

Capparis Spinosa Improves Non-Alcoholic Steatohepatitis Through Down-Regulating SREBP-1c and a PPAR α -Independent Pathway in High-Fat Diet-Fed Rats

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Research Article

Keywords: Capparis spinosa, NASH, SREBP-1c, ACC, PPAR α , CPT1

Posted Date: May 27th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-523948/v1>

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Abstract

Background: Non-alcoholic steatohepatitis (NASH) has become a global medical problem. Currently, there is no approved pharmacologic treatment for this condition. This study aimed to evaluate the ameliorative effects of *Capparis spinosa* (CS) on NASH in comparison to fenofibrate.

Methods: An animal model of NASH was developed using a high-fat (HF) emulsion. Fatty liver rats were treated with aqueous extract of CS fruit or fenofibrate in parallel to the HF for six weeks. Animals were examined for weight gain, serum biochemistry, insulin sensitivity, hepatic triglyceride (TG) content, histopathological changes as well as gene expression of sterol regulatory element-binding protein-1c (SREBP-1c), acetyl-CoA carboxylase (ACC), peroxisome proliferator-activated receptor α (PPAR α) and Carnitine palmitoyltransferase I (CPT1) in liver.

Results: Fasting blood sugar, insulin level, body and liver weight, activities of liver enzymes, hepatic triglyceride (TG) content as well as serum lipids were decreased following six weeks CS and fenofibrate treatments compared to the HF administration alone. Histopathological examinations also showed that liver steatosis, inflammation and hepatic fibrosis were markedly attenuated in response to CS and fenofibrate interventions. At the molecular level, CS treatment down-regulated SREBP1c, ACC and up-regulated CPT1 expression, but did not show a significant effect on PPAR α . In contrast, fenofibrate treatment induced the expression of all studied genes in fatty liver rats.

Conclusions: These findings indicated the favorable therapeutic effects of CS fruit extract on liver damages associated with NASH. The beneficial effects of CS on lipid accumulation and steatosis were comparable to those of fenofibrate.

Background

Nonalcoholic fatty liver disease (NAFLD) is among the most common types of liver disease and is becoming an important public health problem all over the world (1). It is a growing problem in parallel with the increasing prevalence of obesity (2). Nonalcoholic steatohepatitis (NASH) is a progressive type of NAFLD (3). According to the multi-hit theory, many pathogenic drivers contribute to the development of NAFLD and its progression to NASH (4). Among them, impairments in carbohydrate and fatty acid metabolic pathways within the liver are believed to be the leading causes of the disease, leading to excessive accumulation of lipid, enhanced lipid peroxidation and inflammation (5). Several key transcription factors including Sterol Regulatory Element Binding Proteins (SREBP) such as SREBP-1a, SREBP-1c, and SREBP-2, control various genes involved in regulating cholesterol homeostasis and lipid metabolism (6). Acetyl-CoA carboxylase gene is a major downstream target of SREBP-1c and plays a key role in de novo lipogenesis (DNL) (7). In addition to SREBPs, peroxisome proliferator-activated receptors (PPARs) have pivotal roles in glucose and lipid hemostasis and intrahepatic lipid accumulation. They are ligand-activated transcription factors including PPAR α , PPAR γ , and PPAR β/δ subtypes. PPAR- α is expressed dominantly in rat and human liver (8). Numerous genes are regulated by PPAR- α such as

carnitine palmitoyltransferase1A (CPT1A) which is an important metabolic enzyme (9). CPT1A mediates the translocation of long-chain fatty acids across the inner mitochondrial membrane for β -oxidation (10).

Discovering the underlying mechanisms of NASH would identify proper targets for potential pharmacological treatments. Currently, there are no approved therapeutic drugs available for use in patients with NAFLD/ NASH. Thus, there is an urgent need for developing novel medications. Medicinal plants have great potentials for discovering new drugs for NASH treatment.

Capparis spinosa (Caper) belongs to the family of Capparidaceae and is a common medicinal plant used in many parts of the world to treat various diseases(11–13). Its fruit is used in diabetic patients because traditionally it is believed to have hypolipidemic and hypoglycemic effects(14–16). Due to a large number of bioactive compounds, especially polyphenols and alkaloids in its extract, the therapeutic properties of the plant have drawn much attention over the past two decades(13, 15, 17). *Capparis spinosa* consumption has been shown to have anti-inflammatory, anti-diabetic, anti-obesity, and cholesterol-lowering benefits(18–20). Administration of Caper fruit extract has been associated with inhibition of gluconeogenesis and fat accumulation in streptozotocin-induced diabetic rats, thus it might have positive effects on liver health and fatty liver (20). However, its usefulness in NAFLD/ NASH and the possible underlying mechanisms have not yet been elucidated, and it warrants further investigations.

In the current study, we aimed to evaluate the effects of aqueous extract of *Capparis spinosa* (CS) fruit on biomarkers of steatosis in an experimental NASH model. A growing body of evidence showed that NASH is commonly associated with metabolic syndrome and diabetes. So, in this study, a diabetic fatty liver model was generated using a low dose of streptozotocin and a high-fat diet in rats. This animal model is known to mimic various aspects related to fatty liver. We evaluated liver tissue, biochemical parameters, and liver lipid content. We also evaluated potential hepatic gene expression changes with regard to the CS extract effects. Since CS is a natural supply with multiple bioactive compounds such as alkaloids, flavonoids, steroids, terpenoids, and tocopherols, we used the whole extract of the plant to test for its efficacy in NASH.

Methods

Providing CS aqueous extract

Capparis spinosa fruits were picked from wild plants growing in the Shooshtar region in Khuzestan province, southwest Iran. Determination and taxonomy assessment was done by Dr. Fereshteh Gol Fakhrabadi from Ahvaz Jundishapur University of Medical Sciences. Fruits were deposited at the Medicinal Plant Research Center, Ahvaz Jundishapur University of Medical Sciences (Ahvaz, Iran) with the voucher number A1805812FP.

Providing the aqueous extract was done in terms of the approach explained by Jalali et al. (20). In brief, CS fruits were washed with distilled water, dried at 40°C, and then were powdered. To 10 g powdered fruits, 100 ml distilled water was added which stirred for 3 hours. The mentioned mixture was boiled

about 10 min and cooled about 15 min. The aqueous extract was filtered applying a 0.2 mm Millipore filter (Millipore 0.2 mm, St Quentin en Yvelines, France). The obtained filtrate was freeze-dried which stored at -20°C for subsequent utilization. Fresh aqueous extracts were daily reconstituted immediately before the administration. The freeze-dried extract was reconstituted in 1.5 ml of distilled water and orally given to various groups with 20 mg/kg dose, regarding Jalali et al. research. (20).

Providing high-fat emulsion

As mentioned by Yuhong Zou et al., high-fat emulsion (HF) was provided (21). The HF composition is noted in Table 1 which involved 77% fat, 14% total milk powder, and 9% carbohydrates. In present formulation, the source of protein, carbohydrate, and fat are full milk powder, saccharose, and corn oil, respectively. Moreover, HF emulsion was supplemented with the vitamin and mineral mixture. The last emulsion was maintained at 4 ° C, and before utilization was heated and completely mixed in a 40°C water bath.

Table 1
Composition and caloric content of
the high fat emulsion

Corn oil (g)	400
Saccharose (g)	150
Total milk powder (g)	80
Cholesterol (g)	100
Sodium deoxycholate (g)	10
Tween 80 (g)	36.4
Propylene glycol (g)	31.1
Vitamin mixture (g)	2.5
Cooking salt (g)	10
Mineral mixture (g)	1.5
Distilled water (ml)	300
Total energy (kcal/l)	4342

Providing Chemicals

Fenofibrate was bought from the Sinadarou Labs Company (Tehran, Iran). For oral performance, fenofibrate suspension was provided in 0.5% w/v sodium carboxymethylcellulose (Sigma, St. Louis, MO, USA) in distilled water. In 0.1M fresh cold citrate buffer solution at pH 4.2, the streptozotocin (Sigma, St. Louis, MO, USA) was obtained.

Animals and treatments

The male Wistar rats (180 ± 20 g) were prepared by the Experimental Animal Center, Ahvaz Jundishapur University of Medical Sciences. They were located in the plastic cages under the standard environmental situations at a room temperature of $24 \pm 1^\circ\text{C}$ with $55 \pm 5\%$ humidity under 12-h light-dark cycles. Our research was done based on the ethical principal and national norms and standards to conduct the medical research in Iran which confirmed by the Ethics Committee of Research Center & Experimental Animal House, Ahvaz Jundishapur University of Medical Sciences.

At first, 45 rats were randomly (by a random number table) divided into two groups: normal control group (NC group, $n = 18$) and HF group which achieved the high-fat emulsion (10 ml/kg, $n = 27$) orally once per day and free achievement to a saccharose solution (18%). Passing 6 weeks, the rats of HF group were injected intraperitoneally with a freshly provided solution of Streptozotocin (STZ) at a single low dose (30 mg/kg) to induce a more feature model of NASH. In the final part of sixth week, two rats from NC group and HF group were sacrificed, and their liver was transformed to the lab for the pathological investigation to ensure NASH development. After confirming the model, the pharmacological treatments were begun from the seventh experimental week which followed until the final of week 12 on a daily basis. To this objective, HF group was more divided by a random number table into three groups ($n = 8$) to achieve either high-fat emulsion (HF group), high-fat emulsion plus fenofibrate 100mg/kg body weight (HF + FENO group) or CS 20 mg/kg body weight (HF + CS group). Fenofibrate and CS were suspended in 0.5 % carboxymethylcellulose (CMC) solution and were daily given at 10:00 a.m. in a volume equal to 1.5 ml/kg via gavage. The rats in HF group achieved the equal volumes of 0.5 % CMC solution (drug vehicle). Furthermore, NC group was randomly divided into two groups ($n = 8$), involving CS group and NC group which achieved CS (20 mg/kg body weight) and the similar volume of distilled water, respectively. All rats were achieved a standard rodent chow diet and water through study course. At the final of treatments, regarding 14 h fasting, all rats were sacrificed by the intraperitoneal injection of a high dose ketamine-xylazine (90/10mg/kg). The blood samples were gathered via the cardiac puncture. The livers were immediately removed, weighed and washed with normal saline and were flash-frozen in liquid nitrogen which kept at -190°C for further analysis of gene expression. The liver index was computed from the ratio of liver weight/body weight.

Biochemical measurements

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), and glucose in serum were done by the Roche 6000 auto-analyzer applying the corresponding assay kits. The levels of free fatty acids (FFA) and insulin in serum were identified applying a commercial analysis kit and an enzyme-linked immunoassay kit (DRG Diagnostics, Marburg, Germany), respectively, following instructions of manufacturer. Furthermore, the hepatic TG content was enzymatically measured by a colorimetric-specific kit (Cayman Chemical, USA).

Gene expression analysis

Expression of SREBP1-c, acetyl-CoA carboxylase (ACC), PPARα and carnitine palmitoyltransferase 1 (CPT1) mRNA was assessed by the real-time PCR technique. In brief, the total RNA from the frozen livers were separated with the FastPure™ RNA Kit from Takara Bio (Otsu, Japan) and cDNA synthesis was done applying PrimeScript RT reagent Kit (Takara Bio, Otsu, Japan) following the procedures obtained by the manufacturer. Real-time PCR was done with an SYBR Green PCR kit (Takara Bio, Otsu, Japan) on QuantStudio™ 3 Real-Time PCR System (ABI Applied Biosystems) based on the manufacturer’s protocol. Comparative quantification of gene expression was utilized as noted in the manual applying β-actin as an internal control. The primer sequences were indicated in Table 2.

Table 2
Primer sequences of the target and reference genes amplification

Gene	Forward primer	Reverse primer
PPARα	5'- CCCCACTTGAAGCAGATGACC – 3'	5'- CCCTAAGTACTGGTAGTCCGC – 3'
CPT-1	5'-GCTCGCACATTACAAGGACAT-3'	5'-TGGACACCACATAGAGGCAG-3'
SREBP1-c	5'- GGAGCCATGGATTGCACATT – 3	5'- GCTTCCAGAGAGGAGCCCAG – 3
ACC	5'-CGCTGCGGTCAAGTGT-3'	5'-CGTTGGCGTAGTTGTTATT-3'
β-Actin	5'-CCCATCTATGAGGGTTACGC-3	5'-TTTAATGTCACGCACGATTTC-3

Histopathological evaluations

The sections of liver tissue were immediately obtained after the rats were sacrificed. The tissue specimens were fixed in 10% formalin solution. Sections of the liver were dehydrated, embedded with paraffin which were stained with hematoxylin-eosin (HE) for histopathological assessments to survey the hepatic steatosis, inflammation, necrosis, and fibrosis. Steatosis was graded due to hepatocytes percentage including the macrovesicular fat (Grade 1: 0–25%; grade 2: 26–50%; grade 3: 51–75%; grade 4, 76–100%) (22). The degree of inflammation, necrosis, and fibrosis was explained by the mean of 10 various fields in every slide which were grouped with a scale of 0–3 (0: normal; 1: mild; 2: moderate; 3: severe) which was described by Avni et al. (23).

Statistical analysis

Quantitative data were noted as mean ± SD, and qualitative variables were noted as the percentages. One-way analysis of variance (ANOVA) and Tukey's post hoc tests was used to compare quantitative variables. For the categorical data, Chi-square test was done. The significance level for all tests was regarded at $p \leq 0.05$. The analyses were handled applying the GraphPad Prism software version 8.0.2.

Results

The impacts of treatments on liver index and histopathological parameters

Based on Fig. 1.A, after 12 weeks continuous feeding with the high-fat emulsion, HF group rats had a mean body weight of 334.71 ± 4.71 g in comparison with 243.57 ± 6.77 g in control rats, showing a considerable increase in body weight of HF group rats in comparison with control group ($p < 0.001$). CS and fenofibrate treatment almost normalized this increase in body weight in HF + CS and HF + FENO groups. Moreover, liver weights in the HF group were significantly increased ($p < 0.001$) in comparison with NC group. The treatment with CS (HF + CS group) for 42 consecutive days significantly decreased the liver weight in comparison with HF group. Hence, there was no considerable difference between fenofibrate treatment group and HF group was found in the liver weight (Fig. 1.B). The liver index in HF group was considerably higher than that in NC group ($p < 0.01$). CS treatment considerably attenuated this index in comparison with HF group ($p < 0.05$). Moreover, the liver index was increased answering to the fenofibrate treatment ($p < 0.01$) in comparison with high-fat emulsion alone (Fig. 1.C).

Passing 12 weeks of treatment, the liver triglyceride content was markedly increased in HF group in comparison with NC group. CS treatment for six weeks was efficient to inhibit hepatic triglyceride accumulation. Furthermore, the intervention with fenofibrate normalized hepatic TG content in comparison with HF group (Fig. 1.D).

HF-fed rats enhanced the severe degrees of steatosis. The livers of these rats were grossly larger than those in NC group which were beige in color (Fig. 1). The severe steatosis was approved by hematoxylin-eosin staining of liver sections. The high-fat emulsion feeding led to the prominent inflammation, RBCs accumulation, and necrosis in HF group; whereas the livers from rats fed normal diet for 12 weeks were normal (Fig. 1). Passing 6 weeks of CS treatment, considerable enhancement was seen in the steatosis and aspects of necro-inflammation of hepatic tissue. Hence, fenofibrate (a PPAR α agonist) indicated the same impacts to CS and the degrees of steatosis and inflammation were attenuated in the fenofibrate group (Fig. 1).

The impacts of treatments on the biochemical parameters

Liver performance tests

By a signified increase in ALT and AST enzymes, the immense hepatic damages in our NASH model induced by the high-fat emulsion were indicated. Serum levels of ALT and AST in HF group were significantly increased in comparison with rats in NC group (< 0.001) (Table 3). CS and fenofibrate treatments reversed the detrimental impacts of high-fat emulsion and normalized AST and ALT activity to levels in comparison with those in NC group; thus, CS treatment showed more effectiveness in this regard in comparison with the fenofibrate (Table 3).

Table 3

Effect of daily administration of CS (20 mg/kg) and fenofibrate (100mg/kg) on Serum biochemistry parameters in rat model of HFD-induced NASH.

Parameters NC	CS	HF	HF + FENO	HF + CS	P Value			
					NC VS.CS HF VS.NC	HF + FENO VS. HF	HF + CS VS. HF	HF + CS VS. HF + FENO
ALT (U/L) 31.57 ± 3.30	29.27 ± 4.11	70.57 ± 5.82	53.71 ± 14.13	40.38 ± 6.43	ns <0.001	0.0047	< 0. 001	0.0244
AST (U/L) 44.71 ± 3.25	39.97 ± 5.13	106.00 ± 19.07	86.71 ± 6.13	59.00 ± 4.27	ns <0.001	0.0117	< 0. 001	0.002
TG (mg/dl) 44.57 ± 9.14	40.17 ± 8.87	115.10 ± 16.32	38.29 ± 16.66	50.25 ± 25.10	ns < 0.001	< 0. 001	< 0. 001	ns
TC (mg/dl) 71.57 ± 2.63	67.43 ± 3.13	124.1 ± 7.49	82.29 ± 16.74	82.63 ± 8.60	ns <0.001	< 0. 001	< 0. 001	ns
HDL-c (mg/dl) 44.00 ± 2.82	46.63 ± 3.02	27.71 ± 5.03	32.86 ± 7.84	37.75 ± 4.68	ns < 0.001	ns	0.0071	ns
LDL-c (mg/dl) 18.66 ± 3.33	16.66 ± 5.71	71.40 ± 7.25	48.06 ± 11.57	32.83 ± 6.51	ns < 0.001	< 0. 001	< 0. 001	0.0041
FFA (mg/dl) 9.99 ± 3.04	8.76 ± 4.06	29.52 ± 4.37	36.63 ± 6.08	19.86 ± 4.01	ns < 0.001	< 0. 001	0.0014	< 0. 001
FBS (mg/dl) 105.70 ± 7.54	99.31 ± 6.94	254.30 ± 8.95	160.1 ± 20.22	124.10 ± 8.72	ns < 0.001	< 0. 001	< 0. 001	< 0. 001
Insulin (mIU/L) 3.68 ± 1.07	3.08 ± 0.97	3.079 ± 1.088	5.744 ± 0.79	3.327 ± 1.53	ns ns	0.0012	ns	0.0033
HOMO-IR 0.96 ± 0.26	0.87 ± 0.41	1.925 ± 0.68	2.268 ± 0.41	1.04 ± 0.49	ns0.0059	ns	0.0130	0.005
Values are expressed as the mean ± SD of parameters analyzed by one-way ANOVA and Tukey post hoc tests (n = 8). AST, aspartate aminotransferase; ALT, alanine aminotransferase; TG, triacylglycerol; TC, total cholesterol; HDL-c, high density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol; FFA, free fatty acid; FBS, fasting blood sugar; HOMA-IR, homeostatic model assessment of insulin resistance.								

Serum Lipid Profile

Passing 6 weeks feeding with the high-fat emulsion, serum levels of TC, TG, LDL-C, and FFA were significantly increased in comparison with NC group, except for HDL-C. More treatment with CS and fenofibrate for six weeks in addition to the high-fat emulsion led to a diminution of these lipid parameters significantly ($P < 0.001$) and alleviated dyslipidemia in comparison with HF group. In addition, the interventions improved the HDL-C levels (Table 3).

Glycemic indices

High-fat emulsion administration together with a low dose STZ injection resulted in a 2.4-fold increase in the fasting blood glucose (FBG) level in HF group in comparison with control group. Plasma insulin levels in HF group stayed without change which gave rise to a 2-fold increase in the HOMA index in comparison with control group (Table 3). The treatment with CS and fenofibrate significantly ($P < 0.001$) restored the increase in FBG levels. The insulin level did not alter answering to CS vs. HF emulsion alone which led to decreasing HOMA-IR index in CS group in comparison with HF group ($p < 0.05$). While, fenofibrate treatment increased the insulin level which consequently reduced HOMA-IR index in comparison with HF group (Table 3).

Hepatic mRNA expression of SREBP-1c, ACC, PPAR α , and CPT1

Regarding qPCR mRNA expression analysis in the post-treatment livers, we noticed the increased hepatic SREBP1c and ACC mRNA expression in HF group in comparison with NC group (Fig. 2). Fenofibrate treatment significantly reduced the SREBP-1 expression as in comparison with HF group ($p < 0.05$). Hence, CS treatment could significantly and more effectively than fenofibrate reduce the elevated mRNA expression of SREBP-1c following 12 weeks high-fat emulsion feeding ($p < 0.001$). Based on Fig. 2.B, while a considerable reduction in the hepatic mRNA expression of ACC was found in the CS-treated rats ($p < 0.001$), it was not influenced considerably in the fenofibrate group in comparison with HF group.

The high-fat emulsion feeding did not change the mRNA expression of PPAR α and CPT1 and no considerable difference to NC group. Six weeks of the intervention with PPAR α agonist fenofibrate improved the hepatic mRNA levels of PPAR α and its downstream target gene CPT1 considerably compared to HF group (Fig. 2). Thus, a considerable impact of CS on PPAR α expression was not seen. Based on Fig. 2.D, though the CPT1 expression answering to CS treatment was not as much as fenofibrate, it was considerably regarded in comparison with HF group ($p < 0.001$).

Discussion

In the present study, we evaluated the hepatoprotective effects of CS fruit extract in a rat model of HFD-induced NASH in comparison to fenofibrate. We found for the first time that oral administration of CS extract reduced body weight gain, liver index and favorably ameliorated lipid metabolism which led to an overall improvement in the pathologic features of experimentally induced NASH in rats. Specifically, CS

fruit extract decreased the elevated liver enzymes (ALT, AST), improved systemic insulin resistance, and attenuated hepatic lipid accumulation, which were comparable to the effects provided by fenofibrate.

Though NAFLD pathogenesis remains weakly understood, metabolic syndrome is one of the most causative elements in enhancing NAFLD/ NASH. The main factors which signify the metabolic syndrome are obesity, hypertension, hyperglycemia, hypertriglyceridemia, and low levels of HDL-cholesterol (24). A high-fat emulsion and a low-dose STZ were applied to induce NASH in rats. Our NASH model successfully reproduced some typical aspects of NASH like obesity, hyperlipidemia, elevated liver enzymes, hyperglycemia, and insulin resistance. Furthermore, the generic hepatic lesions of NASH, steatosis, and inflammation were also enhanced in this model.

Traditional herbal medicine has recently drawn much attention to the treatment of human diseases, including fatty liver(25–27). ALT and AST are considered as the most sensitive and specific indicators of hepatocellular injury(28–30). We found high levels of these enzymes in the HF group while the administration of CS extract reduced the elevated serum levels of ALT and AST. In accordance with the decrease in liver enzymes, we found CS extract markedly diminished the fat deposition and lipid accumulation in liver tissues based on histopathological evaluations. Our results confirmed that CS administration can ameliorate body weight, liver weight, and liver index in the high fat-fed rats. We also observed significant improvement in steatosis and triglyceride content of the liver tissue of the HF-fed rats treated with the CS extract. A major predisposing factor in the pathogenesis of steatosis and triglyceride accumulation in the liver is insulin resistance which is associated with increased flux of fatty acid from adipose tissue to the liver (30). In our study, the treatment of high fat-fed rats with CS extract decreased the HOMA-IR index that can describe the observed decrease in serum free fatty acid in these animals. These findings indicate the protective and beneficial therapeutic effects of CS on liver damages associated with NASH. These favorable effects of CS on lipid accumulation and steatosis were comparable to those of fenofibrate as revealed by reduced serum ALT and AST, and amelioration of other NASH features. In comparison with CS, which improved glycemic parameters in our NASH model, fenofibrate did not represent beneficial effects on these parameters. This difference suggests that CS and fenofibrate may involve distinct mechanisms to improve NASH. However, in a previous study we showed an important part of these mechanisms in which we observed that FGF21 increased in response to CS treatment thereby reducing steatosis, inflammation, and fibrosis in the NASH animal model (31). To more elucidate the mechanism by which CS ameliorates steatosis, we analyzed the CS extract effects on the expression of the key regulatory genes of hepatic fat content. Among the involved genes, we investigated the changes of PPAR α , CPT-1, SREBP-1c, and ACC expression. SREBP-1c is a crucial transcription factor involved in the regulation of lipid metabolism in the liver. There is credible evidence that a high-fat diet activates SREBP1c and this activation plays an important role in the development and progression of NAFLD. Therefore, inhibition of SREBP1c is considered as a therapeutic target for preventing and treatment of NAFLD (32, 33). In the present study, high-fat diet feeding induced SREBP-1c while CS extract treatment inhibited its expression in our NASH model. These findings suggest the down-regulation of SREBP1c expression as an underlying mechanism by which CS can ameliorate NASH. To confirm the effect of CS on SREBP-1c function, we investigated the expression of acetyl-CoA carboxylase (ACC), as

an important downstream target of this transcription factor, in response to CS. ACC is the committed enzyme in the hepatic de novo lipogenesis (DNL) pathway, which is involved in malonyl-CoA synthesis from carboxylation of acetyl-CoA. Malonyl-CoA is an essential substrate for biosynthesis of fatty acids. Previous studies have shown that inhibition of ACC can significantly ameliorate fatty liver. Thus, pharmacologic inhibition of ACC is considered as an attractive strategy for NAFLD treatment (34). However, it has also been reported that ACC inhibition might result in hypertriglyceridemia through decreasing polyunsaturated fatty acids which further led to SREBP1 induction and decreased PPAR α and CPT1 activity (35). These data indicate the necessity of novel therapies capable of concurrent improvement of hepatic steatosis and normalizing plasma TG. Our results demonstrated that CS extract treatment could down-regulate ACC expression in HF-fed rats with fatty liver that was associated with a decrease in plasma TG.

PPAR α is a dominant transcription factor in liver, regulating numerous pathways involved in lipid metabolism including fatty acid activation, transport of fatty acid to the mitochondria, mitochondrial fatty acid β -oxidation, and lipogenesis (36, 37). There are growing evidences that indicate PPAR α activation can hinder fatty liver development. Hence, pharmacologic PPAR α agonists such as fenofibrate are proposed as therapeutic options for NAFLD (38, 39). In our study, HF feeding did not change PPAR α expression as compared to the NC rats. In accordance with the previous studies (40, 41), fenofibrate treatment remarkably increased PPAR α gene expression. However, we did not find a significant alteration in PPAR α gene expression in response to CS extract treatment in HF-fed rats. This finding suggests that alternative mechanisms independent of PPAR α expression might be involved in the observed CS effects.

For further investigation of the PPAR α pathway in steatosis alleviation by the CS extract, we evaluated CPT1 expression as a target gene of PPAR α . CPT1 plays a key role in the translocation of fatty acids into the mitochondria, which give rise to fatty acid β -oxidation. As a PPAR α agonist, fenofibrate significantly increased CPT1 expression. Likewise, our results indicated that CS extract treatment could significantly up-regulate CPT1 expression in HF-fed rats. But, considering the non-significant effect of CS extract on PPAR α expression, it seems that alternative pathways other than PPAR α such as the AMPK pathway might be involved in the CS-induced CPT1 expression.

Conclusion

This study indicated that therapy with CS extract markedly improves hepatic lipid accumulation and steatohepatitis in high fat-fed rats and these beneficial effects were comparable to fenofibrate effects. The current study also provides new evidence on the molecular mechanism by which CS ameliorates steatosis, which raises new possibilities for the treatment of this condition.

Abbreviations

NAFLD: Non-alcoholic fatty liver disease

NASH: Non-alcoholic steatohepatitis

CS: Capparis spinosa

HF: High fat

TG: Triglyceride

SREBP-1c: Sterol Regulatory Element-Binding Protein-1c

ACC: Acetyl-CoA carboxylase

PPAR α : Peroxisome Proliferator-Activated Receptor α

CPT1: Carnitine Palmitoyltransferase I

DNL: De Novo Lipogenesis

NASH: Nonalcoholic Steatohepatitis

STZ: Streptozotocin

HOMA-IR: Homeostatic Model Assessment of Insulin Resistance

Declarations

Ethics approval and consent to participate

Our study was approved by the Ethics Committee of Research Center & Experimental Animal House, Ahvaz Jundishapur University of Medical Sciences.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by the Ahvaz Jundishapur University of Medical Sciences (grant no. HLRC-9705). The funding source has no role in the design of the study, analysis, and interpretation of data, and publication of the manuscript. This article was extracted from the MSc thesis of Mr. Rasoul Akbari.

Authors' contributions

Narges Mohammadtaghvaei, Hamid Yaghooti and, Mohammad Taha Jalali designed the study. Rasoul Akbari performed all assays. Laya sadat Khorsandi contributed to histological study. Rasoul Akbari analyses the data. Narges Mohammadtaghvaei wrote first draft. Hamid Yaghooti revised the manuscript. Mohammad Taha Jalali contributed to the interpreting the results. The authors read and approved the final manuscript.

Acknowledgements

The authors wish to thank the Ahvaz Jundishapur University of Medical Sciences for financial support of the study.

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Figures

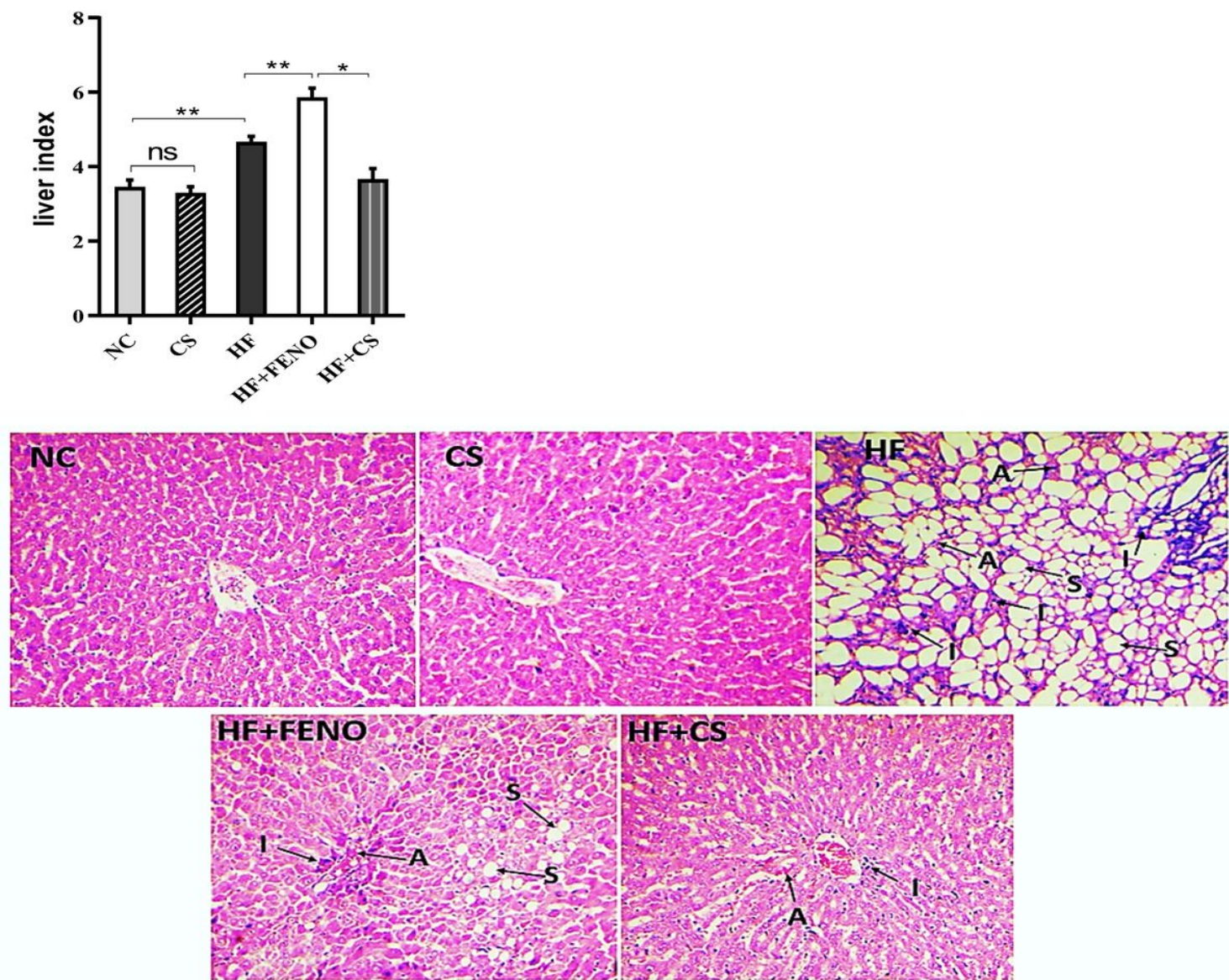
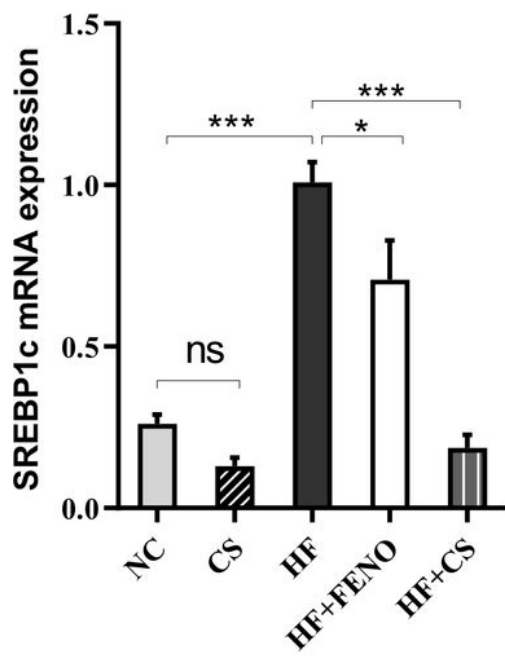
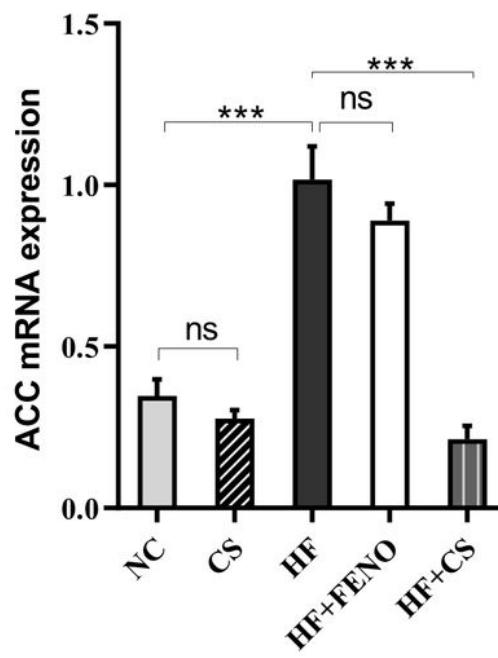


Figure 1

Upper panel: Effect of *Capparis spinosa* fruit extract and fenofibrate on the body weight (A), liver weight (B), liver TG content (C), and liver index (D) in NASH model rats during 12 weeks treatment. Bars represent the mean $\pm$ SD of the variables in each group (n=8). Lower panel: Representative images of hematoxylin-eosin stained sections of liver tissue in different groups at the end of treatments with 40X magnification. Arrows indicate the steatosis lesions. NC, normal control; HF, high fat; CS, *Capparis spinosa*; FENO, fenofibrate. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ and ns: non-significant. a Significantly different from NC at end of week 6 ; b Significantly different from NC after 12 weeks and c significantly different between NC, HF+FENO, and HF+CS vs. HF at end of week 12 ($p < 0.001$).



C



D

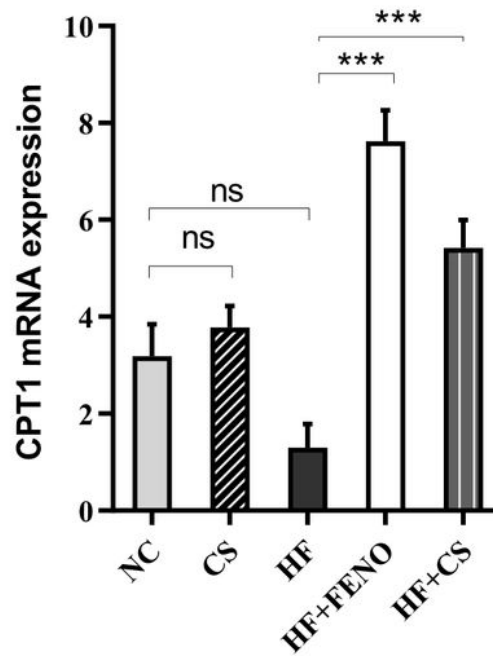
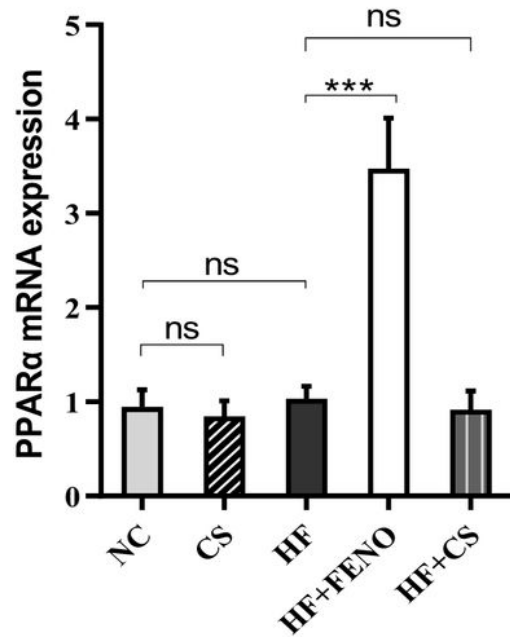


Figure 2

Effects of Capparis spinosa fruit extract (20 mg/kg) and fenofibrate (100mg/kg) on hepatic gene expression in NASH model rats. A, sterol regulatory element binding protein 1c (SREBP1c), B, acetyl-CoA carboxylase (ACC), C, peroxisome proliferator-activated receptor α (PPAR α), D, carnitine palmitoyltransferase 1 (CPT1) mRNA expression. Values are mean $\pm$ SD of fold-changes of mRNA ex-

pression relative to the HF group (n=6). NC, normal control; HF, high fat; CS, Capparis spinosa; FENO, fenofibrate; ns, nonsignificant. * $p < 0.05$; *** $p < 0.001$.