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Peperomia pellucida (L.) Kunth Extract Mitigates Secondhand Smoke-Induced Dyslipidemia by Improving Serum Lipid Profile and Modulating Hepatic SREBP-1c, PPAR-α, and LDLR Expression in Wistar Rats

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Abstract

Secondhand smoke (SHS) exposure is a major environmental risk factor that contributes to dyslipidemia and cardiovascular diseases by disrupting hepatic lipid metabolism. *Peperomia pellucida* (L.) Kunth, a medicinal herb rich in polyphenols and flavonoids, has demonstrated potential lipid-regulating properties, but its efficacy against SHS-induced metabolic disturbances remains underexplored. This study investigated the therapeutic effects of ethanolic extract of *P. pellucida* on SHS-induced dyslipidemia in male Wistar rats by integrating biochemical, molecular, and translational endpoints. Rats were divided into five groups: normal control (NC), *P. pellucida*-only (PP), SHS-exposed control (SHS), SHS + atorvastatin (SHS+ATV), and SHS + *P. pellucida* (SHS+PP). SHS exposure was performed for 4 weeks using a standardized sidestream smoke chamber, followed by 4 weeks of oral treatment. Serum lipid profiles (TC, TG, LDL-C, HDL-C, VLDL-C) and atherogenic index (TC/HDL-C) were measured enzymatically. Hepatic mRNA expression of SREBP-1c, PPAR-α, and LDLR was assessed via RT-qPCR, and corresponding protein levels were quantified using rat-specific ELISA kits. Total phenolic and flavonoid content of the extract were determined spectrophotometrically. SHS exposure induced significant dyslipidemia and upregulated hepatic SREBP-1c while downregulating PPAR-α and LDLR at both mRNA and protein levels ($p < 0.05$). Treatment with *P. pellucida* significantly ($p < 0.05$) restored lipid parameters and normalized gene and protein expression profiles, with comparable efficacy to atorvastatin. The extract showed high phenolic and flavonoid content, supporting its bioactivity. In conclusion, *P. pellucida* ameliorates SHS-induced dyslipidemia through modulation of key hepatic lipid metabolism regulators at transcriptional and translational levels, highlighting its potential as a phytotherapeutic candidate for environmentally triggered metabolic disorders.

Keywords: Tobacco smoke polution; Phytotherapy; Lipid metabolism disorders

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Introduction

The global prevalence of dyslipidemia, a disorder of lipid metabolism characterized by elevated total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol (VLDL-C), along with reduced high-density lipoprotein cholesterol (HDL-C), is escalating at an alarming pace [1]. In 2020, the World Health Organization (WHO) estimated that more than 39% of adults globally are impacted, contributing substantially to the global burden of cardiovascular morbidity and mortality [2]. While conventional risk factors such as diet, obesity, and sedentary lifestyle are well established, environmental exposures, notably secondhand smoke, have emerged as potent but underrecognized contributors to lipid dysregulation and atherosclerosis [3].

Secondhand smoke, characterized by the involuntary inhalation of smoke from burning tobacco products and exhaled mainstream smoke, comprises over 7,000 toxic chemicals, such as nicotine, carbon monoxide, polycyclic aromatic hydrocarbons, and reactive oxygen species [4]. Epidemiological data indicate that secondhand smoke accounts for more than 1.3 million premature deaths each year, disproportionately affecting low- and middle-income countries as well as vulnerable populations, including children and women [5,6]. Beyond its well-documented pulmonary and oncogenic consequences, secondhand smoke exerts profound systemic effects on lipid metabolism, triggering hepatic steatosis, endothelial dysfunction, and pro-atherogenic lipid profiles, often independent of active smoking status [4,7–12].

Mechanistically, secondhand smoke-induced dyslipidemia is increasingly linked to the transcriptional reprogramming of hepatic lipid metabolism genes [13]. Among these, sterol regulatory element-binding protein 1c (SREBP-1c) promotes *de novo* lipogenesis, peroxisome proliferator-activated receptor alpha (PPAR-α) regulates fatty acid oxidation, and the low-density lipoprotein receptor (LDLR) governs plasma LDL-C clearance. Disruption in this triad leads to lipid accumulation, impaired oxidative capacity, and reduced lipoprotein uptake, forming a critical molecular basis for smoke-induced metabolic dysfunction [8,14]. While statins remain the mainstay of therapy, their side effects, cost, and limited action on upstream regulatory networks necessitate the search for alternative, multi-target agents [15].

Peperomia pellucida (L.) Kunth, a medicinal plant traditionally used across Southeast Asia and South America, has gained research interest due to its rich content of flavonoids, alkaloids, and terpenoids with established antioxidant, anti-inflammatory, and hypolipidemic properties [16–19]. Preclinical studies have demonstrated its efficacy in reducing serum lipid levels, improving liver function, and modulating redox balance in models of diet- or toxin-induced metabolic stress [20–22]. However, its therapeutic potential against secondhand smoke-induced dyslipidemia, particularly at the molecular level of gene regulation, remains unexplored.

The present study investigates the protective effects of *P. pellucida* ethanolic extract in a Wistar rat model of secondhand smoke-induced dyslipidemia, focusing on both serum lipid profile (TC, TG, LDL-C, VLDL-C, and HDL-C) and hepatic expression of SREBP-1c, PPAR-α, and LDLR. This study aims to clarify the molecular routes by which *P. pellucida* influences lipid regulation by integrating transcriptional, translational, and biochemical endpoints, hence supporting its potential as a phytotherapeutic candidate for environmentally induced metabolic diseases.

Methods

Plant Collection, Identification, and Extraction

Aerial parts of *P. pellucida* were collected from Laboratory of Herbal Materia Medica, Batu, East Java, Indonesia and authenticated at the same institution, with voucher specimen number: HMM-PP-2025-031 (No. 000.9.3/3693/102.20/2025). The aerial parts were specifically selected because they are widely used in traditional practice for inflammatory and metabolic conditions and are known to be rich in phenolic and flavonoid constituents associated with antioxidant, anti-inflammatory, and lipid-modulating activities [17]. Moreover, recent metabolomic profiling demonstrates organ-specific distributions of secondary metabolites across leaves and stems, supporting the pharmacognostic rationale for focusing on the aerial fraction in a dyslipidemia model [23]. Plant material was cleaned, oven-dried at 50 °C, and ground into a fine powder. Extraction was conducted through maceration in 96% ethanol (1:10 *w/v*) for a duration of 72 hours at ambient temperature, accompanied by intermittent stirring. The extract underwent filtration, concentration via a rotary evaporator (R-300, Büchi Labortechnik AG, Flawil, Switzerland) at 40 °C, and subsequent freeze-drying. The yield was maintained at 4 °C in an amber container [19].

Chemicals

Absolute ethanol (96%, analytical grade), Folin–Ciocalteu reagent, sodium carbonate (Na_2CO_3), aluminum chloride ($AlCl_3$), gallic acid, and quercetin were obtained from Sigma-Aldrich, Merck KGaA, St. Louis, MO, USA and used for phytochemical quantification. Atorvastatin calcium was obtained from Pfizer Indonesia (Jakarta, Indonesia).

For molecular analysis, TRIzol® reagent and the High-Capacity cDNA Reverse Transcription Kit were

procured from Invitrogen™ (Thermo Fisher Scientific, Carlsbad, CA, USA; Cat. No. 15596026). Quantitative reverse transcription polymerase chain reaction (RT-qPCR) was performed using the TaqMan™ Fast Advanced Master Mix (Applied Biosystems™, Thermo Fisher Scientific, Foster City, CA, USA; Cat. No. 4444557) on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA; Cat. No. 1855195).

Enzymatic colorimetric assay kits for serum lipid profile analysis (TC, TG, LDL-C, and HDL-C) were obtained from DiaSys Diagnostic Systems GmbH (Holzheim, Germany). All reagents utilized were of analytical grade, and solutions were prepared with distilled or deionized water.

Phytochemical Quantification of Peperomia pellucida Extract

Total Phenolic Content

The total phenolic content (TPC) of the *P. pellucida* extract was assessed using the Folin–Ciocalteu colorimetric method, as initially outlined by Singleton and Rossi (1965), with slight modifications [24]. In summary, 100 µL of extract solution (1 mg/mL) was combined with 500 µL of 10% (*v/v*) Folin-Ciocalteu reagent. Following a 5-minute incubation at room temperature, 400 µL of a 7.5% (*w/v*) Na_2CO_3 solution was introduced. The mixture was vortexed and incubated in the dark for 30 minutes at ambient temperature to facilitate color development.

A UV-Visible spectrophotometer (UV-1800, Shimadzu Corporation, Kyoto, Japan) was used to detect absorbance at 765 nm. Concentrations of gallic acid ranging from 25 to 200 µg/mL were employed to establish a calibration curve. TPC was quantified in milligrams of gallic acid equivalent per gram of desiccated extract (mg GAE/g extract). All measurements were conducted in triplicate.

Total Flavonoid Content

The aluminum chloride colorimetric assay was employed to assess the total flavonoid content (TFC) [25]. In a 1.5 mL reaction volume, 500 µL of extract (1 mg/mL) was combined with 500 µL of 2% 500 µL of extract (1 mg/mL) was combined with 500 µL of 2% $AlCl_3$ in methanol in a 1.5 mL reaction volume. The absorbance was measured at 415 nm using a UV-Visible spectrophotometer (UV-1800, Shimadzu Corporation, Kyoto, Japan) after a 30-minute incubation in the dark at room temperature. The calibration curve was developed using quercetin at concentrations ranging from 25 to 200 µg/mL. The results were expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g extract). Analyses were conducted on each sample in triplicate.

Experimental Animals and Ethical Approval

From the Animal Research Facility at the Faculty of Medicine, Universitas Wijaya Kusuma Surabaya, Indonesia, 35 male Wistar rats (*Rattus norvegicus*) weighing between 180 and 220 grams and aged 8 to 10 weeks were obtained. Animals were maintained in polycarbonate cages under standard laboratory conditions (22 ± 2 °C, 50-60% humidity, 12-hour light/dark cycle), with continuous access to commercial pellet feed and tap water. Rats were randomly assigned to five experimental groups (n = 7 per group) after a seven-day acclimatization period, as determined by a computer-generated sequence. All experimental protocols were conducted in conformance with institutional animal care policies and were approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Wijaya Kusuma Surabaya, Indonesia, under approval number 7/SLE/FK/UWKS/2025.

Study Design and Group Allocation

The study spanned 8 weeks, consisting of 4 weeks of secondhand smoke exposure followed by 4 weeks of treatment. Animals were divided into the following groups (n = 7/group):

- Normal Control (NC): No secondhand smoke exposure; received vehicle (distilled water) orally for 4 weeks
- *P. pellucida* Control (PP): No secondhand smoke exposure; received *P. pellucida* extract (400 mg/kg BW) orally for 4 weeks
- Secondhand Smoke Control (SHS): Exposed to secondhand smoke for 4 weeks; followed by vehicle for 4 weeks
- Secondhand Smoke + Atorvastatin (SHS+ATV): Exposed to secondhand smoke for 4 weeks; treated with atorvastatin (10 mg/kg BW) orally for 4 weeks
- Secondhand Smoke + *P. pellucida* Extract (SHS+PP): Exposed to secondhand smoke for 4 weeks; treated with *P. pellucida* extract (400 mg/kg BW) orally for 4 weeks

The 400 mg/kg BW dose of *P. pellucida* extract was selected based on previous *in vivo* studies demonstrating significant biological activity and safety at this level [26–28]. Investigators conducting outcome measurements were blinded to group allocation to minimize bias.

Secondhand Smoke (SHS) Exposure Protocol

Rats in the secondhand smoke-exposed groups (SHS, SHS + ATV, SHS + PP) were exposed to sidestream smoke generated from one commercially available cigarette per rat per day, using a custom-built transparent acrylic exposure chamber (each chamber grid accommodates one rat, dimension approx. 40 × 40 × 40 cm), adapted from a validated passive smoking system developed by Cendon et al. (1997) [29]. Smoke was

generated by sidestream delivery, with cigarettes lit sequentially to maintain consistent exposure for each session (one cigarette/rat). Exposures occurred once daily at 09:00, seven days per week, over a total of four weeks.

Sample Collection and Tissue Harvesting

Rats were anesthetized with intraperitoneal administrations of ketamine (75 mg/kg) and xylazine (10 mg/kg), both sourced from Interchemie werken "De Adelaar" B.V., Venray, The Netherlands, after a 12-hour fasting period following the 8-week intervention [30]. The blood was obtained through cardiac puncture, allowed to coagulate, and subsequently centrifuged at 3,000 rpm for 10 minutes at 4°C [31]. Aliquots of serum samples were prepared and stored at –80°C. The liver was excised, rinsed with phosphate-buffered saline, and sectioned. A portion was frozen in liquid nitrogen and subsequently stored at –80°C for molecular analysis.

Biochemical Analysis of Serum Lipid Profile and Atherogenic Index

Serum levels of TC, TG, LDL-C, and HDL-C were assessed using enzymatic colorimetric assay kits from DiaSys Diagnostic Systems GmbH (Holzheim, Germany): Cholesterol FS (Cat. No. 1 1300 99 10 021), Triglycerides FS (Cat. No. 1 1550 99 10 021), LDL-C Direct FS (Cat. No. 1 4131 99 10 021), and HDL-C Direct FS (Cat. No. 1 4100 99 10 021), following the manufacturer's protocols. VLDL-C was determined utilizing the Friedewald equation: $VLDL = \frac{TG}{5}$. The atherogenic index was quantified using the total cholesterol to HDL cholesterol ratio [32]. Assays were performed in duplicate.

RNA Extraction and Quantitative Real-Time PCR (qRT-PCR)

About 30 mg of liver tissue was homogenized in 1 mL of TRIzol® reagent (Invitrogen™, Thermo Fisher Scientific, Carlsbad, CA, USA; Cat. No. 15596026) for total RNA extraction, adhering to the manufacturer's protocol. The purity and concentration of RNA were evaluated spectrophotometrically by measuring A260/A280 ratios with a NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). cDNA synthesis was conducted with 1 µg of total RNA with the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems™, Thermo Fisher Scientific, Waltham, MA, USA; Cat. No. 4368814).

RT-qPCR was performed utilizing TaqMan™ Fast Advanced Master Mix (Applied Biosystems™, Thermo Fisher Scientific, Foster City, CA, USA; Cat. No. 4444557) on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA; Cat. No. 1855195). Specific TaqMan™ Gene Expression Assays (Applied Biosystems™, Foster City, CA, USA) were used for the target genes: Rn01495769_m1 for SREBP-1c, Rn00566193_m1 for PPAR-α, Rn00598442_m1 for LDLR, and Rn00667869_m1 for β-actin, which served as the endogenous control. Thermal cycling parameters were established as follows: 50 °C for 2 minutes, 95 °C for 20 seconds, succeeded by 40 cycles of 95 °C for 1 second and 60 °C for 20 seconds. All samples were analyzed in duplicate. Relative mRNA expression levels were assessed utilizing the $2^{-\Delta\Delta Ct}$ method.

Protein Quantification by Enzyme-Linked Immunosorbent Assay (ELISA)

To validate the transcriptional results and evaluate the translational effects of secondhand smoke exposure and therapy interventions, hepatic protein levels of SREBP-1c, PPAR-α, and LDLR were measured using commercially available ELISA kits. Approximately 50 mg of frozen liver tissue was homogenized in ice-cold RIPA lysis buffer (Thermo Fisher Scientific, Waltham, MA, USA; Cat. No. 89900), augmented with protease and phosphatase inhibitor cocktails (Roche Diagnostics, Mannheim, Germany; Cat. Nos. 11836153001 and 04906837001, respectively). Homogenates were subjected to centrifugation at 12,000 × g for 15 minutes at 4 °C, and the resultant supernatants were harvested. The protein concentrations of the supernatants were quantified with the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA; Cat. No. 23225), with the results for SREBP-1c, PPAR-α, and LDLR levels reported as ng/mg of total protein.

ELISA was conducted utilizing rat-specific kits for SREBP-1c (Cat. No. E2265Ra), PPAR-α (Cat. No. E0283Ra), and LDLR (Cat. No. E1345Ra) from Shanghai Korain Biotech Co., Ltd., Shanghai, China, in accordance with the manufacturers' instructions. Forty microliters of sample or fifty microliters of standard were introduced into each well of pre-coated 96-well microplates and incubated for sixty minutes at 37 degrees Celsius. Following the washing process, biotinylated detection antibodies were successively added, followed by the streptavidin-HRP conjugate. The plates were created using tetramethylbenzidine (TMB) substrate, and the reaction was halted using 2N H_2SO_4. Optical density was assessed at 450 nm utilizing a microplate reader (Synergy HTX, BioTek Instruments, Agilent Technologies, Winooski, VT, USA). All samples were analyzed in duplicate.

Statistical Analysis

The data is presented as the mean ± standard error of the mean (SEM). The normality of data distribution was evaluated using the Shapiro-Wilk test, and the homogeneity of variances was analyzed using Levene's test. For comparisons among multiple groups, a one-

way analysis of variance (ANOVA) was implemented, followed by a Tukey post hoc test to identify pairwise differences. The Kruskal-Wallis test, in conjunction with Dunn's multiple comparison test, was employed as a non-parametric alternative in instances where the data did not meet the criteria for normality or homogeneity. GraphPad Prism version 9.5.1 (GraphPad Software, San Diego, CA, USA) and IBM SPSS Statistics version 26 (IBM Corp., Armonk, NY, USA) were employed to conduct statistical analyses. Statistical significance was determined by a p value of less than 0.05. Two-tailed statistical tests were implemented for all statistical analyses. GraphPad Prism was employed to generate the graphs.

Results

Phytochemical Content of Peperomia pellucida Extract

The TPC and TFC of the ethanolic extract were measured. The TPC was determined to be 189.85 ± 2.53 mg GAE/g extract, while the TFC was 167.62 ± 0.83 mg QE/g extract. These findings validate the existence of a substantial concentration of polyphenolic substances that may confer lipid-modulating effects.

Effect of Peperomia pellucida on Serum Lipid Profile and Atherogenic Index

The effects of *P. pellucida* extract on serum lipid parameters and atherogenic index in secondhand smoke-exposed rats are shown in Figure 1. Four weeks of exposure to secondhand smoke significantly disrupted lipid homeostasis, as evidenced by elevated levels of TC (Figure 1A; $p < 0.0001$ vs. NC), TG (Figure 1B; $p < 0.0001$ vs. NC), LDL-C (Figure 1C; $p < 0.0001$ vs. NC), VLDL-C (Figure 1D; $p < 0.0001$ vs. NC), and a marked reduction in HDL-C (Figure 1E; $p < 0.0001$ vs. NC). These changes culminated in a significant rise in the TC/HDL-C ratio, indicative of heightened atherogenic risk (Figure 1F; $p < 0.0001$ vs. NC).

Treatment with *P. pellucida* extract (SHS+PP group) effectively ameliorated these dyslipidemic alterations. Notably, rats in the SHS+PP group exhibited significantly reduced TC ($p < 0.0001$ vs. SHS), TG ($p = 0.0040$ vs. SHS), LDL-C ($p < 0.0001$ vs. SHS), VLDL-C ($p = 0.0040$ vs. SHS), and TC/HDL-C ratio ($p < 0.0001$ vs. SHS) compared to the SHS group, while HDL-C levels were significantly restored ($p = 0.0028$ vs. SHS). The lipid-modulating effects of *P. pellucida* were comparable to those of atorvastatin in most parameters, with no significant differences observed between the SHS+ATV and SHS+PP groups ($p > 0.05$). Importantly, administration of *P. pellucida* extract alone (PP group) did not significantly differ from the NC group across all measured lipid parameters, confirming its safety profile.

Hepatic mRNA Expression of Lipid Metabolism-Related Genes

Quantitative analysis of hepatic gene expression revealed significant alterations in the mRNA levels of key lipid metabolism regulators, SREBP-1c, PPAR-α, and LDLR, following exposure to secondhand smoke and subsequent treatment with *P. pellucida* extract or atorvastatin. As shown in Figure 2A, secondhand smoke exposure markedly upregulated SREBP-1c mRNA expression compared to the NC and PP groups ($p < 0.0001$), indicating activation of hepatic lipogenesis. Treatment with *P. pellucida* extract (SHS+PP) or atorvastatin (SHS+ATV) significantly attenuated this increase ($p < 0.0001$ vs. SHS), restoring expression to levels statistically indistinguishable from one another ($p > 0.05$).

Conversely, PPAR-α mRNA levels were significantly downregulated in secondhand smoke-exposed rats (Figure 2B; $p < 0.0001$ vs. NC), consistent with impaired fatty acid oxidation. Both *P. pellucida* and atorvastatin treatments significantly upregulated PPAR-α expression ($p = 0.0010$ and $p = 0.0090$ vs. SHS, respectively), although expression remained slightly lower than in the NC group. Similarly, LDLR mRNA expression (Figure 2C) was significantly reduced by secondhand smoke exposure ($p < 0.0001$ vs. NC), suggesting impaired LDL clearance. Treatment with *P. pellucida* or atorvastatin significantly reversed this reduction ($p = 0.0035$ and $p = 0.0014$ vs. SHS, respectively), with no statistical difference between treatment groups ($p = 0.9966$). Notably, administration of *P. pellucida* extract alone (PP group) did not significantly alter the expression of any of the target genes compared to the NC group ($p = 0.6479$), confirming the extract's safety profile in non-exposed animals.

Effect of Secondhand Smoke and Peperomia pellucida on Hepatic Protein Expression of Lipid Metabolism Regulators

To assess the translational impact of secondhand smoke exposure and therapeutic interventions, hepatic protein levels of SREBP-1c, PPAR-α, and LDLR were quantified using rat-specific ELISA and normalized to total protein content. As shown in Figure 3A, secondhand smoke exposure (SHS group) significantly elevated hepatic SREBP-1c protein levels compared to the NC and PP groups ($p < 0.0001$), indicating enhanced lipogenic signaling. Treatment with *P. pellucida* extract (SHS+PP group) significantly attenuated this increase ($p < 0.0001$ vs. SHS), with levels comparable to those in the atorvastatin-treated group (SHS+ATV) ($p = 0.7418$).

Conversely, secondhand smoke exposure resulted in a marked reduction of PPAR-α protein expression rel-

ative to NC and PP groups ($p < 0.0001$), suggesting suppression of fatty acid oxidation pathways (Figure 3B). Administration of *P. pellucida* extract restored PPAR-α levels significantly ($p < 0.0001$ vs. SHS), with no statistically significant difference from the SHS+ATV group ($p = 0.3448$). A similar pattern was observed for LDLR protein levels, with secondhand smoke exposure significantly downregulating hepatic LDLR levels ($p < 0.0001$ vs. NC), indicative of impaired LDL clearance (Figure 3C). Both *P. pellucida* and atorvastatin treatments significantly ameliorated this reduction ($p < 0.001$), restoring LDLR levels toward baseline values.

Discussion

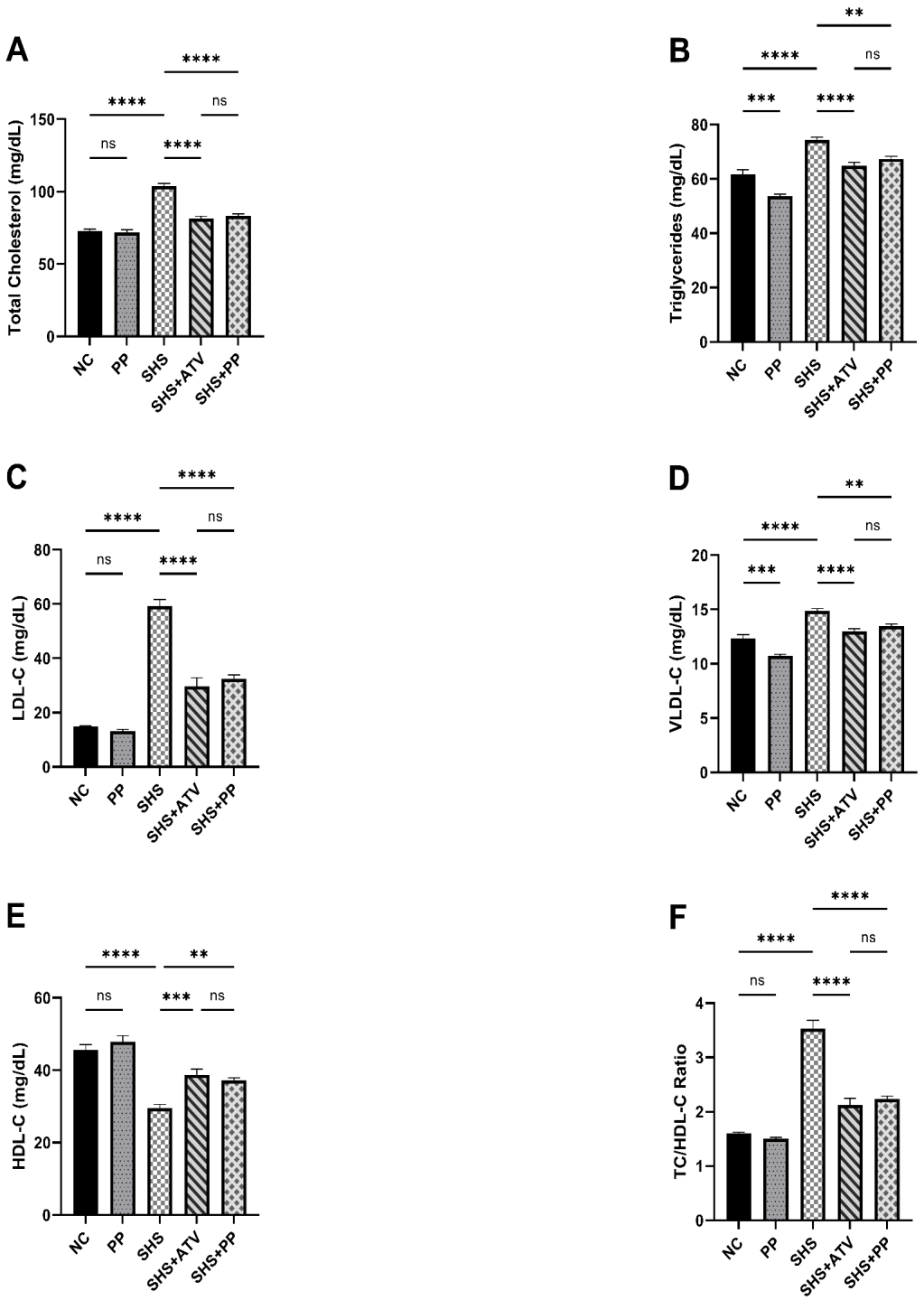

Figure 1. Effects of *Peperomia pellucida* extract on serum lipid profile and atherogenic index in secondhand smoke (SHS)–exposed Wistar rats. Serum levels of (A) total cholesterol (TC), (B) triglycerides (TG), (C) low-density lipoprotein cholesterol (LDL-C), (D) very low-density lipoprotein cholesterol (VLDL-C), (E) high-density lipoprotein cholesterol (HDL-C), and (F) TC/HDL-C ratio were measured after 8 weeks of treatment. Rats were divided into five groups: normal control (NC), *P. pellucida* control (PP), SHS-exposed control (SHS), SHS + atorvastatin (10 mg/kg BW; SHS+ATV), and SHS + *P. pellucida* extract (400 mg/kg BW; SHS+PP). Values are presented as mean ± SEM (n = 7 per group). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test. ****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; ns = not significant.

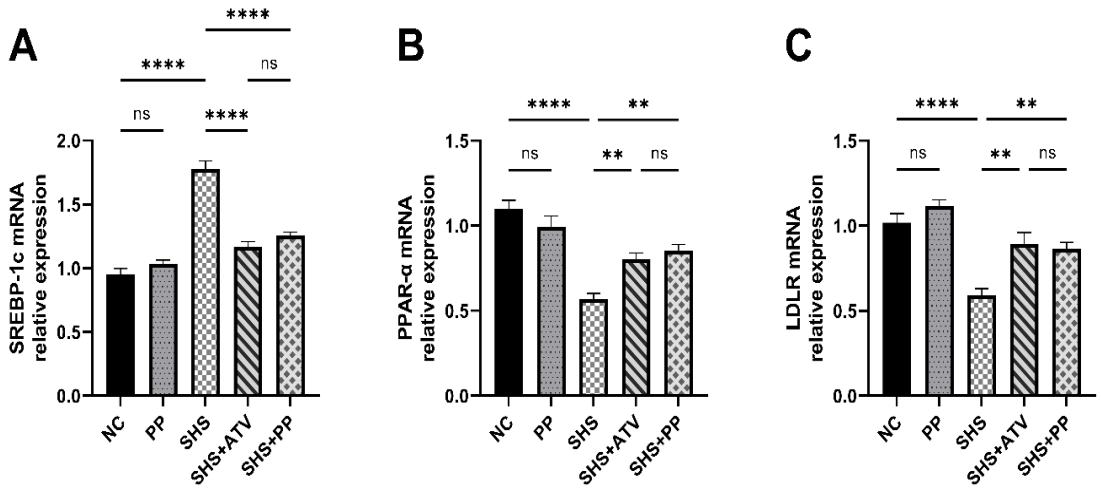

Figure 2. Relative hepatic mRNA expression levels of lipid metabolism–related genes in secondhand smoke-exposed and treated Wistar rats. Quantitative real-time PCR analysis of (A) SREBP-1c, (B) PPAR-α, and (C) LDLR mRNA expression levels in liver tissues of male Wistar rats after 4-week secondhand smoke (SHS) exposure and subsequent treatment with either atorvastatin (10 mg/kg BW) or *Peperomia pellucida* extract (400 mg/kg BW). β-Actin was used as the endogenous control. Gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method and is expressed as fold change relative to the normal control (NC) group. Rats were divided into five groups: normal control (NC), *P. pellucida* control (PP), SHS-exposed control (SHS), SHS + atorvastatin (10 mg/kg BW; SHS+ATV), and SHS + *P. pellucida* extract (400 mg/kg BW; SHS+PP). Values are presented as mean ± SEM (n = 7 per group). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test. ****$p < 0.0001$; **$p < 0.01$; ns = not significant.

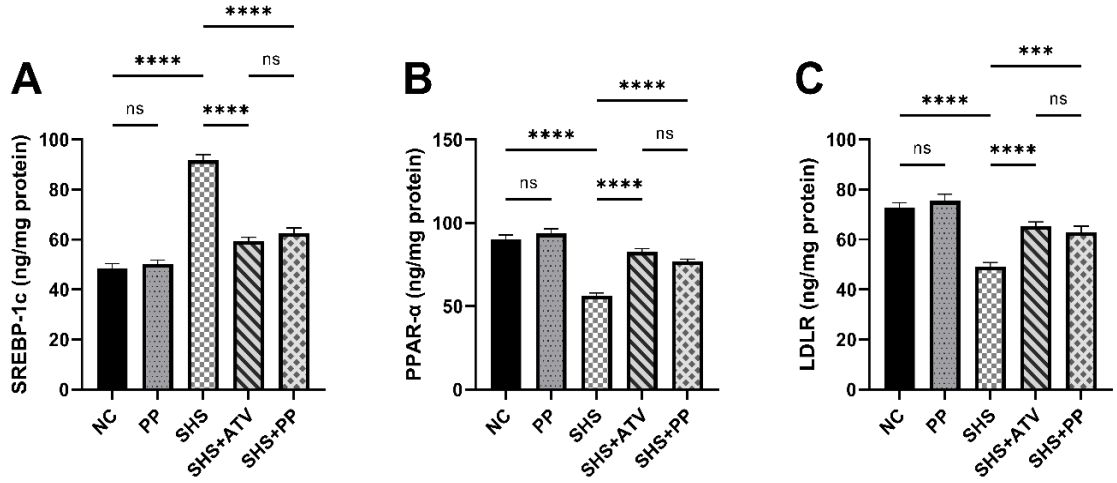

Figure 3. Hepatic protein expression levels of lipid metabolism-related targets in secondhand smoke-exposed and *Peperomia pellucida*-treated Wistar rats. Quantification of (A) SREBP-1c, (B) PPAR-α, and (C) LDLR protein levels in liver tissues of rats following 4-week secondhand smoke (SHS) exposure and subsequent treatment with either atorvastatin (10 mg/kg BW) or *P. pellucida* extract (400 mg/kg BW). Protein concentrations were measured using rat-specific ELISA kits and normalized to total protein content, expressed as ng/mg protein. Rats were divided into five groups: normal control (NC), *P. pellucida* control (PP), SHS-exposed control (SHS), SHS + atorvastatin (10 mg/kg BW; SHS+ATV), and SHS + *P. pellucida* extract (400 mg/kg BW; SHS+PP). Values are presented as mean ± SEM (n = 7 per group). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test. ****$p < 0.0001$; ***$p < 0.001$; ns = not significant.

This study comprehensively elucidates the therapeutic potential of *P. pellucida* ethanolic extract in ameliorating secondhand smoke-induced dyslipidemia through coordinated modulation of hepatic lipid metabolism at both transcriptional and translational levels. Secondhand smoke exposure induced significant derangements in the serum lipid profile, including marked elevations in TC, TG, LDL-C, and VLDL-C, alongside reductions in HDL-C and an increase in the atherogenic index (TC/HDL-C ratio). These alterations are indicative of a pro-atherogenic and metabolically compromised state, consistent with established models of tobacco-induced dyslipidemia [8,33].

At the hepatic molecular level, these alterations were

associated with a notable overexpression of SREBP-1c and a simultaneous downregulation of PPAR-α and LDLR, pointing to a coordinated disruption in lipid synthesis, oxidation, and clearance pathways [34,35]. Importantly, ELISA-based quantification of hepatic SREBP-1c, PPAR-α, and LDLR protein levels revealed trends highly consistent with RT-qPCR data, reinforcing the translational relevance of the observed gene expression changes. Secondhand smoke exposure increased hepatic SREBP-1c protein content while suppressing PPAR-α and LDLR proteins, suggesting not only transcriptional dysregulation, but also post-transcriptional impairment in lipid regulatory signaling.

Treatment with *P. pellucida* extract significantly attenuated these pathological alterations across multiple levels. Biochemically, the extract markedly reduced serum TC, TG, LDL-C, and VLDL-C; while elevating HDL-C and restoring the TC/HDL-C ratio toward a cardioprotective range. These improvements were paralleled by the normalization of hepatic gene expression and protein levels of SREBP-1c, PPAR-α, and LDLR, indicating systemic and hepatic lipid homeostasis restoration. These findings suggest that *P. pellucida* mediates its protective effects via modulation of key hepatic transcriptional regulators and the downstream lipid profile, ultimately reversing secondhand smoke-induced metabolic stress.

The pathophysiological effects of secondhand smoke are attributable to its complex composition of toxicants, including nicotine, carbon monoxide, and polycyclic aromatic hydrocarbons, which generate reactive oxygen species and promote chronic inflammation, leading to dysregulation of hepatic lipid metabolism [4,5,10,11,36]. Upregulation of SREBP-1c under oxidative and lipotoxic stress enhances transcription of enzymes pertinent to *de novo* lipogenesis, including fatty acid synthase (FASN) and acetyl-CoA carboxylase (ACC), thereby increasing hepatic triglyceride accumulation [33,37,38]. Simultaneously, suppression of PPAR-α inhibits mitochondrial β-oxidation, while downregulation of LDLR impairs LDL clearance, together contributing to the observed hypercholesterolemia and hypertriglyceridemia [13,39–41].

P. pellucida intervention reversed these molecular and biochemical abnormalities, indicating a multi-targeted mechanism involving transcriptional reprogramming and hepatic lipid handling. The extract's efficacy is likely attributable to its high total phenolic and flavonoid content, which includes compounds such as quercetin, luteolin, and pellucidin A [25,42]. These polyphenols are known to activate AMP-activated protein kinase (AMPK), which suppresses SREBP-1c while upregulating PPAR-α, thereby balancing lipid anabolism and catabolism [43]. Restoration of LDLR expression at both mRNA and protein levels implies enhanced hepatic LDL uptake and cholesterol clearance [44]. Emerging evidence also suggests that flavonoids can downregulate proprotein convertase subtilisin/kexin type 9 (PCSK9), therefore inhibiting LDLR degradation and further enhancing cholesterol homeostasis, a mechanism worth exploring in future studies [45–47].

Interestingly, the effects of *P. pellucida* were comparable to atorvastatin, a widely used statin known to inhibit HMG-CoA reductase and upregulate LDLR. In this study, both treatments improved lipid indices and gene/protein expression. However, unlike atorvastatin, which may be associated with hepatotoxicity, insulin resistance, and myopathy during long-term use, *P. pellucida* showed no adverse effects in healthy animals [15]. The PP-only group displayed no significant deviation from normal controls in any parameter, underscoring the selective pharmacological activity of the extract under pathophysiological conditions. This adaptogenic effect aligns with the concept of homeostatic phytotherapy, wherein bioactive compounds exert therapeutic effects only in the presence of metabolic disruption [48].

The consistency between serum lipid restoration and hepatic gene/protein normalization further underscores the integrative role of *P. pellucida* in reprogramming lipid metabolism. Its pleiotropic effects, including the inhibition of lipogenesis, the augmentation of fatty acid oxidation, and the facilitation of LDL clearance, highlight its promise as a multitargeted agent for environmental toxin-induced dyslipidemia.

Despite these encouraging results, several limitations should be acknowledged. First, the study did not perform direct quantification of nicotine, carbon monoxide, or polycyclic aromatic hydrocarbons within the exposure chamber, and dose normalization based on lung volume or body weight was not applied. Second, the study did not investigate key upstream and downstream pathways such as AMPK, liver X receptor (LXR), and PCSK9, which are likely involved in the observed effects. Third, the intervention was evaluated at a single dose and duration; future research should explore dose-response relationships, chronic exposure models, and combination therapies. Fourth, while ELISA provided reliable protein-level validation, more mechanistic insights could be obtained through Western blot, immunohistochemistry, or phospho-protein analyses. Finally, comprehensive metabolomic and lipidomic profiling, coupled with isolation and characterization of specific bioactive constituents, is warranted to delineate the extract's full pharmacodynamic profile and facilitate translational development.

Conclusion

Peperomia pellucida effectively attenuated secondhand smoke-induced dyslipidemia in Wistar rats by

improving serum lipid profiles and modulating hepatic expression of key lipid metabolism genes and protein. The extract suppressed SREBP-1c and upregulated PPAR-α and LDLR, suggesting coordinated regulation of lipogenesis, fatty acid oxidation, and cholesterol clearance. These findings support *P. pellucida* as a promising phytotherapeutic candidate for managing environmentally induced lipid disorders. Further studies are warranted to validate its active constituents and translational potential.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgements

None.

Supplementary

Table S1. Effects of *Peperomia pellucida* extract on serum lipid profile and atherogenic index in secondhand smoke (SHS)-exposed Wistar rats.

Variables	NC	PP	SHS	SHS+ATV	SHS+PP
TC (mg/dL)	72.71 ± 1.48	71.86 ± 1.81	103.71 ± 1.84	81.29 ± 1.81	83.00 ± 1.57
TG (mg/dL)	61.71 ± 1.71	53.57 ± 0.84	74.29 ± 1.15	64.86 ± 1.34	67.43 ± 0.95
LDL-C (mg/dL)	14.80 ± 0.28	13.29 ± 0.53	59.29 ± 2.44	29.60 ± 3.24	32.37 ± 1.50
VLDL-C (mg/dL)	12.34 ± 0.34	10.71 ± 0.17	14.86 ± 0.23	12.97 ± 0.27	13.49 ± 0.19
HDL-C (mg/dL)	45.57 ± 1.46	47.86 ± 1.65	29.57 ± 0.97	38.71 ± 1.54	37.14 ± 0.74
TC/HDL-C Ratio	1.60 ± 0.02	1.51 ± 0.02	3.53 ± 0.15	2.13 ± 0.12	2.24 ± 0.05

Data are presented as mean ± SEM (n = 7). NC, normal control; PP, *P. pellucida* control; SHS, secondhand smoke-exposed control; SHS+ATV, secondhand smoke + atorvastatin (10 mg/kg BW/day); SHS+PP, secondhand smoke + *P. pellucida* extract (400 mg/kg BW/day); TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

Table S2. Relative hepatic mRNA expression levels of lipid metabolism-related genes in secondhand smoke-exposed and treated Wistar rats.

Variables (fold of control)	NC	PP	SHS	SHS+ATV	SHS+PP
SREBP-1c	0.95 ± 0.05	1.04 ± 0.03	1.78 ± 0.07	1.17 ± 0.04	1.26 ± 0.03
PPAR-α	1.10 ± 0.05	0.99 ± 0.06	0.57 ± 0.03	0.80 ± 0.04	0.86 ± 0.03
LDLR	1.02 ± 0.05	1.11 ± 0.04	0.59 ± 0.04	0.89 ± 0.07	0.87 ± 0.04

Data are presented as mean ± SEM (n = 7). NC, normal control; PP, *P. pellucida* control; SHS, secondhand smoke-exposed control; SHS+ATV, secondhand smoke + atorvastatin (10 mg/kg BW/day); SHS+PP, secondhand smoke + *P. pellucida* extract (400 mg/kg BW/day); SREB-1c, sterol regulatory element-bind ing protein 1c; PPAR-α, peroxisome proliferator-activated receptor alpha; LDLR, low-density lipoprotein receptor.

Table S3. Hepatic protein expression levels of lipid metabolism-related targets in secondhand smoke-exposed and *Peperomia pellucida*-treated Wistar rats.

Variables (ng/mg protein)	NC	PP	SHS	SHS+ATV	SHS+PP
SREBP-1c	48.56 ± 1.82	50.18 ± 1.51	91.71 ± 2.24	59.26 ± 1.64	62.51 ± 2.13
PPAR-α	90.08 ± 2.72	93.68 ± 2.71	56.21 ± 1.78	82.61 ± 1.94	76.75 ± 1.52
LDLR	72.66 ± 2.07	75.48 ± 2.66	49.21 ± 1.58	65.32 ± 1.69	62.87 ± 2.54

Data are presented as mean ± SEM (n = 7). NC, normal control; PP, P. pellucida control; SHS, secondhand smoke-exposed control; SHS+ATV, secondhand smoke + atorvastatin (10 mg/kg BW/day); SHS+PP, secondhand smoke + P. pellucida extract (400 mg/kg BW/day); SREB-1c, sterol regulatory element-bind ing protein 1c; PPAR-α, peroxisome proliferator-activated receptor alpha; LDLR, low-density lipoprotein receptor.

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