Journal of Ethnopharmacology 150 (2013) 1045–1052

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Modulation of hepatic sterol regulatory element-binding protein-1c-mediated gene expression contributes to *Salacia oblonga* root-elicited improvement of fructose-induced fatty liver in rats

Lei Liu [a,1], Ming Yang [a,1], Xuemei Lin [a], Ying Li [a], Changjin Liu [a,b], Yifan Yang [c], Johji Yamahara [d], Jianwei Wang [e,*], Yuhao Li [c,**]

[a] *Faculty of Basic Medical Sciences, Chongqing Medical University, Chongqing, China*
[b] *College of Laboratory Medicine, Chongqing Medical University, Chongqing, China*
[c] *Endocrinology and Metabolism Group, Sydney Institute of Health Sciences/Sydney Institute of Traditional Chinese Medicine, Sydney, NSW 2000, Australia*
[d] *Pharmafood Institute, Kyoto, Japan*
[e] *Department of Traditional Chinese Medicine, Chongqing Medical University, Chongqing, China*

ARTICLE INFO

Article history:
Received 26 March 2013
Received in revised form
4 July 2013
Accepted 10 October 2013
Available online 21 October 2013

Keywords:
Salacia oblonga
Lipid
Liver
Sterol regulatory element-binding protein-1c
Triglyceride

ABSTRACT

Ethnopharmacological relevance: Salacia oblonga root (SOR) is a traditionally herbal medicine for obesity and diabetes, which are closely associated with fatty liver. To investigate the molecular mechanisms of SOR in the treatment of dietary-induced fatty liver.

Materials and methods: Male rats were co-administered with fructose in drinking water and vehicle or the aqueous-ethanolic extract of SOR (by gavage, once daily) for 10 weeks. Biochemical variables were determined enzymatically or by ELISA. Gene expression was analyzed by Real-Time PCR and/or Western blot.

Results: SOR treatment (20 mg/kg) diminished fructose-induced fatty liver indicated by decreases in excess triglyceride accumulation and the increased vacuolization and Oil Red O staining area in the livers of rats. Importantly, Hepatic gene expression profile revealed that SOR suppressed fructose-stimulated overexpression of sterol regulatory element-binding protein (SREBP)-1/1c mRNA and nuclear protein. In accord, overexpression of SREBP-1c-responsive genes, such as fatty acid synthase, acetyl-CoA carboxylase-1 and stearoyl-CoA desaturase-1, was also downregulated. In contrast, overexpressed nuclear protein of carbohydrate response element binding protein and mRNA of its target gene liver pyruvate kinase were not altered. Additionally, SOR also did not affect expression of peroxisome proliferator-activated receptor-gamma- and -alpha, as well as their target genes, such as carnitine palmitoyltransferase-1a, acyl-CoA oxidase and CD36.

Conclusions: These results suggest that modulation of hepatic sterol regulatory element-binding protein-1c-mediated gene expression contributes to SOR-elicited improvement of fructose-induced fatty liver in rats. Our findings provide a better understanding of SOR in the treatment of obesity and diabetes.

1. Introduction

Nonalcoholic fatty liver disease, the most common cause of liver dysfunction, is gaining increasing recognition as a component of the epidemic of obesity in the world. All features of the metabolic syndrome, including obesity, type 2 diabetes, arterial hypertension and hyperlipidemia, are associated with nonalcoholic fatty liver disease (Postic and Girard, 2008). Fatty liver is the hallmark of nonalcoholic fatty liver disease, which has become an important public health problem due to its high prevalence, potential progression to severe liver disease, and association with cardiometabolic abnormalities (Marchesini et al., 2003; Postic and Girard, 2008). Despite the increasing incidence of nonalcoholic fatty liver disease, there are no therapies currently approved by The Food and Drug Administration of the United States of America for treatment of these common liver disorders.

Abbreviations: ACC, acetyl-CoA carboxylase; ACO, acyl-CoA oxidase; ChREBP, carbohydrate response element binding protein; CPT, carnitine palmitoyltransferase; FAS, fatty acid synthase; LPK, liver pyruvate kinase; NEFA, non-esterified fatty acids; PPAR, peroxisome proliferator-activated receptor; SCD, stearoyl-CoA desaturase; SOR, *Salacia oblonga* root; SREBP, sterol regulatory element-binding protein; WAT, white adipose tissue

* Correspondence to: Department of Traditional Chinese Medicine, Chongqing Medical University, 1 Yixueyuan Road, Yuzhong District, Chongqing 400016, China. Tel.: +86 18602326815; fax: +86 23 65712064.
** Correspondence to: Sydney Institute of Health Sciences, Level 5, 545 Kent Street Sydney NSW 2000 Australia. Tel.: +61 2 9261 2289; fax: +61 2 9261 2903.
E-mail addresses: wjwcq@yahoo.com.cn (J. Wang), yuhao@sitcm.edu.au, yuhaoli@hotmail.com (Y. Li).
[1] These authors contributed equally to this work.

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http://dx.doi.org/10.1016/j.jep.2013.10.020

Increasing consumption of sugars is one of the main contributing factors to the obesity epidemic. Fructose, a monosaccharide, has now become a major constituent of our modern diet. It is well known that chronic overconsumption of fructose results in fatty liver, dyslipidemia, insulin resistance, and obesity in animals and humans (Johnson et al., 2009; Tappy and Lê, 2010). Fructose, by providing large amounts of hepatic triose-phosphate as precursors for fatty acid synthesis, is highly lipogenic (Tappy and Lê, 2010). Recent findings suggest that increase in hepatic de novo lipogenesis plays an important role in fructose-induced fatty liver and hypertriglyceridemia (Stanhope et al., 2009; Tappy and Lê, 2010). Sterol regulatory element-binding protein (SREBP)-1c and carbohydrate response element binding protein (ChREBP) are well-known important proteins that may mediate hepatic de novo lipogenesis (Postic and Girard, 2008; Tappy and Lê, 2010). A high-fructose diet may stimulate SREBP-1c and ChREBP through currently-unknown mechanisms (Tappy and Lê, 2010). In addition, peroxisome proliferator-activated receptor (PPAR)-α and -γ, two members of the ligand-activated nuclear receptor superfamily, are also important transcription factors that are involved in lipid and glucose metabolism (Evans et al., 2004).

Salacia species is a traditionally herbal medicine for obesity and diabetes (Chandrasena, 1935). It has been demonstrated that the root of *Salacia oblonga* Wall. (*Celastraceae*) (SOR), one of the *Salacia* species, improves obesity- and diabetes-associated disorders, such as hyperlipidemia, fatty liver, cardiac steatosis and hypertrophy and fibrosis, as well as renal fibrosis (Li et al., 2004; Huang et al., 2006a, 2006b, 2008; Li et al., 2008; He et al., 2011; Wang et al., 2012). Although the PPAR-α activator property may be involved (Huang et al., 2006a; Rong et al., 2008), the underlying lipid metabolism-regulating mechanisms of SOR remain largely unknown.

In the present study, we tested the effects of an aqueous-ethanolic extract of SOR on fructose-induced fatty liver and further investigated the underlying mechanisms in rats.

2. Materials and methods

2.1. Preparation and identification of the aqueous-ethanolic extract of SOR

SORs were collected in Tamil Nadu, India. A voucher specimen was deposited in Pharmafood Institute, Kyoto, Japan (voucher specimen No: PS0083). SOR was identified carefully by Professor Johji Yamahara, who is an expert in taxonomy. The aqueous-ethanolic extract used in the present study was prepared. Briefly, dried SOR materials were ground into crude powder, immersed in 7 volumes of 50% ethanol (50 °C) with intermittent shaking for 5 h, and filtrated. The residue was extracted for additional 2 times using the same method. The combined filtrate was evaporated under reduced pressure below 45 °C. The yield of the extract was 20%, which was much higher than that of water extract (6.5%) (Li et al., 2004; Huang et al., 2008). The constituents of *Salacia* are numerous and may vary depending on the species and place of origin. It has been found that SOR contains mangiferin, salacinol, kotalanol and kotalagenin 16-acetate (Li et al., 2008). The aqueous-ethanolic extract was quantified by a HPLC method to contain 2.1% mangiferin, a prominent component considered suitable for the quality control of *Salacia* species and its products (Yoshikawa et al., 2001). This content was higher than those of the water extracts (0.74–1.4%) (Li et al., 2004; Huang et al., 2008; Rong et al., 2008; Wang et al., 2012).

2.2. Animals and treatment protocols

All animal procedures were in accordance with the 'Principles of laboratory animal care' (http://grants1.nih.gov/grants/olaw/references/phspol.htm) and were approved by the Animal Ethics Committee, Chongqing Medical University, China.

Male Sprague-Dawley rats weighing 210–230 g and the standard chow were supplied by the laboratory animal center, Chongqing Medical University, China. Rats were housed in a temperature controlled facility (21 ± 1 °C, 55 ± 5% relative humidity) with a 12-h light/dark cycle. Animals were allowed free access to water and the standard chow for at least 1 week prior to starting the experiments.

It is known that sugar-sweetened nonalcoholic beverages, such as soft drinks, appear as the major source of fructose for all classes of age considered, except for children younger than 6 year and adults older than 50 year (Tappy and Lê, 2010). Thus, fructose in drinking water was used in the present study, as described previously (Roglans et al., 2002; Gao et al., 2012; Wang et al., 2013; Liu et al., 2013). It has been demonstrated that SOR extract at higher dosages (>50 mg/kg) has pleiotropic pharmacological activities via multiple targets, such as α-glucosidase PPAR-α, PPAR-γ and angiotensin II type 1 receptor (Li et al., 2004, 2008; Huang et al., 2006a, 2006b, 2008; Girón et al., 2009; Shimada et al., 2011, He et al., 2011). It has been demonstrated that activation of PPAR-α by fenofibrate not only upregulates the genes responsible for fatty acid β-oxidation, but also strongly increases hepatic de novo lipogenesis by inducing the expression of hepatic lipogenic genes via the hepatic SREBP-1c pathway (Oosterveer et al., 2009). In our preliminary experiments, we found that the aqueous-ethanolic extract at 20 mg/kg did not affect intestinal alpha-glucosidase activity in mice (data not shown). The aims of the present study were to examine the effect of SOR on fructose-induced fatty liver and to further investigate the underlying new hepatic mechanisms. In order to dissociate the known mechanisms, we selected lower dosages of SOR extract in the present study. 24 rats were divided into 4 groups ($n=6$ per group): (1) water control, free access to water; (2) fructose control, free access to 10% fructose solution (w/v, preparation every day); (3) fructose SOR extract 5 mg/kg, free access to 10% fructose solution; and (4) fructose SOR extract 20 mg/kg, in which the fructose concentration was adjusted once per 3 days based on the results in the fructose control during the previous 3 days. There was no difference in body weight between the groups before treatments commenced. Animals in SOR extract-treated groups were administered SOR extract 5 or 20 mg/kg (suspended in 5% Gum Arabic solution, gavage once daily) for 10 weeks, respectively. The rats in the corresponding water- and fructose-control groups received vehicle (5% Gum Arabic) alone. All rats had free access to the standard chow. To avoid stress and more accurately monitor fructose intake, 2 rats were housed in a cage. The consumed chow and fructose solution were measured per 2 rats daily and the intake of fructose was calculated. On day 70, rats were deprived of chow, but still had free access to water (Group 1) or fructose solution (Group 2–4) overnight. Blood samples were collected by retroorbital venous puncture under ether anesthesia at 9:00–12:00 am for determination of plasma concentrations of total cholesterol (kit from Kexin Institute of Biotechnology, Shanghai, China), triglyceride (Triglyceride-E kit, Wako, Osaka, Japan), non-esterified fatty acid (NEFA) (NEFA-C kit, Wako, Osaka, Japan), glucose (kit from Kexin Institute of Biotechnology, Shanghai, China) and insulin (kit from Morinaga Biochemical Industries, Tokyo, Japan). Immediately, animals were weighed and killed by prompt dislocation of the neck vertebra. Liver was collected and weighed. The ratio of liver weight to body weight was calculated. Segments of liver were snap frozen in liquid nitrogen and stored at −80 °C for subsequent determination of gene/protein expression and triglyceride content.

2.3. Determination of triglyceride content in liver

Triglyceride content in liver was determined as described previously (Rong et al., 2010b). Briefly, 100 mg of tissue was homogenized and extracted with 2 ml of isopropanol. After centrifugation

(3000 rpm), the triglyceride content in supernatants was determined enzymatically (Wako, Osaka, Japan).

2.4. Histological examination

A portion of liver was fixed with 10% formalin and embedded in paraffin. Three-micron sections were cut and stained with hematoxylin and eosin for examination of liver histology (BX-51, Olympus Corporation, Tokyo, Japan). To further confirm lipid droplet accumulation, six-micron frozen sections were stained with Oil Red O. Forty fields in three individual sections were randomly selected, and the Oil Red O-stained area and the total tissue area were measured using an ImageJ 1.43 analyzing system. The ratio of the Oil Red O-stained area to the total tissue area was calculated (%).

Table 1
Primer sequences for real time PCR assays.

Gene	Forward primers	Reverse primers
β-actin	ACGGTCAGGTCATCACTATCG	GGCATAGAGGTCTTTACGGATG
ACC-1	AACATCCCGCACCTTCTTCTAC	CTTCCACAAACCAGCGTCTC
ACO	CCCAAGACCCAAGAGTTCATTC	TCACGGATAGGGACAACAAAGG
CD36	AACCCAGAGGAAGTGGCAAAG	GACAGTGAAGGCTCAAAGATGG
ChREBP	GAAGACCCAAAGACCAAGATGC	TCTGACAACAAAGCAGGAGGTG
CPT-1a	CTGCTGTATCGTCGCACATTAG	GTTGGATGGTGTCTGTCTCTTCC
FAS	ACCTCATCACTAGAAGCCACCAG	GTGGTACTTGGCCTTGGGTTTA
LPK	GACCCGAAGTTCCAGACAAGG	ATGAGCCCGTCGTCAATGTAG
PPAR-α	GTCATCACAGACACCCTCTCCC	TGTCCCCACATATTCGACACTC
PPAR-γ	GCCCTTTGGTGACTTTATGGAG	GCAGCAGGTTGTCTTGGATGT
SCD-1	CAGTTCCTACACGACCACCACTA	GGACGGATGTCTTCTTCCAGAT
SREBP-1c	CTGTCGTCTACCATAAGCTGCAC	ATAGCATCTCCTGCACACTCAGC

Sequences: 5′ to 3′.

2.5. Real-time PCR

Real time PCR was performed as described previously (Gao et al., 2012; Wang et al., 2013). Total RNA was isolated from livers of individual rats using TRIzol (Takara, Dalian, China). cDNA was synthesized using M-MLV RTase cDNA Synthesis Kit (Takara, Dalian, China) according to the manufacturer's instructions. Real time PCR was performed with the CFX 96 Real Time PCR Detection System (Biorad Laboratories Inc, Hercules, CA, USA) using the SYBR® Premix Ex Taq™ II (Takara, Dalian, China). The sequences of primers are shown in Table 1. The gene expression from each sample was analysed in duplicates and normalized against the internal control gene β-actin. Levels in water control rats were arbitrarily assigned a value of 1.

2.6. Western blot

Western blot was performed as described previously (Gao et al., 2012). Nuclear protein was prepared individually from livers using the NE-PER nuclear and cytoplasmic extraction reagent kit (Pierce Biotechnology, Rockford, IL, USA), according to the manufacturer's instructions. Protein concentration was determined using the Bradford method (Bio Rad Laboratories, Hercules, CA, USA) using bovine serum albumin as a standard. Nuclear protein (30 μg) was subjected to SDS-PAGE analysis on a 10% gel. Proteins were electrotransferred onto Polyvinylidene Fluoride Membrane (Amersham, Buckinghamshire, UK). SREBP-1 (dilution 1:200, Santa Cruz Biotechnology, Santa Cruz, CA, USA), ChREBP (dilution 1:1000, Novus Biologicals, Littleton, CO, USA) and stearoyl-CoA desaturase (SCD)-1 (dilution 1:200, Santa Cruz

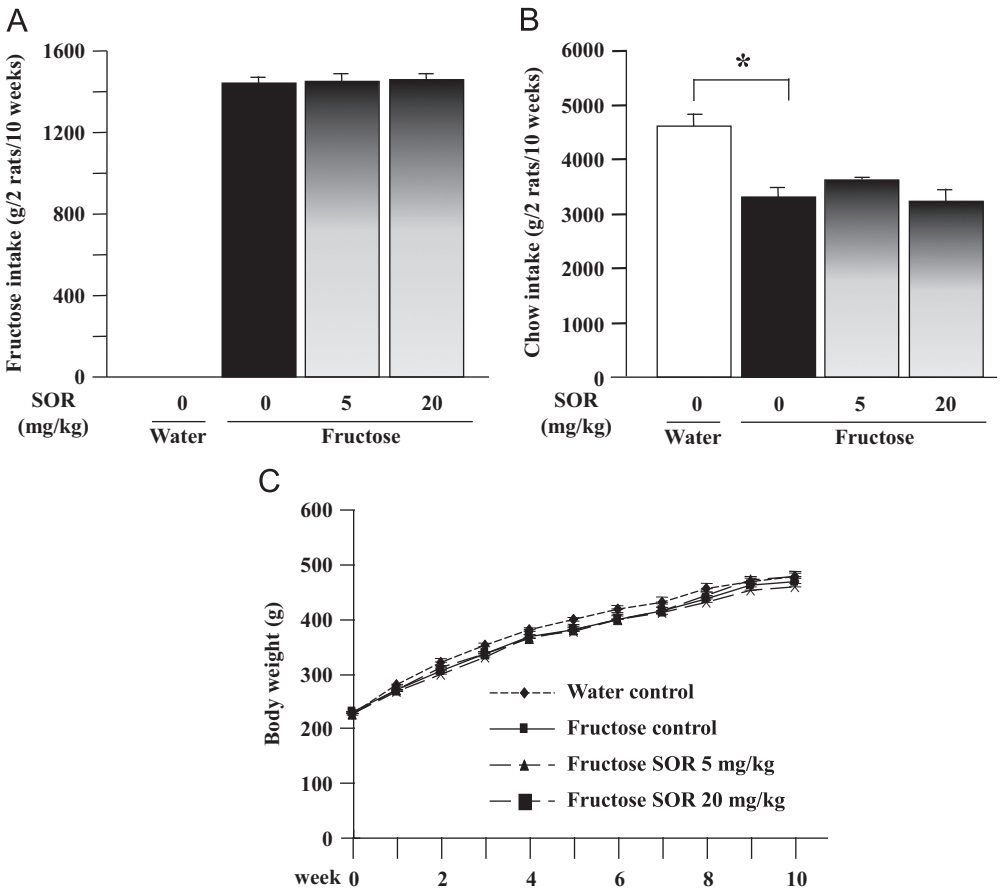

Fig. 1. Intakes of fructose (A) and laboratory chow (B), and body weight (C) in water control, fructose control and fructose *Salacia oblonga* root (SOR)-treated rats. Data are means ± SEM ($n=6$ each group). *$P < 0.05$.

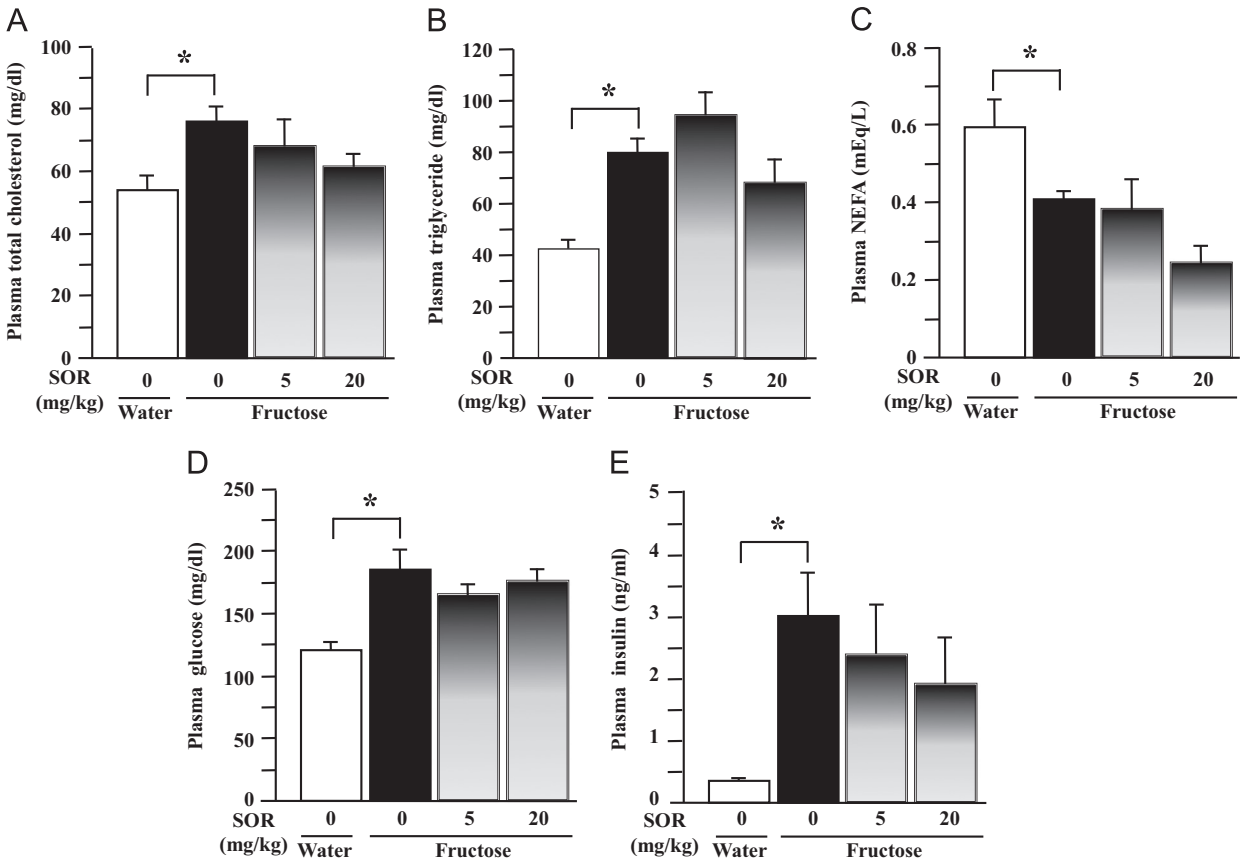

Fig. 2. Plasma total cholesterol (A), triglyceride (B), NEFA (C), glucose (D) and insulin (E) concentrations in water control, fructose control and fructose *Salacia oblonga* root (SOR)-treated rats. Data are means ± SEM ($n=6$ each group). *$P<0.05$.

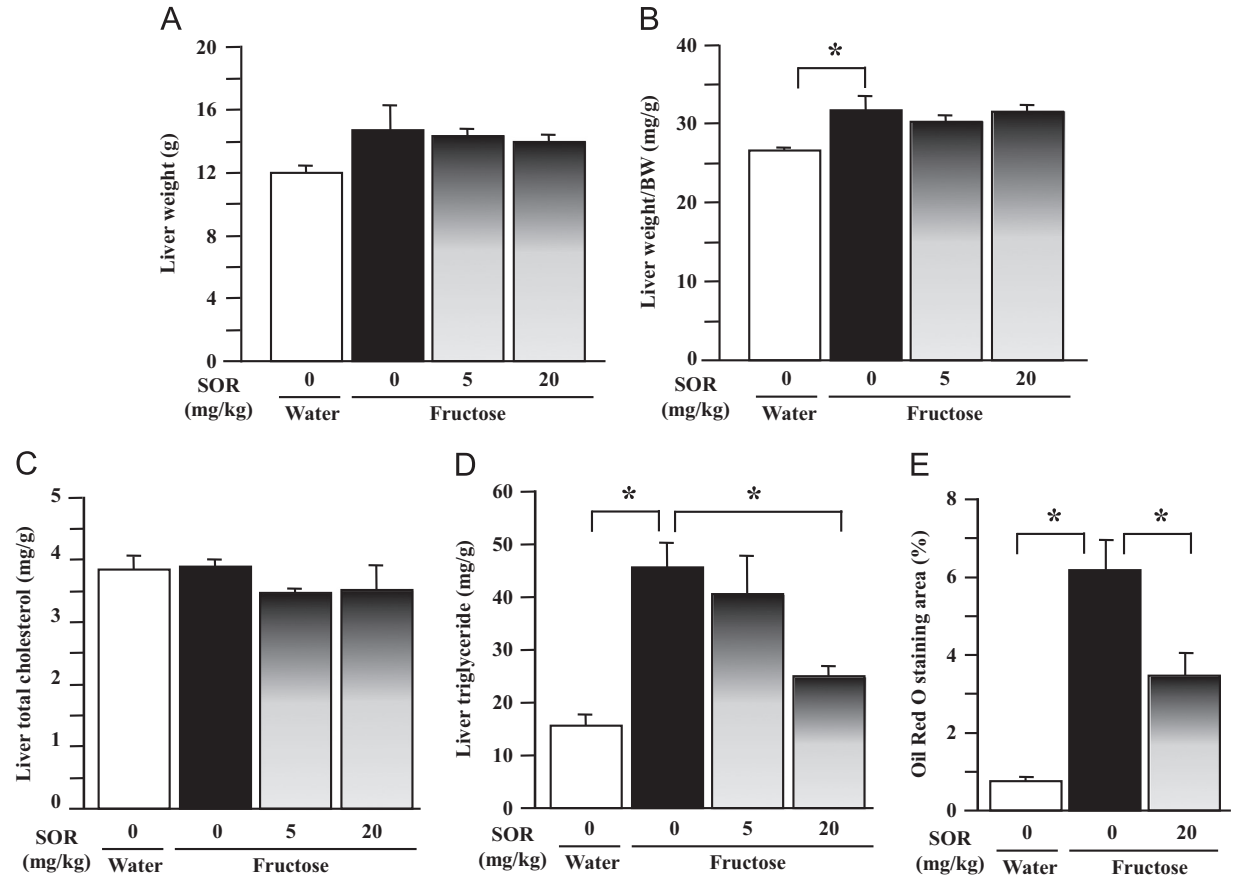

Fig. 3. Liver weight (A), the ratio of liver weight to body weight (B), Liver total cholesterol content (C), liver triglyceride content (D) and Oil Red O staining area (E) in water control, fructose control and fructose *Salacia oblonga* root (SOR)-treated rats. Data are means ± SEM ($n=6$ each group). *$P<0.05$.

Biotechnology, Santa Cruz, CA, USA) were detected with a goat polyclonal antibody and rabbit polyclonal antibody, respectively. Detection of signals was performed using the ECL Western blot detection kit (Pierce Biotechnology, Rockford, IL, USA) with anti-goat and anti-rabbit horseradish peroxidase-conjugated IgG (dilution 1:5000, Santa Cruz Biotechnology, Santa Cruz, CA, USA) as second antibody, respectively. Polyclonal rabbit Lamin A/C antibody (dilution 1:1000, Cell Signaling Technologies, Beverly, MA, USA) was used as loading control to normalize the signal obtained for nuclear SREBP-1 and ChREBP proteins. Mouse monoclonal β-Actin antibody (dilution 1:1000, Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used as loading control to normalize the signal obtained for SCD-1 protein. The immunoreactive bands were visualized by autoradiography and the density was evaluated using ImageJ 1.43. Levels in water control rats were arbitrarily assigned a value of 1.

2.7. Data analysis

All results are expressed as means ± SEM. Data were analyzed by ANOVA using the StatView software, and followed by The Student–Newman–Keuls test to locate the differences between groups. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Intakes of fructose and chow, and body weight in rats

The amount of consumed fructose is important for the development of fatty liver. In our preliminary experiments, we found that the rats treated with SOR extract at 20 mg/kg, but not 5 mg/kg, consumed more 10% fructose solution, compared to the rats treated with vehicle (data not shown). The present study focuses on the effect of SOR extract on fructose-induced fatty liver and the underlying hepatic mechanisms. In order to exclude the influence of the variability in intake of fructose, the fructose consumption in SOR extract (20 mg/kg)-treated rats was regulated to that in fructose controls. The results showed that fructose control rats ate less chow, compared to water controls, (Fig. 1B). There was no significant difference in intakes of fructose (Fig. 1A) and chow (Fig. 1B) between fructose control and fructose SOR-treated groups. All groups did not show difference in body weights (Fig. 1C).

3.2. Blood biochemical parameters in rats

Compared to water control rats, fructose controls showed higher plasma concentrations of total cholesterol (Fig. 2A), triglyceride

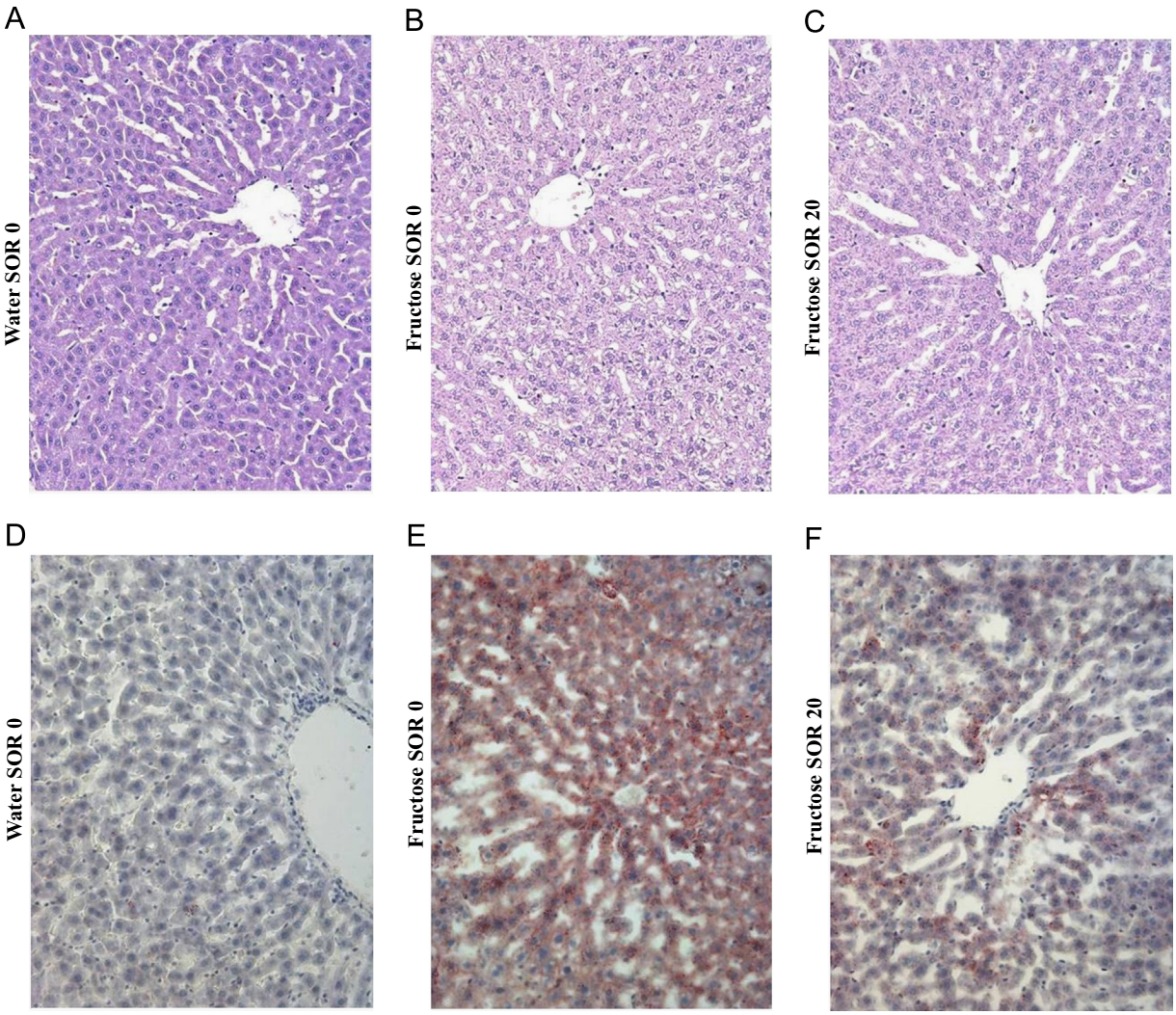

Fig. 4. Representative images showing histology of liver (Hematoxylin and eosin–staining, (A)–(C); Oil Red O staining, (D)–(F). X200) in water control, fructose control and fructose *Salacia oblonga* root (SOR)-treated rats.

(Fig. 2B), glucose (Fig. 2D) and insulin (Fig. 2E), whereas plasma NEFA concentration (Fig. 2C) was lower. SOR extract treatment at 5 and 20 mg/kg did not significantly affect plasma concentrations of total cholesterol, triglyceride, NEFA, glucose and insulin (Fig. 2A–E).

3.3. Liver-associated parameters in rats

Although fructose feeding did not significantly affect liver weight (Fig. 3A) and hepatic total cholesterol content (Fig. 3C), it increased the ratio of liver weight to body weight (Fig. 3B). Pronouncedly, hepatic triglyceride content in fructose control group was increased to 3-folds of that of water control group (Fig. 3D). In accord with this finding, fructose feeding increased vacuolization (Fig. 4B) and Oil Red O staining area (Fig. 3E, Fig. 4A, D and E), indicative of fructose-induced excess hepatic lipid droplet accumulation. SOR extract treatment (both dosages) did not alter liver weight (Fig. 3A), the ratio of liver weight to body weight (Fig. 3B) and hepatic total cholesterol content (Fig. 3C). However, SOR extract at 20 mg/kg substantially decreased hepatic triglyceride content (Fig. 3D). Consistently, vacuolization (Fig. 4C) and Oil Red O staining area (Fig. 3E and Fig. 4F) in liver were also reduced. Low dosage (5 mg/kg) of SOR extract showed minimal effect on these variables.

3.4. Hepatic gene/protein expression in rats

As the treatment with SOR extract at 5 mg/kg showed negative effects on all phenotypic parameters, comparisons in gene/protein expression are restricted to water control, fructose control and fructose SOR 20 mg/kg groups.

By real-time PCR fructose feeding increased mRNA levels of SREBP-1c (Fig. 5A), fatty acid synthase (FAS) (Fig. 5C), acetyl-CoA carboxylase (ACC)-1 (Fig. 5D), SCD-1 (Fig. 5E), ChREBP (Fig. 6A) and liver pyruvate kinase (LPK) (Fig. 6C). The increased contents of nuclear SREBP-1 (Fig. 5B) and ChREBP (Fig. 6B) proteins and SCD-1 protein (Fig. 5F) were further demonstrated by Western blot analysis. After SOR extract treatment (20 mg/kg) pronounced suppression of mRNAs encoding SREBP-1c (Fig. 5A), FAS (Fig. 5C), ACC-1 (Fig. 5D) and SCD-1 (Fig. 5E) was noted. The results of protein expression further confirmed the suppression of SREBP-1 (Fig. 5B) and SCD-1 (Fig. 5F) by SOR extract treatment. Although SOR extract treatment also decreased ChREBP mRNA level (Fig. 6A), it did not significantly alter nuclear ChREBP protein content (Fig. 6B). Consistent with the finding in nuclear ChREBP protein expression, the target gene LPK (Fig. 6C) mRNA level was also without significant change.

Ten-week fructose feeding did not alter mRNA levels of PPAR-γ, PPAR-α and CD36, but downregulated hepatic expression of carnitine palmitoyltransferase (CPT)-1a and acyl-CoA oxidase (ACO) (Fig. 6D). SOR extract treatment was without effect on expression of these genes (Fig. 6D).

4. Discussion

The present results clearly demonstrated that treatment of rats with the aqueous-ethanolic SOR extract decreased fructose

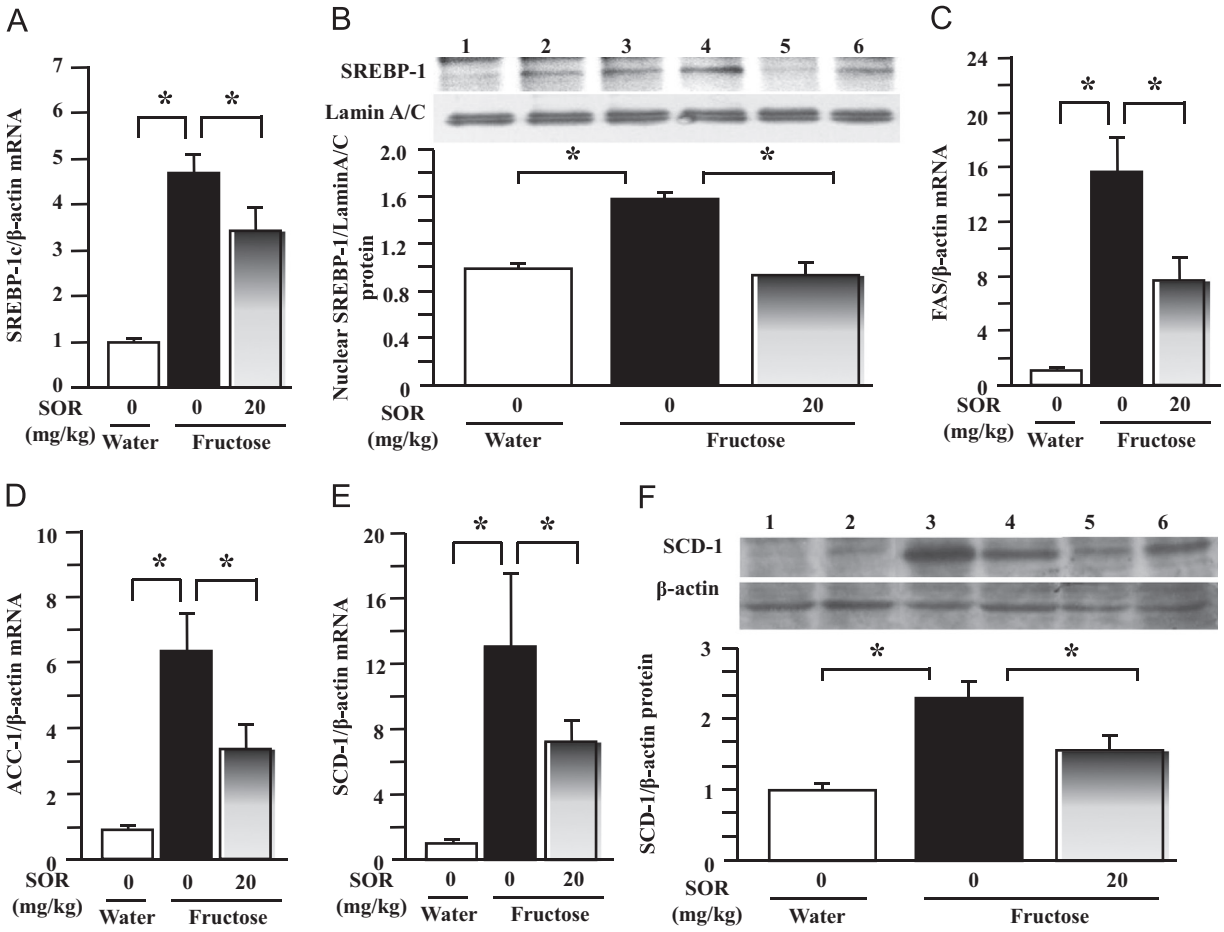

Fig. 5. Hepatic expression of mRNAs encoding sterol regulatory element-binding protein (SREBP)-1c (A), fatty acid synthase (FAS) (C), acetyl-CoA carboxylase (ACC)-1 (D) and stearoyl-CoA desaturase (SCD)-1 (E), and proteins of nuclear SREBP-1 ((B), Lane 1 and 2, water control; Lane 3 and 4, fructose control; Lane 5 and 6, fructose SOR 20 mg/kg) and SCD-1 (F, Lane 1 and 2, water control; Lane 3 and 4, fructose control; Lane 5 and 6, fructose SOR 20 mg/kg) in water control, fructose control and fructose *Salacia oblonga* root (SOR)-treated rats. Data are means ± SEM ($n=6$ each group). *$P<0.05$.

feeding-induced excess hepatic triglyceride accumulation and the increased vacuolization and Oil Red O staining area in the livers rats. These findings suggest that SOR diminishes fructose-induced fatty liver in rats.

SREBP-1c is the principal inducer of de novo hepatic lipogenesis by modulating important lipogenic enzymes, such as ACC, FAS and SCD-1 (Postic and Girard, 2008; Tappy and Lê, 2010). It has been demonstrated that deletion of SREBP-1 ameliorated fatty liver, accompanied by suppression of the overexpression of hepatic SREBP-1c downstream lipogenic genes, such as FAS and SCD-1 in ob/ob mice (Yahagi et al., 2002). Many herbal medicines and their active components have been reported to improve nonalcoholic fatty liver disease. Some herbal extracts and their components, such as *Magnolia officinalis*, *Gentiana manshurica*, green tea, resveratrol, honokiol and caffeine, have been demonstrated to improve alcohol ingestion-induced fatty liver by suppressing the increased maturation of hepatic SREBP-1c (Ding et al., 2012). We have recently demonstrated that oleanolic acid, a naturally-occurring triterpenoid, improves fructose-induced fatty liver via the hepatic SREBP1c pathway (Liu et al., 2013). In the present study, SOR extract treatment did not affect chow and fructose intakes and body weight, and was with minimal effect on plasma concentrations of total cholesterol, triglyceride, NEFA and glucose. However, this treatment substantially suppressed fructose feeding-stimulated hepatic overexpression of both SREBP-1c mRNA and nuclear SREBP-1 protein. Further, the overexpression of SREBP-1c downstream genes ACC-1, FAS and SCD-1, was also downregulated. Thus, these results suggest that modulation of hepatic SREBP-1c-mediated expression of the genes responsible for de novo fatty acid synthesis contributes to the anti-steatotic effect of SOR in rats.

ChREBP is also involved in hepatic lipid synthesis (Uyeda and Repa, 2006; Postic and Girard, 2008). Although ChREBP is localized in the cytosol, the endogenous ChREBP protein is addressed into the nucleus in response to a high-carbohydrate diet in liver of mice (Uyeda and Repa, 2006). Thus, ChREBP in the nucleus is a key determinant of its functional activity (Uyeda and Repa, 2006). ChREBP, but not SREBP-1c, is responsible for transcription of LPK (Yamashita et al., 2001; Stoeckman and Towle, 2002; Ishii et al., 2004). We have recently demonstrated that treatment with the extract of ginger, another important traditional medicine and spice, improves fructose-induced fatty liver and hypertriglyceridemia, accompanied by suppression of overexpression of hepatic nuclear ChREBP protein, but not SREBP-1c in rats (Gao et al., 2012). In the present study, SOR extract treatment did not significantly alter the nuclear ChREBP protein level, although ChREBP mRNA expression was decreased. Further, overexpressed ChREBP-targeted gene LPK mRNA and hypertriglyceridemia were also without significant change after SOR extract treatment. Thus, these findings suggest that the ChREBP-mediated pathway may play a less role in the anti-steatotic effect of SOR extract.

PPAR-α and -γ are also involved in hepatic lipid accumulation by regulating the genes, such as CPT-1a, ACO, and CD36 (Evans et al., 2004). We have previously demonstrated the aqueous extract of SOR at the higher dosage (100 mg/kg) as a hepatic PPAR-α activator (Huang et al., 2006a). SOR was also reported to increase glucose transporter 4-mediated glucose uptake in L6 rat myotubes, in which

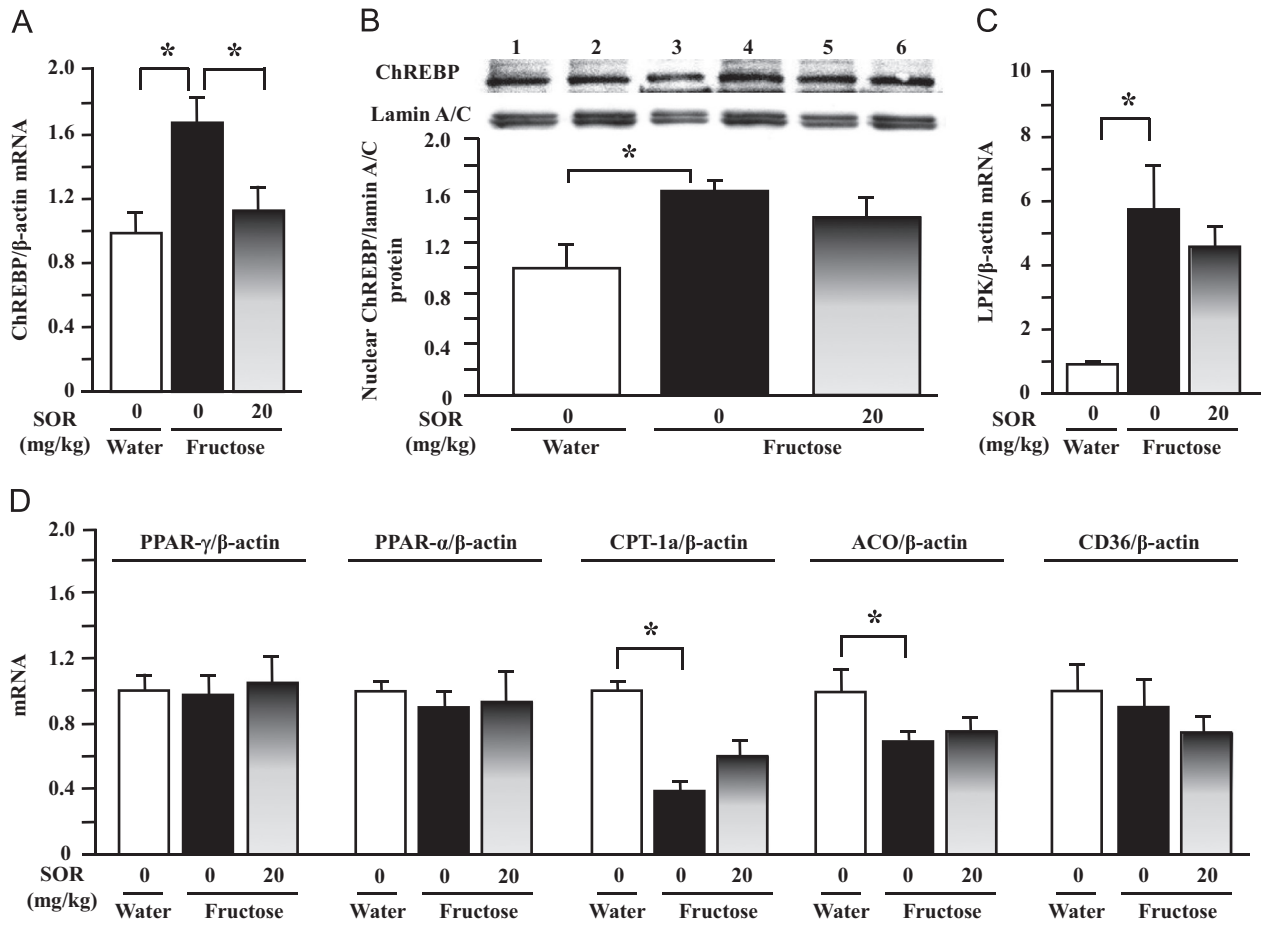

Fig. 6. Hepatic expression of mRNAs encoding carbohydrate response element binding protein (ChREBP) (A), liver pyruvate kinase (LPK) (C), peroxisome proliferator-activated receptor (PPAR)-γ, PPAR-α, carnitine palmitoyltransferase (CPT)-1a, acyl-CoA oxidase (ACO) and CD36 (D), and nuclear ChREBP protein ((B), Lane 1 and 2, water control; Lane 3 and 4, fructose control; Lane 5 and 6, fructose SOR 20 mg/kg) in water control, fructose control and fructose *Salacia oblonga* root (SOR)-treated rats. Data are means ± SEM ($n=6$ each group). *$P < 0.05$.

PPAR-γ was involved (Girón et al., 2009). *Salacia reticulata*, another *Salacia* species, was shown to inhibit PPAR-γ-mediated gene expression in 3T3-L1 adipocytes (Shimada et al., 2011). However, SOR extract at 20 mg/kg in the present study neither affected mRNA levels of PPAR-α and its target genes CPT-1a and ACO, nor altered expression of PPAR-γ and its downstream gene CD36 in the livers of fructose-fed rats. Thus, the present findings dissociate the modulation of hepatic SREBP-1c pathway with the PPAR-α and -γ modulator properties in the anti-steatotic effect of SOR in fructose-fed rats. It is possible that the experimental conditions, especially different dosages and tissues, are involved in the discrepancy in PPAR-α- and -γ-mediated gene expression.

In conclusion, our present results suggest that modulation of hepatic sterol regulatory element-binding protein-1c-mediated gene expression contributes to *Salacia oblonga* root-elicited improvement of fructose-induced fatty liver in rats. Our findings provide a better understanding of *Salacia oblonga* in the treatment of obesity and diabetes.

Acknowledgements

This work was financially supported by National Natural Science Foundation of China (Grant 30970872), China, and R&D Agency for Curative Natural Products (a Japanese government-registered non-profit organization), Kyoto, Japan.

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