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Page: [O108-120]

Original research article

Unveiling the mechanism of madecassic acid in nonalcoholic fatty liver disease: a network pharmacology and experimental validation

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ABSTRACT

Madecassic acid (MA), a triterpenoid derived from *Centella asiatica*, exhibits diverse pharmacological properties. Despite its potential, the mechanisms underlying its effects on insulin resistance and lipid metabolism remain unclear. This study aimed to elucidate the pharmacological properties of MA in nonalcoholic fatty liver disease (NAFLD) via network pharmacology and experimental validation. A network pharmacology analysis was conducted to identify potential therapeutic targets of MA in NAFLD. Target genes were retrieved from GeneCards and other relevant databases, and a component–target–disease network was constructed. Furthermore, the effect of MA on the *in vitro* model of NAFLD, insulin resistance, and oxidative stress were determined in HepG2 and Raw 264.7 cells. Network pharmacology analysis revealed that MA involves anti-inflammation and lipid metabolic pathways. Experimental validation confirmed the ability of MA to suppress proinflammatory gene expression (*IL-6*, *Tnf-α*, *Mcp-1*), reduce oxidative stress, and modulate lipid metabolism dysregulation by ameliorating *Srebp-1c* and *Fasn* overexpression, preventing *Cpt-1* downregulation, and enhancing glucose utilization. Our findings suggest that MA is a potential therapeutic option for NAFLD and its associated complications.

Keywords: madecassic acid, inflammation, NAFLD, lipid metabolism, network pharmacology

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1. Introduction

Nonalcoholic fatty liver disease (NAFLD) and its more severe form, nonalcoholic steatohepatitis (NASH), are increasingly prevalent chronic liver diseases.[1] These conditions are closely linked to a complex interplay of metabolic factors, including excessive energy consumption, oxidative stress, inflammation, and insulin resistance.[2,3]

Insulin resistance is characterized by decreased tissue responsiveness to circulating insulin.[4] It is closely associated with oxidative stress and low-grade inflammation, driven by macrophage infiltration into the adipose and hepatic tissue in individuals with obesity.[5] This infiltration leads to an increase in circulating inflammatory cytokines such as TNF-*α*, *IL-6*, IL-1β, INF-γ, and free fatty acids.[5-7] Moreover, hepatic recruitment of macrophages promotes NASH via CCR2.[8] Multiple studies provide the link between insulin resistance and metabolic disorders, such as type 2 diabetes, dyslipidemia, and hypertension,[9] highlighting its role in driving NAFLD progression through glucotoxicity and lipotoxicity.[10-12]

Excessive reactive oxygen species (ROS) can damage cellular components, activate inflammatory pathways, and impair mitochondrial function,[13] leading to the exacerbation of insulin resistance.[11,14] Additionally, studies have consistently shown that insulin resistance is associated with downregulated AMP-activated protein kinase (AMPK) and upregulated its downstream lipogenic genes, such as sterol regulatory element binding protein 1c (Srebp-1c) and fatty acid synthase (Fasn).[15,16] Moreover, chronic low-grade inflammation and oxidative stress can trigger endoplasmic reticulum (ER) stress,[17] further upregulating Srebp-1c, and contributing to hepatic lipid accumulation.[18-20] Therefore, attenuating oxidative stress, reducing inflammatory responses, and inhibiting *de novo* lipogenesis represent promising therapeutic strategies for mitigating NAFLD progression.

Madecassic acid (MA), a naturally occurring pentacyclic triterpenoid primarily isolated from *Centella asiatica*, is also present in several edible plants including gynura, basil, and daylily.[21] This compound exhibits a wide range of pharmacological activities, such as antibacterial,[22] anticancer,[23,24] antioxidant,[25] and anti-inflammatory properties.[26] Notably, Hsu et al. reported that MA effectively reduces ROS, improves glycemic control, and lowers triglyceride and cholesterol levels in a diabetic mouse model.[27] These findings align with a more recent study by Wang et al., which demonstrated that MA ameliorates oxidative stress and inflammation in streptozotocin-induced diabetic rats.[28] Given these findings, MA may offer therapeutic benefits for fatty liver disease. However, its specific molecular mechanism in improving lipid metabolism remains a limited study.

This study aimed to elucidate the underlying mechanisms of the potential therapeutic effects of MA on the NAFLD model using network pharmacology, a systems biology approach to drug discovery. Our approach involved identifying potential molecular targets of MA from SwissTarget Prediction and retrieving target genes related to NAFLD from the GeneCard database, and subsequently applying computational tools to identify key molecular interactions relevant to the treatment of NAFLD. By employing this method, we aimed to identify key molecules and pathways involved in the therapeutic action of MA, providing valuable insights to guide the design of targeted *in vitro* experiments. This integrative approach not only reveals complex molecular and pathway interactions but also establishes a strong foundation for subsequent experimental validation, thereby enhancing the translational potential of our findings.

2. Materials and Methods

2.1 Materials

Dulbecco's modified eagle medium (DMEM) was purchased from Gibco (Life Technologies, Eugene, OR, USA). Fetal

bovine serum (FBS) was obtained from HyClone Laboratories (South Logan, UT, USA). The nonessential amino acids and TRIzol® reagent were obtained from Invitrogen (Life Technologies, Eugene, OR, USA). MA was purchased from ChemFaces Biochemical Co., Ltd. (Wuhan, China). iScript Reverse Transcription Supermix and SsoFast EvaGreen SuperMix were obtained from Bio-Rad (Hercules, CA, USA). Palmitic acid (PA) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Network pharmacology

MA targets were obtained by uploading MA Canonical SMILES to the SwissTarget Prediction database (http://www.swisstargetprediction.ch). NAFLD-associated targets were retrieved from the GeneCards database (https://www.genecards.org) using the keyword "nonalcoholic fatty liver disease." The target sets for MA and NAFLD were then analyzed using VENNY 2.1 (https://bioinfogp.cnb.csic.es/tools/venny) to carry out union and intersection operations, and the results were visualized as a Venn diagram. A protein-protein interaction (PPI) network was constructed using the STRING database (version 11.0) (https://string-db.org) based on the *Homo sapiens* model, with an interaction confidence threshold of 0.7. The nodes of the network represented proteins (targets), and edges represented their interactions. Core target proteins were identified and ranked using the cytoHubba plugin (version 0.1) in Cytoscape based on the Degree topological algorithm. KEGG enrichment analysis was conducted using the ClueGO plugin (version 2.5.1) in Cytoscape.

2.3 Cell culture and treatments

The HepG2 cells (American Type Culture Collection, Rockville, MD, USA) were maintained in DMEM supplemented with 1 g/L glucose (5.5 mM glucose), L-glutamine, and supplemented with 10% FBS, and nonessential amino acids, HEPES 12.5 mmol/L. Murine macrophage (RAW 264.7) cells (ATCC, Rockville, MD, USA) were cultured at 37°C in DMEM. The culture medium of both cell lines was supplemented with 10% FBS, penicillin (100 U/mL), and gentamycin (50 µg/mL). The cells were incubated in a humidified 5% CO_2 atmosphere at 37°C and cultured in a completed medium until they reached 70% confluence. The medium was renewed every 2–3 days. The cells were used for assays after an overnight starvation serum-free media.

2.4 Cell viability assay

Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetra zolium bromide (MTT) assay. Briefly, the cells were inoculated into 96-well plates and treated with various MA concentrations ranging from 10 nM to 100 µM, and the duration was 24 h. Then, the tetrazolium compound, MTT, was added and incubated at 37°C for 4 h. Absorbance (optical density) was measured using a spectrophotometer at 540 nm (TECAN, Sunrise™, Switzerland). Viability was calculated as the percent normalized to the control.

2.5 Phorbol myristate acetate (PMA) - induced superoxide anion ($O_2^{\bullet-}$) formation

To assess the antioxidant effects of MA, a phorbol myristate acetate (PMA)-induced $O_2\bullet-$ generation was adapted from a study by Prawan et al.[29] Briefly, Raw 264.7 cells were pretreated with various MA concentrations for 3 h at 37°C before being exposed to 0.25 µM of PMA for 15 min. The $O_2^{\bullet}-$ levels in the control, PMA-stimulated, and MA-pretreated cells were then measured via lucigenin-enhanced chemiluminescence.

2.6 Palmitic acid (PA) - induced inflammation

To investigate the anti-inflammatory effects of MA, we utilized a PA-induced inflammation model in Raw 264.7 cells, as described by Korbecki et al.[30] The cells were incubated with or without various MA concentrations, followed by PA (250 µM) stimulation. The expression levels of proinflammatory genes were then analyzed using real-time quantitative reverse-transcription polymerase chain reaction (qRT-PCR).

2.7 Glucose utilization assay

To induce insulin resistance, HepG2 cells were exposed to high glucose (HG; 30 mM glucose) as previously described by Boonloh et al.[31] The effects of MA on glucose utilization in insulin-resistant HepG2 cells were then evaluated. Briefly, the cells were cultured in a high-glucose medium to mimic the hyperglycemic condition and incubated with or without various MA concentrations. Glucose depletion in the cultured medium was measured at various time points using a glucometer (ACCU-CHEK®, Roche Diagnostics, UT, USA).

2.8 Palmitic acid (PA) - induced lipid metabolism dysregulation

To investigate the effect of MA on lipid metabolism disorders, we employed a PA-induced lipid metabolism dysregulation model, as described by Xiao et al.[32] HepG2 cells were starved for 18 h and then cultured in medium containing PA (250 µM) with or without various MA concentrations for 24 h. Total RNA was extracted, and the expression levels of lipid homeostasis genes (*Srebp-1c*, *Fasn*, and *Cpt-1*) were measured using qRT-PCR.

2.9 Quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR)

Total RNA was isolated from cultured cells using TRIzol® reagent according to the manufacturer's protocols. cDNA synthesis was initiated from 2 µg of total RNA with iScript Reverse Transcription SuperMix®, employing a thermal cycling program of 5 min at 25°C, 30 min at 42°C, and a final step of 5 min at 85°C in a C1000 thermal cycler (Bio-Rad). qRT-PCR was performed using the LightCycler®480 Real-Time PCR instrument (Roche Applied Science). Each 15-µL reaction contained 4 µL of cDNA template, 0.5 µM of each primer, 7.5 µL of PCR SuperMix, and 2 µL of sterile water. The cycling conditions included an initial denaturation at 95°C for 3 min, followed by 40 cycles for the genes of interest, which consist of denaturation (95°C for 15 s), annealing (60°C for 30 s), and extension (72°C for 5 min). The primer sequences are presented in Tables 1 and 2. Gene expression levels were normalized to the housekeeping; *β-actin* or *Gapdh*, and relative fold changes were calculated using the standard curve method. The specificity of amplification was verified by melting curve analysis.

Table 1 Nucleotide sequences of primers used for PCR *(Homo sapiens).*

Genes	Forward primer	Reverse primer	PCR product
β-actin	5′-TGGAACGGTGAAGGTGACAG-3′	5′-AACAACGCATCTCATATTTGGAA-3	125 bp
Srebp-1c	5′-GCCATGGATTGCACTTT-3′	5′-CAAGAGAGGAGCTCAATG-3′	181 bp
Fasn	5′-CATCCAGATAGGCCTCATAGAC-3′	5′- CTCCATGAAGTAGGAGTGGAAG-3′	391 bp
Cpt-1	5′-GATTTTGCTGTCGGTCTTGG-3′	5′-CTCTTGCTGCCTGAATGTGA-3′	193 bp

Table 2 Nucleotide sequences of primers used for PCR *(Mus musculus).*

Genes	Forward primer	Reverse primer	PCR product
Gapdh	5'- AGGTCGGTGTGAACGGATTTG-3'	5'- TGTAGACCATGTAGTTGAGGTCA-3'	123 bp
Il-6	5'- GACAAAGCCAGAGTCCTTCAGA-3'	5'- GTGACTCCAGCTTATCTCTTGGT-3'	75 bp
Mcp-1	5'- AGGTCCCTGTCATGCTTCTG -3'	5'- GCTGCTGGTGATCCTCTTGT -3'	167 bp
Tnf-α	5'- CCAGACCCTCACACTCAGATC-3'	5'- CACTTGGTGGTTTGCTACGAC-3'	79 bp

2.10 Statistical analysis

All data are expressed as means ± standard error of the mean (SEM). Statistical comparisons between normally distributed mean values were performed using a one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. A *P*-value < 0.05 was deemed statistically significant.

3. Results

3.1 Network pharmacology of MA on NAFLD

A total of 100 molecular targets of MA were predicted using the SwissTargetPrediction database, whereas 8,031 targets associated with NAFLD were retrieved from the GeneCards database. Through a Venn diagram analysis (Fig. 1A), 92 potential targets of MA against NAFLD were identified from the intersection of these datasets. To gain further insights into the interactions between these targets, a PPI network of all intersected targets was constructed and visualized using the STRING database. The high-confidence interactions (full STRING network) are shown in Fig. 1B, illustrating a network of 92 nodes and 144 edges, where each edge represents an interaction between two targets. The average node degree, representing the average number of connections per protein, was 3.13, whereas the local clustering coefficient, indicating the cohesiveness of the nodes, was 0.48.

To identify the key targets within the cluster, the PPI network was further analyzed using Cytoscape. The targets were ranked based on their Degree parameter (Degree centrality), which corresponds to the number of interactions each node has. Higher Degree values indicate hub proteins, which are likely to play pivotal roles within the network. The top 10 targets with the highest Degree values are listed in Fig. 2A. KEGG enrichment analysis was then conducted to investigate the involvement of candidate targets in biological pathways. The analysis revealed that the candidate targets were involved in pathways related to inflammation (arachidonic acid metabolism and IL-17 signaling pathway) and lipid/glucose metabolism (PPAR signaling pathway and insulin resistance) (Fig. 2B).

Overall, this analysis provided insights into how MA may influence the progression of NAFLD by targeting specific proteins and pathways involved in metabolic and inflammatory processes. The identification of key hub targets and pathways indicates the biological relevance of MA and its potential therapeutic application for NAFLD. These findings guided the design of *in vitro* experiments, focusing on two major aspects: 1.) evaluation of the impact of MA on inflammation, which included measurement of superoxide anion levels and proinflammatory gene expression, and 2.) assessment of glucose and lipid metabolism, which involved glucose utilization and lipogenic gene expression.

3.2 Effect of MA on cell viability

The effect of MA on cell viability was performed to determine a concentration range that did not cause overt cytotoxicity. Concentrations of MA of ≤ 50 μM in both cells resulted in cell viability greater than 90% and were subsequently employed in further experiments (Fig. 3).

3.3 MA reduced the $O_2^{\bullet-}$ level in PMA-challenged-Raw 264.7 model

PMA significantly stimulated $O_2^{\bullet-}$ production compared with the control group. However, with a pronounced initial effect, MA exhibited a dose-dependent reduction in $O_2^{\bullet-}$ levels (Fig. 4). Notably, MA possesses antioxidant properties, as shown by PMA with concentrations higher than 10 μM significantly decreased $O_2^{\bullet-}$ levels when compared with the PMA-treated group.

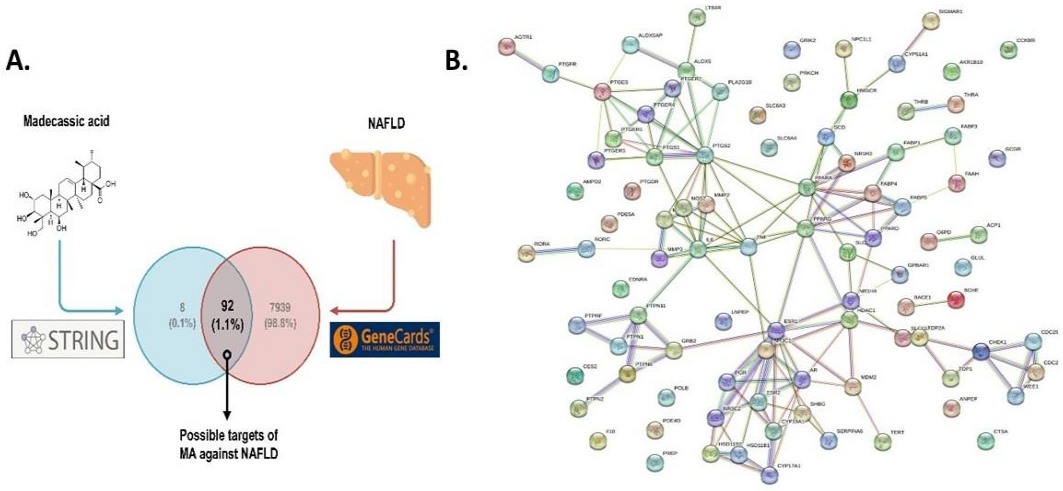

Fig. 1. Venn diagram showing the intersection of MA and NAFLD targets (A) and PPI network of the intersected 92 potential MA targets against NAFLD (B).

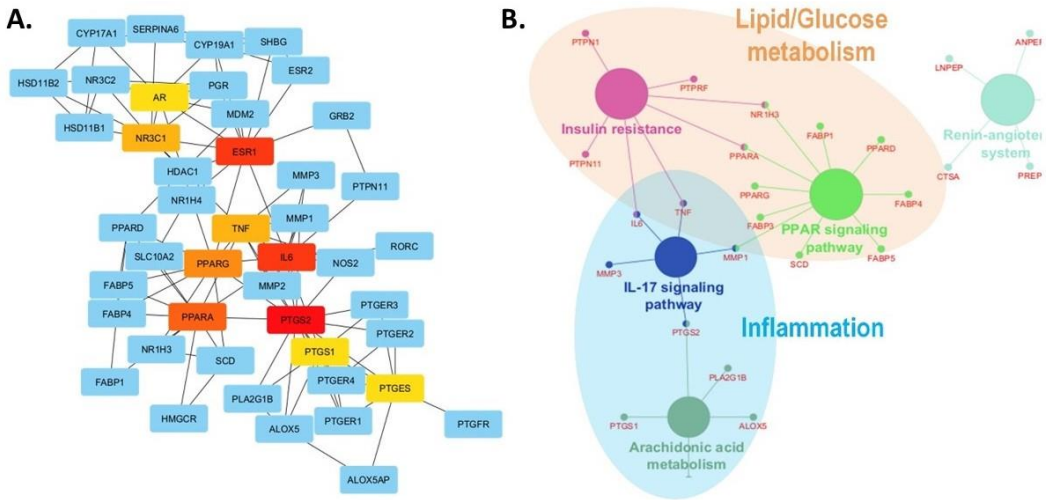

Fig. 2. The top 10 targets of MA against NAFLD ranked from red to yellow (A) and KEGG enrichment analysis (B).

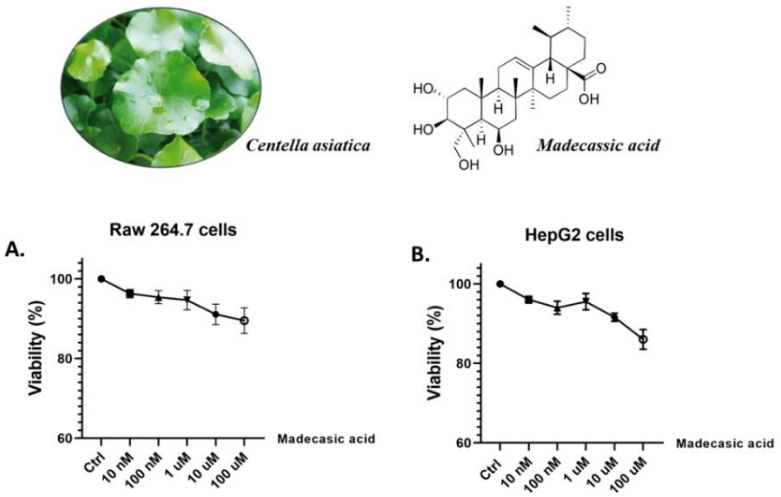

Fig. 3. Effect of MA on cell viability; Raw 264.7 cells (A) and HepG2 cells (B) were pretreated with different MA concentrations (varying from 10 nM to 100 μM) for 24 h.

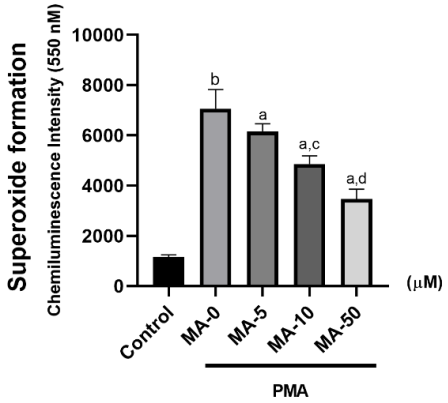

Fig.4. PMA-induced $O_2^{\bullet-}$ formation in murine macrophage cells. The data are expressed as means ± SEM; $^{a}P < 0.05$, $^{b}P < 0.01$, compared with the Con group; $^{c}P < 0.05$, $^{d}P < 0.01$ compared with the PMA-control group.

3.4 MA inhibited palmitic acid-induced pro-inflammatory gene expression

Low-grade chronic inflammation is associated with insulin resistance in metabolic syndrome. To explore the anti-inflammatory effects of MA, RAW 264.7 cells were stimulated with PA. PA-induced cells exhibited increased mRNA levels of *Mcp-1*, *Il-6*, and *Tnf-α*. The MA treatment significantly decreased the expression of these proinflammatory genes in a dose-dependent manner (Fig. 5A–C), suggesting that MA attenuates the cellular injuries and inflammatory status caused by PA.

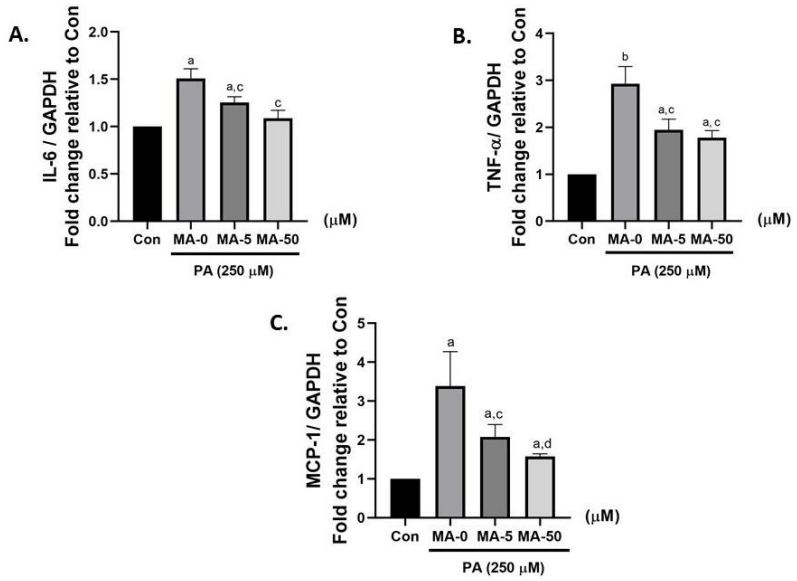

Fig. 5. Effects of MA on the expression levels of inflammatory genes *Mcp-1*(A), *Tnf-α* (B), *Il-6* (C) in PA-induced Raw 264.7 cells. The data are expressed as means ± SEM; $^{a}P < 0.05$, $^{b}P < 0.01$, compared with the Con group; $^{c}P < 0.05$, $^{d}P < 0.01$ compared with the PMA-control group.

3.5 MA enhanced glucose utilization in insulin resistant HepG2 cells

Insulin resistance is often characterized by impaired glucose uptake. When cultured in a low-glucose medium (5.5-mM glucose), HepG2 cells exhibited a progressive increase in glucose consumption over time. After a 32-h incubation, the glucose levels in the medium decreased to below the detection limit (<10 mg/dL; N/A). Contrarily, the HepG2 cells in a high-glucose condition (30-mM glucose) exhibited a significantly slower rate of glucose disappearance, particularly during the 24–32-h interval, indicating impaired glucose utilization (Table 3). Treatment with MA or metformin improved glucose utilization in HepG2 cells, particularly at higher MA concentrations (Table 3).

Table 3 The effect of MA on glucose utilization of HepG2 cells in high glucose-medium.

Time (h)	0	18	24	32
Control (normal)	95.2 ± 0.6	72 ± 1.2	59.75 ± 1.5	N/A
HG control	446.2* ± 1.5 [a]	426.3[†,‡] ± 2.9 [b,a]	416.4 ± 1.5 [c,a]	414.5 ± 1.2 [c,a]
HG-MA 5	446.2 ± 1.5 [a]	418.3[‡] ± 2.3 [b,b]	401.3 ± 2.1 [c,b]	395.8 ± 1.9 [c,b]
HG-MA10	446.2 ± 1.5 [a]	402.3 ± 1.3 [b,c]	392.3 ± 0.9 [c,c]	378.3 ± 1.8 [c,c]
HG-MA50	446.2 ± 1.5 [a]	404.3 ± 2.4 [b,c]	389.0 ± 1.6 [c,c]	369.5 ± 2.3 [d,d]
HG-Met	446.2 ± 1.5 [a]	383.3 ± 1.8 [b,d]	367.8 ± 1.3 [c,d]	342.0 ± 2.4 [d,e]

*Data is expressed as mean ± SEM, from 3 determinations. [†]Comparisons for the period of incubation times within the same treatment. [‡]Comparisons for the treatments within the same period of incubation time. Values with the same letter are not significantly different, *P < 0.05.*

3.6 MA prevented PA-induced derangement in lipogenic gene expression

This study determined whether MA could attenuate these deranged expressions. HepG2 cells were treated with PA and various MA concentrations for 24 h. The results indicated that cells cultured challenged with PA (250 μM) exhibited significantly increased *Srebp-1c* and *Fasn* expressions compared with those in the normal culture (Con group).

The MA treatment effectively restored the levels of lipogenic gene expression, significantly suppressing *Srebp-1c* and *Fasn* overexpression and preventing the downregulation of *Cpt-1* in PA-exposed HepG2 cells (Fig. 6A–C). These findings are consistent with those from the study by Hsu et al., which reported the efficacy of madecassic and rotundic acids in ameliorating hyperglycemia and hypertriglyceridemia.[27]

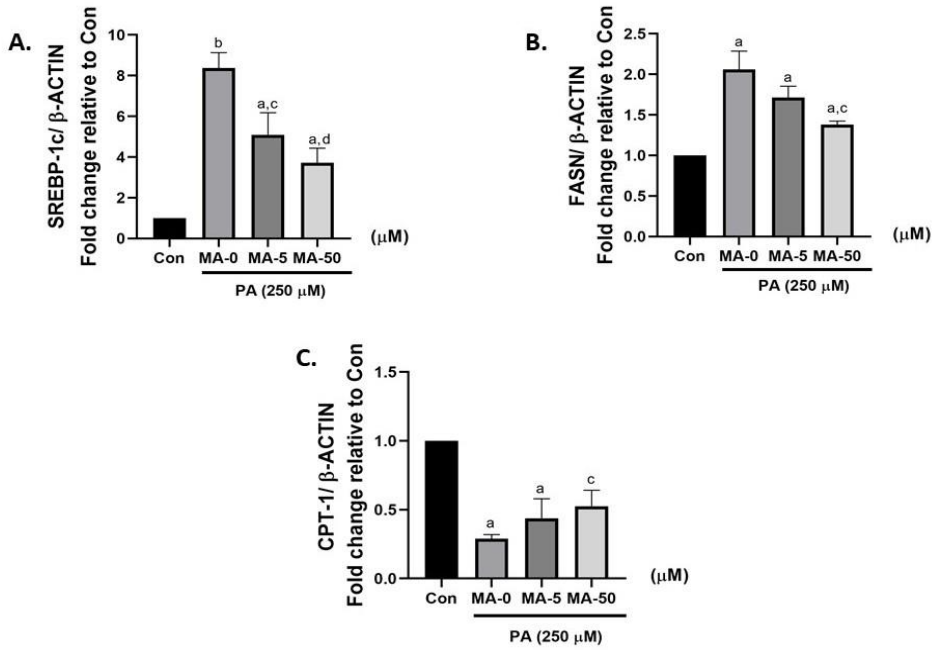

Fig. 6. MA inhibited PA-induced derangement in the expression of lipogenic genes. The relative mRNA expression levels of Srebp-1c (A), Fasn (B), and Cpt-1 (C) were determined via qRT-PCR. The data are expressed as means ± SEM; [a] $P < 0.05$, [b] $P < 0.01$, compared with the Con group; [c] $P < 0.05$, [d] $P < 0.01$ compared with the PA-control group.

4. Discussion

NAFLD or hepatic steatosis and its more severe form, NASH, is becoming a major public health concern worldwide, mainly due to the increasing prevalence of obesity and metabolic disorders.[1,33] Steatosis is characterized by abnormal lipid accumulation in the liver. These conditions are often associated with a complex interplay of oxidative stress, inflammation, insulin resistance, and lipid metabolism dysregulations.[2,3] Despite the effectiveness of conventional treatments, including lifestyle modifications and pharmacological interventions, their limitations have attracted a growing interest in alternative therapies. Natural compounds are increasingly recognized for their potential to provide safer and more sustainable therapeutic options.

MA, a natural triterpenoid compound obtained from *Centella asiatica*, has shown promise owing to its hepatoprotective properties,[34] making it an appealing candidate

for addressing gaps in current NAFLD interventions. This study aimed to elucidate the mechanisms by which MA exerts its therapeutic effects on NAFLD, leveraging network pharmacology. To elucidate the mechanism by which MA attenuates metabolic derangements and hepatic lipid accumulation, we utilized various models of hepatic injuries, particularly PA-treated HepG2 and Raw 264.7 cells. PA has been reported to induce cellular stress, increase intrahepatic lipid accumulation,[35,36] and promote the generation of free radicals and inflammatory responses,[30,37,38] eventually leading to cell injury, ER stress, and cell death.[39]

The network pharmacology analysis revealed that MA may be involved in hepatic steatosis and NASH through inflammatory modulation and lipid/glucose metabolism. The interplay between these processes is complex, as increased lipid metabolism (lipogenesis) can lead to glucose intolerance,[12] and both factors subsequently exacerbate inflammation, thereby creating a self-perpetuating cycle.

In our *in vitro* model, we proposed that increased cellular metabolism of both glucose and lipids results in excessive ROS production, which further triggers inflammation. To evaluate the antioxidant effects of MA, we employed PMA, a potent activator of PKC, to induce the production of $O_2^{\bullet-}$, a ROS associated with numerous diseases, including neurodegenerative disorders, cardiovascular diseases, and cancers.[14,40,41] Since elevation of $O_2^{\bullet-}$ and ROS induced by NOX4, or PA has been shown to impair insulin sensitivity by inactivating AKT. Suppression of ROS rescues insulin sensitivity.[42] In the present study, PMA was used to activate NOXs leading to the formation of ROS. Results showed that MA has antioxidant activity in suppressing ROS formation, which may reduce insulin resistance.

Furthermore, inflammation can worsen glucose intolerance, thereby continuing the cycle of lipogenesis. To investigate this stress condition, superoxide anion, a byproduct of high cellular metabolism, was generated in the Raw 264.7 cell model to mimic the ROS produced during elevated glucose and lipid metabolism. Our results demonstrated that MA could dose-dependently decrease the expression of pro-inflammatory genes such as *Il-6, Tnf-α,* (identified as hub genes in the network analysis) as well *as Mcp-1*. These findings suggest that MA alleviates inflammation by reducing ROS levels, thereby suppressing the inflammatory response, a fundamental cause of insulin resistance, ER stress, and hepatic lipid accumulation.

We extended our investigation further to evaluate the effect of MA on both glucose and lipid metabolism. To this end, HepG2 cells were treated with high glucose to model glucose metabolism and with PA to study lipid metabolism. Exposure to PA can serve as a model for lipotoxicity.[43] Typically, lipotoxicity-induced hepatocyte injury is mediated by oxidative stress, mitochondrial dysfunction, c-Jun N-terminal kinase activation, and the release of proinflammatory cytokines,[44] such as TNF-α, IL-6, and IL-1 β; subsequent activation of TLR4 perpetuates a cycle of inflammation.[3] PA-induced lipid accumulation is a complex process that involves multiple mechanisms. PA inhibits fatty acid oxidation, resulting in the buildup of lipids.[45] Furthermore, PA can stimulate lipogenesis and the production of new fatty acids, thereby contributing to lipid accumulation.[43] In addition, it impairs insulin signaling, leading to reduced glucose uptake and increased lipid synthesis, further exacerbating hepatic lipid accumulation.[43] Consistent with other studies, we observed that PA-exposed HepG2 cells increased the expressions of *Srebp-1c* and *Fasn*, which are key players in lipogenesis. MA treatment markedly reduced lipogenesis, as indicated by the downregulation of lipogenic gene expression, whereas it preserved the expression of the fatty acid oxidation gene. Moreover, our results indicated that MA significantly improved

glucose intolerance, as evidenced by enhanced glucose utilization.

These findings highlight the dual role of MA in mitigating metabolic dysfunction by enhancing glucose utilization and suppressing lipid accumulation. Overall, our results suggest that MA, by reducing ROS levels, may attenuate inflammation and improve both glucose and lipid metabolism, highlighting its promise as a therapeutic option for NAFLD management. However, studies in HepG2 culture cells may inherit many limitations due to the nature of the cells in culture, further investigation to validate the findings in other models including *in vivo* is required.

5. Conclusion

Collectively, our findings suggest that MA has the potential to alleviate insulin resistance, inflammation, and normalized lipid metabolism, the important of metabolic changes in NAFLD. Thus, MA is a promising candidate for development as a dietary supplement or nutraceutical to mitigate the severity of NAFLD, one of the metabolic diseases.

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Conflicts of Interest

The authors declare there is no potential conflict of interest relevant to this article.

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