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Antilipogenic Effect of *Cirsium japonicum* var. *maackii* According to Harvest Time and Plant Part in High-Fat Diet-Induced Nonalcoholic Fatty Liver Disease

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ABSTRACT

The global rise in obesity has been closely linked to an increasing incidence of nonalcoholic fatty liver disease (NAFLD), a major metabolic disorder. In this study, we evaluated the lipid-lowering and hepatoprotective effects of *Cirsium japonicum* var. maackii (CJ) harvested at different times, spring (CJ-sp), fall (CJ-fall), and from pappus (CJ-pap), a tuft of bristles in CJ, in a high-fat diet (HFD) mouse model. Male C57BL/6 mice were assigned to either a normal control diet (NOR) or HFD, with treatment groups receiving CJ-sp, CJ-fall, CJ-pap (100 and 200 mg/kg), cirsimaritin (Cirsi, 25 mg/kg), or milk thistle (MT, 100 mg/kg). Administration of the CJ extract markedly reduced body weight gain, food efficiency ratio (FER), and serum lipid parameters compared with the HFD control (CTL). Among the extracts, CJ-sp and CJ-pap most effectively alleviated hepatic steatosis, as evidenced by decreased fat deposition and reduced inflammatory changes in liver tissue. In addition, CJ-treated mice displayed significantly lower white adipose tissue mass and smaller adipocyte size than CTL mice. These improvements were associated with the downregulation of hepatic lipogenic genes and activation of fatty acid oxidation pathways. Collectively, CJ supplementation improved metabolic homeostasis and protected against HFD-induced obesity and liver dysfunction. Notably, CJ-sp and CJ-pap exerted greater efficacy than other CJ fractions or MT, suggesting their potential application as functional food ingredients for the prevention of NAFLD.

1 | Introduction

Nonalcoholic fatty liver disease (NAFLD) has emerged as a major metabolic disorder closely associated with obesity and lifestyle-related conditions, posing a substantial public health burden due to its rising global incidence and severe outcomes [1]. The disease represents a heterogenous spectrum that progresses from simple lipid accumulation within hepatocytes to more advanced pathological states, including nonalcoholic steatohepatitis (NASH), fibrosis, and ultimately cirrhosis [2]. At the cellular level, excessive hepatic triglyceride (TG) deposition occurs when fatty acid uptake and synthesis outweigh oxidation and export processes, particularly via very-low-density lipoprotein (VLDL) [3, 4]. Thus, therapeutic approaches aimed at restoring lipid

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balance, by suppressing hepatic steatosis and normalizing metabolic flux, are essential to slow NAFLD progression [4, 5].

Despite extensive investigation, no pharmacological agent has yet received official regulatory approval for NAFLD therapy. Existing strategies under development generally focus on antioxidants, insulin sensitizers, and lipid-lowering compounds. For instance, vitamin E has demonstrated benefits in nondiabetic NASH patients in recent clinical trials [6], whereas statins have been linked with reduced hepatic morbidity and mortality in NAFLD cohorts [7]. Moreover, sodium-glucose cotransporter 2 (SGLT2) inhibitors such as dapagliflozin and canagliflozin improve hepatic metabolism and reduce fat accumulation [8]. Nevertheless, these options remain experimental or limited to specific patient subgroups. In contrast, traditional herbal medicines, which are naturally derived, multitargeted, and widely accessible, have gained attention as promising complementary approaches for managing NAFLD [9].

Cirsium japonicum var. maackii (CJ) is a perennial herb widely used in East Asian traditional medicine for inflammatory, metabolic, and neoplastic disorders, including diabetes and chronic inflammation [10–13]. Phytochemical profiling has identified several flavonoids in CJ, among which cirsimaritin (Cirsi) exhibits potent anti-inflammatory and lipid-modulating effects [14]. Recent evidence indicates that CJ extracts and Cirsi mitigate metabolic dysfunctions and fatty liver changes induced by high-fat diets (HFDs) [15]. However, the molecular mechanism underlying these hepatoprotective actions remains incompletely understood, and the influence of harvest season or plant part on CJ's efficacy has rarely been explored.

Therefore, this study compared the protective effects of CJ leaves harvested in spring (CJ-sp) and fall (CJ-fall), as well as pappus (CJ-pap), in a HFD-induced NAFLD mouse model. Treatment with CJ-sp, CJ-fall, CJ-pap, or Cirsi significantly improved metabolic indices, including body weight, liver mass, and serum lipid profiles. Histological and molecular assessments demonstrated marked suppression of hepatic steatosis and downregulation of lipogenic regulators such as acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), peroxisome proliferator-activated receptor gamma (PPARγ), and sterol regulatory element-binding protein 1c (SREBP1c). Notably, CJ-sp and CJ-pap exerted superior activity compared with other extracts, indicating that seasonal timing and tissue specificity are key determinants of CJ's therapeutic potential. These results provide novel insight into the optimization of CJ-based interventions for preventing and managing obesity-related fatty liver disease.

2 | Materials and Methods

2.1 | Preparation of CJ Extracts

Dried samples of CJ, harvested in spring (CJ-sp), fall (CJ-fall), and from pappus (CJ-pap), were obtained from a certified medicinal herb supplier (Imsil Herbal Medicine, Korea). The plant material was collected from the farm of Imsil Herbal Medicine (35.527976° N, 127.337058° E), Imsil, Republic of Korea. Each sample was ground and extracted with 70% ethanol (1 L, 1:10, w/v) using a Gyeongseo Extractor Cosmos-600 (Inchon, Korea) for 3 h. The filtrates were concentrated under reduced pressure, lyophilized, and stored at −20°C until use. The extraction yield of the CJ extracts was 22.6% for CJ-sp, 19.3% for CJ-fall, and 6.1% for CJ-pap. For phytochemical profiling, authentic standards, cirsimarin (Cat. no. N048-04), hispidulin (N051-07), and cirsimaritin (N049-01) (Natural Product Institute of Science and Technology, Korea) were used for high-performance liquid chromatography (HPLC) calibration.

2.2 | HPLC Analysis Conditions

HPLC analysis was performed to identify the contents of cirsimarin, hispidulin, and cirsimaritin in CJ. HPLC analysis was conducted using a YMC Pack-Pro C18 column (250 × 4.6 mm, 5 μm). The standard compounds were prepared by dissolving 1 mg of each standard compound (cirsimarin, hispidulin, and cirsimaritin) in 2 mL of HPLC-grade methanol to generate a concentration of 500 ppm. For the samples (CJ-sp, CJ-fall, and CJ-pap), 30 mg of each was dissolved in 1 mL of the same solvent to generate a concentration of 30,000 ppm. The solutions were dissolved in an ultrasonic shaker for 15 min or until properly homogenized. The solutions were then filtered using a 0.45 μm polytetrafluoroethylene (PTFE) filter. To generate a calibration curve, the standard solutions were diluted into 6 concentrations (31.25–500 ppm). Chromatograms of each sample were collected under the same conditions (Table 1). The HPLC method was validated by determining the limits of detection (LOD) and quantification (LOQ) which were calculated based on signal-to-noise ratios of 3 and 10, respectively.

2.3 | Animals and Experimental Protocol

Male C57BL/6 mice (7 weeks) were maintained under controlled conditions (25 ± 2°C; 50%–55% humidity; 12-h light/dark). After one week of acclimatization, animals were randomly assigned to 10 groups ($n = 10$ per group): a normal diet group (NOR, D12450J, Research Diets, USA; 10% kcal from fat), a HFD control group (CTL, D12492, Research Diets, 60% kcal from fat), and HFD-fed groups treated with CJ-sp, CJ-fall, CJ-pap (100 or 200 mg/kg body weight), cirsimaritin (cirsi, 25 mg/kg), or milk thistle (MT, 100 mg/kg). The detailed composition of the experimental diets (% kcal and g/kg) is provided in Supporting Table S1. All samples were suspended in 0.5% carboxymethyl cellulose (CMC) and administered orally for 12 weeks. Body weight and feed intake were recorded weekly. The study complied with the National Institutes of Health (Korea) guidelines and was approved by the Daejeon University Institutional Animal Care and Use Committee (IACUC) (DJUARB2018-029). At the end of treatment, animals were fasted overnight and anesthetized with 2%–2.5% isoflurane. After euthanasia, blood and tissues (liver and white adipose tissue [WAT] depots) were collected, weighed, frozen in liquid nitrogen, and stored at −80°C.

2.4 | Quantification of Serum Metabolic Parameters and Hepatic TGs

Serum alanine aminotransferase (ALT), aspartate aminotransaminase (AST), total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), TG, free fatty acids (FFA), glycerides, and lactate dehydrogenase (LDH) were analyzed using an automated clinical chemistry analyzer (Hitachi-720, Japan). Hepatic lipids were extracted following Avtanski et al. [10] and dissolved in isopropanol for TG

TABLE 1 | HPLC conditions.

Item	Condition
Equipment	Waters Alliance e2695 Separations Module, USA Waters 2489 UV/Vis Detector, USA
Column	YMC Pack-Pro C18 column (250 × 4.6 mm × 5 μm)
Column oven temperature	30°C
Injection volume	10 μL
Mobile phase	Gradient [0.5% Acetic acid in water (A) and ACN (B)
Flow rate	1.00 mL/min
Detector wavelength	UV 278 nm

quantification (Roche Accutrend Plus, Germany). In some experiments, serum tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were determined by the indicated enzyme-linked immunosorbent assay (ELISA) kits.

2.5 | RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA was isolated (RNAsol B reagent, Tel-Test, USA), and 1 μg was reverse-transcribed (DNA synthesis kit, Thermo Scientific, USA). Amplification was performed on an Applied Biosystems 7500 Real-Time PCR system (Applied Biosystems, USA) under the following conditions: an initial denaturation at 94°C for 10 min, followed by 40 cycles of 95°C for 15 s and 45°C for 60 s. Primer efficiency was experimentally determined for all primer sets, and melt curve analysis was performed to confirm amplification specificity. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal control for the comparative Ct method. Expression levels of SREBP1c, FAS, ACC, fatty acid-binding protein 4 (FABP4, also known as aP2), CCAAT/enhancer-binding protein beta (C/EBPβ), PPARγ, peroxisome proliferator-activated receptor alpha (PPARα), carnitine palmitoyltransferase 1A (CPT1A), and uncoupling protein 2 (UCP2) were calculated by the $2^{-\Delta\Delta Ct}$ method. The primer sequences, efficiency values, and melt curve results are presented in Table 2.

2.6 | Histological Analysis

For histopathological analysis, liver sections fixed in 10% neutral buffered formalin were embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (H&E). The stained sections were observed under a Leica DM IL LED microscope (Leica, Wetzlar, Germany). Liver histology was evaluated using the NAFLD Activity Score (NAS), which consists of hepatic steatosis, lobular inflammation, and hepatocyte ballooning. Steatosis was scored from 0 to 4 based on the percentage of lipid-laden hepatocytes (Grade 0: no steatosis, grade 1: < 25%, Grade 2: 26%–50%, Grade 3: 51%–75%, and Grade 4: 76%–100%). The degree of lobular inflammation was histologically quantified by counting inflammatory foci in 20 consecutive high-power fields (×40 objective) (Grade 0: no foci, Grade 1: < 2 foci, and Grade 2: ≥ 2 foci). Hepatocyte ballooning was evaluated on a scale of 0–2 (Grade 0: none, Grade 1: few ballooned cells, and Grade 2: numerous ballooned cells/marked ballooning).

TABLE 2 | qRT-PCR primer sequences.

Gene	Primer	Sequence (5′ ⟶ 3′)	Efficiency (%)	Melt curve (Tm, °C)
SREBP1c	Forward Reverse	AGCCTGGCCATCTGTGAGAA CAGACTGGTACGGGCCACAA	98.3	85 (single peak)
FAS	Forward Reverse	CTGAGATCCCAGCACTTCTTGA GCCTCCGAAGCCAAATGAG	107.6	85 (single peak)
ACC	Forward Reverse	GAATCTCCTGGTGACAATGCTTATT GGTCTTGCTGAGTTGGGTTAGCT	97.1	85 (single peak)
FABP4 (aP2)	Forward Reverse	GGATTTGGTCACCATCCGGT CCAGCTTGTCACCATCTCGT	110	81.5 (single peak)
C/EBPβ	Forward Reverse	AAGCTGAGCGACGAGTACAAGA GTCAGCTCCAGCACCTTGTG	101	80.5 (single peak)
PPARγ	Forward Reverse	AGAGGGCTGAGCGTAGGTAA AAAGGAGGGGCCTAGAGGAG	91.7	84 (single peak)
PPARα	Forward Reverse	GCCTGTCTGTCGGGATGT GGCTTCGTGGATTCTCTTG	110	79.5 (single peak)
CPT1A	Forward Reverse	CTTCCAAGGCAGAAGAGTGGG GAACCTTGGCTGCGGTAAGAC	96.8	75 (single peak)
UCP2	Forward Reverse	TTCAAATGAGATTGTGGGAAAAT ACCGATACAGTACAGTACAGTA	93.3	79 (single peak)

2.7 | Statistical Analysis

Statistical analysis was performed using GraphPad Prism 5 software (GraphPad Software, San Diego, CA, USA). Data are presented as the mean ± standard error of the mean (SEM) of triplicate experiments. Statistical significance was determined using one-way ANOVA and Tukey's post hoc test, and p values of less than 0.05 were considered statistically significant.

3 | Results

3.1 | Seasonal and Anatomical Variation in Flavonoid Composition of CJ

Numerous studies have reported that the levels of bioactive compounds in medicinal plants fluctuate depending on the harvest season and plant maturity [16–18]. To assess these differences in CJ, the contents of cirsimarin, hispidulin, and cirsimaritin, the key flavonoids, were quantified using HPLC analysis (Figure 1(a)). Distinct retention times were observed for cirsimarin (26.7 min), hispidulin (32.0 min), and cirsimaritin (34.7 min) under the optimized HPLC conditions (Table 1). All three markers were detected in ethanol extracts from spring leaves (CJ-sp), fall leaves (CJ-fall), and pappus (CJ-pap) at corresponding peaks (Figure 1(c)). Calibration curves for each compound displayed high linearity ($R^2 \geq 0.997$), confirming analytical reliability. Quantitative analysis revealed that CJ-sp contained the greatest amount of cirsimaritin (3.99 ± 0.03 mg/g), followed by CJ-fall (3.80 ± 0.03 mg/g), while CJ-pap exhibited a markedly lower concentration (0.23 ± 0.00 mg/g) (Figure 1(b)). Trace amounts of hispidulin were detected only in CJ-pap. These results suggest that the biosynthesis or accumulation of these flavonoids varies with plant maturity, showing marked restriction in the pappus compared with the leaves.

3.2 | CJ Extract Attenuates Body Weight Gain and Improves Serum Lipid Profiles in HFD-Fed Mice

Mice fed an HFD exhibited progressive body weight gain over a period of 12 weeks compared with the NOR group (Figure 2(a)). Treatment with CJ-sp, CJ-fall, or CJ-pap significantly reduced body size and final body weight relative to the HFD control (CTL) group (Figures 2(b), 2(c), and 2(d)). Although food intake remained comparable among groups, the food efficiency ratio (FER) was notably lower in the CJ-sp and MT group (Figures 2(e) and 2(f)). Other treatment groups also showed modest reductions in FER though not statistically significant. These findings suggest that CJ administration decreases fat accumulation efficiency rather than food consumption.

Furthermore, the HFD group showed marked elevation in serum TG and nonesterified fatty acids (NEFA), confirming lipid dysregulation. CJ supplementation reversed these increases, especially in CJ-sp (200 mg/kg) and CJ-pap (200 mg/kg) groups (Figures 2(g) and 2(h)). TC was also reduced, while the HDL/TC ratio increased in most CJ-treated groups (Figures 2(i) and 2(j)). Conversely, the LDL/TC ratio declined, with the greatest improvement observed in high-dose CJ-sp and CJ-pap groups (Figure 2(k)). Collectively, CJ extracts ameliorated HFD-induced dyslipidemia, with spring and pappus-derived extracts showing superior efficacy compared with fall-harvested samples.

3.3 | CJ Extract Protects Against Hepatic Lipid Accumulation and Injury

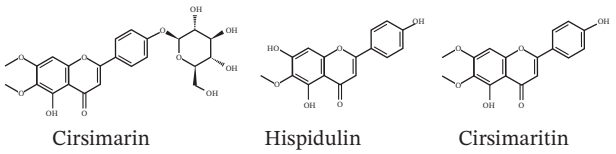
Liver weights were significantly elevated in the HFD group relative to the NOR group, whereas CJ treatment reversed this increase (Figures 3(a) and 3(b)). Hepatic TG levels mirrored this trend, decreasing substantially after CJ-sp (200 mg/kg), CJ-fall (200 mg/kg), CJ-pap (100 and 200 mg/kg), and MT administration (Figure 3(c)). Histological examination using H&E and Oil Red O staining revealed severe steatosis and lipid droplet accumulation in HFD livers. CJ-treated mice, particularly those receiving CJ-sp (200 mg/kg), CJ-fall (200 mg/kg), CJ-pap (100 and 200 mg/kg), and MT, exhibited a marked reduction in lipid deposition and improved hepatic architecture (Figures 3(d) and 3(e)). Consistent with these histological observations, quantitative assessment using the NAS demonstrated that administration of CJ extracts significantly reduced NAS scores, with the most pronounced effects observed in the CJ-sp (200 mg/kg), CJ-fall (200 mg/kg), CJ-pap (100 and 200 mg/kg) groups (Figure 3(f)). Serum biomarkers of hepatic damage and tissute damage (ALT, AST, and LDH) were also reduced after CJ-sp, CJ-fall, CJ-pap, and Cirsi administration (Figures 3(g), 3(h) and 3(i)). Moreover, proinflammatory cytokines TNF-α and IL-6, which were elevated in the HFD group, were significantly suppressed by CJ and Cirsi (Figures 3(j) and 3(k)). These data indicate that the CJ extract mitigates both steatosis and inflammation-induced hepatic injury.

3.4 | CJ Extract Reduces Adipose Tissue Hypertrophy in White Fat Depots

To further examine CJ antiobesity activity, multiple WAT depots were analyzed. HFD-fed mice showed a significant increase in overall fat mass compared with controls (Figure 4(a)). CJ treatment lowered the weights of subcutaneous (sWAT), retroperitoneal (rpWAT), and mesenteric (mWAT) adipose tissues across most groups, whereas epididymal WAT reduction was most evident in CJ-pap-treated mice (Figure 4(b)). Histological analysis demonstrated reduced adipocyte size and decreased frequency of hypertrophic cells in CJ-treated groups (Figure 4(c)). Quantitative assessment of adipocyte size revealed a significant increase in the proportion of smaller adipocytes (10–30 μm) in the CJ-treated groups, while no significant differences were detected in the 30–50 μm size range among all groups. The number of larger adipocytes (50–70 and 70–90 μm) tended to be elevated in HFD-fed groups compared with the NOR group. Notably, the frequency of very large adipocytes ($\geq$ 90 μm) was significantly reduced in the CJ-treated groups compared with the CTL group (Figure 4(d)). These results imply that CJ supplementation restrains adipocyte enlargement and adipose expansion in HFD-induced obesity.

3.5 | CJ Downregulates Hepatic Lipogenic Genes and Attenuates the mRNA Expression of Genes Associated With Lipogenesis and Enhances Fatty Acid Oxidation

To elucidate the role of CJ in hepatic lipid metabolism, hepatic mRNA expression of lipid metabolism-related genes was examined by qRT-PCR. The HFD group showed robust upregulation of lipogenic regulators, SREBP1c, FAS, ACC, FABP4, C/EBPβ, and PPARγ, relative to normal controls. Administration



Sample	Content (mg/g DW)		
	Cirsimarin	Hispidulin	Cirsimaritin
CJ_sp	6.14 ± 0.05	trace	3.99 ± 0.03
CJ_fall	6.39 ± 0.05	trace	3.80 ± 0.03
CJ_pap	0.72 ± 0.01	0.55 ± 0.00	0.23 ± 0.00

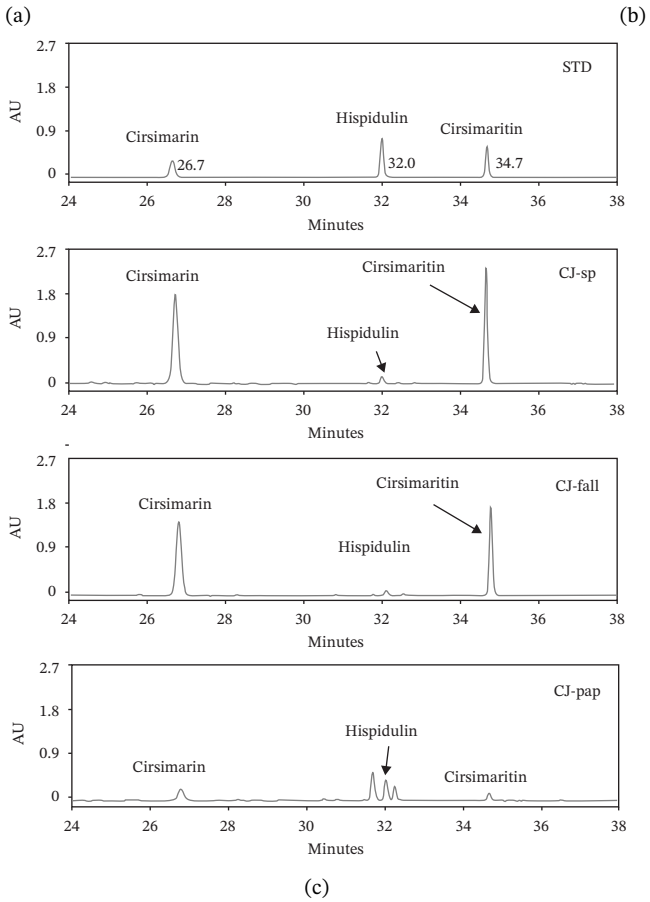


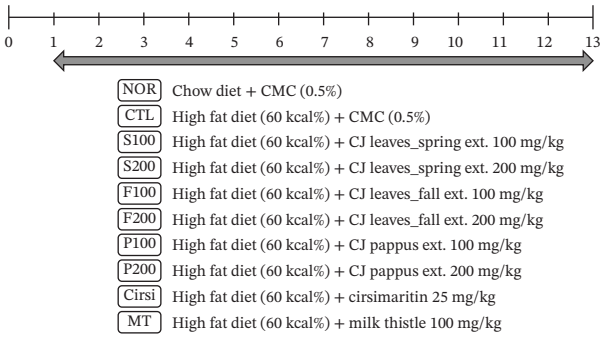
FIGURE 1 | HPLC analysis of *C. japonicum* ethanol extract and quantification of major flavonoids. (a) Chemical structures of the representative flavonoids, including cirsimarin, hispidulin, and cirsimaritin, identified in *C. japonicum*. (b) Quantitative analysis of cirsimarin, hispidulin, and cirsimaritin in *C. japonicum* extracts, comparing different harvest times (spring versus autumn) and plant parts (leaf versus pappus). (c) Representative high-performance liquid chromatography (HPLC) chromatograms of the mixed flavonoid standards and the ethanol extract of *C. japonicum*. Peaks corresponding to each compound were identified based on the retention times of the standards.

of CJ extracts or Cirsi markedly suppressed these transcripts to near-normal levels (Figures 5(a), 5(b), 5(c), 5(d), 5(e), and 5(f)). In contrast, the expression of genes promoting fatty acid oxidation (PPARα and UCP2) and CPT1A increased following CJ-sp (200 mg/kg), CJ-pap (200 mg/kg), and Cirsi treatment (Figures 5(g), 5(h), and 5(i)). Collectively, these findings demonstrate that CJ modulates hepatic lipid homeostasis by inhibiting lipogenesis and activating oxidative pathways, with the most potent effects observed in CJ-sp and CJ-pap groups.

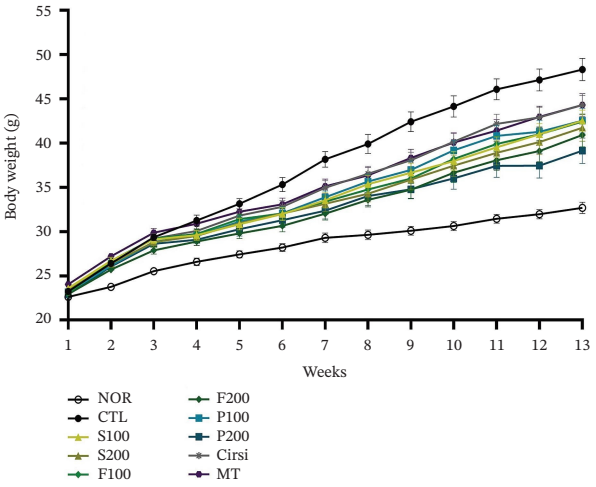
4 | Discussion

NAFLD arises from complex metabolic and environmental interactions that disrupt hepatic lipid regulation [11]. Approximately 20 percent of the cases progress to NASH and fibrosis or cirrhosis when uncontrolled [15]. Although lifestyle modifications such as caloric restriction and exercise remain the cornerstone of therapy, pharmacological interventions are being explored to achieve faster improvements in hepatic function [19, 20]. Farnesoid X receptor (FXR) agonists (e.g., obeticholic acid) and TNF-α antagonists (e.g., pentoxifylline) have shown some benefits in trials but lack long-term validation [21, 22]. Hence, the search for safe and effective agents remains a pressing priority.

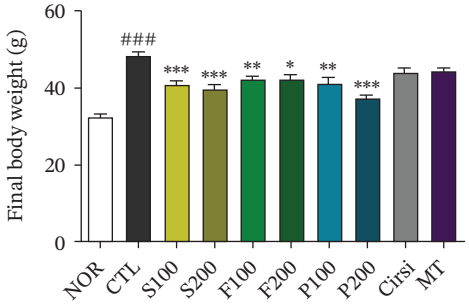
Natural herbal remedies are increasingly recognized for their multitarget actions and favorable safety profiles in chronic liver disorders [23–26]. Unlike single-compound drugs, whole extracts can modulate several pathways simultaneously with low toxicity risks [27]. Given that inflammation drives both hepatic steatosis and fibrosis in NAFLD [28, 29], we investigated the antisteatotic potential of CJ using an HFD model. Prior reports suggested that CJ attenuates metabolic dysfunction. However, the impact of harvest season and anatomical plant part remains poorly understood. Our study demonstrated distinct differences in



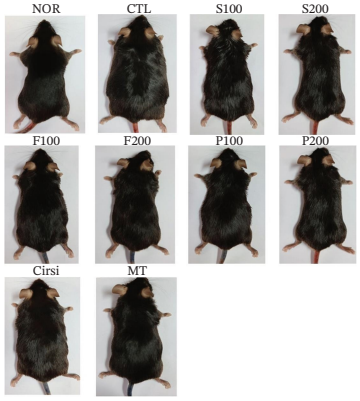
(a)



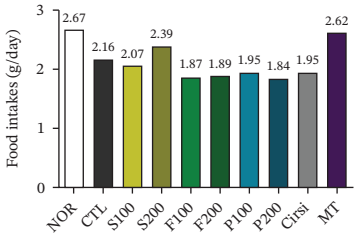
(b)



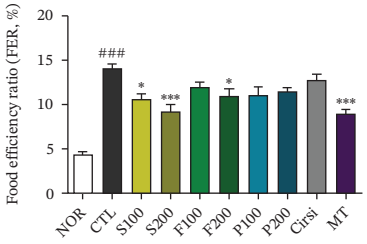
(c)



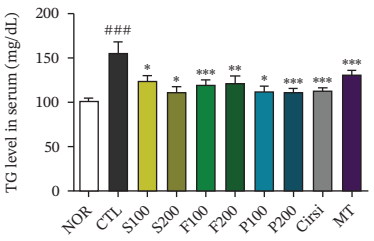
(d)



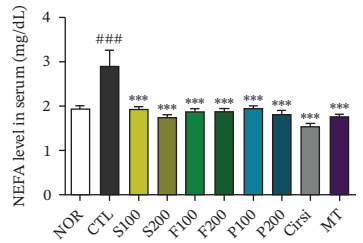
(e)



(f)



(g)



(h)

FIGURE 2 | (Continued)

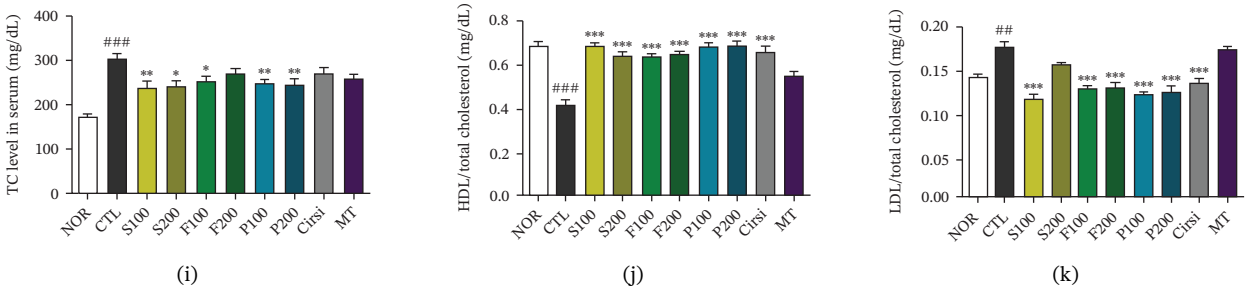


FIGURE 2 | Antiobesity effects of *C. japonicum* extract harvested at different times and from distinct plant parts in high-fat diet (HFD)-induced mice. (a) Schematic diagram of the experimental design. Male C57BL/6 mice were subjected to an HFD for 13 weeks to induce obesity, followed by oral administration of various *C. japonicum* (CJ) extracts (100 mg or 200 mg/kg/day). Extracts were derived from spring (CJ-sp), autumn (CJ-fall), and the pappus (CJ-pap). (b) Body weight gain was monitored weekly throughout the treatment period. Data are presented as mean ± standard error of the mean (SEM) ($n = 10$). (c) Final body weight measurements at the end of the treatment period. Bars represent mean ± SEM ($n = 10$). (d) Representative images of mice from each experimental group at the end of the treatment period demonstrate a visible difference in body size and adiposity. (e–k) Measurement of obesity-related phenotypic parameters and metabolic organ indices: (e) food intake; (f) food efficiency ratio (FER); (g) serum triglycerides (TG); (h) serum nonesterified fatty acids (NEFA); (i) Serum total cholesterol (TC); (j) Serum high-density lipoprotein cholesterol (HDL-c)/TC ratio; and (k) serum low-density lipoprotein cholesterol (LDL-c)/TC ratio. All data are presented as mean ± SEM ($n = 10$). Statistical comparisons were conducted using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ versus the CTL group, and $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ versus NOR after Student's *t*-test.

bioactivity between spring leaves and the pappus, highlighting the importance of phytochemical composition. Feeding an HFD (≈60% kcal from fat) for 12 weeks produced a reproducible NAFLD phenotype in mice [30–33]. This response establishes a suitable model for the early stages of the disease. The FER of mice receiving the CJ-sp and CJ-fall diets was lower than that of the HFD group (Figure 2(f)). This observation may be attributed to the interaction of nearly all amino acids with primary and secondary products of oxidized lipids, which reduces the digestive utilization of proteins, amino acids, and fats and may subsequently impact weight gain [34, 35].

Imbalances in lipid homeostasis, including the synthesis, degradation, and transport of associated lipoprotein particles, result in abnormal lipid levels. Despite being essential components of the body, elevated levels of TG and cholesterol are recognized risk factors for heart disease, stroke, dyslipidemia, and obesity [36, 37]. Furthermore, obesity chronically disrupts hepatic lipid metabolism, which may lead to various metabolic comorbidities [38]. Our results demonstrated that CJ administration lowered body weight gain and hepatic TG accumulation, consistent with suppressed lipogenesis and improved lipid turnover. Notably, the administration of CJ significantly downregulated hepatic lipogenesis-related genes (ACC, FAS, PPARγ, SREBP1c, FABP4, and C/EBPβ) and upregulated fat-oxidation markers (PPARα, CPT1A, and UCP2). By improving glucose tolerance and insulin sensitivity, CJ administration appears to suppress hepatic lipid synthesis via the downregulation of SREBP1c and PPARγ. Notably, while PPARγ improves insulin sensitivity, it can also promote lipogenesis through the activation of SREBP1, leading to the upregulation of FAS and ACC [39–41]. Thus, the suppression of PPARγ expression may provide dual benefits in NAFLD by reducing both insulin resistance and hepatic lipid accumulation [42]. These modulations suggest that CJ acts by rebalancing lipid homeostasis rather than simply reducing intake or absorption. Furthermore, reduced serum ALT/AST and inflammatory cytokines indicate that CJ ameliorates both steatosis and inflammatory injury.

Phytochemical analyses revealed that flavonoid content in CJ varied with season and plant morphology. The highest levels of cirsimarin and cirsimaritin were found in spring leaves, suggesting a seasonal increase in flavonoid biosynthesis potentially driven by developmental or environmental factors. Seasonal variation in flavonoid accumulation is closely associated with changes in environmental conditions, including light quality and intensity, ultraviolet (UV) exposure, temperature, and other abiotic stressors that modulate plant secondary metabolism. Flavonoids are synthesized through the phenylpropanoid/flavonoid biosynthetic pathway [43], in which enzymes such as phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), and chalcone isomerase (CHI) play crucial roles. Given that light- and UV-responsive signaling pathways enhance flavonoid biosynthesis and that these metabolites are significant in photoprotection and antioxidant defense mechanisms [44, 45], our findings demonstrate that the increased accumulation of cirsimarin and cirsimaritin in spring serves as a seasonally dependent adaptive response during periods of active growth.

Interestingly, pappus, typically regarded as waste material, also contained substantial levels of cirsimarin, suggesting its potential pharmacological value and industrial utilization. Although the present study did not definitively identify the specific flavonoids responsible for the observed anti-inflammatory and lipid-modulating effects, previous studies suggest that *Cirsium*-derived flavonoids target multiple inflammatory pathways relevant to NAFLD. Specifically, cirsimaritin isolated from CJ suppresses LPS-induced NO, TNF-α, and IL-6 production by inhibiting IκBα/Akt-associated signaling, as well as c-fos and STAT3 activation [46]. In addition, cirsimaritin has been reported to modulate oxidative stress–related mechanisms, further supporting its role in inflammatory modulation [47]. Furthermore, flavonoids have been widely reported to influence lipid metabolism-related pathways, including the regulation of key transcription factors such as SREBP-1c and PPARs [48]. However, as the current study was conducted at the extract level, the specific contributions of individual flavonoids and their precise

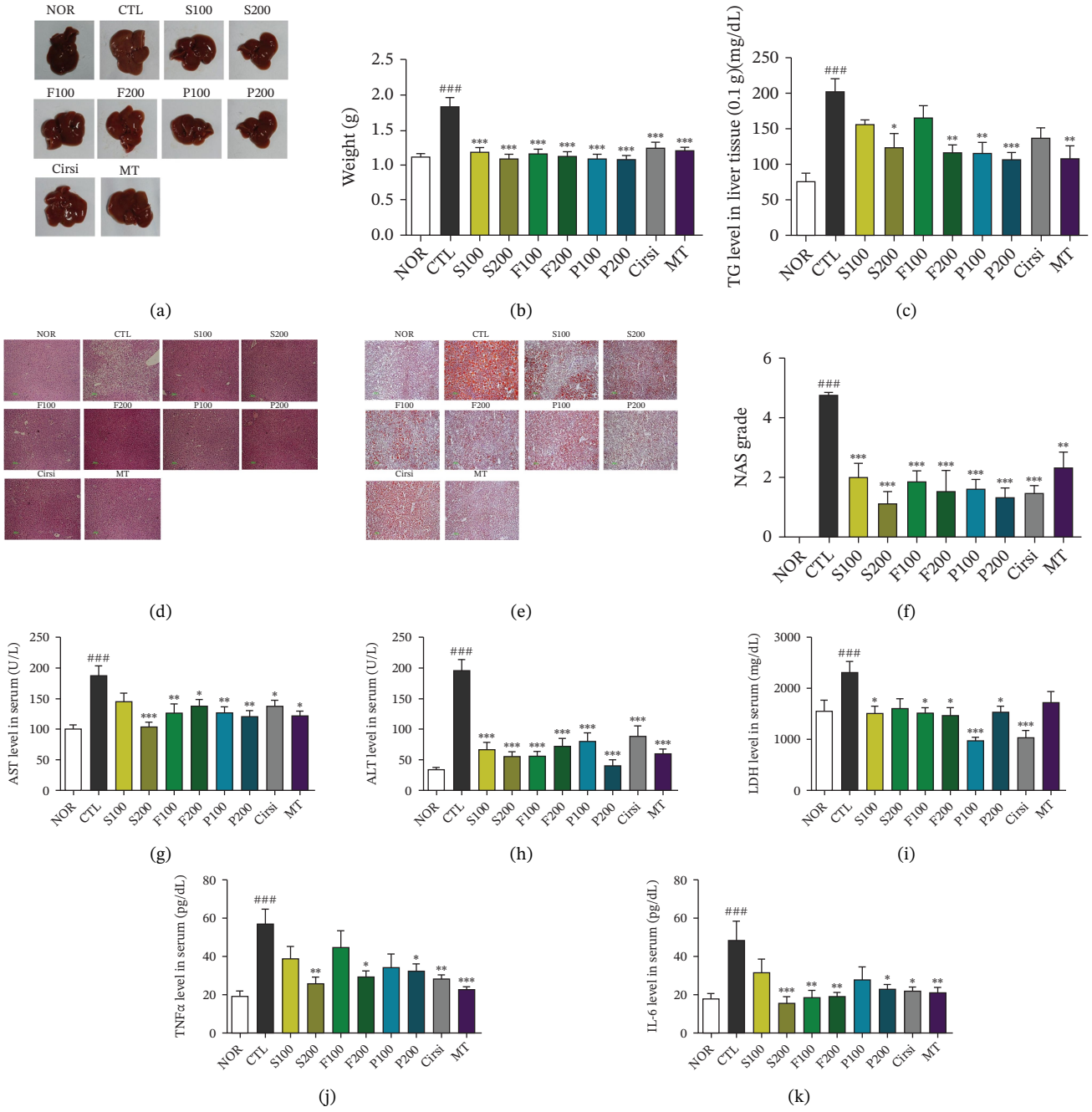


FIGURE 3 | Hepatoprotective effects of *C. japonicum* extracts in HFD-induced mice. Mice were subjected to an HFD to induce NAFLD and were treated with ethanol extracts of *C. japonicum* harvested at different times and from different plant parts. (a) Representative images of liver tissues collected at the end of the experiment from each treatment group. (b) Liver weight and (c) hepatic TG levels were significantly elevated in the HFD group, while treatment with *C. japonicum* extracts resulted in attenuation of these effects. (d–f) Representative microscopic images of liver sections stained with hematoxylin and eosin (H&E) (d) and oil red O (e), demonstrating reduced hepatocellular lipid accumulation and hepatic ballooning in *C. japonicum*-treated groups. Scale bar = 100 μm. Serum levels of liver injury markers and proinflammatory cytokines were evaluated to assess the hepatoprotective and anti-inflammatory effects of each extract. (f) NAFLD Activity Score (NAS) of liver tissues based on histological evaluation of steatosis, lobular inflammation, and hepatocyte ballooning. (g) Serum aspartate aminotransferase (AST) levels, (h) serum alanine aminotransferase (ALT) levels, (i) serum lactate dehydrogenase (LDH) levels, (j) serum tumor necrosis factor-α (TNF-α) levels, and (k) serum interleukin-6 (IL-6) levels were measured. All data are presented as mean ± SEM ($n = 10$). Statistical comparisons were performed using one-way ANOVA followed by Tukey's post hoc test. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ versus the CTL group, and $^{\#\#\#}p < 0.001$ versus the NOR group after Student's *t*-test.

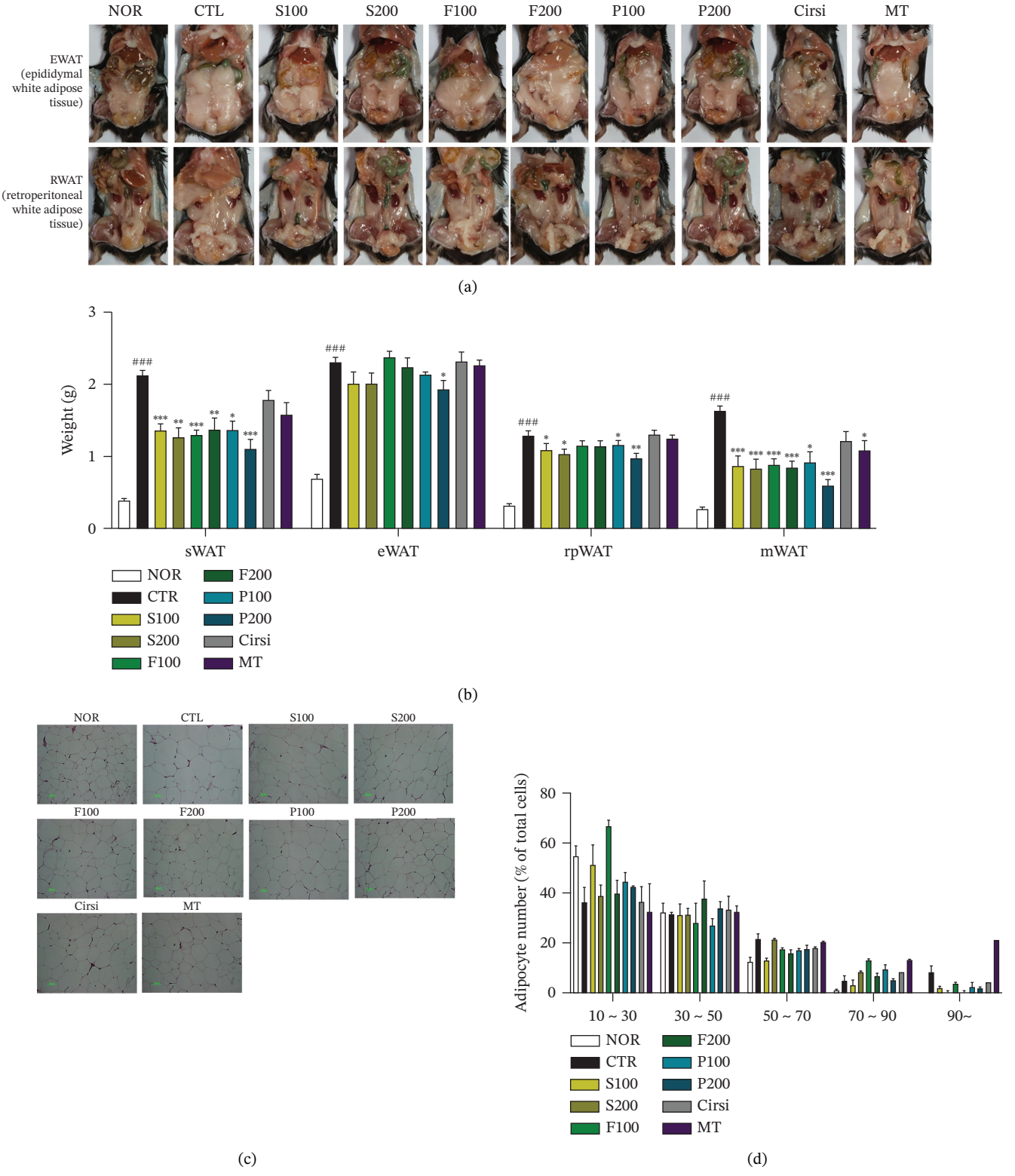


FIGURE 4 | Effects of *C. japonicum* extracts on visceral adiposity and adipocyte morphology in HFD-induced mice. HFD-induced mice were administered *C. japonicum* extracts obtained from different harvest times and plant sources, followed by an assessment of visceral white adipose tissue (WAT) accumulation and adipocyte morphology. (a) Representative images of epididymal white adipose tissue (eWAT) and retroperitoneal white adipose tissue (rpWAT) collected from mice in each experimental group. (b) Quantification of subcutaneous WAT (sWAT), eWAT, rpWAT, and mesenteric WAT (mWAT) weight. (c) Histological analysis of eWAT. Representative H&E-stained tissue sections demonstrating adipocyte morphology at × 200 magnification. Scale bar = 100 μm. (d) Quantification of adipocyte size and number was evaluated. All data are presented as mean ± SEM ($n = 10$).

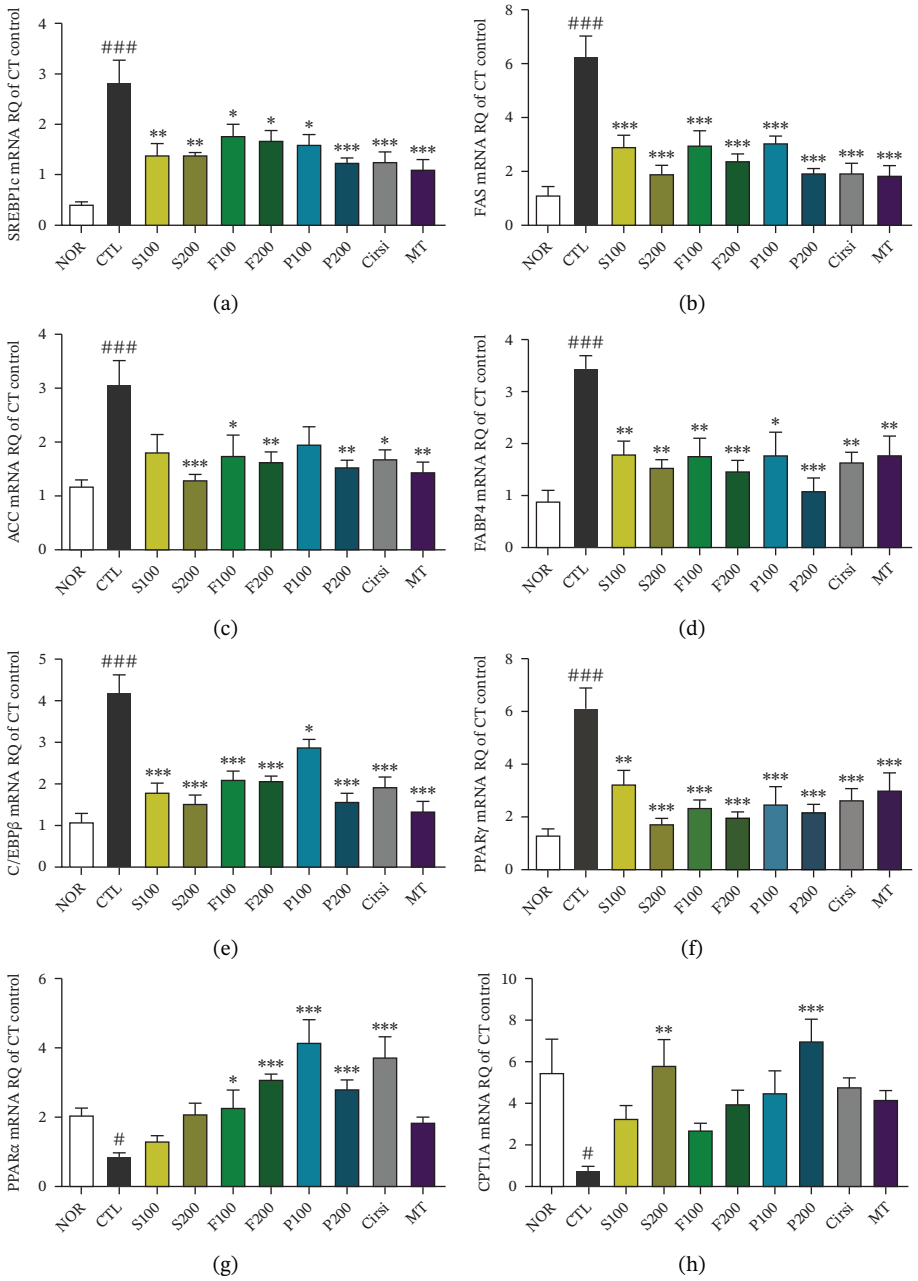
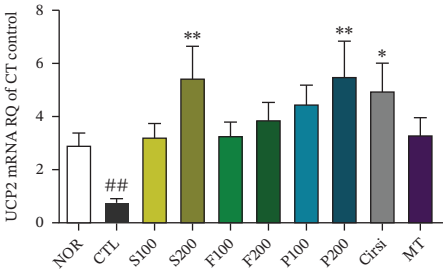


FIGURE 5 | (Continued)



(i)

FIGURE 5 | Effects of *C. japonicum* extracts on mRNA expression of hepatic lipid metabolism-related genes in the liver tissue of HFD-induced mice. The regulatory effects of *C. japonicum* extracts on hepatic lipogenesis and fatty acid oxidation were quantified by measuring the mRNA expression levels of key genes involved in adipogenesis, lipid synthesis, and mitochondrial fatty acid metabolism, utilizing quantitative reverse transcription polymerase chain reaction (RT-qPCR) in liver tissue. Relative quantification (RQ) of mRNA expression was normalized to housekeeping genes and presented as fold change relative to the CTL group. (a) mRNA expression of sterol regulatory element-binding protein 1c (SREBP1c), (b) fatty acid synthase (FAS), (c) acetyl-CoA carboxylase (ACC), (d) fatty acid-binding protein 4 (FABP4, also known as aP2), (e) CCAAT/enhancer-binding protein beta (C/EBPβ), (f) peroxisome proliferator-activated receptor gamma (PPARγ), (g) peroxisome proliferator-activated receptor alpha (PPARα), (h) carnitine palmitoyltransferase 1A (CPT1A), and (i) uncoupling protein 2 (UCP2). All data are presented as mean ± SEM ($n = 10$). Statistical comparisons were made using one-way ANOVA followed by Tukey's post hoc test. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ versus CTL group, and $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ versus the NOR group after Student's *t*-test.

molecular targets remain to be fully elucidated. Collectively, the therapeutic effects of CJ in NAFLD may be associated with the combined action of its flavonoid components, including flavonoids, which may collectively contribute to both anti-inflammatory and lipid metabolic regulatory effects.

5 | Conclusion

Our findings indicate that CJ regulates lipid metabolism through transcriptional control of lipogenic and oxidative pathways, thereby attenuating NAFLD progression. The results also underscore the importance of optimal harvest season and plant part selection in maximizing the pharmacological efficacy of CJ-based preparations. Future work should investigate the specific signaling mechanisms and pharmacokinetic profiles of its bioactive flavonoids.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data used to support the findings of this study are available on request from the corresponding authors.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Supporting Information.** Supporting Table S1. Composition of experimental diets, including macronutrient distribution (% kcal) and ingredient composition (g/kg).