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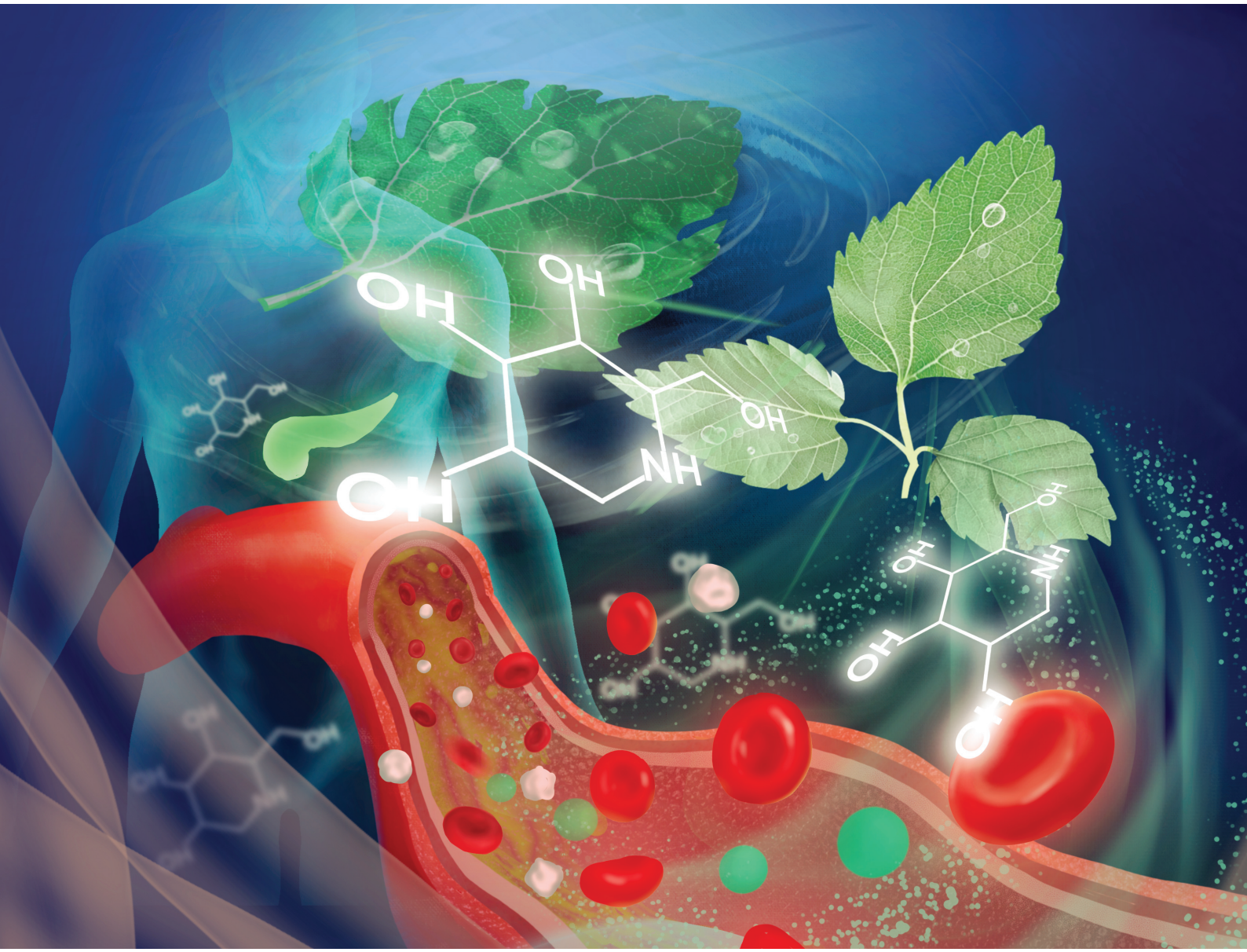

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REVIEW ARTICLE

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REVIEW

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Effect of mulberry leaf or mulberry leaf extract on glycemic traits: a systematic review and meta-analysis†

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Mulberry leaf (ML) and mulberry leaf extract (MLE) have numerous biological properties, such as regulating sugar and lipid metabolism, reducing blood glucose, and increasing insulin secretion. The aim of this study was to perform a systematic review and meta-analysis of randomized clinical trials to examine the effect of ML/MLE supplementation on glycemic traits in adults, including fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c), and fasting plasma insulin (FPI). Twelve clinical trials (615 participants) fulfilled the eligibility criteria for the present meta-analysis, which included sensitivity analysis and GRADE (grading of recommendations assessment, development, and evaluation) certainty. Based on the heterogeneity between included studies, a random effects model was applied in the meta-analysis, and the results are expressed as WMD (weighted mean differences) with 95% CI (confidence intervals). Meta-analysis showed that ML/MLE supplementation resulted in a significant reduction in FBG by −0.47 mmol L^{-1}, HbA1c by −2.92 mmol mol^{-1}, and FPI by −0.58 μIU mL^{-1}. In addition, subgroup analysis indicated that long-term supplementation of ML/MLE (≥8 weeks) was more effective for regulation of the glycemic traits in the non-healthy and baseline FPG >6.1 mmol L^{-1} subgroups. Glycemic regulation by ML/MLE may be attributed to the phytochemicals they contain, which are mainly 1-deoxynojirimycin, flavonoids, phenolics, and polysaccharides.

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1. Introduction

The leaves of the mulberry (*Morus alba* L.), which belongs to the class Magnoliopsida, order Rosales, family Moraceae, and genus Morus., are commonly used as traditional Chinese medicine and are incorporated into functional foods such as tea, beverages, noodles, and salad. Mulberry is mainly grown in Asian countries including China, Japan, and Korea.[1–3] Mulberry leaf-derived products in the form of powders, tea, extracts, tablets, and capsules are now available as functional foods and dietary supplements for controlling blood glucose.[4–6]

Mulberry leaves (ML) are rich in bioactive components, such as 1-deoxynojirimycin (1-DNJ 5.47%), resveratrol (0.39%), chlorogenic acid (1.21%), astragaloside (0.04%), and scopoletin (0.09%).[7] Kaempferol-3-*O*-rutinoside, quercetin, morin, coumaric acid, gallic acid, caffeic acid, ferulic acid, and polysaccharides are also present in ML.[8–11] The bioactive components in ML are mainly divided into flavonoids,[12–14] phenolics,[13,15,16] phytosterols,[17,18] terpenes,[17,18] γ-aminobutyric acid,[19,20] alkaloids,[21–23] and polysaccharides.[23–26] 1-DNJ is one of the primary alkaloids in ML, and it is an inhibitor of α-glucosidase, which results in the inhibition of the elevation of postprandial blood glucose.[27] Anti-oxidant, anti-inflammatory, anti-inflammatory, and anti-obesity effects have been observed after oral administration of 1-DNJ.[28] These active components in ML play an important role in the treatment of metabolic diseases including diabetes, dyslipidemia, obesity, atherosclerosis, and hypertension.[29]

Diabetes mellitus (DM) has become a major global problem with a significant health impact in many countries due to changes in lifestyle and dietary habits.[30] Type 2 diabetes mellitus (T2DM) is the most common of the three types of DM. It is linked to an inadequate production of insulin and/or insulin action, resulting in chronic hyperglycemia and abnormal

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metabolism of carbohydrates, lipids, and lipoproteins.[31] Predominant insulin resistance, accompanied by deficient hormone activity, leads to a decrease in its secretion.[32] Diabetes control entails essential changes in lifestyle and behaviors in addition to the process of disease control and management.[33] According to a World Health Organization (WHO) report, most of the world population prefers to use herbal medicine for the prevention and treatment of diseases due to fewer side effects and higher acceptance.[34] There have been numerous reports describing how mulberry leaf and mulberry leaf extract (MLE) result in significant hypoglycemic effects for those consuming high-fat and high-sugar diets or streptozotocin-induced diabetic animals and cells. 1-DNJ can attenuate hepatic injury by lowering glucose in T2DM db/db mice.[35] Studies have found that MLE intervention significantly suppresses the peak level of glucose in T2DM patients and non-T2DM subjects.[36,37]

Several reviews have previously been published and focused on the active principles in ML and their functional properties.[3,18,23,28,38,39] However, there has been no meta-analysis on the effect of ML/MLE on glycemic levels in clinical trials. The aim of the present study was to analyze randomized controlled trials (RCTs) and assess evidence of the effects of ML/MLE on fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c), and fasting plasma insulin (FPI) in healthy people, diabetics, obese, and other people consuming ML/MLE as compared with the controls.

Twelve eligible studies included all randomized and controlled clinical trials on this topic and were chosen for analysis of the effects of various factors on FBG, HbA1c, and FPI after ML/MLE supplementation, including physical condition, duration time, baseline FBG, female/total, and intervention type. We further evaluated the medicinal potential of ML/MLE for the control of glycemic traits.

2. Methods

This systematic review and meta-analysis were prepared and conducted according to PRISM (preferred reporting items for systematic reviews and meta-analysis) statements to ensure rigorous methodology and reporting.[40]

2.1. Identification of relevant studies

Two independent researchers (Z. S. and F. C.) conducted electronic searches in PubMed, Embase, Web of Science, and the CNKI library for studies published between January 1970 and July 2022. After deleting duplicate results, two investigators (Q. X. and B. X.) independently scanned the titles and abstracts from studies. The full text was independently obtained by the other two investigators (Y. W. and K. L.). Differences were decided by a third investigator (A. C.). The search strategy included the following keywords: ("mulberry leaf" OR "mulberry leaves" OR "*Morus indica* L." OR "1-deoxynojirimycin" OR "1-DNJ") and ("diabetes" OR "diabetic" OR "type 2 diabetes mellitus" OR "T2DM" OR "glucose" OR "blood sugar" OR "insulin" OR "iletin" OR "Ins" OR "glycated hemoglobin" OR "HbA1c" OR "glycosylated hemoglobin" OR "GHB"). The search was limited to human studies with full text. Duplicates were removed by filters used in the retrieval process. The reference lists of the relevant studies were inspected to identify any additional published studies not identified by the literature searches.

2.2. Study selection

The inclusion criteria were: (1) the study design was RCT, including parallel rows and crossover interventions; (2) participants were aged between 18 to 75 years old and had unlimited medical conditions, including health, overweight, obesity, or diabetes; (3) the intervention duration was not less than 2 weeks; (4) the data were presented as mean values with standard deviation (SD) or standard error (SE) values at baseline or endpoint or both for the outcomes investigated; (5) outcomes included FBG, and/or FPI, and/or HbA1c; (6) in studies with ≥ three intervention arms, of which two or three were eligible, only the eligible arms were included; (7) controlled trials of ML/MLE *versus* non-ML/MLE were included regardless of energy control. The exclusion criteria were: (1) studies did not use ML/MLE; (2) there were other interfering factors and multi-component interventions that prevented clarification of the effects of ML/MLE.

2.3. Data extraction

For studies that fulfilled the inclusion criteria, two investigators (W. C. and K. L.) extracted relevant subjects and intervention characteristics, including: (1) general information, *e.g.*, authors, regions, year of publication; (2) basic characteristics, *e.g.*, design, the number of dropouts; (3) intervention details, *e.g.*, the type and dosage of ML/MLE, treatment duration; (4) characteristics of the participants, *e.g.*, health conditions, gender, average age, average body mass index (BMI); and (5) outcomes, *e.g.*, FBG (mmol L^{-1}), HbA1c (mmol mol^{-1}), and FPI (μIU mL^{-1}) between pre and post intervention.

2.4. Quality assessment

According to the Cochrane risk of bias tool,[41] the methodological quality of the included studies was independently assessed by two authors (C. C. and W. C.). Any discrepancy was resolved after consultation with a third reviewer (A. C.). The following domains were considered: random sequence generation, allocation concealment, blinding (participants and investigators), incomplete outcome data, selective outcome reporting, and other sources of bias. Each category was assigned a high risk of bias, unclear risk of bias, or low risk of bias, based on the available information, and was presented in the included studies. We defined a study as having an overall high risk of bias if it was assessed as having a high risk in at least one out of six domains (we did not consider the item "other source of bias"). Low risk of bias was assigned if a study scored as low risk in all six domains. Otherwise, we considered the study to have an unclear risk of bias.

2.5. Statistical analysis

To perform the comparison, the glucose level was converted from mg dL^{-1} to mmol L^{-1} according to the conversion formula (1 mg dL^{-1} = 1/18 mmol L^{-1}), and the insulin level was converted from pmol L^{-1} to mIU L^{-1} or μIU mL^{-1}, according to the conversion formulae (1 pmol L^{-1} = 6.965 mIU L^{-1}, 1 pmol L^{-1} = 6.965 μIU mL^{-1}).[42] The HbAlc level was converted from % to mmol mol^{-1} according to eqn (1):

$$\text{HbA1c (mmol mol}^{-1}) = [\text{HbA1c (\%)} - 2.15] \times 10.929 \quad (1)$$

When multiple scales were used to measure the same symptoms, the scale with the highest clinical reliability was used for the meta-analysis. If there were multiple treatment groups in the study, we chose the group with the highest concentration as the active treatment group, and the lowest concentration was in the control treatment group. The entire process of statistical analyses was carried out using Review Manager 5.4 software.

A random-effects model was used for calculation of the effect size. The effect size consisted of weighted mean difference (WMD) and 95% CI (confidence intervals) between the outcomes of the intervention and control groups using a generic inverse-variance random effects model. A two-sided *p*-value of 0.05 was considered statistically significant. Heterogeneity between studies was assessed using the Cochrane *Q* and I^2 statistics. The threshold of heterogeneity interpretation corresponds to the Cochrane Collaboration (I^2 0–40%: might be considered irrelevant; 30–60%: moderate heterogeneous; 50–90%: massive heterogeneity; 75–100%: considerable heterogeneity).[43] Sensitivity analysis was used to recalculate its effect by deleting each study, which assessed the source of heterogeneity.

Subgroup analyses were used to evaluate the impact of certain factors, and they were performed according to the following variables: physical condition (healthy or non-healthy), duration time (<8 weeks or ≥8 weeks), baseline FBG (≤6.1 mmol L^{-1} or >6.1 mmol L^{-1}), and intervention type (MLE or ML (including tea and powder)). Changes from baseline to endpoint were used for the analysis of FBG, HbA1c, and FPI. The changes in the mean and standard deviation were calculated according to eqn (2) and (3), respectively.

$$\Delta\bar{X} = \bar{X}_2 - \bar{X}_1 \quad (2)$$

$$\Delta\text{SD} = \sqrt{\text{SD}_1{}^2 + \text{SD}_2{}^2 - 2 \times \text{corr} \times \text{SD}_1 \times \text{SD}_2} \quad (3)$$

where $\bar{X}_1$ and $\bar{X}_2$ denote the mean at the baseline and endpoint, respectively. The correlation coefficient is 0.4, and SD_1 and SD_2 denote the standard deviations at the baseline and endpoint, respectively.

2.6. Grading the certainty of evidence for major comparisons and outcomes

We graded the certainty of evidence of relevant outcomes based on current GRADE (grading of recommendations assessment, development, and evaluation) guidance.[44] Developed to grade the overall certainty of a body of evidence, this approach incorporates five key domains: (1) risk of bias, (2) inconsistency, (3) indirectness, (4) imprecision of the evidence, and (5) other considerations. Two reviewers (K. L. and Q. X.) assessed each domain for each selected outcome and resolved differences by a third reviewer (A. C.). Grades of evidence included high, moderate, low, and very low certainty.

3. Results

3.1. Study selection

Fig. 1 illustrates the literature search and screening process performed for this systematic review. From a total of 1158 records from our initial database searches, 423 duplicates were removed. Then, based on the title and abstract, 678 records were excluded because they did not meet our criteria. Next, 13 suitable articles were chosen for full-text assessment. Among these, one article was excluded due to glycemic indices that were not reported. Finally, 12 eligible studies were included for the meta-analysis and systematic review.

3.2. Characteristics of the eligible studies

The basic characteristics of the 12 studies[36,45–55] are listed in Table 1. These studies included 326 participants in the intervention group (IG) and 325 participants in the control group (CG), and were published between 2001 and 2022. One study was conducted in the USA,[49] one was in Italy,[52] and the others were in Asian countries. In total, the results included the outcomes of 12 FBG, 11 HbA1c, and 5 FBI that were reported from 12 individual treatment arms. The ages of the participants ranged from 18 to 75 years, and the duration time of the intervention ranged from 4 to 24 weeks. These studies were conducted using healthy,[45–47,52,53] T2DM,[36,49,50,54,55] obese,[51] and hyperlipidemic[48] subjects.

3.3. Quality assessment

Fig. 2 shows a summary of the risk of bias, as determined using the Cochrane risk of bias tool. In the RCTs, 7 biases in three studies were estimated as low risk.[49,50,53] Random sequence generation (selection bias) in all studies was considered as low risk. High risk of bias was not assessed in two studies.[47,51] The low, unclear, and high risk of bias in allocation concealment was 50%, 33.3%, and 16.7%; the low, unclear, and high risk of bias in blinding of participants and personnel was 58.3%, 13.3%, and 33.3%; the low, unclear risk of bias in the blinding of outcome assessment was 66.7% and 33.3%; the low, unclear, and high risk of bias in selective reporting was 58.3%, 16.7%, and 25.0%; the low, unclear risk of other bias was 33.3% and 66.7%, respectively.

3.4. Intervention effects

3.4.1. Fasting blood glucose (FBG). Twelve studies, including 810 subjects, considered FBG as an outcome. The meta-analysis showed that the FBG levels in the intervention group (WMD: −0.47 mmol L^{-1}, 95% CI: −0.84 to −0.10 mmol L^{-1},

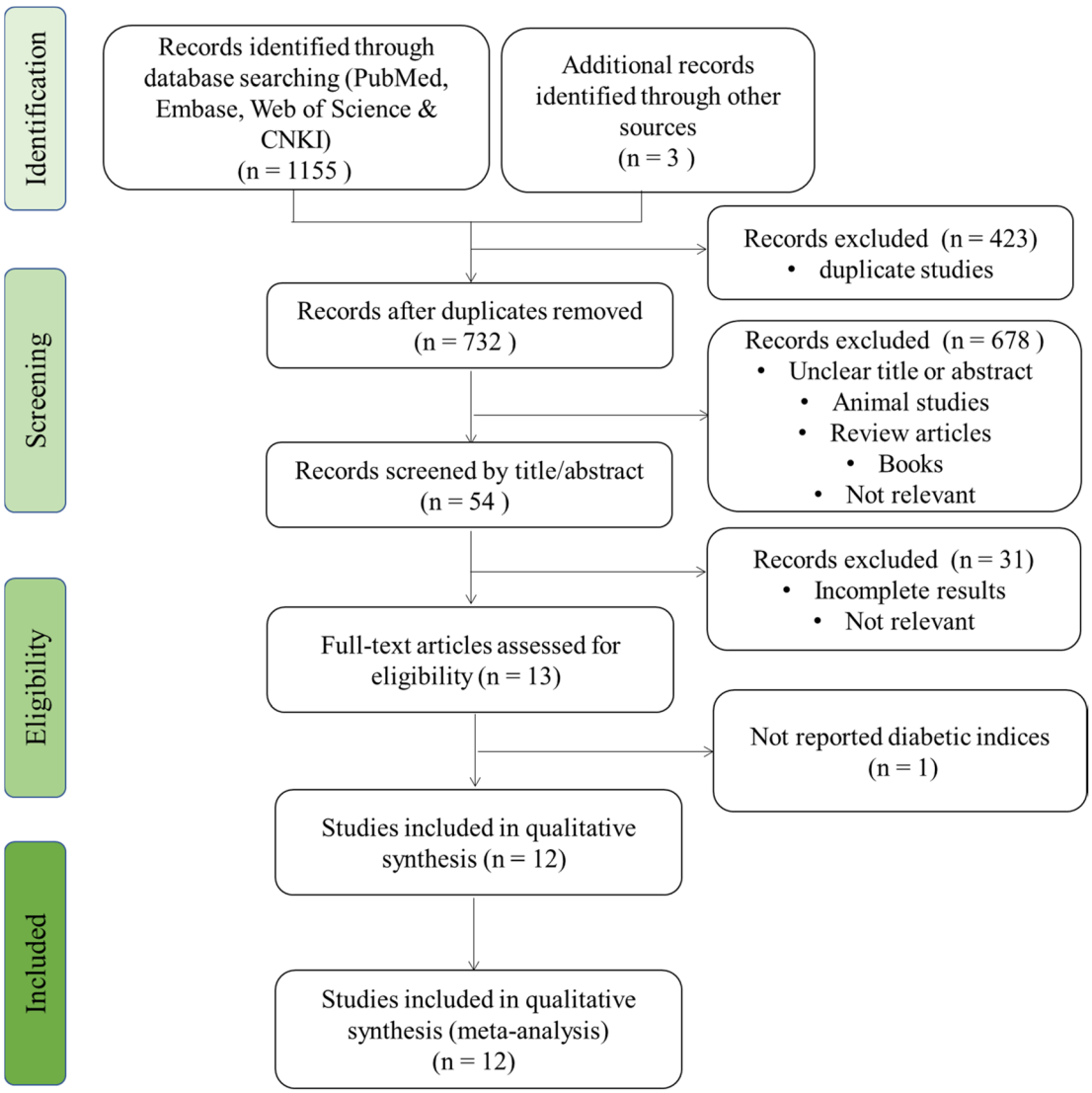

Fig. 1 PRISMA flow diagram for a systematic review.

p = 0.01) were significantly decreased compared with the control group (Fig. 3A). There was significant overall heterogeneity with I^2 = 96.0% (p < 0.00001).

A subgroup analysis according to physical condition (healthy or non-healthy), duration time (<8 weeks or ≥8 weeks), baseline FBG (≤6.1 mmol L^{-1} or >6.1 mmol L^{-1}), and intervention type (MLE or ML) was performed to determine the effect of ML/MLE on FBG, as shown in Table 2. The results showed that the FBG level significantly decreased in the non-healthy (−0.80 mmol L^{-1}), baseline FBG >6.1 mmol L^{-1} (−0.91 mmol L^{-1}) and MLE intervention subgroups (−0.47 mmol L^{-1}). However, there were no statistical differences in the healthy (−0.02 mmol L^{-1}), baseline FBG ≤6.1 mmol L^{-1} (−0.03 mmol L^{-1}), and ML intervention subgroups (−0.44 mmol L^{-1}). The meta-analysis according to duration time showed that the FBG level significantly decreased when the duration time was ≥8 weeks (−0.54 mmol L^{-1}), or <8 weeks (−0.62 mmol L^{-1}). ML/MLE oral administration obviously reduced the FBG level at baseline in non-healthy subjects ($p \leq 0.05$), such as T2DM, pre-diabetic, and hyperlipidemic subjects.

The test showed that there is high heterogeneity in the non-healthy (I^2 = 95%, p < 0.00001), duration time ≥8 weeks (I^2 = 91%, p < 0.00001), duration time <8 weeks (I^2 = 98%, p < 0.00001), baseline FBG >6.1 mmol L^{-1} (I^2 = 93%, p < 0.00001), and MLE intervention subgroups (I^2 = 97%, p < 0.00001). The heterogeneity was massive in the ML intervention subgroup (I^2 = 70%, p = 0.07), but the heterogeneity was considered irrelevant in the healthy (I^2 = 0%, p = 0.67) and baseline FBG ≤6.1 mmol L^{-1} subgroups (I^2 = 0%, p = 0.70). Based on the FBG, tests of the subgroup differences for physical condition, duration time, baseline FBG, and intervention type were 0.04, 0.86, 0.03, and 0.93, respectively. The results showed that the physical condition and baseline FBG level were the important

Table 1 Characteristics of the studies included in this meta-analysis

Study ID	Country	Gender (F/M)	Age (years)	Health conditions	Duration (weeks)	Intervention		Mean age		Mean BMI		Indicator	Design
						Type	Dose	CG	IG	CG	IG		
Andallu *et al.*, 2001 [36]	India	12/12	40–60	T2DM	4	Capsules	3 g	—	—	—	—	HbA1C, FBG	R
Aramwit *et al.*, 2011 [45]	Thailand	46	20–60	Healthy	4	ML tablets	2.29 g MLE (3.303 mg 1-DNJ)	32.83 ± 1.99	—	24.16 ± 2.04	—	HbA1C, FBG	R
Kim *et al.*, 2015 [46]	Korea	19/19	20–65	Healthy	4	18 tablets	5 g MLE	50.16 ± 7.83	53.00 ± 7.20	25.93 ± 3.86	24.69 ± 2.19	HbA1C, FBG, FPI	R, PC, DB
Kimura *et al.*, 2007 [47]	Japan	6/19	—	Healthy	38 d	DNJ powder	1.2 g	24.7 ± 1.0		21.3 ± 0.6		FBG, FPI	PC
Kojima *et al.*, 2010 [48]	Japan	9/10	20–64	Hyperlipidemia	12	DNJ-rich MLE	Capsules (36 mg DNJ)	79.2 ± 10.8	77.1 ± 11.0	28.0 ± 3.7	27.2.0 ± 3.5	HbA1C, FBG, FPI	R
Qi *et al.*, 2018 [54]	China	70/104	30–85	T2DM	4	MLE	1 g	—	—	—	—	HbA1C, FBG	R
Riche *et al.*, 2017 [49]	USA	14/10	—	T2DM	12	MLE capsules	2500 mg (1000 mg MLE)	56 ± 7.0	57 ± 5.5	—	—	HbA1C, FBG	R, PC
Taghizadeh *et al.*, 2022 [50]	Iran	42/15	35–70	T2DM	12	MLE	600 mg	52.6 ± 6.95	46.2 ± 20.1	31.1 ± 3.9	30.7 ± 3.3	HbA1C, FBG, FPI	R, DB, PC
Thaipitakwong *et al.*, 2020 [51]	Thailand	37/17	18–50	Obesity	16	ML powder	4.6 g (12 mg DNJ)	52 ± 8.22	53.14± 5.48	31.61 ± 5.85	30.06 ± 4.06	HbA1C, FBG, FPI	R
Trimarco *et al.*, 2015 [52]	Italy	22/24	18–70	Healthy	8	MLE	200 mg	—	59.5 ± 6.3	26.8 ± 3.6	—	HbA1C, FBG, FPI	R, PC, DB
Yang *et al.*, 2019 [55]	China	49/51	32–63	T2DM pre-diabetes	24	ML tea	5 g	43.58 ± 10.12	43.2 ± 10.72	—	—	HbA1C, FBG	
Yasumoto *et al.*, 2022 [53]	Japan	24/23	20–64	Healthy	4	MLE beverage	0.7% MLE	46.4 ± 10.2	43.3 ± 13.7	22.7 ± 3.1	21.3 ± 1.6	FBG, HbA1C	R, PC, DB

MLE, mulberry leaf extract; ML, mulberry leaf; BMI, body mass index; IG, intervention group, CG, control group; R, randomized; PC, placebo controlled; DB, double blind; T2DM, type 2 diabetes mellitus; M, male; F, female; FBG, fasting blood glucose; HbA1C, glycosylated hemoglobin; FPI, fasting plasma insulin.

parameters that significantly affected the inhibitory effect of ML/MLE on FPG in participants. Sensitivity analysis for FBG showed that the overall estimate was affected by the exclusion of the study conducted by Qi *et al.*[54] (WMD: −0.31 mmol L^{-1}; 95% CI: −0.66 to 0.03 mmol L^{-1}) (Table S2†).

3.4.2. Glycosylated hemoglobin (HbA1c). Except for the study by Kojima *et al.*,[47] 11 studies including 639 participants reported HbA1c as an outcome. Compared with the control diets, there was a significant tendency for ML/MLE consumption to result in lower HbA1c levels (WMD: −2.92 mmol mol^{-1}; 95% CI: −5.66 to −0.18 mmol mol^{-1}, p = 0.04) (Fig. 3B). The overall results showed that there is high heterogeneity, with I^2 = 92% (p < 0.00001).

A subgroup analysis of HbA1c was conducted, as shown in Table 2. According to the meta-analysis of physical condition, ML/MLE consumption was more effective in reducing HbA1c for the non-healthy condition (−4.70 mmol mol^{-1}), compared with the healthy condition (−0.14 mmol mol^{-1}). Similarly, ML/MLE significantly decreased the HbA1c level when baseline FBG was >6.1 mmol L^{-1} (−5.61 mmol mol^{-1}), but there was no significant effect in the baseline FBG ≤6.1 mmol L^{-1} (−0.17 mmol mol^{-1}) subgroup. There was no statistical difference in the HbA1c level when the duration time <8 weeks (−5.33 mmol mol^{-1}), or duration time ≥8 weeks (−1.06 mmol mol^{-1}). The test showed that there was high heterogeneity in the non-healthy (I^2 = 94%, p < 0.00001), baseline FBG >6.1 mmol L^{-1} (I^2 = 92%, p < 0.00001), duration time <8 weeks (I^2 = 96%, p < 0.00001), and MLE intervention subgroups (I^2 = 93%, p < 0.00001. There was moderate heterogeneity at baseline FBG ≤6.1 mmol L^{-1} (I^2 = 51%, p = 0.08), but the heterogeneity was considered irrelevant in the healthy (I^2 = 0%, p = 0.90), duration time ≥8 weeks (I^2 = 23%, p = 0.26), and ML intervention subgroups (I^2 = 0%, p = 0.69). The subgroup differences of physical condition, duration time, baseline FBG level, and intervention type were 0.04, 0.21, 0.04, and 0.62, respectively.

The results showed that the physical condition and baseline FBG were the important parameters that had a significant effect on HbA1c after ML/MLE consumption. Sensitivity analysis for HbA1C showed that the overall estimate was affected by the exclusion of the study conducted by Qi *et al.*[54] (WMD: −1.42 mmol mol^{-1}; 95% CI: −2.18 to −0.65 mmol mol^{-1}) (Table S2†).

3.4.3. Fasting plasma insulin (FPI). The data on FPI were reported in 5 studies[46,48,50–52] that included 214 subjects. As shown in Fig. 3C, ML/MLE consumption resulted in a significant reduction in FPI levels (WMD: −0.58 μIU mL^{-1}; 95% CI: −2.78 to 1.62 μIU mL^{-1}, p = 0.60). The overall results show that there was moderate heterogeneity, with I^2 = 55% (p = 0.06).

As shown in Table 2, due to the lack of eligible studies reporting FPI as an outcome, the subgroup analysis indicated that there were no statistical differences (p > 0.05) in the FPI level for the physical condition (healthy or non-healthy), duration time (<8 weeks or ≥8 weeks), baseline FBG (≤6.1 mmol L^{-1} or >6.1 mmol L^{-1}), or intervention type (MLE or ML). The heterogeneity of the overall test was irrelevant for the non-

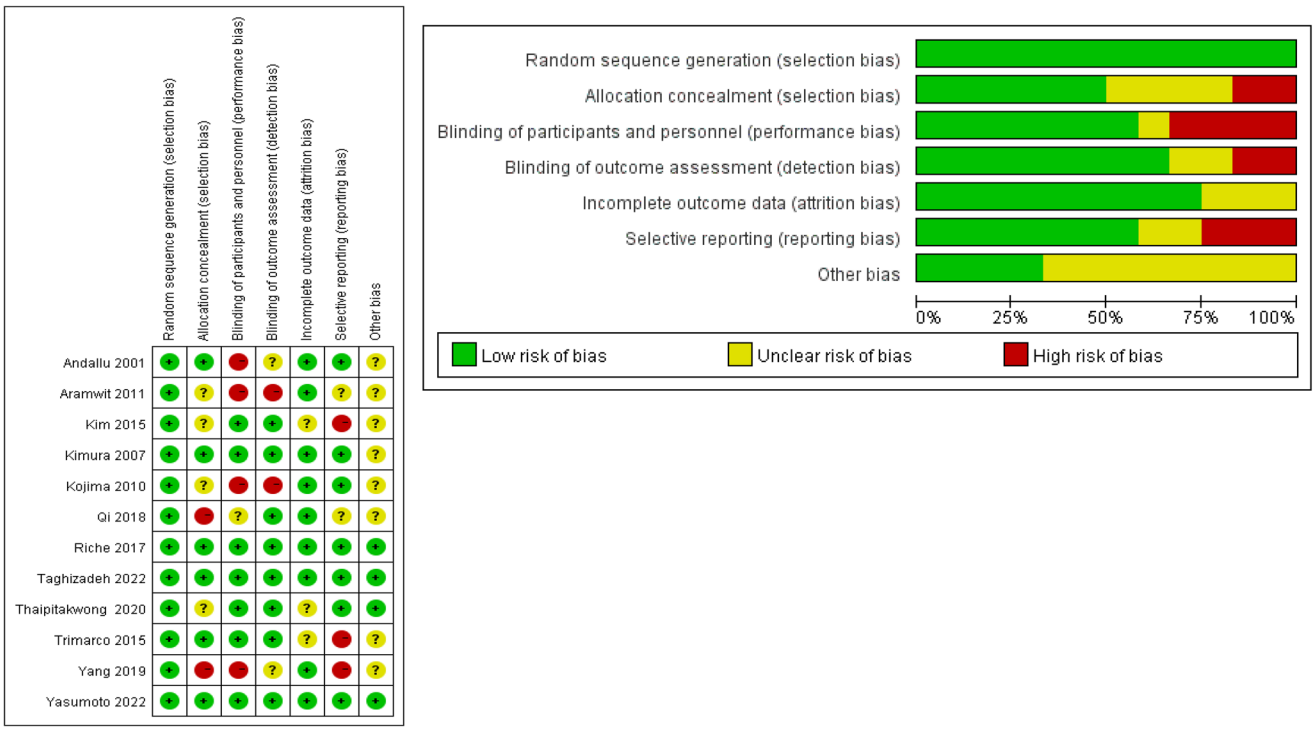

Fig. 2 Risk of bias assessment.

Table 2 Effect of mulberry leaf extract on FBG, HbAlc, and FPI in various subgroups

	FBG				HbAlc				FPI			
Subgroups	*N*	WMD (95% CI)	I^2 (%)	*p*	*N*	WMD (95% CI)	I^2 (%)	*p*	*N*	WMD (95% CI)	I^2 (%)	*p*
Physical conditions												
Healthy	189	−0.02 [−0.07, 0.02]	0	0.32	177	0.14 [−1.30, 1.01]	0	0.81	84	0.48 [−2.87, 3.83]	66	0.78
Others	462	−0.80 [−1.53, −0.06]	95	0.03	462	−4.70 [−8.77, −0.62]	94	0.02	130	−1.80 [−4.22, 0.62]	0	0.14
Duration time												
<8 weeks	351	−0.62 [−1.21, −0.03]	98	0.04	339	−5.33 [−11.94, 1.29]	96	0.11	38	1.76 [0.54, 2.98]	—	0.005
≥8 weeks	384	−0.54 [−1.18, 0.10]	91	0.10	300	−1.06 [−2.22, 0.10]	23	0.07	176	1.80 [−3.85, 0.25]	0	0.09
Baseline FBG												
≤6.1 (mmol L^{-1})	224	−0.03 [−0.07, 0.02]	0	0.24	212	−0.17 [−1.51, 1.16]	51	0.80	119	−1.53 [−3.90, 0.85]	0	0.21
>6.1 (mmol L^{-1})	437	−0.91 [−1.71, −0.11]	93	0.03	427	−5.61 [−10.91, −0.32]	92	0.04	95	0.03 [−4.15, 4.21]	75	0.99
Intervention type												
MLE	497	−0.47 [−0.90, −0.04]	97	0.03	485	−3.08 [−6.85, 0.69]	93	0.11	160	−0.69 [−3.39, 2.02]	64	0.62
ML	154	−0.44 [−1.07, 0.19]	70	0.17	154	−2.08 [−3.30, −0.87]	0	0.0008	54	−0.79 [−4.69, 3.11]	—	0.69

MLE, mulberry leaf extract; ML, mulberry leaf; FBG, fasting blood glucose; HbA1C, glycosylated hemoglobin; FPI, fasting plasma insulin.

healthy (I^2 = 0%, p = 0.81), duration time <8 weeks (I^2 = 0, p = 0.93), and baseline FBG ≤6.1 mmol L^{-1} (I^2 = 0%, p = 0.89) subgroups. The overall test yielded moderately heterogeneous results in the healthy (I^2 = 66%, p = 0.09), baseline FBG >6.1 mmol L^{-1} (I^2 = 75%, p = 0.04), and MLE intervention (I^2 = 64%, p = 0.04) subgroups. Based on the FPI, the subgroup differences in physical condition, duration time, baseline FBG, and intervention type were 0.28, 0.003, 0.53, and 0.97, respectively. Sensitivity analysis for FPI showed that the overall estimate was affected by the exclusion of the study conducted by Kim *et al.*[46] (WMD: −1.80 μIU mL^{-1}; 95% CI: −3.85 to 0.25 μIU mL^{-1}) (Table S2†).[46]

3.5. GRADE assessment

GRADE assessment showed that there was considerable variation in the methodological quality of the included trials, with several quality indicators not fully discussed in many publications. For the 12 eligible studies, only 4 trials (33.3%) reported the randomization double-blind method used, and masking of participants was not possible in most trials due to the intervention characteristics. All trials reported the eligibility criteria, and the intervention and control group were similar at baseline. In summary, GRADE evidence profiles of outcomes are shown in Table S3.† FBG and HbA1c were found to have a very low certainty that statistically and

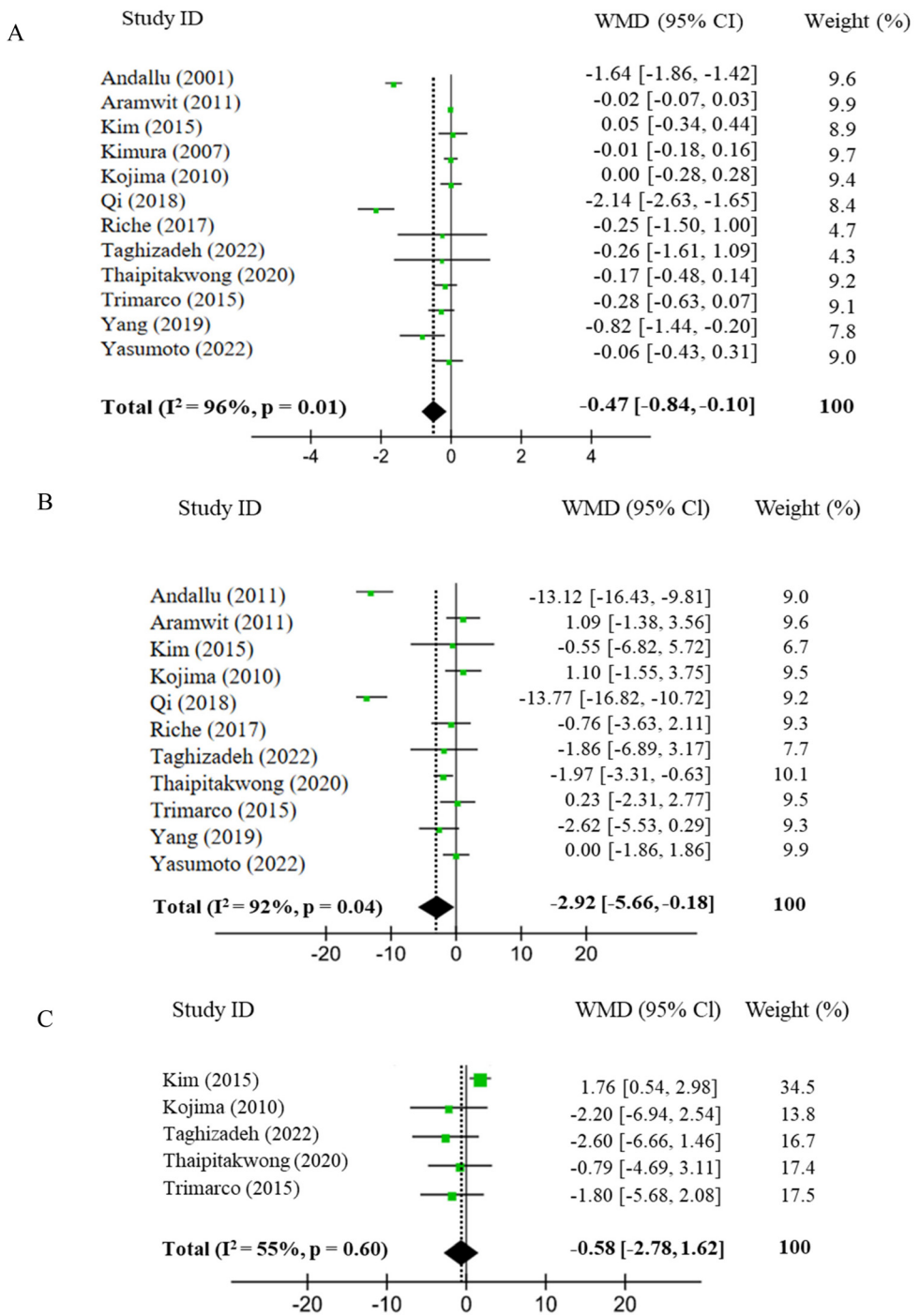

Fig. 3 Forest plots of the effects of mulberry leaf extract on glycemic indices. (A) FBG (fasting blood glucose); (B) HbAlc (glycosylated hemoglobin); (C) FPI (fasting plasma insulin).

clinically decreased with ML/MLE supplementation, but FPI was evaluated as having a low evidence certainty.

4. Discussion

The genus Morus includes several species, such as *Morus alba* and *M. nigra*, which are both native to Asia, and *M. rubra* of North American origin. With a wide distribution in the world, there have been various reports of different medicinal properties for this genus in temperate and tropical areas.[39] Diabetes is associated with increased oxidative stress. It is important to control the glycemic response so that clinical outcomes and quality of life can be improved, especially for patients with metabolic disorders.[56] Several therapeutic strategies such as natural extracts have been developed with the

objective of improving the glycemic response.[57] There has been interest in MLE in the scientific community, and ML and MLE are used for hypoglycemia, obesity, and T2DM[39] because they are rich in phenolics, flavonoids, and polysaccharides, especially 1-DNJ, which is a unique active ingredient in ML.[58,59]

To the best of our knowledge, this is the first meta-analysis to examine the effects of ML/MLE on glycemic traits. A total of 12 RCTs including 5 healthy subjects and 7 non-healthy subjects were eligible for the present study. Subgroup analysis indicated that supplemental ML/MLE significantly decreased the levels of FBG, HbAlc, and FPI in trials with non-healthy subjects, which mainly included those with T2DM, pre-diabetes, obesity, and hyperlipidaemia ($p < 0.05$), but not in healthy subjects ($p > 0.05$). The effect of ML/MLE on subjects with high baseline FBG (>6.1 mmol L^{-1}) is more obvious compared with the normal baseline FBG (≤6.1 mmol L^{-1}). Tests of the subgroup differences indicated that physical condition and baseline FBG level were the important conditions, which had a significant effect on FPG and HbA1c in participants who consumed ML/MLE. Short- and long-term intervention with ML/MLE affected the glycemic index. In all, more than 8 weeks of intervention with ML/MLE supplementation was recommended. The above results showed that physical conditions and baseline FBG level were the important parameters that significantly affected the levels of FBG, HbAlc, and FPI in participants who supplemented with ML/MLE.

The clinical trial and virtual analysis showed that a hydroalcoholic extract of black mulberry (*M. nigra*) leaf may be efficient in decreasing FPG and HbA1c in diabetic patients.[60] The study conducted with 37 adult participants proved that the difference in the positive incremental area under the curve ((pIAUC) for glucose concentration over 120 minutes) for 125, 250, and 500 mg mulberry extract compared with placebo was −6.1% ($p = 0.316$), −14.0% ($p = 0.022$), and −22.0% ($p < 0.001$), respectively.[61] 1-DNJ is an effective inhibitor of α-glucosidase, which is an enzyme that breaks down starch and other disaccharides into glucose. Thus, 1-DNJ can block carbohydrate absorption in the body, inhibiting any increases in blood sugar to achieve the effect of prevention and treatment of DM.[62,63]

The physiological properties of MLE were also affected by the different composition and content of the active components contained in MLE obtained from different extraction methods.[63] MLE reduced the glycemic indexes of four common dietary carbohydrates, which were maltose, sucrose, maltodextrin, and glucose, by 53.11%, 33.51%, 31.00%, and 8.12%, respectively.[67] The intervention time and intervention dose of ML/MLE were the main factors that affected the level of FBG and HbA1c. HbA1c is mainly used to assess the blood glucose in the body over a three-month period, and there are various confounding factors when measuring HbAlc.[68] Additionally, both types of ML possess unique viscous dietary fibers that become thick when mixed with liquids, resulting in a reduced rate of glucose digestion and absorption.[64–66]

The studies examining the effect of ML/MLE on glycemic traits were mostly based on mice or cell models. MLE was orally administered for 8 weeks to female Wistar rats that were fed a high-cholesterol diet to evaluate its ability to decrease obesity, dyslipidemia, and insulin resistance (IR). Significant results were observed, with the occurrence of decreases in body weight gain, hypercholesterolemia, hypertriglyceridemia, and atherogenic indices, based on lipid profile, coronary artery, glucose level, and the insulin resistance index.[69] 1-DNJ in ML enhanced the conversion from white preadipocytes to beige adipocytes by activating AMPK, and then increased energy consumption.[70] Compared with normal control rats, the diabetic rats in the mulberry leaf polysaccharide treatment group exhibited increased oral glucose tolerance and restored levels of glycogen.[71] Flavonoids from ML regulated the suppression of factor associated suicide (FAS) and glucose transporter 4 (GLUT4) *via* the insulin receptor substrate/phosphatidylinositol 3-kinase/protein kinase B (IRS1/PI3K/AKT) pathway, which alleviated the glycolipid metabolic abnormalities in 3T3-L1 adipocytes.[72] These effects are closely associated with reducing hepatic fat accumulation, suppressing adipocyte lipolysis, increasing adiponectin receptor expression, improving peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT/enhancer binding protein (C/EBPα) action, restoring the balance of SOD/CAT and GSH/GSSG, regulating the adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK), IR, PI3K, and AKT pathways, and regulating the NF-κB signaling pathways *via* decreasing inflammation.[29]

Hence, some studies demonstrated that ML and MLE confer antioxidant and antidiabetic effects, which are efficacious in the correction of hyperglycemia and the increased secretion of insulin,[73,74] mainly by regulating hepatic enzymes involved in cellular glucose metabolism[75,76] and by activating the expression of insulin receptors.[35] Others mention that administering MLE could be considered as a potential adjunct therapy for the management of diabetes because it reduces the level of glucose in fasting blood and plasma glucose.[77] The glycemic index is affected by multiple factors, such as intestinal digestion, absorption efficiency of carbohydrates,[78] lifestyle factors, medications,[79,80] and the gut microbiome.[81] ML and MLE are strong antioxidants with numerous biological and therapeutic properties such as regulation of sugar metabolism, protection of islet cells, modulation of lipids, protection against oxidative stress, enhanced mitochondrial functioning, enhanced glycolysis in peripheral tissues, and decreased gluconeogenesis in the liver, which contribute to their anti-diabetic, anti-atherosclerotic, and anti-hypertensive effects.[29,39] MLE may be developed as a potential anti-diabetic agent, and the possible mechanisms involved in the regulation of diabetes by ML/MLE are summarized and shown in Fig. 4.

5. Strengths, limitations, and implications

There are several strengths in this systematic review and meta-analysis. (1) We performed a comprehensive and reproducible search. (2) The selection process of the eligible literature was

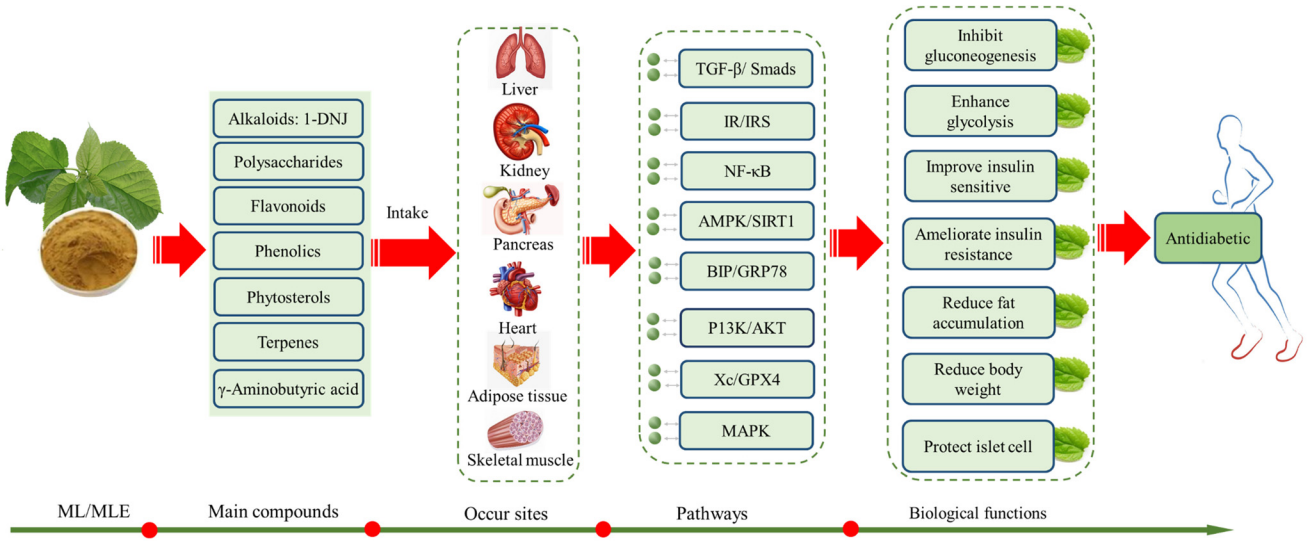

Fig. 4 Possible mechanisms involved in the regulation of diabetes by ML/MLE. *Notes*: MLE, mulberry leaf extract; 1-DNJ, 1-deoxynojirimycin; TGF-β, transforming growth factor-β; IR, insulin resistance; IRS, insulin receptor substrate; NF-κB, nuclear factor-κ-gene binding; AMPK, adenosine 5′-monophosphate (AMP)-activated protein kinase; SIRT1, NAD-dependent deacetylase sirtuin-1; BIP, immunoglobulin heavy chain binding protein in pre-B cells; GRP78, glucose regulated protein 78 kDa; PI3K, phosphatidylinositol 3 kinase; AKT, protein kinase B; GPX4, glutathione peroxidase 4; MAPK, mitogen-activated protein kinase.

strictly and carefully performed. (3) Collation and synthesis of all the available evidence from 12 studies was performed, and it included 326 subjects in intervention studies and 325 subjects in control studies. (4) We included an assessment of overall study quality using the Cochrane Collaboration risk of bias tool. (5) The main and subgroup analysis on the effect of ML/MLE on glycemic traits was systematically researched.

However, there were several limitations to our meta-analysis. As we mentioned, the impacts of confounding factors, including racial and ethnic background, energy intake, and lifestyle variables on the efficacy of ML/MLE, remained unclear. Furthermore, subgroup evidence was downgraded due to the small number of included studies. Therefore, the overall interpretation of the results is difficult due to the aforementioned bias. Thus, there are some limitations in this meta-analysis that should be considered in interpreting the results. This meta-analysis is based on studies that included different subjects, with the following differences: (1) the extraction and purification method used for ML/MLE; (2) digestion and absorption of ML/MLE in the human digestive system; (3) dietary patterns; (4) rates and degrees of obesity, pre-diabetes, T2DM, and hyperlipidemia; (5) prevalence of confounding factors; (6) limitation of generalizability due to most of the studies being conducted in Asia; (7) with the details of the dietary assessment used, some heterogeneity is expected between studies.

Most of the included studies adjusted for lifestyle factors such as overweight and obesity, physical exercise, smoking, and alcohol consumption that are common risk factors for glycemic traits, as well as other possible confounding factors. In addition, the rigorous GRADE standards were used to systematically appraise the methodological quality of all included trials. Begg's test, Egger's test, and regression analysis were not performed in this study due the inability of Review Manager 5.4 software to assess the bias analysis and linear relationship of the included data.

Future trials should focus on the development of high quality and increased effectiveness to translate recommendations into practice. Confounding factors such as different dietary compliance, lifestyle factors, absorption efficiency, production process, storage, and different geographical locations and botanical species of mulberry also need to be considered. Additionally, there are pitfalls associated with glycemic measurements and their false-positive/false-negative results. In this situation, using other alternative indexes is desirable.

6. Conclusions

The present meta-analysis demonstrated that intake of ML/MLE significantly reduced the levels of FBG, HbA1c, and FPI. We also found that ML/MLE intake by non-healthy subjects might have more significant effects than those in healthy subjects. The baseline FBG was the important parameter that was affected on the glycemic level by ML/MLE. Additionally, long-duration supplementation of ML/MLE is more beneficial for the regulation of the glycemic indices. However, further studies should be performed on diabetic and prediabetic patients to determine the effect of ML/MLE supplementation on impaired glycemic control.

In conclusion, the findings of the present study raise the possibility that supplementation of the diet with ML/MLE or DNJ could potentially assist in suppressing FBG and HbA1c levels, which may be of benefit to patients with impaired

glucose tolerance or diabetes mellitus. However, it should be noted that this study is preliminary in nature, and that the results are tentative.

Abbreviations

MLE	Mulberry leaf extract
ML	Mulberry leaf (leaves)
RCTs	Randomized controlled trials
PRISM	Preferred reporting items for systematic reviews and meta-analysis
BMI	Body mass index
IG	Intervention group
CG	Control group
R	Randomized
PC	Placebo controlled
DB	Double blind
WMD	Weight mean difference
CI	Confidence intervals
T2DM	Type 2 diabetes mellitus
M	Male
F	Female
FBG	Fasting blood glucose
HbA1c	Glycosylated hemoglobin
FPI	Fasting plasma insulin
1-DNJ	1-Deoxynojirimycin
GRADE	Grading of recommendations assessment, development, and evaluation
TGF-β	Transforming growth factor-β
IR	Insulin resistance
IRS	Insulin receptor substrate
NF-κB	Nuclear factor-κ-gene binding
AMPK	Adenosine 5′-monophosphate-activated protein kinase
SIRT1	Sirtuin-1
BIP	Immunoglobulin heavy chain binding protein in pre-B cells
GRP78	Glucose regulated protein 78 kDa
Pl3K	Phosphatidylinositol 3-kinase
AKT	Protein kinase B
GPX4	Glutathione peroxidase
FAS	Factor associated suicide
GLUT4	Glucose transporter 4
GSH	L-Glutathione
GSSG	Glutathione oxidized
CAT	Catalase
SOD	Superoxide dismutase
PPARγ	Peroxisome proliferator-activated receptor γ
C/EBPα	CCAAT/enhancer binding proteins α.

Author contributions

Anwei Cheng (A. C.) and Fachun Wan (F. W.) designed the research and provided the funding. Zhaoyue Sun (Z. S.) and Fuchun Chen (F. C.) researched the database and checked the results. Qian Xiao (Q. X.), Ben Xu (B. X.), and Yunfu Wang (Y. W.) scanned the titles and abstracts from studies. Wenyu Cui (W. C.), Kaiyun Luo (K. L.), and Caifang Cui (C. C.) extracted and analyzed the data. Wenyu Cui (W. C.) and Kaiyun Luo (K. L.) drafted the manuscript. Anwei Cheng (A. C.), Weijun Shen (W. S.), and Qian Xiao (Q. X.) contributed to writing, reviewing, or revising the paper.

Conflicts of interest

No potential conflicts of interest have been reported by the authors.

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