected [33]. Such discrepancy could be explained by the variances in the baseline levels of those markers in participants with NAFLD and patients with diabetes, as well as differences in study design.

Collectively, regarding the effectiveness of flavonoid supplementation on NAFLD, the present meta-analysis suggested that dihydromyricetin and hesperidin demonstrated the most potency among the flavonoids investigated in previous RCTs, exerting beneficial effects on liver function, lipid profile and inflammation. Regarding effective doses of flavonoid supplementation on liver function, lipid profile and inflammation, the dose of ≥500 mg/d was more effective in reducing AST, GGT, TC, and TNF-α concentrations, compared with the dose of <500 mg/d. It is noteworthy that the RCTs included in this meta-analysis have assessed safety markers and not found any issue, including side effects. However, discretion is warranted to avoid the possible adverse effects due to flavonoid overdose [40]. Further studies are needed to ascertain the long-term safety of supplemental flavonoids.

The limitations of this meta-analysis need to be acknowledged. Specifically, the discrepancy in the inclusion criteria of individual RCTs resulted in heterogeneity in the participant profile with different baseline parameters related to NAFLD. Notably, differences in study design (flavonoid type, dosage, intervention duration, and ethnicity) might contribute to heterogeneity among studies.

5. Conclusions

This meta-analysis demonstrated that flavonoid supplementation contributes to the alleviation of NAFLD through regulating liver function (ALT, AST, GGT, CK-18M30, steatosis score), modulating lipid metabolism (TG, TC, LDL-C), and attenuating inflammation (TNF-α, NF-κB) (Fig. 7). Although multiple pathways implicated in the etiology of NAFLD make the treatment challenging, this meta-analysis indicated that

flavonoids seem to suit seamlessly in the amelioration of NAFLD, through the regulation of various pathways. Notably, the present systematic review provides new insights into how flavonoids alleviate NAFLD, meanwhile facilitating the development of comprehensive information for future well-designed RCTs. Identification of the best structural analogues among flavonoid subclasses should now follow by establishing structure activity relationship (SAR) studies using enzyme-based assays or ideally animal models.

Authors' contributions

L.L. and K.J. designed the study. K.J., L.L. and F.D. selected the final included studies, extracted data, and carried out the meta-analysis. L.L. and K.J. interpreted the results and wrote the article. N.J., C.B., M.A.F., H.L., X.L. and J.X. provided critical revisions of the final manuscript. All authors have read and approved the final manuscript.

Conflicts of Interest

The authors have declared no conflict of interest.

Data Availability Statement

Data is contained within the article or supplementary material.

References

[1] Van De Wier, B., Koek, G. H., Bast, A., Haenen, G. R. M. M., *Critical Reviews in Food Science and Nutrition.* **2017**, *57*, 834-855.

[2] Wong, V. W., Adams, L. A., de Ledinghen, V., Wong, G. L., *Nat Rev Gastroenterol Hepatol.* **2018**, *15*, 461-478.

[3] Dietrich, P., Hellerbrand, C., *Best Practice & Research Clinical Gastroenterology.* **2014**, *28*, 637-653.

[4] Harrison, S. A., Gawrieh, S., Roberts, K., Lisanti, C. J.*, et al.*, *Journal of Hepatology.* **2021**, *75*, 284-291.

[5] Sachdeva, S., Khandait, H., Kopel, J., Aloysius, M. M.*, et al.*, *SN Comprehensive Clinical Medicine.* **2020**, *2*, 2726-2729.

[6] Hoffmann, C., Gerber, P. A., Cavelti-Weder, C., Licht, L.*, et al.*, *Hormone and Metabolic Research.* **2022**, *54*, 522-531.

[7] Ahmed, M., Ahmed, M. H., *World J Clin Cases.* **2021**, *9*, 4133-4142.

[8] Hekmatdoost, A., Shamsipour, A., Meibodi, M., Gheibizadeh, N.*, et al.*, *Int J Food Sci Nutr.* **2016**, *67*, 1024-1029.

[9] Ghaemi, A., Taleban, F. A., Hekmatdoost, A., Rafiei, A.*, et al.*, *Hepat Mon.* **2013**, *13*, e15227.

[10] Van De Wier, B., Koek, G. H., Bast, A., Haenen, G. R., *Critical Reviews in Food Science and Nutrition.* **2017**, *57*, 834-855.

[11] Li, L., Qin, Y., Xin, X., Wang, S.*, et al.*, *Biomedicine & Pharmacotherapy.* **2023**, *164, 114991*.

[12] Wang, K., Tan, W., Liu, X., Deng, L.*, et al.*, *Biomed Pharmacother.* **2021**, *137*, 111326.

[13] Meng, W., Zhao, Z., Chen, L., Lin, S.*, et al.*, *Foods.* **2022**, *11,* 2169.

391 [14] Chen, L., Yao, M., Fan, X., Lin, X.*, et al.*, *eFood.* **2020**, *1*, 188-195.
392 [15] Câmara, J. S., Albuquerque, B. R., Aguiar, J., Corrêa, R. C. G.*, et al.*, *Foods.* **2021**,
393 *10*, 37.
394 [16] Maleki, S. J., Crespo, J. F., Cabanillas, B., *Food Chem.* **2019**, *299*, 125124.
395 [17] Shen, N., Wang, T., Gan, Q., Liu, S.*, et al.*, *Food Chemistry.* **2022**, *383*, 132531.
396 [18] de Oliveira, D. A. F., Diniz, S. N., Pereira, R. M. S., Gonçalves, I. D.*, et al.*, *Journal*
397 *of Food Biochemistry.* **2022**, *46*, e13999.
398 [19] Peng, M.-f., Tian, S., Song, Y.-g., Li, C.-x.*, et al.*, *Journal of Ethnopharmacology.*
399 **2021**, *273*, 113947.
400 [20] Shin, J. H., Jung, J. H., *Clinics and Research in Hepatology and Gastroenterology.*
401 **2017**, *41*, 17-24.
402 [21] Zhong, Q.-w., Wu, Y.-y., Xiong, F., Liu, M.*, et al.*, *British Journal of Nutrition.* **2021**,
403 *125*, 460-470.
404 [22] Jakubczyk, K., Skonieczna-Żydecka, K., Kałduńska, J., Stachowska, E., et al.,
405 Nutrients. 2020, 12, 2435.
406 [23] Naeini, F., Namkhah, Z., Ostadrahimi, A., Tutunchi, H., Hosseinzadeh-Attar, M. J.,
407 *Advances in Nutrition.* **2020**, *12*, 413-428.
408 [24] Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G., *Physical Therapy.* **2009**, *89*, 873-
409 880.
410 [25] Julian PT Higgins, J. S., Matthew J Page, Jonathan AC Sterne, *Cochrane.* **2019**.
411 [26] Schwingshackl, L., Knuppel, S., Schwedhelm, C., Hoffmann, G.*, et al.*, *Adv Nutr.*
412 **2016**, *7*, 994-1004.
413 [27] Higgins, J., Thompson, S. G., Decks, J. J., Altman, D. G. J. B. M. J., **2003**, *327*,
414 p.557-560.

[28] Mousavi, S. M., Mokhtari, P., Asbaghi, O., Rigi, S.*, et al.*, *Crit Rev Food Sci Nutr.* **2021**, *62*, 6315-6327.

[29] Kalopitas, G., Antza, C., Doundoulakis, I., Siargkas, A.*, et al.*, *Nutrition.* **2021**, *83*, 111092.

[30] Kalantari, H., Shahshahan, Z., Hejazi, S. M., Ghafghazi, T., Sebghatolahi, V. J. J. o. R. i. M. S., *Journal of Research in Medical Sciences*. **2011**, *16*, 287-290.

[31] Olaleye, M. T., Amobonye, A. E., Komolafe, K., Akinmoladun, A. C., *Saudi J Biol Sci.* **2014**, *21*, 486-492.

[32] Mavromati, M., Jornayvaz, F. R., *International Journal of Molecular Sciences.* **2021**, *22,* 12797.

[33] Liu, F., Sirisena, S., Ng, K., *Crit Rev Food Sci Nutr.* **2021**, 1-27.

[34] Hu, Y., Xu, J., Chen, Q., Liu, M.*, et al.*, *BMC Complement Med Ther.* **2020**, *20*, 257.

[35] Kamel, A. M., Farag, M. A., J Med Food. 2022, 25, 931-942.

[36] Petrescu, M., Vlaicu, S. I., Ciumărnean, L., Milaciu, M. V.*, et al.*, *Medicina.* **2022**, *58,* 641.

[37] Al-Khayri, J. M., Sahana, G. R., Nagella, P., Joseph, B. V.*, et al.*, *Molecules.* **2022**, *27,* 2901.

[38] Chen, L., Yao, M., Fan, X., Lin, X.*, et al.*, *eFood.* **2020**, *1*, 188-195.

[39] Barreby, E., Chen, P., Aouadi, M., *Nature Reviews Endocrinology.* **2022**, *18*, 461-472.

[40] Galati, G., O'Brien, P. J., *Free Radical Biology and Medicine.* **2004**, *37*, 287-303.

[41] Cheraghpour, M., Imani, H., Ommi, S., Alavian, S. M.*, et al.*, *Phytother Res.* **2019**, *33*, 2118-2125.

[42] Aller, R., Izaola, O., Gomez, S., Tafur, C.*, et al.*, *European Review for Medical and Pharmacological Sciences.* **2015**, *19*, 3118-3124.

[43] Yari, Z., Cheraghpour, M., Alavian, S. M., Hedayati, M.*, et al.*, *Eur J Clin Nutr.* **2021**, *75*, 99-111.

[44] Zhang, P. W., Chen, F. X., Li, D., Ling, W. H., Guo, H. H., *Medicine (Baltimore).* **2015**, *94*, e758.

[45] Hosseinikia, M., Oubari, F., Hosseinkia, R., Tabeshfar, Z.*, et al.*, *Nutrition & Food Science.* **2020**, *50*, 1279-1293.

[46] Wah Kheong, C., Nik Mustapha, N. R., Mahadeva, S., *Clin Gastroenterol Hepatol.* **2017**, *15*, 1940-1949.

[47] Pasdar, Y., Oubari, F., Zarif, M. N., Abbasi, M.*, et al.*, *Clin Nutr Res.* **2020**, *9*, 11-19.

[48] Amanat, S., Eftekhari, M. H., Fararouei, M., Bagheri Lankarani, K., Massoumi, S. J., *Clin Nutr.* **2018**, *37*, 1210-1215.

[49] Anushiravani, A., Haddadi, N., Pourfarmanbar, M., Mohammadkarimi, V., *Eur J Gastroenterol Hepatol.* **2019**, *31*, 613-617.

[50] Chen, S., Zhao, X., Wan, J., Ran, L.*, et al.*, *Pharmacol Res.* **2015**, *99*, 74-81.

[51] Hassan, S., Ramin, G., Mohammad, K. A., Zahra, H. Y., *Caspian J Intern Med.* **2020**, *5*, 9-12.

[52] Loguercio, C., Andreone, P., Brisc, C., Brisc, M. C.*, et al.*, *Free Radic Biol Med.* **2012**, *52*, 1658-1665.

461 **Table**

462 **Table 1.** Characteristics of the studies included in the meta-analysis.

Study	Country	Population			Intervention Group	Control Group	Duration (weeks)	Main Results
		N (IG/CG)	Age	BMI				
[41]	Iran	25/24	18-70	IG: 31.70 ± 5.21 CG: 33.00 ± 5.03	hesperidin, 1 g/d + LMP	placebo (starch), 1 g/d + LMP	12	↑: LDL-C, AST ↓: ALT, NF-κB, hs-CRP, TNF-α, fibrosis score, steatosis score ND: BMI, WHR, GGT, FBS, insulin, HOMA-IR, HDL-C
[42]	Spain	18/18	18-67	IG: 36.8 ± 7.9 CG: 35.0 ± 7.4	silybum, 540.3 mg/d; VE, 36 mg/d + LMP	LMP	12	↓: AST, ATG, GGT ND: BMI, TG, HOMA-IR
[43]	Iran	22/21	18-70	IG:31.07 ± 4.38 CG:33.06 ± 5.14	hesperidin, 1 g/d + LMP	LMP	12	↑: AST ↓: fibrosis score, steatosis score, ALT, FBS, insulin, HOMA-IR, BMI, hs-CRP, TNF-α ND: GGT, QUICKI, LDL-C, HDL-C, NF-κB
[44]	China	37/37	25-65	IG: 27.10 ± 3.20 CG: 27.3 ± 3.5	anthocyanin, 320 mg/d	placebo, 320 mg/d	12	↓: ALT, AST, MPO, insulin, CK-18M30, HOMA-IR ND: BMI, TC, HDL-C, LDL-C
[45]	Iran	39/39	18-65	IG: 29.90 ± 3.10 CG: 30.8 ± 4.2	quercetin, 500 mg/d	placebo, 500 mg/d	12	↓: TG, TNF-α, TC ND: BMI, ALT, WHR, ALT, AST, GGT
[46]	Malaysia	49/50	>18	IG: 30.0 ± 4.0 CG: 31.0 ± 4.6	silymarin, 2100 mg/d + LMP	placebo, 2100 mg/d + LMP	48	↓: ALT, AST, GGT ND: HOMA-IR, TG, TC, HDL-C, LDL-C
[47]	Iran	39/39	18-65	25-40	quercetin, 500 mg/d	placebo, 500 mg/d	12	↑: RBC ↓: mean corpuscular volume, MCH, ferritin

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Table 1. Continued.

Study	Country	Population			Intervention Group	Control Group	Duration (weeks)	Main Results
		N (IG/CG)	Age	BMI				
[48]	Iran	41/41	18-69	IG: 29.09 ± 4.67 CG: 27.83±4.51	genistein, 250 mg/d + LMP	cornstarch, 250 mg/d + LMP	8	↓: FBS, HOMA-IR, IL-6, TNF-α, MDA ND: BMI, WHR, TG, LDL-C, HDL-C, AST, ALT
[49]	Iran	30/30	18-65	IG: 25.10 ± 3.7 CG: 26.1 ± 3.1	silymarin, 140 mg/d + LMP	placebo, 140 mg/d + LMP	12	↓: BMI ND: TG, HDL-C, LDL-C, FBS, AST, ALT
[50]	China	30/30	20-60	IG: 25.5 ± 2.87 CG: 25.6 ± 2.26	dihydromyricetin, 600 mg/d	placebo, 600 mg/d	12	↓: BMI, TC, TG, HOMA-IR, CK-18M30, TNF-α, FGF21 ND: TG, HDL-C, LDL-C, FBS, AST, ALT
[51]	Iran	33/31	IG: 43.6 ± 8.3 CG: 39.36 ±10.5	IG: 27.4 ± 1.70 CG: 27.5 ± 1.90	silymarin, 210 mg/d + LMP	placebo, 210 mg/d + LMP	8	↓: AST, ALT
[52]	Italy	55/53	18-65	IG: 29.9 ±4.6 CG: 29.3 ± 4.4	silybin, 94 mg/d; PC, 194 mg/d; VE, 89.28 mg/d + LMP	extra white saccharine, 94 mg/d; PC, 194 mg/d; VE, 89.28 mg/d + LMP	48	↓: AST, AST, HOMA-IR ND: TG, TC

G = gender; N = number; IG = Intervention Group; CG = Control Group; VE = vitamin E; VD = vitamin D; LMP = lifestyle modification program; PC = phosphatidylcholine; ND = no significant differences; ↑ = significant increase for intervention group; ↓ = significant decrease for intervention group; LDL-C = low-density lipoprotein cholesterol; AST = aspartate aminotransferase; ALT = alanine aminotransferase; CK-18M30 = cytokeratin 18-M30; NF-κB = nuclear factor-κB; hs-CRP = high-sensitivity C-reactive protein; TNF-α = tumor necrosis factor-α; BMI = body mass index; WHR = waist-to-hip ratio; GGT = γ-glutamyl transpeptidase; FBS = fasting blood sugar; HOMA-IR = homeostatic model assessment of insulin resistance; HDL-C = high-density lipoprotein cholesterol; QUICKI = quantitative insulin sensitivity check index; MPO = myeloperoxidase; TC = total cholesterol; TG = triglycerides; RBC = red blood cell; MCH = mean corpuscular hemoglobin; IL-6 = interleukin- 6; FGF21 = fibroblast growth factor 21.

Figure Legends

Figure 1. The analysis of keywords co-occurrences on fatty liver and flavonoids in research during 2001–2023 from Scopus (collected on May 14th 2023).

Figure 2. Flow diagram of the study selection.

Figure 3. Forest plots from the meta-analyses of randomized controlled trials investigating the effects of flavonoids compared to control group on liver function: A) ALT; B) AST; C) GGT; D) CK-18M30; E) fibrosis score; F) steatosis score.

Figure 4. Forest plots from the ma-analyses of randomized controlled trials investigating the effects of flavonoids compared to control group on lipid profile: A) TG; B) LDL-C; C) HDL-C; D) TC.

Figure 5. Forest plots from the meta-analyses of randomized controlled trials investigating the effects of flavonoids compared to control group on inflammation: A) TNF-α; B) hs-CRP; C) NF-κB.

Figure 6. Forest plot from the meta-analyses of randomized controlled trials investigating the effects of flavonoids compared to control group on insulin resistance: A) HOMA-IR; B) insulin; C) FBS.

Figure 7. The alleviation of NAFLD by flavonoids through various risk factors. ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = γ-glutamyl transpeptidase; CK-18M30 = cytokeratin 18-m30; TG = triglycerides; TC = total cholesterol; LDL-C = low-density lipoprotein cholesterol; TNF-α = tumor necrosis factor-α; NF-κB = nuclear factor-κB; DMY = dihydromyricetin; SIL = silymarin; HES = hesperidin; GEN = genistein.

Supporting Information

Appendix A1. Fig 1. Research trend for publications retrieved by searching Scopus database for the keyword flavonoids and fatty liver as from the year 2000 till 2022.

Appendix A1. Fig 2. Pie chart showing papers retrieved by subject area by searching Scopus database for flavonoids and fatty liver from 2000 till now.

Appendix A2. Search term.

Appendix A3. A) Risk of bias summary. B) Risk of bias graph.

Appendix A4. Subgroup analysis of biochemical biomarkers.

Appendix A5. Forest plot, sensitivity analysis, funnel plot, Egger's regression of liver function.

Appendix A6. Forest plot, sensitivity analysis, funnel plot, Egger's regression of lipid profile.

Appendix A7. Forest plot, sensitivity analysis, funnel plot, Egger's regression of inflammation.

Appendix A8. Forest plot, sensitivity analysis, funnel plot, Egger's regression of insulin resistance.

Appendix A9. Sensitivity analysis of one by one deletion method.

Appendix A10. Forest plot for subgroup analysis.