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Cinnamic acid ameliorates diet-induced hyperlipidemia in Wistar rats through antioxidant and gene expression modulatory effects

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ABSTRACT

Background: Diet- induced hyperlipidemia is strongly associated with metabolic disorders and cardiovascular diseases. This study explored the ameliorating effects of cinnamic acid (CA) on high- fat diet (HFD)- induced hyperlipidemia in Wistar rats.

Methods: Male Wistar rats were arranged into four groups based on their feeding pattern: control, HFD, control + CA, or HFD + CA. CA was orally administered every day (50 mg/kg body weight). The feeding was continued for 8 weeks, after which the rats were sacrificed, and oxidative stress-associated parameters such as MDA, NO, AOPP, GSH, SOD, and catalase, lipid profiles, and liver enzyme levels were investigated in the serum. Using hepatic tissues, the mRNA levels of several proteins related to the metabolism, uptake, transportation, and storage of lipid was also explored.

Results: Administration of CA significantly ($p<0.05$) mitigated HFD- induced oxidative stress and increased hepatic enzyme activity. It also prevented the HFD- induced increased mRNA levels of SREBP-2, PPARγ, HMGCR, and Apo-B100. HFD- mediated suppression of LDLR, ABCA1, and Apo-A1 mRNA was significantly ($p<0.05$) increased by feeding CA. All these positive effects resulted in the reduction of liver weight, adipose tissue weight, and overall body weight, and pro- atherogenic lipid levels, except TG, along with an increase in HDL cholesterol levels.

Conclusion: CA positively influences HFD- induced hyperlipidemia and adiposity in Wistar rats through its antioxidant, hepatoprotective, and gene expression modulatory effects.

INTRODUCTION

High-fat diet-induced oxidative stress, mitochondrial dysfunction, and inflammation disturb the normal patterns of glucose and lipid metabolism, leading to hyperglycemia and hyperlipidemia [1]. The vital characteristics of hyperlipidemia are increased low-density lipoprotein cholesterol (LDLC), total cholesterol (TC), and triacylglycerol (TG), together with reduced high- density lipoprotein cholesterol (HDLC) in the blood. These abnormalities of lipid levels are the primary contributors to the pathogenesis of cardiovascular diseases (CVDs) [2]. Therefore, reducing TG, TC, and LDLC with simultaneous augmentation of HDLC is the primary goal for the management of hyperlipidemia and obesity-related health issues. Although synthetic lipid-lowering drugs are generally prescribed to manage hyperlipidemia and obesity, their adverse effects have contributed to a growing demand for antihyperlipidemic drugs with less toxicity and higher efficacy [3]. Recognizing the demand for safer and more effective alternatives to synthetic drugs, exploring plant-derived remedies may offer a potential solution to combat hyperlipidemia and obesity-related complications.

Cinnamon has been valued for centuries not only for its culinary use as a spice but also for its therapeutic properties, particularly in the prevention and cure of disorders connected to metabolic syndrome. Clinical studies on human volunteers and patients have suggested that dietary supplementation of cinnamon offers multiple benefits, including prevention of inflammation and oxidative stress as well as reduction of blood pressure, lipids, and glucose [4, 5]. Researchers have attributed these therapeutic effects of cinnamon to its essential oil, which contains a diverse array of bioactive phytochemicals, including cinnamaldehyde, eugenol, coumarin, tannins, lignin, flavonoids, and volatile oils [6]. Recent investigations using animal models of diseases have revealed that cinnamic acid and related compounds offer a broad range of therapeutic activities against cancer, diabetes, neurodegeneration, hyperlipidemia, and microbial infections [7]. Animal models have also reported that the consumption of cinnamic acid and its derivatives reduces the levels of harmful lipids, including LDLC, TG, TC, and VLDLC, and increases the levels of beneficial lipids, such as HDLC [8-10]. Researchers have credited these positive effects of cinnamic acid and its metabolites on humans and animals to their anti-inflammatory, antioxidant, hypolipidemic, and antihyperglycemic activities [11]. Apart from humans and other animals. In vitro investigations have revealed that cinnamic acid can lower palmitic acid- and oleic acid-induced triglyceride (TG) and overall lipid deposition in HepG2 cells [12]. This compound also suppressed palmitic acid-induced increases in the expression of fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), and carnitine palmitoyltransferase 1 (CPT-1) in NIT-1 cells, a mouse cell line obtained from the pancreatic β-cells [13].

Despite these promising in vivo and in vitro effects, the molecular mechanisms via which cinnamic acid modulates lipid metabolism, homeostasis, and storage remain elusive. For example, the effects of cinnamic acid on the expression of peroxisome proliferator- activated receptors, liver X receptors, sterol-regulatory element binding proteins (SREBPs), LDL-receptors, HMG-CoA reductase, and apolipoproteins remain unexplored. Therefore, an elaborate mapping of transcription factors, enzymes, receptors, and related proteins may help to understand the effects of this compound on lipid transportation, metabolism, and distribution. In this study, we explored the antihyperlipidemic effect of cinnamic acid in HFD- induced hyperlipidemic Wistar rats, focusing on its antioxidant, hepatoprotective, and gene expression modulatory activities.

METHODS

Chemicals and reagents

Cinnamic acid, thiobarbituric acid, trichloroacetic acid, sulfanilamide, and malondialdehyde tetrabutylammonium salt (analytical standard), were purchased from Wako Chemicals (Chuo-ku, Osaka, Japan). The commercial kits used for the assay of total cholesterol, HDLC, LDLC, and TG levels, and liver enzymes' activity were obtained from Abnova (Taipei, Taiwan). Reagents for RNA isolation, purification, conversion to cDNA, and SYBR Green Master Mix for performing quantitative real- time PCR were purchased from Thermo Fisher Scientific, USA. The oligonucleotides used as primers were purchased from Promega Corporation, South Korea. Other reagents and chemicals required for the assessment of nitric oxide, malondialdehyde, advanced oxidation protein products, catalase, superoxide dismutase, and reduced glutathione were procured from Scharlau, Spain.

Experimental design and animal feeding

Healthy 8–9-week-old male Wistar rats were reared in separate cages and placed in a facility maintained at a comfortable temperature (22–25°C) with a 12 h light/dark cycle with random access to food and water. The well-being and comfort of the rats were ensured via a protocol approved by the IACUC of North South University, Bangladesh (ACE-029-2023). The rats were arbitrarily arranged into 4 groups, each of which consisted of 7 animals. The groups were as follows:

Control: Consumed a normal diet and water

HFD: Consumed a high-fat diet and water

Control + CA: Consumed a normal diet with 50 mg/kg/day cinnamic acid

HFD + CA: Consumed an HFD with 50 mg/kg/day cinnamic acid

The composition of AIN-76A described by the American Institute of Nutrition was used as a framework for the preparation of the control diet and HFD, which are described in detail in Supplementary Table 1. The quantity of ingredients was maintained to ensure that each kg of a normal diet (Control) contained 3782 kcal, with fat providing 11.9% [14]. On the other hand, each kg of HFD contained 5152 kcal of fat energy, with fat contributed 52.4%. Feeding was continued for 8 weeks during which the food consumption pattern and its effect on body weight were monitored every day previously outlined procedure [15].

Collection of feces, serum, liver, and adipose tissue

After 8 weeks of treatment, rats were euthanized via ketamine injection (100 mg/kg, SC) and sacrificed for blood, tissue, and organ collection. The serum was separated by centrifugation (at 8000 × g) for 15 minutes and stored at -18 °C for later study. The fatty tissue attached to the surface of the liver was separated, and the weight of the wet liver was recorded. The peritoneal, epididymal, and mesenteric adipose tissues were cautiously collected, rinsed with cooled PBS, and weighed according to a previous method [16].

Measurement of oxidative stress-related parameters

The concentration of malondialdehyde (MDA) was measured to evaluate the degree of lipid peroxidation. This was performed by the spectrophotometric detection of a 1:2 complex between MDA and TBA (thiobarbituric acid) at 533 nm [17]. The nitric oxide concentration was quantified after mixing the serum with a reagent containing 1.0% (w/v) sulfanilamide in an acidic solution and 1% (w/v) N-(1) naphthyl ethylenediamine in water. The absorbance of the mixture was measured at 546 nm, and the level of nitric oxide in the serum was computed using a standard curve [18]. The advanced oxidation protein product (AOPP) level was estimated by a spectrophotometer at 340 nm according to an earlier method [19]. The level of reduced glutathione (GSH) in the plasma sample was quantified using Ellman's reagent, which involves measuring the absorbance of a yellow anion of thionitrobenzoic acid (TNB) at 410 nm [20]. Superoxide dismutase (SOD) activity was recorded at 560 nm following the procedure established by Kakkar et al. [21]. Catalase activity was assayed using a commercial kit (Abcam Ltd. (Cambridge, UK), which measures the concentration of remaining hydrogen peroxide as a function of enzymatic activity. In this method, unreacted H_2O_2 reacts with a probe to produce a complex that can be measured at 570 nm [22].

Assessment of plasma lipid and hepatic enzyme levels

The concentrations of total cholesterol (TC), triacylglycerol (TG), low-density lipoprotein cholesterol (LDLC), and high-density lipoprotein cholesterol (HDLC) were assayed in plasma samples using diagnostic kits for the corresponding lipids purchased from Abnova Ltd. (Taipei, Taiwan). For the measurement of hepatic marker enzymes, including alanine transaminase (ALT), alkaline phosphatase (ALP), and aspartate transaminase (AST), commercial diagnostic kits from the same company were used.

Quantification of gene expression

An RNA extraction kit (Thermo- Fisher Scientific, USA) was used to isolate mRNA from the hepatic tissue. After the concentration of mRNA was measured with a NanoDrop2000 (Bio-Rad, USA), 1 µg of mRNA from each sample was used as a template to generate complementary DNA using a cDNA synthesis kit (Promega Corporation, South Korea) in a thermal cycler. This cDNA was then used for quantitative real- time PCR using the GoTaq® qPCR Master Mix (Promega Corporation, South Korea). To quantify the mRNA levels of the target genes, primers were designed using the Primer3 Plus online tool and are listed in Supplementary Table 2. The quantitative RT PCR was conducted using the program developed by Khan et al. [23] in a Thermal Cycler Dice Real Time System Single (Takara, Japan). The data were collected and evaluated using software provided by the same manufacturer. To measure the transcript level of a target protein in a particular sample, the transcript level of β-actin in the same sample was used as a control.

Histology

For the histological evaluation of the effect of CA on HFD-induced hyperlipidemia, the hepatic tissues of the rats from each group were fixed in 10% neutral buffered formalin (NBF) and subsequently treated with ethanol and xylene according to a previous method [24]. The tissues were carefully embedded in the paraffin slab. The tissues were then cut using a microtome into thin (5 µm) transparent slices, which were subsequently attached to glass slides and then stained with hematoxylin/eosin (H&E). All slides with stained tissues were photographed using an optical microscope (Zeiss Axioscope, Germany) at 40x magnification. The photomicrographs were further analyzed using ImageJ software, and the area covered by the lipids was measured.

Statistical analysis

All tests, including body weight changes, biochemical assays, and mRNA level quantification, were performed in sextuplicate (n = 6). The results are shown as the mean ± standard error of the mean (SEM). For the detection of significant differences between the two groups, values were evaluated by one- way ANOVA, followed by Dunnett's multiple comparisons using Graph Pad Prism. The number of rats in each group (n = 6) and the total number of rats for the whole study (6*4 = 24) were determined based on power calculation using G*Power version 3.1. The expected effect size of 1.4 with a significance level (α) less than 0.05 and power level (1- β) greater than 95% ensured the scientific validity of the observed effects and justified the ethical issue regarding the animal number used in the study.

RESULTS

Effects of cinnamic acid on body weight, liver weight, and fat weight

Consumption of high-fat diet (HFD) resulted in a significant ($p<0.05$) increase in the body weight of the rats compared to the normal diet (control) consuming group. The same pattern of weight change was also observed in the weight of liver adipose tissues, such as mesenteric, epididymal, and peritoneal fat. CA consumption (50 mg/ kg/ day) markedly ($p<0.05$) prevented HFD- mediated increases in body and liver weight. Compared with the HFD- fed rats, the mesenteric and peritoneal fat deposition in HFD- fed rats was also significantly lower. CA consumption did not significantly attenuate the HFD-mediated increase in epididymal fat weight.

HFD consumption also resulted in increased fat content in feces. However, the consumption of CA with a HFD did not significantly ($p<0.05$) affect fecal fat content (Fig. 1).

Effects of cinnamic acid on HFD-induced oxidative stress

The concentrations of oxidative stress markers and antioxidant enzyme activity were evaluated in the blood of all four groups of rats. Feeding an HFD resulted in a significant ($p<0.05$) increase in the levels of malondialdehyde (MDA), nitric oxide (NO), and advanced oxidation protein products (AOPP). However, an HFD resulted in a decrease in the level of reduced glutathione (GSH) with a reduction in the activity of enzymes such as superoxide dismutase (SOD) and catalase. Oral administration of CA to HFD-fed rats resulted in significant ($p<0.05$) reductions in MDA and NO levels and an increase in GSH levels. The catalytic power of SOD and catalase was

also increased significantly ($p<0.05$) by CA consumption in HFD-fed rats. Feeding CA to rats with normal diet did not alter the abovementioned parameters (Fig. 2).

Effects of cinnamic acid on HFD-induced hepatic stress and hyperlipidemia

Feeding an HFD resulted in a significant ($p < 0.05$) increase in the activity of hepatic enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) (ALT, AST, and ALP) in comparison to the normal diet-fed rats. The oral administration of 50 mg/kg/day CA for 8 weeks significantly ($p < 0.05$) suppressed the serum ALT and ALP levels in the HFD + CA group compared with the HFD group. However, CA administration in the same pattern for 8 weeks did not alter the HFD-mediated increase in AST levels (Fig. 3A-C).

The HFD caused an increase in serum triglyceride (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDLC) levels compared with those of rats fed a normal diet (control group). However, the level of HDLC decreased in rats that consumed an HFD. In contrast, rats fed an HFD plus CA exhibited significant ($p < 0.05$) reductions in TC and LDLC. Feeding CA was not effective in reducing HFD-induced elevated TG levels. The HFD-mediated decrease in HDLC levels was significantly ($p<0.05$) increased by the oral consumption of CA. The consumption of CA by control diet-fed rats did not affect any of these lipid parameters (Fig. 3D-G).

Effects of cinnamic acid on transcription factors

This part of the current study aimed to explore the effects of an HFD and CA on the expression of crucial transcription factors connected to lipid metabolism and adipogenesis, such as sterol regulatory element binding proteins 1a, 1c, and 2 (SREBP-1a, SREBP-1c, and SREBP2), liver X receptor α (LXRα), peroxisome proliferator-activated receptor γ (PPARγ), and CCAAT enhancer binding protein α (C/EBPα). Compared with the control diet, the HFD significantly ($p<0.05$) increased SREBP-1a (2.2-fold), SREBP-1c (1.57-fold), SREBP2 (1.87-fold), LXRα (1.61-fold), PPARγ (1.63-fold), and C/EBPα (2.01-fold) expression. CA consumption reduced the HFD-mediated increased expression of SREBP2 and PPARγ. The downregulation of HFD-induced increases in the mRNA expression of SREBP-1a, SREBP-1c, LXRα, and C/EBPα was not statistically significant (ns). In all these cases, the consumption of CA by the control group did not cause any changes in the expression of any of these 6 transcription factors (Fig. 4).

The impact of cinnamic acid on the expression of downstream proteins

This part of our study aimed to evaluate the effects of CA on the expression of lipid metabolism, cellular uptake, efflux, and transportation-related proteins. The transcript levels of HMG-CoA reductase (HMGCR), low-density lipoprotein receptor (LDLR), fatty acid synthase (FAS), ATP-binding cassette transporter protein A1 (ABCA1), apolipoprotein (A1Apo-A1), and apolipoprotein B100 (Apo-B100) were quantified. In HFD-fed rats, the transcript levels of HMGCR (1.76-fold), FAS (1.86-fold), and Apo-B100 (1.84-fold) increased significantly ($p<0.05$), indicating increased cholesterol and lipid synthesis. CA treatment with an HFD reduced the expression of HMGCR and Apo-B100. However, HFD-induced augmented FAS gene expression was not significantly ($p<0.05$) reduced by treatment with CA. The expression of LDLR (0.462-fold), ABCA1 (0.52-fold), and Apo-A1 (0.41-fold) was markedly ($p<0.05$) downregulated by HFD consumption. CA feeding with an HFD significantly ($p<0.05$) prevented the downregulation of LDLR, ABCA1, and Apo-A1 (Fig. 5).

Effect of cinnamic acid on HFD-induced hepatic fat accumulation

The effect of varying dietary conditions on the accumulation of liver fat was evaluated by hematoxylin and eosin staining of the hepatic tissues. ImageJ software was used to measure the area covered by lipid droplets. As revealed in the photomicrographs, the number and size of lipid droplets (LDs) increased markedly ($p<0.05$) in response to a high-fat diet (HFD) compared to the normal diet (Control). CA consumption reduced the number and size of HFD-induced lipid droplets (LD) as revealed in the photomicrographs (Fig. 6A-6D). HFD feeding also promoted the accumulation of lipids in the hepatic tissue, as revealed in the area covered by lipid droplets. The HFD-mediated increase in lipid accumulation was also significantly ($p<0.05$) reduced by CA feeding (Fig. 6E). However, oral CA administration to the control group did not change the size

of lipid droplets or the area occupied by lipids (Fig. 6).

Figures

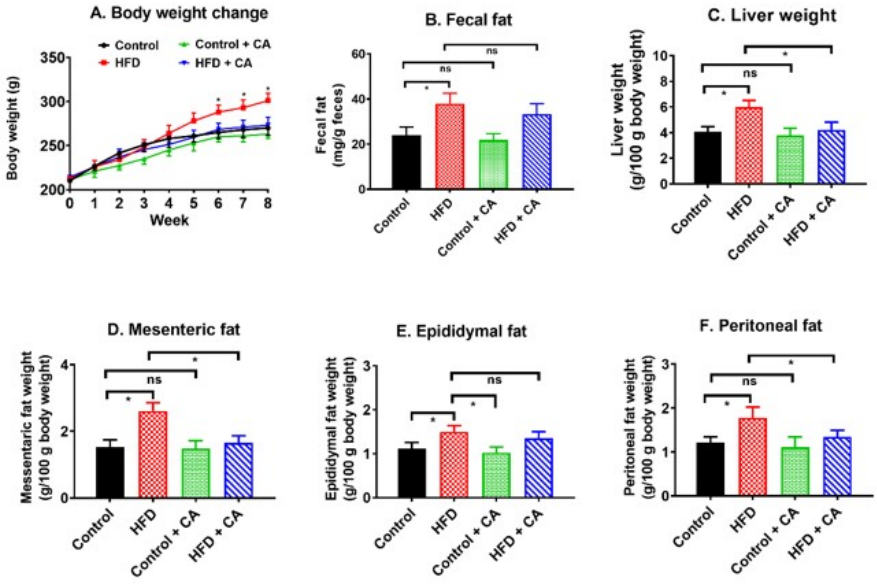

Fig 1: Impact of cinnamic acid (CA) on HFD-induced increased (A) body weight, (B) fecal fat content, (C) liver weight, (D) mesenteric fat, (E) epididymal fat, and (F) peritoneal fat. Four groups of rats were fed with one of the following four diets: normal diet (Control), high-fat diet (HFD), normal diet with CA (Control + CA), or HFD with CA (HFD + CA). Body weight changes were recorded throughout the experimental period (8 weeks); stool was collected before the day of sacrifice, and fecal fat content was measured. Liver weight, mesenteric fat, epididymal fat, and peritoneal fat were collected immediately after the rats were sacrificed. The values are presented as the mean ± SEM, n = 6. For comparisons, a one-way ANOVA was conducted, followed by Dunnett's multiple comparison test. Significantly different mean values are indicated by an asterisk (*) when p<0.05

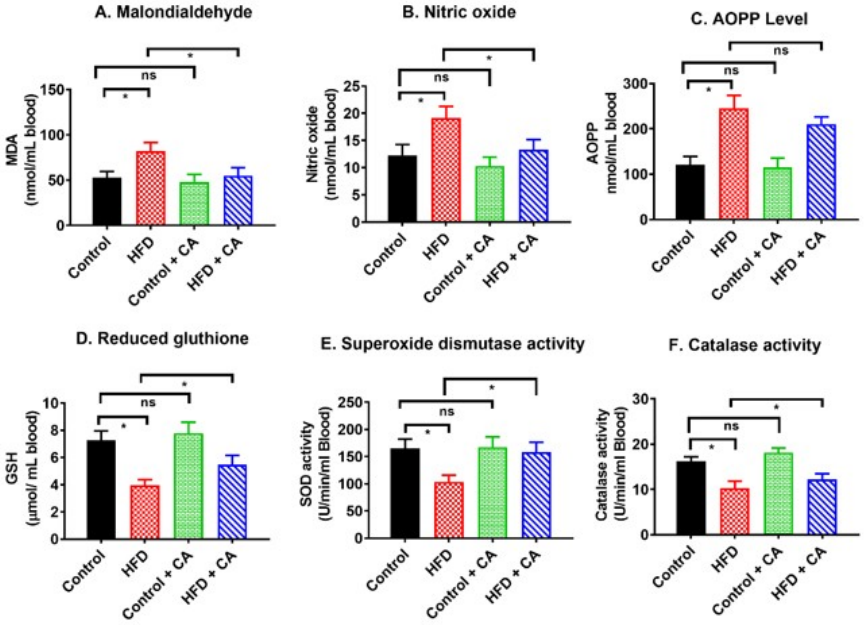

Fig 2: Impacts of cinnamic acid (CA) on oxidative stress-related parameters such as (A) MDA content, (B) NO content, (C) AOPP content, (D) GSH content, (E) SOD activity, and (F) catalase activity. Four groups of rats were fed with one of the following four diets: normal diet (Control), high-fat diet (HFD), normal diet with CA (Control + CA), or HFD with CA (HFD + CA). The aforementioned parameters (A-F) were measured in serum samples from rats in the corresponding groups. The values are presented as the mean ± SEM, n = 6. For comparisons, a one-way ANOVA was conducted, followed by Dunnett's multiple comparison test. Significantly different mean values are indicated by an asterisk (*) when p<0.05.

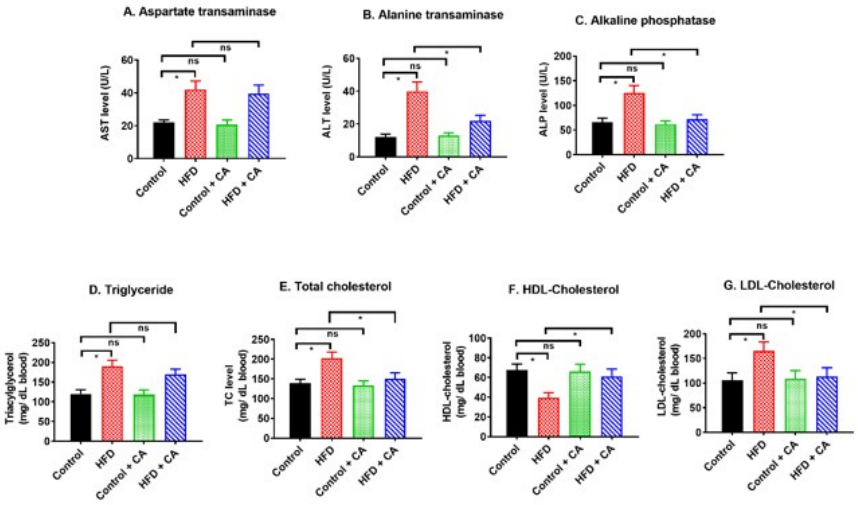

Fig 3: Impact of cinnamic acid (CA) on hepatic enzyme activity and plasma lipids levels. Four groups of rats were fed with one of the following four diets: normal diet (Control), high-fat diet (HFD), normal diet with CA (Control + CA), or HFD with CA (HFD + CA). The activity of hepatic enzymes such as (A) AST, (B) ALT, and (C) ALP and plasma lipid-related parameters including (D) TG, (E) TC, (F) HDLC, and (G) LDLC were measured in the serum samples from the rats in the corresponding groups. The values are presented as the mean ± SEM, n = 6. For comparisons, a one-way ANOVA was conducted, followed by Dunnett's multiple comparison test. Significantly different mean values are indicated by an asterisk (*) when p<0.05.

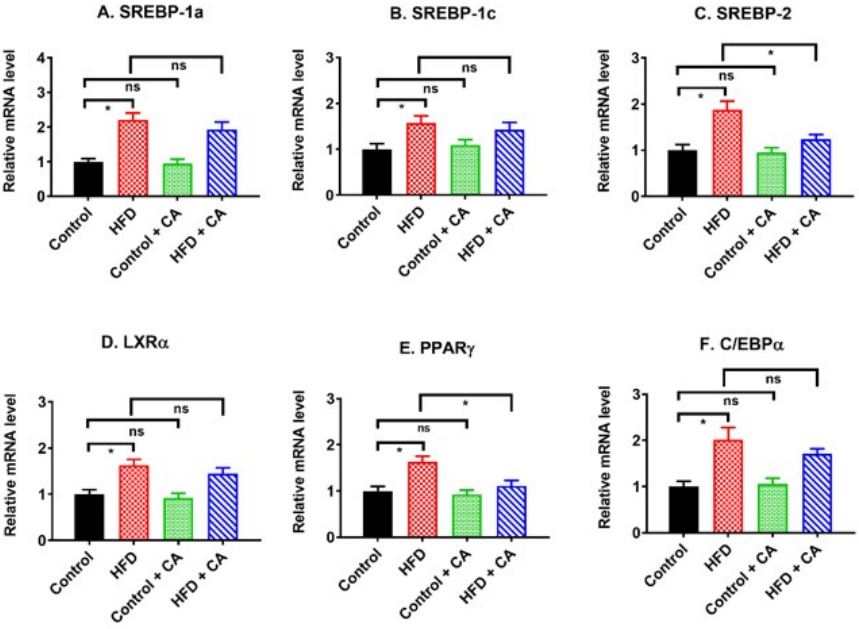

Fig 4: Impact of cinnamic acid (CA) feeding on the transcript levels of (A) SREBP-1a, (B) SREBP-1c, (C) SREBP2, (D) LXRα, (E) PPARγ, and (F) C/EBPα. Four groups of rats were fed with one of the four diets as mentioned: normal diet (Control), high-fat diet (HFD), normal diet with CA (Control + CA), or HFD with CA (HFD + CA). To quantify transcript levels, total mRNA was collected from the hepatic tissue of six rats from each group. The mRNA was converted to cDNA, and then real-time PCR was performed. The values are presented as the mean ± SEM, n = 6. For comparisons, a one-way ANOVA was conducted, followed by Dunnett's multiple comparison test. Significantly different mean values are indicated by an asterisk (*) when p<0.05.

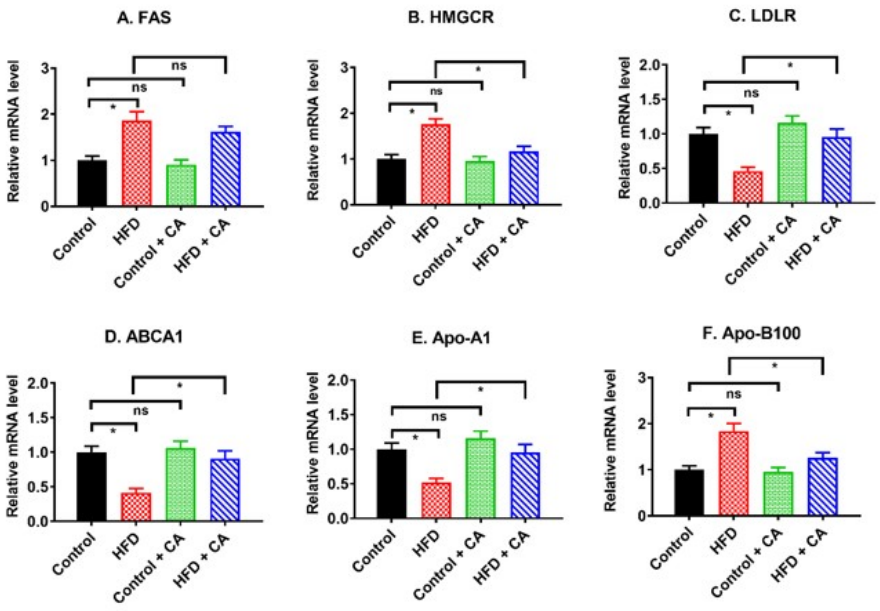

Fig 5: Impact of cinnamic acid (CA) feeding on the expression of (A) HMGCR, (B) LDLR, (C) FAS, (D) ABCA1, (E) Apo-A1, and (F) Apo-B100. Four groups of rats were fed one of the following four diets: normal diet (Control), high-fat diet (HFD), normal diet with CA (Control + CA), or HFD with CA (HFD + CA). To quantify transcript levels, total mRNA was collected from the hepatic tissue of six rats from each group. The mRNA was converted to cDNA, and then real-time PCR was performed. The values are presented as mean ± SEM, n = 6. For comparisons, one-way ANOVA was conducted followed by Dunnett's multiple comparison test. Significantly different mean values are indicated by an asterisk (*) when p<0.05.

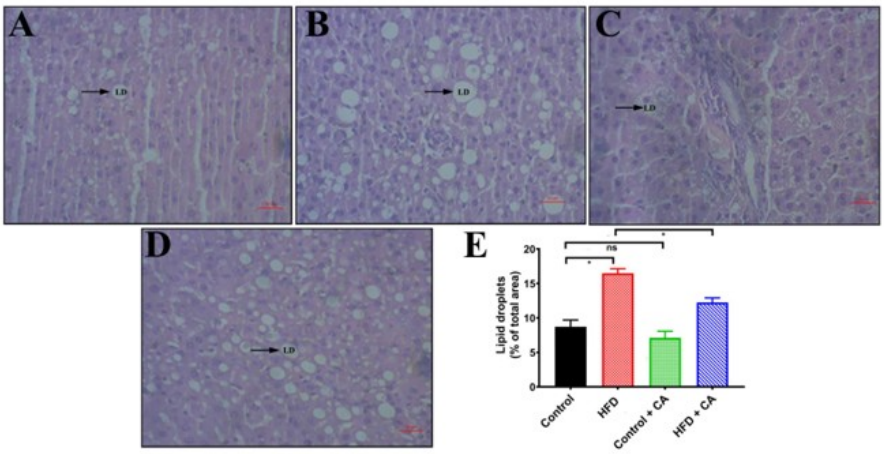

Fig 6: Photomicrographs (A-D) showing the accumulation of lipids in the hepatic tissue of Wistar rats fed with any one of the following diets: normal diet (Control), high-fat diet (HFD), normal diet with CA (Control + CA), or HFD with CA (HFD + CA). Lipid droplets (LDs) were captured at 40x magnification by fluorescence microscopy (Carl Zeiss). Additionally, the percentage of the area taken up by lipid droplets (E) was measured using ImageJ software. To determine statistical significance, the percent area was compared using one-way ANOVA followed by Dunnett's post hoc test and expressed as mean ± SEM (n = 6). Significantly different mean values are indicated by an asterisk (*) when p<0.05.

DISCUSSION

A high-fat diet (HFD) induces oxidative stress and metabolic abnormalities in many animals, including Wistar rats [25]. HFD reduces the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx), thereby diminishing the degradation of peroxide and superoxide, which increases the level of reactive oxygen species (ROS) [26]. An increased level of ROS intensifies chronic inflammation together with lipid and carbohydrate metabolism abnormalities, which may lead to hyperlipidemia and hyperglycemia [27]. In the present study, we also observed that HFD consumption intensified oxidative stress in parallel with a decrease in antioxidant enzyme activity and reduced glutathione (GSH) levels. Feeding with cinnamic acid (CA) restored the activity of antioxidant enzymes and GSH, as

reflected by reduced levels of malondialdehyde (MDA), nitric oxide (NO), and advanced oxidation protein product (AOPP) (Fig. 2). The observations of the current study are consistent with an earlier investigation in which CA prevented gentamicin- induced oxidative stress in rats by lowering NO and MDA levels while stimulating the catalytic activity of glutathione peroxidase and catalase [28]. In addition, our findings (Fig. 2) are in agreement with another investigation in which CA mitigated oxidative stress and restored antioxidant enzyme activity in streptozotocin-induced diabetic rats [29].

In this study, HFD-induced oxidative stress was also accompanied by increased blood glucose as revealed in the oral glucose tolerance test (Supplementary Fig.). HFD- induced oxidative stress also increased the harmful lipids along with a decrease in the level of HDL cholesterol (Fig. 3). In contrast, the CA-mediated reduction in oxidative stress was coupled with reduced levels of blood glucose (Supplementary Fig.), LDLC, and TC, with a parallel increase in HDLC levels (Fig. 3). However, CA did not significantly ($p<0.05$) reduce the HFD-induced increase in TG levels (Fig. 3). Nevertheless, the cholesterol-lowering effect of CA resulted in a reduction of lipid accumulation in the mesenteric, and peritoneal adipose tissue (Fig. 1). Furthermore, CA reduced liver weight (Fig. 1), hepatic fat accumulation as well as restored hepatic enzyme activity (Fig. 3), indicating improvement in liver health. These observations support the idea that the antioxidant properties of CA are crucial to its antihyperlipidemic, antihyperglycemic, and hepatoprotective effects. In agreement with our findings, researchers have reported that CA, owing to its antioxidant properties, can reduce HFD- induced increases in body weight and plasma lipid levels [30, 31]. However, depending on its antioxidant and hepatoprotective effects, the lipid- lowering effects of CA cannot be comprehensively explained. Therefore, to further explore the hypolipidemic effects of CA, we examined the expression of crucial proteins involved in lipid transport, cellular uptake, and metabolism.

In mammalian cells, hyperlipidemia involves the activation of signal transduction mechanisms triggered by several transcription factors. Among these factors, sterol regulatory element binding proteins (SREBPs), liver X receptors (LXRs), and peroxisome proliferator- activated receptors (PPARs) are of pivotal importance [32, 33]. The intracellular and extracellular lipid levels in mammals are heavily influenced by SREBPs, which is consists of three members: SREBP-1a, SREBP-1c, and SREBP-2 [34]. The overexpression of SREBP-1a and SREBP-1c in rodents caused the transcriptional activation of downstream genes involved in the biosynthesis of fatty acids and triglycerides (TG) [35, 36]. In this study we also observed increased transcript levels of SREBP-1a, SREBP-1c, and FAS, which also resulted in a significant ($p<0.05$) increase in plasma TG levels, in HFD- consuming rats (Figs 3 and 4). The consumption of CA could not reduce the HFD-induced increase in TG levels might be attributable to the inefficacy of CA in suppressing the expression of SREBP-1a and SREBP-1c (Fig. 4). Our observations are in agreement with those of a recent study by Horton et al., which reported that the downregulation of both SREBP-1a and SREBP-1c is crucial for lowering fatty acid and triglyceride levels in the plasma [37]. This led us to hypothesize that the lipid- controlling mechanism of CA extends beyond TG regulation. Therefore, we evaluated the expression of the third member, SREBP-2, to determine whether CA exerts its lipid-lowering effects by modulating cholesterol biosynthesis. SREBP-2, by enhancing the expression of HMG- CoA reductase (HMGCR) and LDL receptor (LDLR) promotes the biosynthesis and cellular uptake of cholesterol from the blood [38, 39]. In this study, CA significantly ($p<0.05$) downregulated the expression of SREBP-2 (Fig. 4) and HMGCR (Fig. 5) in HFD- consuming rats. However, HFD- mediated downregulation of LDL receptor expression was significantly ($p<0.05$) increased in the CA-treated group (Fig. 5). The augmented expression of LDLR in rats consuming CA appears to stem from its inability to suppress the expression of LXRα (Fig. 4). Researchers have reported that increased expression of LXRα helps to upregulate the expression of the LDLR [40]. In this study, we also observed HFD-induced upregulation of PPARγ and C/EBPα, which are master regulators of adipogenesis, and enhanced the deposition of fat in the liver and adipose tissues [41]. Therefore, the observed increase in liver fat accumulation, adipose tissue weight, and overall body weight (Fig. 1) in rats fed an HFD might be attributable to the upregulation of PPARγ. Oral administration of CA to HFD-fed rats significantly ($p<0.05$) reduced the expression of PPARγ and fat accumulation in the mesenteric and peritoneal adipose tissues (Fig. 1) and liver (Fig. 6).

In lipid homeostasis, hyperlipidemia, and adipogenesis, SREBP-2 works with LXRα and PPARγ to control cholesterol efflux and lipoprotein biosynthesis [42]. Hence, observing the ability of CA to suppress the expression of SREBP-2 and PPARγ, we predicted that its antioxidant and gene expression modulatory effects might have altered the expression of cholesterol efflux protein as well as the constituent proteins of LDL and HDL. Subsequently, we investigated the expression

of ATP-binding cassette transporter A1 (ABCA1), which enhances the efflux of cholesterol, making it available for Apo-A1 to form HDLC [43]. The observed downregulation of ABCA1 and Apo-A1 might be attributable to HFD-induced oxidative stress, as reported in previous studies [12, 44]. Feeding CA significantly ($p<0.05$) ameliorated oxidative stress and thereby upregulated the expression of ABCA1 and Apo-A1 (Fig. 5). This observation also suggests that the CA-mediated downregulation of SREBP-2 contributed to the upregulation of ABCA1, as reported in previous studies [43, 45]. Moreover, the increased expression of ABCA1 and Apo-A1 is attributable to the inability of CA to suppress the gene expression of LXRα (Fig. 4). In agreement with this observation, Lee and Tontonoz reported that adequate levels and activity of LXRα are necessary for upregulating the expression of ABCA1 for elevating the levels of HDLC in the blood [46, 47]. Elevated levels of HDLC, promoting reverse cholesterol transport (RCT), suppress the expression of ApoB-100 [48]. In this study, we also observed HFD-induced augmented Apo-B100 expression, which was significantly ($p<0.05$) suppressed by the feeding of CA, contributing to the reduction of LDLC. In summary, CA effectively diminishes HFD-induced oxidative stress, hyperlipidemia, and adipogenesis by modulating crucial transcription factors and their downstream proteins involved in cholesterol metabolism and adipogenesis.

Cinnamic acid (CA) can significantly ($p<0.05$) diminish HFD-induced oxidative stress, hyperglycemia, and hepatic enzyme activity, which in turn positively affects liver health, fat accumulation in fat depots, as well as lipid metabolism and homeostasis. It also suppresses the expression of transcription factors such as SREBP-2 and PPARγ, which are upstream transcription factors that regulate the expression of several crucial enzymes, receptors, cholesterol efflux proteins, and constituent proteins of LDLC and HDLC. Thus, CA reduces cholesterol biosynthesis by downregulating the expression of HMG-CoA reductase and enhances the clearance of LDL cholesterol by stimulating the expression of LDL receptors. Furthermore, by upregulating the expression of ABCA1 and Apo-A1, CA enhances the efflux of cholesterol and consequently increases the level of HDL cholesterol in the blood. In contrast, CA by downregulating the expression of Apo-B100, suppresses the level of LDL cholesterol in the circulation. However, CA did not suppress the expression of SREBP-1a, SREBP-1c, and FAS, thereby not suppressing the HFD-induced elevated levels of triglyceride. Taking all these observations together, we concluded that the antihyperlipidemic and antiadipogenic effects of CA are mediated through the suppression of SREBP-2, PPARγ, HMG CoA-reductase, and Apo-B100, along with the upregulation of LDL-receptor, ABCA1, and Apo-A1. Although this study showed ameliorating effects of CA through the modulation of genetic factors at the mRNA level, the changes in these factors at the protein level remain unknown. Therefore, further exploration of the above-mentioned genetic factors at the protein level in Wistar rats and other animal models of hyperlipidemia would enrich the learning of the mechanistic pathways and provide a stronger foundation for designing future clinical trials.

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AUTHOR CONTRIBUTIONS

SAK Experimental design, biochemical investigation, data analysis; RF, AMS, TT, & FI Biochemical investigation, animal handling; NMK, IPE, & RK data analysis and manuscript writing; MAA Conceptualization, experimental design; FK Conceptualization, experimental design, writing manuscript, supervision. All authors have revised the manuscript and approved the final version of the manuscript.

Declarations

We declare that we have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Availability of data and materials

Data are available upon request

Competing interests

The authors declare no competing interests.

REFERENCES

1. Jiang S, Liu H, Li C. Dietary regulation of oxidative stress in chronic metabolic diseases. Foods, (2021); 10(8): 1854.
2. Soppert J, Lehrke M, Marx N, Jankowski J, Noels H. Lipoproteins and lipids in cardiovascular disease: from mechanistic insights to therapeutic targeting. Advanced Drug Delivery Reviews, (2020); 159: 4-33.
3. Ruscica M, Ferri N, Santos RD, Sirtori CR, Corsini A. Lipid lowering drugs: present status and future developments. Current Atherosclerosis Reports, (2021); 23: 1-13.
4. Sarmadi B, Musazadeh V, Dehghan P, Karimi E. The effect of cinnamon consumption on lipid profile, oxidative stress, and inflammation biomarkers in adults: An umbrella meta-analysis of randomized controlled trials. Nutrition, Metabolism and Cardiovascular Diseases, (2023); 33(10): 1821-35.
5. Maierean SM, Serban M-C, Sahebkar A, Ursoniu S, Serban A, Penson P, et al. The effects of cinnamon supplementation on blood lipid concentrations: A systematic review and meta-analysis. Journal of Clinical Lipidology, (2017); 11(6): 1393-406.
6. Guo J, Jiang X, Tian Y, Yan S, Liu J, Xie J, et al. Therapeutic Potential of Cinnamon Oil: Chemical Composition, Pharmacological Actions, and Applications. Pharmaceuticals, (2024); 17(12): 1700.
7. Ruwizhi N, Aderibigbe BA. Cinnamic acid derivatives and their biological efficacy. International Journal of Molecular Sciences, (2020); 21(16): 5712.
8. Nair A, Preetha Rani M, Salin Raj P, Ranjit S, Rajankutty K, Raghu K. Cinnamic acid is beneficial to diabetic cardiomyopathy via its cardioprotective, anti-inflammatory, anti-dyslipidemia, and antidiabetic properties. Journal of Biochemical and Molecular Toxicology, (2022); 36(12): e23215.
9. Nouni C, Theodosis-Nobelos P, Rekka EA. Antioxidant and hypolipidemic activities of cinnamic acid derivatives. Molecules, (2023); 28(18): 6732.
10. Alsoodeeri FN, Alqabbani HM, Aldossari NM. Effects of cinnamon (Cinnamomum cassia) consumption on serum lipid profiles in albino rats. Journal of Lipids, (2020); 2020(1): 8469830.
11. Hariri M, Ghiasvand R. Cinnamon and Chronic Diseases: Drug Discovery from Mother Nature, (2016); 929: 1-24.
12. Shavva VS, Bogomolova AM, Nikitin AA, Dizhe EB, Oleinikova GN, Lapikov IA, et al. FOXO1 and LXRα downregulate the apolipoprotein AI gene expression during hydrogen peroxide-induced oxidative stress in HepG2 cells. Cell Stress and Chaperones, (2017); 22(1): 123-34.
13. Zhao L, Jiang S-j, Lu F-e, Xu L-j, Zou X, Wang K-f, et al. Effects of berberine and cinnamic acid on palmitic acid-induced intracellular triglyceride accumulation in NIT-1 pancreatic β cells. Chinese Journal of Integrative Medicine, (2016); 22: 496-502.
14. Speakman JR. Use of high-fat diets to study rodent obesity as a model of human obesity. International Journal of Obesity, (2019); 43(8): 1491-2.
15. Kuddus SA, Tasnim Z, Shohag MH, Yasmin T, Uddin MS, Hossain MM, et al. Dillenia Indica Fruit Extract Suppressed Diet-induced Obesity in Rats by Down-regulating the mRNA Level of Proadipogenic Transcription Factors and Lipid Metabolizing Enzymes. Current Nutrition & Food Science, (2021); 17(4): 433-47.
16. Yang W, Liu J, Zheng Y, Qu J, Tang X, Bai H, et al. Study on chronic toxicity of rhubarb extract in Sprague-Dawley rats. Heliyon, (2022); 8(10): e10907.
17. Gazwi HS, Hassan MS, Ismail HA, El-Naem GFA, Tony SK. The hypoglycemic and hypolipidemic effects of polyphenol-rich strawberry juice on diabetic rats. Plant Foods for Human Nutrition, (2023); 78(3): 512-9.
18. Naseri M, Mianroodi RA, Pakzad Z, Falahati P, Borbor M, Azizi H, et al. The effect of Melissa officinalis L. extract on learning and memory: Involvement of hippocampal expression of nitric oxide synthase and brain-derived neurotrophic factor in diabetic rats. Journal of Ethnopharmacology, (2021); 276: 114210.
19. Rahman MM, Shahab NB, Miah P, Rahaman MM, Kabir AU, Subhan N, et al. Polyphenol-rich leaf of Aphanamixis polystachya averts liver inflammation, fibrogenesis and oxidative stress in ovariectomized Long-Evans rats. Biomedicine & Pharmacotherapy, (2021); 138: 111530.
20. Battal A, Dogan A, Uyar A, Demir A, Keleş ÖF, Celik I, et al. Exploring of the ameliorative effects of Nerium (Nerium oleander L.) ethanolic flower extract in streptozotocin induced diabetic rats via biochemical, histological and molecular aspects. Molecular Biology Reports, (2023); 50(5): 4193-205.
21. Kakkar P, Das B, Viswanathan P. A modified spectrophotometric assay of superoxide dismutase. Indian Journal of Biochemistry and Biophysics, (1984); 21(2): 130-2.
22. Hasan R, Lasker S, Hasan A, Zerin F, Zamila M, Parvez F, et al. Canagliflozin ameliorates renal oxidative stress and inflammation by stimulating AMPK–Akt–eNOS pathway in the isoprenaline-induced oxidative stress model. Scientific Reports, (2020); 10(1): 14659.
23. Khan F, Syeda PK, Nartey MNN, Rahman MS, Islam MS, Nishimura K, et al. Pretreatment of cultured preadipocytes with arachidonic acid during the differentiation phase without a cAMP-elevating agent enhances fat storage after the maturation phase. Prostaglandins & Other Lipid Mediators, (2016); 123: 16-27.
24. Miah P, Mohona SBS, Rahman MM, Subhan N, Khan F, Hossain H, et al. Supplementation of cumin seed powder prevents oxidative stress, hyperlipidemia and non-alcoholic fatty liver in high fat diet fed rats. Biomedicine & Pharmacotherapy, (2021); 141: 111908.

25. Lasker S, Rahman MM, Parvez F, Zamila M, Miah P, Nahar K, et al. High-fat diet-induced metabolic syndrome and oxidative stress in obese rats are ameliorated by yogurt supplementation. Scientific Reports, (2019); 9(1): 20026.
26. van der Pol A, van Gilst WH, Voors AA, van der Meer P. Treating oxidative stress in heart failure: past, present and future. European Journal of Heart Failure, (2019); 21(4): 425-35.
27. Nita M, Grzybowski A. The role of the reactive oxygen species and oxidative stress in the pathomechanism of the age-related ocular diseases and other pathologies of the anterior and posterior eye segments in adults. Oxidative Medicine and Cellular Longevity, (2016); 2016(1): 3164734.
28. Babaeenezhad E, Nouryazdan N, Nasri M, Ahmadvand H, Sarabi MM. Cinnamic acid ameliorate gentamicin-induced liver dysfunctions and nephrotoxicity in rats through induction of antioxidant activities. Heliyon, (2021); 7(7): e07465.
29. Anlar HG, Bacanli M, Çal T, Aydin S, Ari N, Bucurgat ÜÜ, et al. Effects of cinnamic acid on complications of diabetes. Turkish Journal of Medical Sciences, (2018); 48(1): 168-77.
30. Kasetti RB, Nabi SA, Swapna S, Apparao C. Cinnamic acid as one of the antidiabetic active principle (s) from the seeds of Syzygium alternifolium. Food and Chemical Toxicology, (2012); 50(5): 1425-31.
31. Mnafgui K, Derbali A, Sayadi S, Gharsallah N, Elfeki A, Allouche N. Anti-obesity and cardioprotective effects of cinnamic acid in high fat diet-induced obese rats. Journal of Food Science and Technology, (2015); 52: 4369-77.
32. Bravo-Ruiz I, Medina MÁ, Martínez-Poveda B. From food to genes: transcriptional regulation of metabolism by lipids and carbohydrates. Nutrients, (2021); 13(5): 1513.
33. Fatehi-Hassanabad Z, Chan CB. Transcriptional regulation of lipid metabolism by fatty acids: a key determinant of pancreatic β-cell function. Nutrition & Metabolism, (2005); 2: 1-12.
34. Xiaoping Z, Fajun Y. Regulation of SREBP-mediated gene expression. Chinese Journal of Biotechnology, (2012); 28(4): 287.
35. Xu X, So J-S, Park J-G, Lee A-H. Transcriptional control of hepatic lipid metabolism by SREBP and ChREBP. Seminars in Liver Disease, (2013); 33(4): 301-11.
36. Duan Y, Gong K, Xu S, Zhang F, Meng X, Han J. Regulation of cholesterol homeostasis in health and diseases: from mechanisms to targeted therapeutics. Signal Transduction and Targeted Therapy, (2022); 7(1): 265.
37. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. The Journal of Clinical Investigation, (2002); 109(9): 1125-31.
38. Xue L, Qi H, Zhang H, Ding L, Huang Q, Zhao D, et al. Targeting SREBP-2-regulated mevalonate metabolism for cancer therapy. Frontiers in Oncology, (2020); 10: 1510.
39. Madison BB. Srebp2: A master regulator of sterol and fatty acid synthesis1. Journal of Lipid Research, (2016); 57(3): 333-5.
40. Zhang Y, Breevoort SR, Angdisen J, Fu M, Schmidt DR, Holmstrom SR, et al. Liver LXRα expression is crucial for whole body cholesterol homeostasis and reverse cholesterol transport in mice. The Journal of Clinical Investigation, (2012); 122(5): 1688-99.
41. Yan H, Li Q, Li M, Zou X, Bai N, Yu Z, et al. Ajuba functions as a co-activator of C/EBPβ to induce expression of PPARγ and C/EBPα during adipogenesis. Molecular and Cellular Endocrinology, (2022); 539: 111485.
42. Moslehi A, Hamidi-Zad Z. Role of SREBPs in liver diseases: a mini-review.
43. Attie AD, Kastelein JP, Hayden MR. Pivotal role of ABCA1 in reverse cholesterol transport influencing HDL levels and susceptibility to atherosclerosis. Journal of Lipid Research, (2001); 42(11): 1717-26.
44. Lim CY, Mat Junit S, Abdulla MA, Abdul Aziz A. In vivo biochemical and gene expression analyses of the antioxidant activities and hypocholesterolaemic properties of Tamarindus indica fruit pulp extract. Plos One, (2013); 8(7): e70058.
45. Zeng L, Liao H, Liu Y, Lee T-S, Zhu M, Wang X, et al. Sterol-responsive element-binding protein (SREBP) 2 down-regulates ATP-binding cassette transporter A1 in vascular endothelial cells: a novel role of SREBP in regulating cholesterol metabolism. Journal of Biological Chemistry, (2004); 279(47): 48801-7.
46. Lee J-Y, Parks JS. ATP-binding cassette transporter AI and its role in HDL formation. Current Opinion in Lipidology, (2005); 16(1): 19-25.
47. Lee SD, Tontonoz P. Liver X receptors at the intersection of lipid metabolism and atherogenesis. Atherosclerosis, (2015); 242(1): 29-36.
48. Olofsson S-O, Wiklund O, Borén J. Apolipoproteins AI and B: biosynthesis, role in the development of atherosclerosis and targets for intervention against cardiovascular disease. Vascular Health and Risk Management, (2007); 3(4): 491-502.