

Molecular and Morphological Improvements in White Adipose Tissue Mediated by Polyphyllin I in High-Fat Diet-Induced Obesity

Mejoras Moleculares y Morfológicas en el Tejido Adiposo Blanco Mediadas por Polifilina I en Obesidad Inducida por Dieta Rica en Grasas

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SUMMARY: Obesity is a growing global health problem linked to insulin resistance (IR), dyslipidemia, and hepatic steatosis. Polyphyllin I (PPI), a steroidal saponin, has exhibited diverse pharmacological effects, but its anti-obesity and hepatoprotective potential remain unclear. This study investigated the anti-obesity, hypolipidemic, anti-hyperglycemic, and hepatoprotective effects of PPI in high-fat diet (HFD)-induced obesity in rats, with a focus on the role of AMP-activated protein kinase (AMPK) activation. Rats were distributed into six groups and treated with either a basal diet, a PPI control, an HFD, an HFD plus PPI (2 or 5 mg/kg), or an HFD plus PPI (5 mg/kg) plus the AMPK inhibitor, dorsomorphin. Oxidative stress, inflammatory markers, and expression of lipid-regulatory genes were analyzed. Histopathological evaluation of white adipose tissue (WAT) was performed using H&E staining. PPI significantly reduced the body weight, fat mass, fasting glucose, insulin, and lipid profile in HFD-fed rats, independent of food intake. It activated WAT antioxidant enzymes (SOD, GSH, and catalase) and decreased WAT MDA and serum TNF- α and IL-6. PPI increased phosphorylation of AMPK, PPAR α , and ACC and downregulated SREBP1 and FAS. These effects were reversed by dorsomorphin. Histopathological analysis revealed marked improvements in adipocyte morphology and reduced inflammatory infiltration in WAT. PPI exerted potent anti-obesity, anti-inflammatory, antioxidant, and hepatoprotective effects through activation of AMPK and modulation of lipid metabolism, supported by histopathological evidence. The results suggest PPI could be a powerful tool in managing obesity and related metabolic health.

KEY WORDS: AMP-activated protein kinase; Saponins; Polyphyllin I; Obesity; Lipogenesis.

INTRODUCTION

Obesity is a critical public health challenge and a leading factor in morbidity and mortality worldwide, strongly associated with type 2 diabetes, hypertension, and specific cancers (Ahmed *et al.*, 2025). In Saudi Arabia, the adoption of unhealthy Western lifestyles has increased obesity prevalence to 10 %, the highest in the region (Alrasheed *et al.*, 2025). Excessive caloric intake contributes to liver diseases (Schiavo *et al.*, 2025). High-fat diet (HFD) promotes adipogenesis and suppresses lipolysis, regulated by SREBP-1, PPAR α , PPAR γ , and C/EBP α , while upregulating ACC and FAS and downregulating HSL (Xu *et al.*, 2024). White adipose tissue (WAT) accumulation correlates with insulin resistance (IR) and inflammation, predisposing to Non-Alcoholic Fatty Liver Disease (NAFLD) and Non-Alcoholic Steatohepatitis (NASH). Chronic overnutrition increases the production of reactive oxygen species (ROS) and enhances

the inflammatory signaling, particularly NF- κ B, leading to cytokine release and metabolic impairment (Chen *et al.*, 2025).

AMP-activated protein kinase (AMPK) activation is a promising strategy for the treatment of IR, NAFLD, and NASH (Wang *et al.*, 2025). AMPK is activated during energy deficits and promotes glucose uptake and fatty acid oxidation (Choi *et al.*, 2026). Suppressed AMPK activity is associated with insulin resistance, glucose intolerance, and WAT hypertrophy (Saadati *et al.*, 2025). AMPK activation inhibits lipogenesis, stimulates lipolysis (Wang *et al.*, 2025), and regulates SREBP1, ACC, CPT I, PLIN1, SIRT1, and HSL (Liang *et al.*, 2024).

Polyphyllin I (PPI) is a potent saponin and the primary bioactive constituent of *Paris polyphylla*. It is highly valued

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in modern pharmacological research for its broad-spectrum therapeutic potential, mainly as a natural anti-cancer agent (Yang *et al.*, 2024). PPI has shown cytoprotective effects through its anti-inflammatory and antioxidant activities (Huang *et al.*, 2020; Yang *et al.*, 2022; Kang *et al.*, 2024). PPI was reported to activate AMPK to induce cell autophagy, which inhibits the growth of non-small cell lung cancer (NSCLC) cells (Wu *et al.*, 2020), and to improve diabetes-related symptoms in gestational diabetes mellitus (GDM) mice (Cai *et al.*, 2022). However, its anti-obesity and hepatoprotective effects in HFD-fed rats remain unexplored. This study investigates the anti-obesity, hypolipidemic, anti-hyperglycemic, and hepatoprotective effects of PPIs in HFD-induced obesity in rats and the possible role of AMPK.

MATERIAL AND METHOD

Animals

This study used albino rats (*Rattus norvegicus*) weighing 100 ± 15 g, obtained from King Saud University, Saudi Arabia. The rats were housed in a controlled environment (20–22 °C, 40–60 % humidity) with a 12-hour light-dark cycle to ensure their well-being. They had unrestricted access to fresh water throughout the study. All procedures adhered to ethical guidelines and national regulations to minimize distress and promote animal health.

Diet composition

To induce obesity in rats, an HFD was administered, sourced from Research Diets Inc. (Cat. No. D12492). The HFD contained 35 % fat, had a caloric density of 5.24 kcal/g, incorporated soybean oil, and provided 72.8 g sucrose/773.85 g of diet. In comparison, the standard diet (Cat. No. D12450K) was much lower in fat (4.3 %) and calories (3.85 kcal/g) and lacked sucrose. Both diets were nutritionally balanced, with essential ingredients such as casein, cornstarch, and vitamins. Rats were acclimatized for one week before starting the diet to minimize stress, and the HFD protocol has been successfully used in previous studies to induce obesity over 16 weeks (Al-Harbi, 2024).

Drugs

Natural PPI powder with a purity of ≥ 99 % (Cat. No. PHL80560), dorsomorphin (Cat. No. P5499), and low-viscosity carboxymethylcellulose (CMC) (Cat. No. C5678) were purchased from Sigma Aldrich, USA. PPI and dorsomorphin were freshly prepared by dissolving the powders in a 0.5 % CMC/DMSO solution to get the final concentrations.

Experimental groups and design

The rats were distributed into six groups, with eight animals in each group. The groups were a control group maintained on a basal diet and administered a 0.5 % CMC solution as a control vehicle; a PPI control group administered 5 mg/kg of PPI; an HFD-fed group that received HFS and CMC; an HFD+PPI at 2 mg/kg or 5 mg/kg (Kang *et al.*, 2024); and a last group that was administered HFD+PPI (5 mg/kg) + dorsomorphin (Compound C, an AMPK inhibitor) at 2.5 mg/kg. Treatments, including vehicle and PPI, were administered three times per week for 16 weeks via gavage, while dorsomorphin was injected intraperitoneally, always two hours before PPI administration.

Body weight, BMI, and Lee index estimation in rodent models

Nutritional intake and body mass index (BMI) were calculated. The Lee index for obesity was calculated by dividing the cube root of body weight (g) by body length (cm) and multiplying by 1000; values exceeding 310 were considered indicative of obesity.

Blood and WAT collection

The rats underwent an overnight fasting period, followed by anesthesia with ketamine (80 mg/kg) and xylazine (10 mg/kg). Blood was collected via cardiac puncture into non-additive and EDTA tubes for serum and plasma, respectively. The samples were allowed to clot for 30 minutes, then centrifuged at $1200 \times g$ for 10 minutes, and stored at -20 °C. Euthanasia was performed in accordance with ethical guidelines using cervical dislocation. Subcutaneous, mesenteric, peritoneal, and epididymal adipose tissues were dissected, weighed, and frozen at -80°C for further analysis. Parts of adipose tissues were processed for H&E staining for histological examination.

Extraction of protein from WAT and biochemical analysis

Total proteins were extracted from 100 mg mesenteric adipose tissue using a specialized commercial protein extraction kit (Catalog ID AT-022, Invent Biotechnologies, MN, USA) according to the manufacturer's instructions. To prevent protein degradation and dephosphorylation, 5 μ L of protease/phosphatase inhibitor (Cat. No. 5872, Cell Signaling Technology, USA) was also added during the extraction procedure. Colorimetric quantification of protein was conducted using the Quick Start Bradford Protein Assay kit (Catalog ID 5000201EDU, Bio-Rad, Germany). Total levels of tumor necrosis factor-alpha (TNF- α) and interleukin-6 (IL-6) were measured using ELISA kits (Cat. No.

AE13958RA Abebio and Cat. No. NB-22-69655-96 Biotech chemicals, Köln – Germany). The total levels of malondialdehyde (MDA) (lipid peroxides), superoxide dismutase-1 (SOD1/Cu-Zn), reduced glutathione (GSH), and catalase levels in total mesenteric tissue homogenates were quantified using other ELISA kits (Cat. Nos. ER1878, ER0332, ER0274, ER0264, Fine Test, Hubei, China). Total levels of AMPK α 1 and its phosphorylated variants were assessed in total protein extract with specific rat ELISA kits (Cat. IDs LS-F55289 and LS-F36060; LS Bio, MA, USA). The p-ACC in total cell protein homogenates was analyzed using an ELISA kit (cat. no. abx255889, Abxexa, IL, USA). All used kits were rat-specific, and all analyses were performed in duplicate with $n = 8$ samples/group.

Biochemical analysis in plasma and serum

Serum levels of TNF- α and interleukin-6 (IL-6) were measured using the same ELISA kits used to measure their levels in adipose tissue. Various assays and ELISA kits specific for rats were provided from BioTrend Chemicals (Köln, Germany) to measure levels of plasma glucose (Cat. Nos. CC-81693), HbA1C (Cat. No. CC-80300), and insulin (Cat. Nos. NB-22-65611-96) and serum levels of free fatty acids (FFAs) (Cat. No. EK10453-48T) and total cholesterol (CHOL) were measured with an ELISA kit (Cat. Nos. EK11090-48T and EK7038-96T). Serum levels of low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were measured using the assay form (Cat. Nos. CC-79960 and CC-79970, BioTrend Chemicals, Köln, Germany).

Real-time polymerase chain reaction (qPCR) for AMPK and lipid metabolism-related genes

Total RNA was isolated from the liquid nitrogen-well-preserved mesenteric tissue samples using the RNeasy Mini Kit, specific to the adipose tissue (Catalog ID 74804, QIAGEN, Hilden, Germany), following the manufacturer's guidelines. For cDNA synthesis, 1 μ g of RNA was reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Catalog No. 20531) in a 20 μ L reaction, incubated at 37 °C for 60 minutes and 95 °C for 5 minutes. Quantitative real-time PCR (qPCR) was performed with the CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA), using a 20 μ L reaction mix containing 10 μ L of 2x SYBR Green PCR Master Mix (Applied Biosystems), 0.5 μ M of each primer, and 2 μ L of cDNA. The PCR conditions were an initial denaturation at 95 °C for 10 minutes, followed by 40 cycles of 95 °C for 15 seconds and 60 °C for 1 minute. Gene expression was calculated using the $\Delta\Delta$ Ct method with β -actin as the reference gene (Elsayed *et al.*, 2022). The

primers used were AMPK α 2 (NM_023991.1), forward: GTGGATCGCCAAATTATGCA, reverse: AACCTCAGGACCCGCATACA (product size: 65 bp); SREBP1c (NM_001276707.1), forward: CGGGACAGCTTAGCCTCTACA, reverse: CGGCCACAAGAAGTAGATCA (product size: 21 bp); FAS (NM_017332.1), forward: CCACAGGACAAGCCCATCTT, reverse: TCGGAGACAGTTCACCAAGC (product size: 159 bp); PPAR γ (NM_001145366.1), forward: AAGTTTGAGTTTGCTGTGAAGTTCA, reverse: CGATGGGCTTCACGTTTCAG (product size: 121 bp); and β -actin (NM_031144.3), forward: AGGCCCTCTGAACCCTAAG, reverse: CAGCCTGGATGGCTACGTACA (product size: 96 bp).

Histological study

Mesenteric WAT samples were initially fixed in formalin, dehydrated in graded ethanol, and then embedded in paraffin. 5-7 μ m-thick sections were mounted onto slides, deparaffinized with xylene, and rehydrated through a descending ethanol gradient. Hematoxylin & eosin staining was performed, followed by dehydration, clearing with xylene, and mounting with coverslips. Histological analysis was conducted by examining the slides under an Olympus CX23 light microscope (Olympus Corporation, Tokyo, Japan).

Statistical analysis

Statistical analysis of all data was done utilizing GraphPad Prism (Version 8). The Kolmogorov-Smirnov normality test was applied. One-way ANOVA followed by Tukey's post hoc test (assuming equal variance) was used to determine significant differences ($p < 0.05$). Data are expressed as means $\pm$ standard deviation (SD) for all datasets.

RESULTS

Impact of PPI on Obesity Parameters

Treatment with PPI significantly reduced the HFD-induced increases in body weight and weight gain and in fat accumulation, with lower mesenteric, subcutaneous, and peritoneal fat, despite similar food intake. The effect that was reversed by dorsomorphin administration (Table I).
Effect of PPI on Metabolic Parameters

Treatment with PPI significantly reduced the HFD-induced increase in fasting glucose, insulin levels, and HOMA-IR. The administration of dorsomorphin reversed these effects. (Table II).

Table I. Effect of polyphyllin I (PPI) on the body weight, food intake, and serum lipid profile in control.

Parameter	Control	Control + PPI (5 mg/kg)	HFD	HFD + PPI (2 mg/kg)	HFD + PPI (5 mg/kg)	HFD + PPI (5 mg/kg) + dorsomorphin (2.5 mg/kg)
Initial body weights (g)	114.4 ± 9.5	101.2 ± 10.4	103.2 ± 13.5	108.5 ± 14.9	110.3 ± 9.5	106.3 ± 11.4
Final body weights (g)	483.6 ± 49.6	405.5 ± 35.6 ^a	594.5 ± 66.3 ^{ab}	502.3 ± 44.5 ^{abc}	455.4 ± 51.3 ^{bcd}	615.4 ± 66.2 ^{abde}
Weight gain (%)	365.8 ± 30.5	303.2 ± 26.5 ^a	498.5 ± 44.3 ^a	395.3 ± 42.3 ^{abc}	345.4 ± 33.5 ^{bcd}	553.2 ± 55.9 ^{abde}
Average food intake (kg/last month/8 rats)	6.5 ± 0.63	6.8 ± 0.92	9.4 ± 1.2 ^{ab}	9.1 ± 1.4 ^{ab}	10.1 ± 1.5 ^{ab}	8.9 ± 1.4 ^{ab}
BMI (g/cm ²)	0.63 ± 0.09	0.67 ± 0.07	0.91 ± 1.1 ^a	0.79 ± 0.09 ^{ab c}	0.65 ± 0.07 ^{bcd}	0.89 ± 0.09 ^{abde}
Lee index	276.3 ± 21.4	269.4 ± 24.5 ^a	343.4 ± 31.9 ^{ab}	294.3 ± 25.6 ^{abc}	263.4 ± 23.7 ^{bcd}	338.9 ± 35.4 ^{abde}
Mesenteric fat (g)	8.5 ± 0.8.5	7.1 ± 0.64 ^a	13.5 ± 1.4 ^{ab}	11.1 ± 1.6 ^{abc}	8.8 ± 0.76 ^{bcd}	12.8 ± 1.5 ^{abde}
Subcutaneous fat (g)	10.3 ± 0.13	8.4 ± 0.85 ^a	18.4 ± 1.3 ^{ab}	14.6 ± 1.1 ^{abc}	10.8 ± 1.4 ^{bcd}	19.4 ± 2.2 ^{abde}
Peritoneal fat (g)	7.7 ± 0.65	7.3 ± 0.58	12.5 ± 1.42 ^{ab}	9.4 ± 0.85 ^a	7.5 ± 0.63 ^{cd}	11.8 ± 1.1 ^{abde}
Epididymal fat (g)	4.8 ± 0.47	4.4 ± 0.57	7.8 ± 0.77 ^a	6.3 ± 0.65 ^a	4.6 ± 0.66 ^{cd}	7.5 ± 0.07 ^{abde}
Total fat weight (g)	30.7 ± 2.1	25.8 ± 2.4 ^a	51.6 ± 3.6 ^a	40.2 ± 3.9 ^a	31.5 ± 1.9 ^a	48.9 ± 3.8 ^{abde}

Data were analyzed by a One-way ANOVA test followed by Tukey's t-test. All data are presented as mean ± standard deviation (n=8). Significant differences are indicated by superscript letters: a (vs. control), b (vs. PPI 2 mg/kg), c (vs. HFD), d (vs. HFD + PPI 2 mg/kg), e (vs. HFD + PPI 5 mg/kg).

Table II. Effect of polyphyllin I (PPI) on some diabetic markers and lipid profile in control and high-fat. diet (HFD) rats.

Parameter	Control	Control + PPI (5 mg/kg)	HFD	HFD + PPI (2 mg/kg)	HFD + PPI (5 mg/kg)	HFD + PPI (5 mg/kg) + dorsomorphin (2.5 mg/kg)
Fasting glucose (mg/dL)	108.4 ± 10.5	87.6 ± 7.9 ^a	200.4 ± 22.5 ^{ab}	167.4 ± 15.6 ^{abc}	113.3 ± 14.5 ^{bcd}	193.4 ± 18.6 ^{abde}
Fasting Insulin (ng/mL)	4.5 ± 0.68	4.1 ± 0.53	7.9 ± 0.65 ^{ab}	6.1 ± 0.54 ^{abc}	4.8 ± 0.63 ^{bcd}	7.6 ± 0.81 ^{abde}
HOMA-IR	1.2 ± 0.22	0.86 ± 0.05 ^a	3.84 ± 0.47 ^{ab}	2.58 ± 0.47 ^{abc}	1.29 ± 0.27 ^{bcd}	2.75 ± 0.39 ^{abde}
Serum HbA1C (%)	4.2 ± 0.67	4.17 ± 0.52 ^a	9.3 ± 0.82 ^{ab}	6.9 ± 0.54 ^{abc}	4.5 ± 0.39 ^{cd}	8.9 ± 0.73 ^{abde}
Serum Triglycerides (mg/dL)	77.6 ± 8.4	64.3 ± 5.8 ^a	188.9 ± 16.7 ^{ab}	103.7 ± 10.4 ^{abc}	74.5 ± 6.8 ^{bcd}	178.4 ± 19.4 ^{abde}
Serum Cholesterol (mg/kg)	88.5 ± 7.6	65.7 ± 5.9 ^a	284.4 ± 25.6 ^{ab}	183.4 ± 16.5 ^{abc}	102.8 ± 11.4 ^{abcd}	265.5 ± 28.4 ^{abde}
Serum LDL-c (mg/dL)	41.2 ± 5.1	31.2 ± 3.7 ^a	125.4 ± 13.4 ^{ab}	76.5 ± 7.4 ^{abc}	43.4 ± 5.4 ^{bcd}	114.5 ± 14.3 ^{abde}
Serum FFA (nmol/L)	223.4 ± 19.4	185.6 ± 16.9 ^a	544.5 ± 43.5 ^{ab}	404.5 ± 38.6 ^{abc}	294.7 ± 22.4 ^{abcd}	566.5 ± 58.7 ^{abde}

Data were analyzed by a one-way ANOVA test followed by Tukey's t-test. All data are presented as mean ± standard deviation (n=8). Significant differences are indicated by superscript letters: a (vs. control), b (vs. PPI 2 mg/kg), c (vs. HFD), d (vs. HFD + PPI 2 mg/kg), e (vs. HFD + PPI 5 mg/kg).

Table III. Effect of polyphyllin I (PPI) on serum enzymes of liver function in control.

Parameter	Control	Control + PPI (5 mg/kg)	HFD	HFD + PPI (2 mg/kg)	HFD + PPI 5 mg/kg)	mg/kg) + dorsomorphin (2.5 mg/kg)
ALT (U/L)	44.3 ± 4.8	41.4 ± 6.3	174.3 ± 20.4 ^{ab}	110.3 ± 9.8 ^{abc}	55.2 ± 4.1 ^{abcd}	185.4 ± 19.3 ^{abde}
AST (U/mL)	33.4 ± 4.5	35.6 ± 4.1	88.7 ± 7.8 ^{ab}	63.4 ± 5.8 ^{abc}	31.9 ± 3.7 ^{bcd}	91.7 ± 10.5 ^{abde}
γ-GTT (U/L)	16.7 ± 1.6	18.6 ± 1.6	66.8 ± 5.3 ^{ab}	43.4 ± 3.8 ^{abc}	24.5 ± 3.2 ^{bcd}	61.6 ± 5.7 ^{abde}

Data were analyzed by a one-way ANOVA test followed by Tukey's t-test. All data are presented as mean ± standard deviation (n=8). Significant differences are indicated by superscript letters: a (vs. control), b (vs. PPI 2 mg/kg), c (vs. HFD), d (vs. HFD + PPI 2 mg/kg), e (vs. HFD + PPI 5 mg/kg).

Impact of PPI on Liver Enzyme Levels

Treatment with PPI significantly reduced the HFD-induced increase in liver enzyme levels (ALT, AST, γ-GTT). These effects were reversed by dorsomorphin (Table III).

Antioxidant Effects of PPI in WAT

PPI treatment significantly reduced the HFD-induced increase in MDA levels in WAT and increased the levels of GSH, SOD, and catalase, indicating an antioxidant protective effect. These effects were reversed by dorsomorphin (Fig. 1).

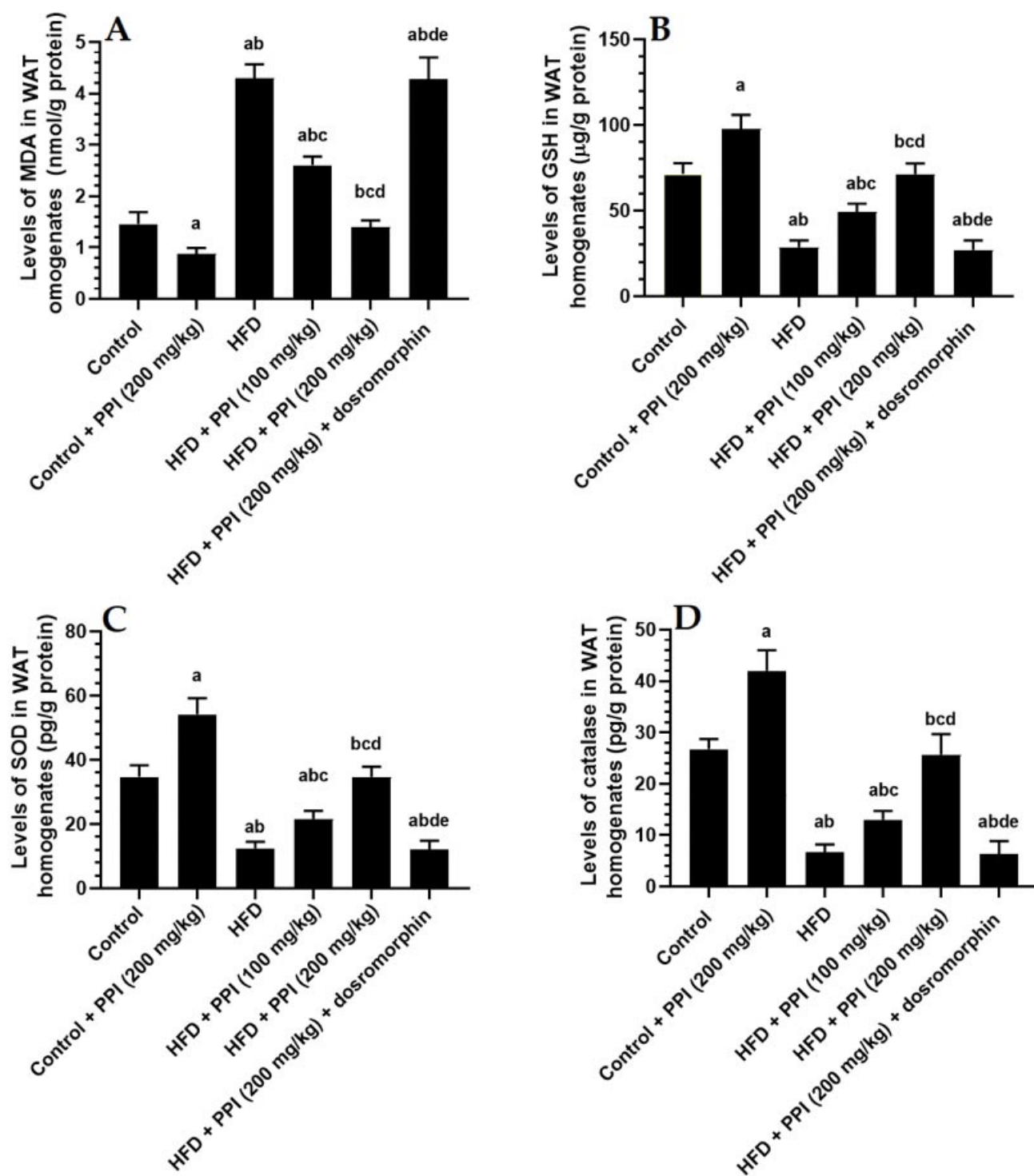


Fig. 1. Effect of polyphyllin I (PPI) on antioxidant levels in the white adipose tissue (WAT) of high-fat diet (HFD)-fed rats. Data were analyzed by a one-way ANOVA test followed by Tukey's t-test. All data are presented as mean $\pm$ standard deviation (n=8). Significant differences are indicated by superscript letters: a (vs. control), b (vs. PPI 2 mg/kg), c (vs. HFD), d (vs. HFD + PPI 2 mg/kg), e (vs. HFD + PPI 5 mg/kg).

Anti-inflammatory Effects of PPI and Dorsomorphin in WAT. PPI treatment significantly reduced the HFD-induced

increase in both WAT and serum IL-6 and TNF. These effects were reversed by dorsomorphin (Fig. 2).

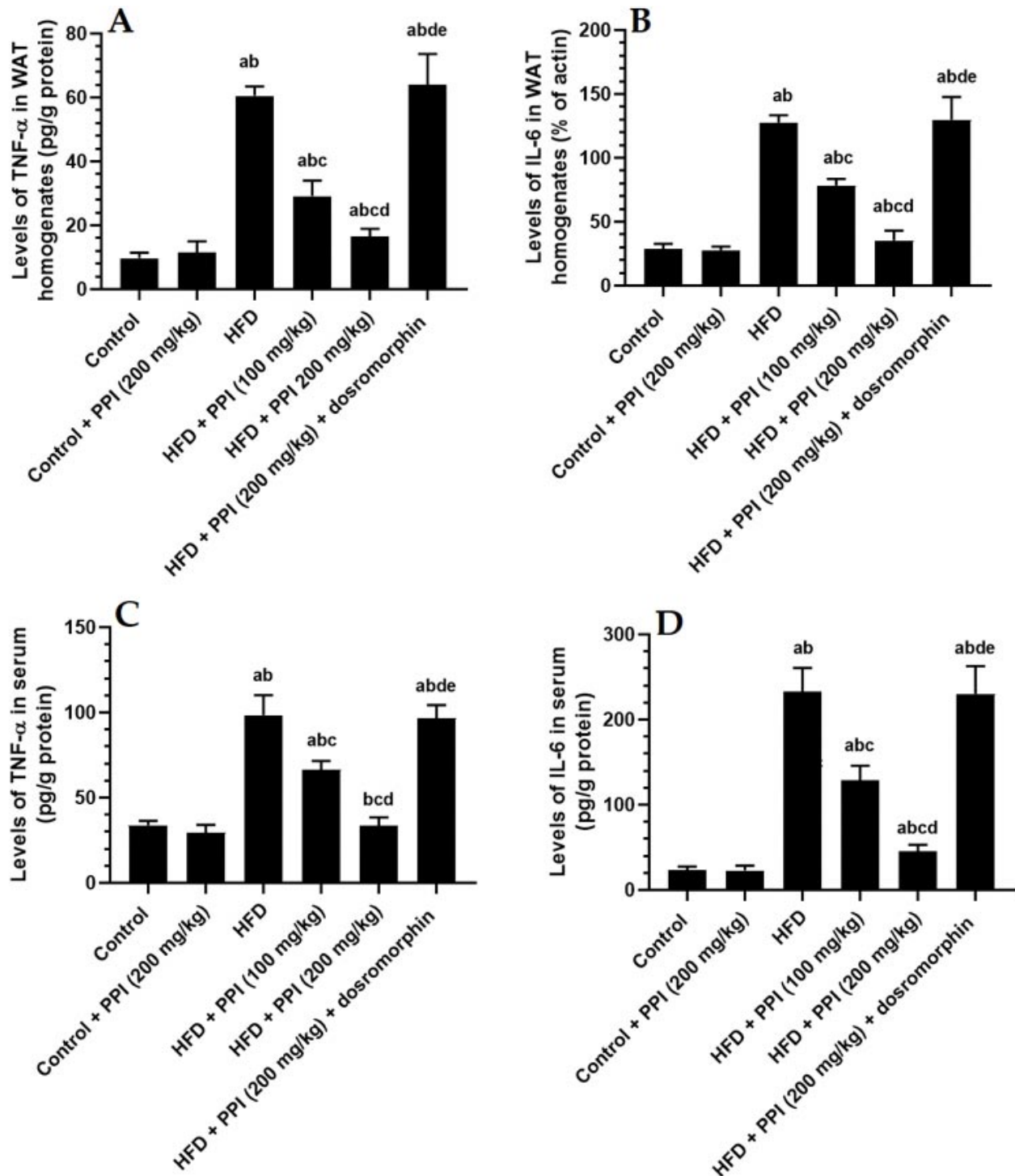


Fig. 2. Effect of polyphyllin I (PPI) on the levels of inflammatory markers in the serum and white adipose tissue (WAT) of high-fat diet (HFD)-fed rats. Data were analyzed by a one-way ANOVA test followed by Tukey's t-test. All data are presented as mean $\pm$ standard deviation (n=8). Significant differences are indicated by superscript letters: a (vs. control), b (vs. PPI 2 mg/kg), c (vs. HFD), d (vs. HFD + PPI 2 mg/kg), e (vs. HFD + PPI 5 mg/kg).

AMPK Activation and Its Modulation by PPI in WAT.
PPI significantly restored the HFD-induced decreases in

AMPK mRNA and phosphorylated AMPK protein levels in WAT. This effect was reversed by dorsomorphin (Fig. 3).

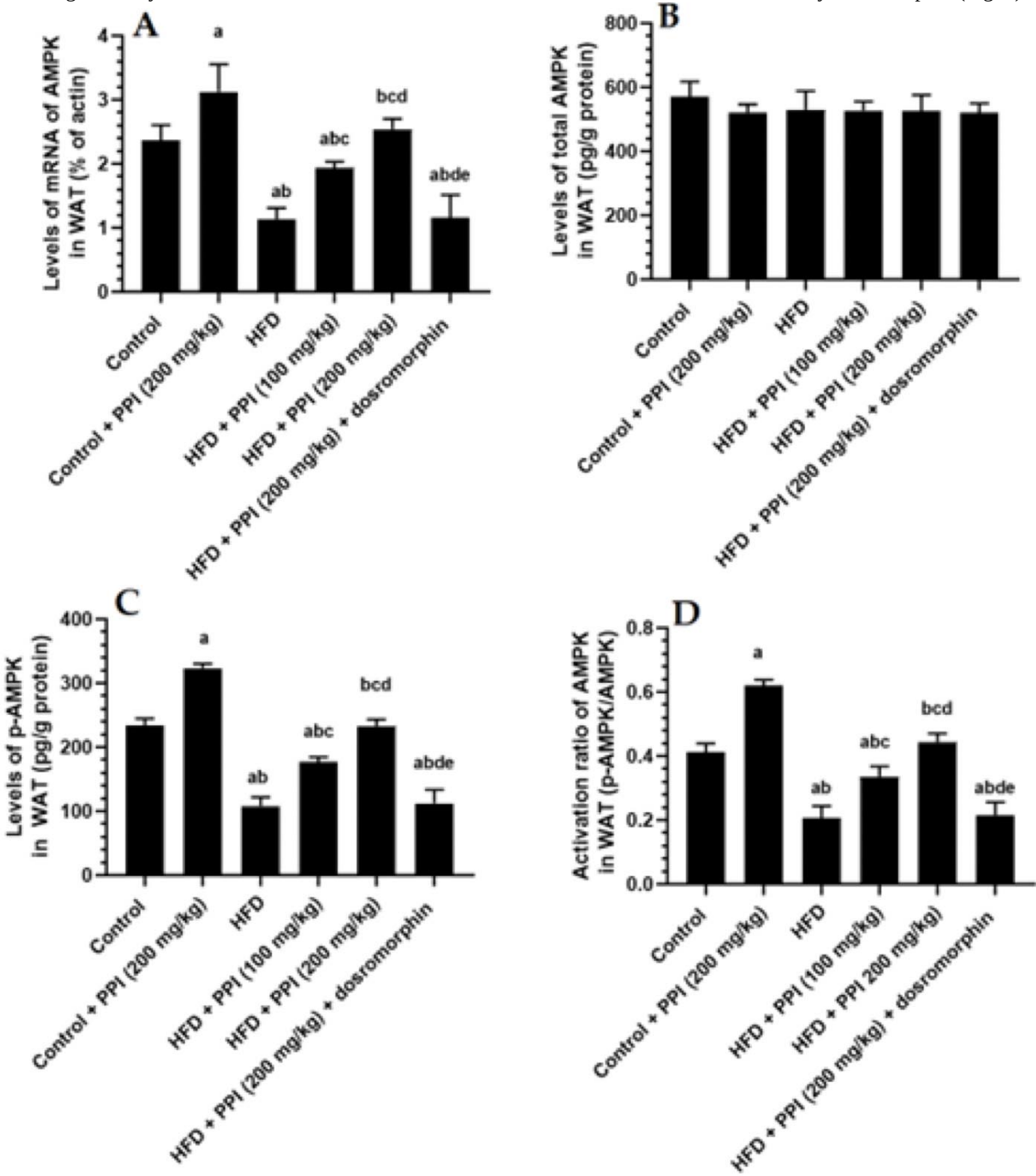


Fig. 3. Effect of polyphyllin I (PPI) on the levels of AMPK in the white adipose tissue (WAT) of high-fat diet (HFD)-fed rats. Data were analyzed by a One-way ANOVA test followed by Tukey's t-test. All data are presented as mean $\pm$ standard deviation (n=8). Significant differences are indicated by superscript letters: a (vs. control), b (vs. PPI 2 mg/kg), c (vs. HFD), d (vs. HFD + PPI 2 mg/kg), e (vs. HFD + PPI 5 mg/kg).

Effects of PPI on WAT lipid metabolism. PPI significantly reduced the HFD-induced increase in mRNA expression of SREBP1 and FAS, while increasing the levels of p-ACC

and PPAR in WAT. This effect was reversed by dorsomorphin (Fig. 4).

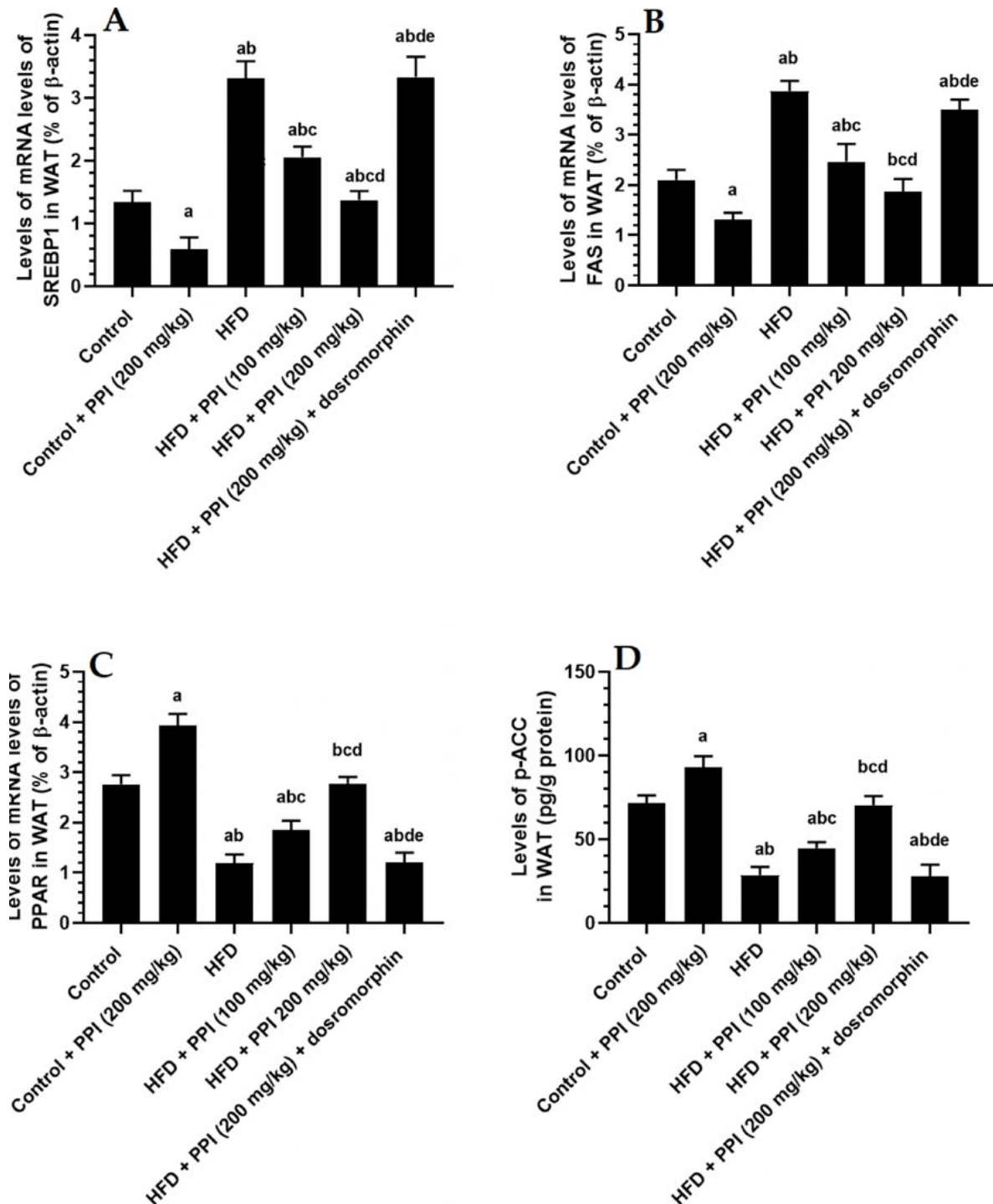


Fig. 4. Effect of polyphyllin I (PPI) on the expression levels of lipid metabolism-related genes in the white adipose tissue (WAT) of high-fat diet (HFD)-fed rats. Data were analyzed by a One-way ANOVA test followed by Tukey's t-test. All data are presented as mean $\pm$ standard deviation (n=8). Significant differences are indicated by superscript letters: a (vs. control), b (vs. PPI 2 mg/kg), c (vs. HFD), d (vs. HFD + PPI 2 mg/kg), e (vs. HFD + PPI 5 mg/kg).

Effects of PPI on WAT morphology

HFD caused adipocyte hypertrophy and increased

inflammatory cell infiltration. This effect was reversed by PPI (Fig. 5).

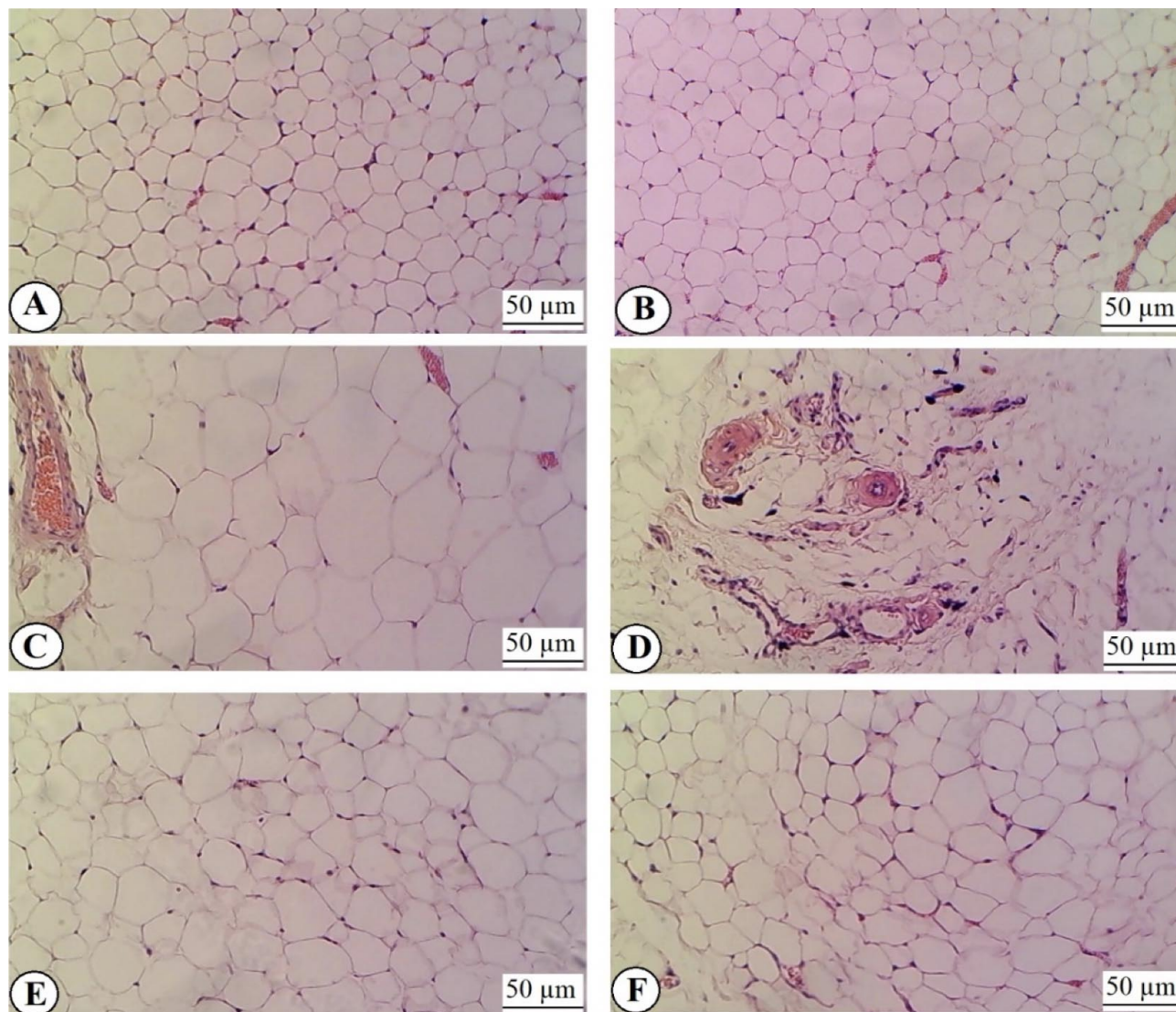


Fig. 5. Histological images of the white adipose tissue (mesenteric fat). A and B were taken from control and control + PPI (5 mg/kg) and show normally sized adipocytes. C and D were taken from HFD rats and show hypertrophic adipocytes and increased inflammatory cell infiltration. E and F were taken from HFD at 2 mg/kg and 5 mg/kg, respectively, and show a progressive decline in adipocyte size, with decreased inflammation. H&E (5 x)

DISCUSSION

Medicinal plants have long been recognized for their potential to enhance human health, particularly in the management of metabolic disorders. Plant-derived flavonoids and saponins modulate obesity by targeting adipose tissue function, insulin sensitivity, oxidative stress, and inflammation (Xue *et al.*, 2025). Despite this growing body of evidence, the anti-obesity effects of Polyphyllin I

(PPI) were not previously studied. The present study provides the first evidence that PPI significantly reduces body weight and adiposity while exerting marked anti-hyperglycemic and hypolipidemic effects in HFD-induced obesity. These effects occurred independently of food intake and were dose-dependent, suggesting that PPI directly modulates metabolic pathways rather than appetite or caloric consumption.

Adipose tissue can maintain systemic energy homeostasis by regulating glucose and fat metabolism and functioning as an endocrine organ (Duan *et al.*, 2025). The HFD rodent model closely mimics human obesity by inducing excessive weight gain, white adipose tissue (WAT) expansion, dyslipidemia, insulin resistance, and chronic low-grade inflammation (Celebi *et al.*, 2025). In agreement with these reports, HFD-fed rats in the current study exhibited increased body weight, elevated BMI and Lee index, impaired glucose tolerance, hyperinsulinemia, elevated HOMA-IR, dyslipidemia, and increased circulating free fatty acids. Moreover, WAT inflammation was evident from elevated TNF- α and IL-6 levels, reflecting obesity-associated inflammatory responses (Xu *et al.*, 2024). Importantly, PPI treatment reversed all these metabolic abnormalities, providing strong evidence for its anti-obesity and metabolic regulatory potential.

In the current study, HFD feeding significantly increased lipid peroxidation, inflammatory cytokine levels, and oxidative activity in WAT, reflecting oxidative stress overload. Oxidative stress and inflammation are tightly interconnected mechanisms that drive adipose tissue dysfunction and IR in obesity. Chronic overnutrition increases the production of reactive oxygen species (ROS) and enhances the inflammatory signaling, particularly NF- κ B, leading to cytokine release and metabolic impairment (Chen *et al.*, 2025). Treatment with PPI significantly suppressed the inflammation and oxidative stress by reducing IL-6, TNF- α , and MDA levels while enhancing endogenous antioxidant defense. Interestingly, these effects were not reversed by dorsomorphin, indicating that PPI exerts antioxidant and anti-inflammatory actions through AMPK-independent mechanisms. This is consistent with previous studies reporting that PPI nanocomposite hydrogel markedly enhances the healing of diabetic wounds by modulating the wound inflammatory microenvironment, with multifaceted therapeutic activities, e.g., anti-inflammatory, antioxidant, and antibacterial (Yang *et al.*, 2025). PPI attenuated cerebral neuroinflammation by enhancing autophagy-dependent M2 microglial polarization in a mouse model of middle cerebral artery occlusion, oxygen-glucose deprivation, and reoxygenation (Kang *et al.*, 2024). PPI regulates macrophage activity in rheumatoid arthritis by modulating polarization and inhibiting JAK2/STAT3 signaling (Yu *et al.*, 2025). PPI suppressed apoptosis, senescence, inflammatory IL-6 and TNF- α , and extracellular matrix degradation of osteoarthritic chondrocytes (Liu *et al.*, 2025). PPI improved myocardial function in the myocardial ischemia/reperfusion model in rats via its anti-inflammatory activity, as demonstrated by suppression of IL-6, TNF- α , and iNOS, increased antioxidant markers SOD and GSH, and inhibition of NF- κ B phosphorylation (Huang *et al.*, 2020). However, in higher doses, Polyphyllin I intervention was

found to induce oxidative stress and suppress the proliferation of hepatocellular carcinoma (HCC) cells (Yang *et al.*, 2024).

Adipogenesis and lipogenesis are key processes underlying adipose tissue expansion in obesity. SREBP1 is a master regulator of fatty acid and triglyceride synthesis, while PPAR α promotes fatty acid oxidation. In obese states, elevated SREBP1 and suppressed PPAR α expression favor lipid accumulation. In the present study, HFD-fed rats exhibited increased expression of SREBP1, FAS, and ACC, along with reduced PPAR α expression and ACC phosphorylation, confirming enhanced lipogenesis and reduced fatty acid oxidation (Xu *et al.*, 2024). PPI treatment significantly reversed these alterations, suppressing lipogenesis gene expression while enhancing fatty acid oxidation in a dose-dependent manner. PPI improved lipid dysfunction in hypoxia/reoxygenation (H/R)-induced H9c2 cells and in the coronary artery disease (CAD) rat model established by high-fat feeding. PPI attenuated myocardial dysfunction, myocardial cell apoptosis, lipid profile (LDL-C, TG, and TCH), and expression of lipogenesis markers (FAS, ACC, C/EBP α , and PPAR- γ), while increasing the serum HDL-C and expression of fat catabolism markers (ATGL, HSL, adiponectin, and Cpt1c) (Yang *et al.*, 2022).

We found that PPI has an anti-hyperglycemic effect in high-fat diet-fed rats. This is consistent with previous studies reporting that PPI can activate AMPK to improve diabetes-related symptoms, blood glucose, and glucose tolerance and to decrease serum inflammatory responses in gestational diabetes mellitus (GDM) mice through targeting the AMPK pathway (Cai *et al.*, 2022).

In this study, AMPK was shown to be increased with the high-fat diet. AMPK is a critical regulator of lipid metabolism and suppresses lipogenesis by inhibiting ACC and downregulating SREBP1 activity (Saadati *et al.*, 2025; Wang *et al.*, 2025; Choi *et al.*, 2026). PPI markedly increased AMPK phosphorylation in WAT, and its effects on lipogenic and oxidative genes were abolished by dorsomorphin, confirming AMPK-linked modulation of lipid metabolism. These findings align with previous evidence that PPI could directly activate AMPK to induce cell autophagy, which suppresses the proliferation of non-small cell lung cancer (NSCLC) cells (Wu *et al.*, 2020), and to improve diabetes-related symptoms in gestational diabetes mellitus (GDM) mice (Cai *et al.*, 2022).

In this study, PPI was found to be hepatoprotective against a high-fat diet, as evidenced by reduced liver enzyme levels. This is consistent with previous studies reporting that Paridis Rhizoma, via its main ingredients, polyphyllins I, II, VI, and VII, could exhibit hepatoprotective effects against

CCL4-induced liver fibrosis through the STAT3, SRC, VEGFA, and AKT1 pathways. Paridis Rhizoma could enhance liver function, regulate inflammatory responses, and decrease liver necrosis (Hong *et al.*, 2025). Polyphyllin VII was reported to exhibit hepatoprotective effects against liver fibrosis by inducing ferroptosis of hepatic stellate cells (Jiang *et al.*, 2025). Polyphyllin I was shown to be the most active compound of the Gupi Xiaoji Prescription (GXP) formula's components in the treatment of hepatitis B virus (HBV)-related liver cancer in HCC-derived HepG2.2.15 (Yu *et al.*, 2021). Polyphyllin I intervention was found to suppress the proliferation of hepatocellular carcinoma (HCC) cells via inducing ferroptosis and mitochondrial disruption through the Nrf2/HO-1/GPX4 axis (Yang *et al.*, 2024). On the other hand, some previous research reported the hepatotoxicity of Polyphyllin I and II derived from *Paris polyphylla* in the human liver cell line L-02 and in zebrafish. These effects were associated with disorders of the cholesterol biosynthetic pathway. PPI could directly bind with squalene epoxidase (SQLE), a rate-limiting enzyme in cholesterol synthesis, thereby disrupting the cholesterol biosynthesis pathway (Li *et al.*, 2023). According to ADMETlab predictions, several active components of *Paris polyphylla* demonstrate potential hepatotoxic effects (Liang *et al.*, 2026). This discrepancy concerning the hepatoprotective effects of PPI may be attributable to differences in experimental models, dosages, and treatment durations. Further in vivo studies are needed to explore the hepatoprotective effects of PPI fully.

CONCLUSION

Our results provide novel evidence that PPI exerts significant anti-obesity, anti-hyperglycemic, and hypolipidemic effects through the activation of AMPK and independent mechanisms that reduce oxidative stress and inflammation in WAT. This highlights the therapeutic potential of PPI as a promising approach to managing obesity, insulin resistance, and dyslipidemia. Further studies are needed to elucidate its clinical applications and therapeutic potential in human subjects.

Study Limitations

One limitation of this study is the reliance on rodent models, which can't mimic the complexity of human obesity, metabolic disorders, and hepatic conditions such as NAFLD. While the findings regarding the effects of PPI on dyslipidemia, oxidative stress, inflammation, and adiposity in HFD rats are promising, these results may not directly translate to human physiology. Additionally, the potential impact of PPI on hepatic lipid regulation and NAFLD in these rats requires further investigation, as our current study did not examine these effects in depth. Although the AMPK

inhibitor dorsomorphin supported AMPK activation, the exact mechanisms of PPI action, particularly in the liver, remain unclear. Furthermore, while the study suggests an AMPK-dependent mechanism, the lack of detailed investigation into the specific dose-response relationships, as well as the use of different animal models (e.g., rats vs. zebrafish), introduces variability that warrants further exploration. Finally, *in vitro* findings regarding the hepatotoxicity of PPI, reported in certain studies, contrast with our in vivo results, indicating that different experimental conditions, such as treatment duration and dosage, may influence the outcomes. These limitations should be addressed in future studies to confirm the therapeutic potential of PPIs for the management of obesity and dyslipidemia.

ELDAI ABDALLA, A. M.; ELDAI SALIH, A. G. K.; MUTWAKIL, H. M. M.; ELSAYED, H. R. H.; SIDEEG, A. M. B.; ALARABI, T. G. M.; HARUN, N. A. & OSMAN, R. M. A. Mejoras moleculares y morfológicas en el tejido adiposo blanco mediadas por polifilina I en obesidad inducida por dieta rica en grasas. *Int. J. Morphol.*, 44(4):1228-1239, 2026.

RESUMEN: La obesidad es un problema de salud global creciente, vinculado a la resistencia a la insulina (RI), la dislipidemia y la esteatosis hepática. La polifilina I (PPI), una saponina esteroidea, ha mostrado diversos efectos farmacológicos, pero su potencial antiobesidad y hepatoprotector aún no está claro. Este estudio investigó los efectos antiobesidad, hipolipemiantes, antihiper-glucémicos y hepatoprotectores de los IBP en la obesidad inducida por una dieta alta en grasas (DAG) en ratas, con un enfoque en el papel de la activación de la proteína quinasa activada por AMP (AMPK). Las ratas se distribuyeron en seis grupos y se trataron con una dieta basal, un control de IBP, una DAG, una DAG más IBP (2 o 5 mg/kg), o una DAG más IBP (5 mg/kg) más el inhibidor de AMPK, dorsomorfina. Se analizaron el estrés oxidativo, los marcadores inflamatorios y la expresión de genes reguladores de lípidos. La evaluación histopatológica del tejido adiposo blanco (TAB) se realizó mediante tinción con H&E. Los IBP redujeron significativamente el peso corporal, la masa grasa, la glucosa en ayunas, la insulina y el perfil lipídico en ratas alimentadas con DAG, independientemente de la ingesta de alimentos. Activó las enzimas antioxidantes del tejido adiposo blanco (SOD, GSH y catalasa) y disminuyó el MDA del tejido adiposo blanco y el TNF- α e IL-6 séricos. El PPI aumentó la fosforilación de AMPK, PPAR α y ACC, y redujo la expresión de SREBP1 y FAS. Estos efectos fueron revertidos por la dorsomorfina. El análisis histopatológico reveló mejoras significativas en la morfología de los adipocitos y una menor infiltración inflamatoria en el tejido adiposo blanco. El PPI ejerció potentes efectos antiobesidad, antiinflamatorios, antioxidantes y hepatoprotectores mediante la activación de AMPK y la modulación del metabolismo lipídico, respaldados por evidencia histopatológica. Los resultados sugieren que el PPI podría ser una herramienta poderosa para el manejo de la obesidad y la salud metabólica relacionada.

PALABRAS CLAVE: Proteína quinasa activada por AMP; Saponinas; Polifilina I; Obesidad; Lipogénesis.

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