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Dose-dependent anticholesterol effects of Fuji apple simplicia in a rat model of metabolic syndrome

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ABSTRACT

Background: Metabolic syndrome-associated dyslipidemia increases cardiovascular disease risk. Fuji apples (*Malus domestica*) contain bioactive compounds that may modulate cholesterol metabolism.

Objective: To evaluate dose-dependent anticholesterol effects of Fuji apple simplicia in metabolic syndrome rats.

Methods: Thirty male Wistar rats were allocated into five groups (n=6): negative control, positive control (metabolic syndrome without treatment), and three treatment groups receiving simplicia at 150, 300, or 450 mg/200 g body weight/day for 28 days. Metabolic syndrome was induced using high-fat diet and streptozotocin-nicotinamide injection. Total cholesterol was measured using enzymatic colorimetric assay and analyzed using mixed ANOVA.

Results: Simplicia produced significant dose-dependent cholesterol reductions compared to positive control ($p<0.001$). Reductions were 71.77 mg/dL (34.09%), 100.53 mg/dL (47.78%), and 112.02 mg/dL (52.75%) at doses of 150, 300, and 450 mg/200 g body weight/day, respectively.

Conclusion: Fuji apple simplicia exhibits significant anticholesterol activity in metabolic syndrome rats through a dose-dependent mechanism, supporting its potential as a complementary intervention for hypercholesterolemia management in metabolic syndrome.

Keywords: anticholesterol, hypercholesterolemia, lipid metabolism, Malus domestica, metabolic syndrome

Introduction

Metabolic syndrome represents a critical global public health challenge, characterized by interconnected metabolic abnormalities that substantially elevate cardiovascular disease and type 2 diabetes mellitus risk. Rising prevalence is driven by rapid urbanization, high-energy dietary patterns, increasing obesity rates, and sedentary lifestyles [1–3]. Global prevalence estimates range from 10% to 84%, reflecting considerable geographic variation [2]. In Indonesia, metabolic syndrome affects approximately 39% of adults, with prevalence remaining stable between 2013 and 2018 [4–6]. Recent surveillance from the 2023 Indonesian Health Survey suggests a continued upward trajectory, with higher prevalence among women [7].

Metabolic syndrome is clinically defined by central obesity, insulin resistance, elevated blood pressure, atherogenic dyslipidemia, and hyperglycemia [8,9]. These interconnected derangements collectively amplify serious cardiovascular event risk—including myocardial infarction and stroke—accelerate type 2 diabetes progression, and contribute to increased all-cause mortality [2,10,11]. Among the cardinal features, dyslipidemia, particularly elevated total cholesterol and low-density lipoprotein cholesterol, plays a critical role in atherosclerotic cardiovascular disease pathogenesis.

The absence of unified pharmacological approaches targeting all metabolic syndrome components has intensified research into natural bioactive compounds as adjunctive interventions [3,12]. Plant-derived phytochemicals, particularly those abundant in fruits and vegetables, demonstrate cardioprotective properties through multiple mechanisms [13]. Flavonoids, polyphenols, and carotenoids exert anti-inflammatory effects and ameliorate metabolic syndrome-associated abnormalities [14].

Apples (*Malus domestica*) have attracted considerable research attention due to their rich polyphenol and pectin content, both possessing lipid-modulating properties [15]. Clinical and experimental evidence indicates that whole apple and apple-derived product consumption reduces total cholesterol and low-density lipoprotein cholesterol concentrations [16,17]. The responsible bioactive constituents include polyphenolic compounds that modulate cholesterol metabolism through multiple pathways, and soluble fiber (pectin) that influences intestinal cholesterol absorption and bile acid excretion.

However, most previous investigations have focused on fresh apples, processed products (juice, extracts), or isolated bioactive fractions, with limited examination of whole apple simplicia—dried, powdered whole fruit retaining the complete phytochemical matrix. Furthermore, few studies have evaluated apple interventions specifically in metabolic syndrome models, where multiple concurrent abnormalities may influence treatment response. The dual-induction model combining high-fat diet with streptozotocin-nicotinamide (STZ-NA) injection provides a clinically relevant representation of human metabolic syndrome, yet has rarely been employed for apple-based interventions. Additionally, dose-response relationships for whole apple simplicia remain poorly defined.

This study evaluated the dose-dependent effect of Fuji apple (*Malus domestica*) simplicia on total cholesterol levels in a rat model of metabolic syndrome induced by combined high-fat diet and STZ-NA injection. The findings may provide preliminary evidence supporting apple-based dietary interventions as adjunctive approaches to lipid management in metabolic syndrome.

Methods

Ethical approval

This study was approved by the Health Research Ethics Committee of Dr. Moewardi Hospital, Surakarta, Indonesia (Approval No. 1.479/VII/HREC/2025, issued on 9 July 2025). All experimental procedures involving animals were conducted in strict accordance with institutional guidelines and international ethical standards for laboratory animal research.

Preparation of fuji apple simplicia

Fuji apples (*Malus domestica*) were procured from Istana Buah, Sleman, Special Region of Yogyakarta, Indonesia. The entire fruit, excluding seeds and cores, were processed into simplicia through a standardized protocol. Fresh apples were initially washed under running tap water to eliminate surface contaminants, then sliced into uniform thickness to ensure consistent drying. The sliced material was dried in a cabinet dryer maintained at 40 °C until the residual moisture content reached 0.02 (w/w). Following drying, samples were cooled to ambient temperature in airtight containers to prevent moisture reabsorption. The dried material was subsequently pulverized into coarse powder using a mechanical grinder and passed through a 60-mesh sieve to obtain uniform particle size. The collected powder was weighed and stored in sealed containers at room temperature until use [18–20].

Animal housing and maintenance

Male Wistar rats were maintained in solid-bottom polypropylene cages equipped with soft, absorbent bedding material (corncob, wood shavings, or paper-based substrate). Bedding was replaced at regular intervals to minimize ammonia accumulation and maintain hygienic conditions. Environmental parameters were controlled as follows: temperature

22 to 24 °C, relative humidity 40 to 46%, and adequate ventilation to ensure air quality. Cages were positioned in areas with minimal direct light exposure, consistent with the nocturnal behavioral patterns of rats. A 12 hour light-dark cycle was applied to preserve circadian rhythms. Housing density did not exceed six animals per cage, in accordance with established animal welfare guidelines [21].

Dosage calculation and justification

The dosage regimen was established through allometric scaling from human consumption data. The recommended apple intake for adults is two fruits per day, or approximately 360 g [22]. Conversion of fresh apple mass to dried powder yields approximately 40 to 50 g from 300 g of fresh fruit [23]. Interspecies dose conversion from humans to rats was performed using the Laurence and Bacharach coefficient [24], yielding an equivalent rat dose of approximately 0.9 g. Safety considerations were informed by previous toxicity evaluations demonstrating no adverse effects at doses up to 400 mg/200 g body weight (BW) [25]. Based on these pharmacokinetic and toxicological considerations, three experimental doses were selected: 150 mg/200 g BW/day, 300 mg/200 g BW/day, and 450 mg/200 g BW/day. The volume of oral administration was determined according to Ritschel's recommendations for laboratory rodents [26]. All doses were adjusted proportionally to individual body weight to maintain dosimetric accuracy.

Experimental design and study protocol

This experimental investigation employed a pretest-posttest control group design with a total duration of 68 days, structured as follows: adaptation period (days 1 to 7), metabolic syndrome induction phase (days 8 to 36), and treatment intervention phase (days 39 to 68).

Thirty male Wistar rats with initial body weights ranging from 180 to 195 g were randomly allocated into five experimental groups (n=6 per group): (i) Normal control: animals received standard BR AD-2 pellet feed and water ad libitum throughout the study period without metabolic syndrome induction; (ii) negative control: animals received standard feed and water ad libitum, with metabolic syndrome induced through high-fat diet (HFD) administration (days 8 to 35) followed by combined streptozotocin-nicotinamide (STZ-NA) injection on day 36, but without simplicia treatment; (iii) treatment groups: animals underwent identical metabolic syndrome induction as Group K2, followed by oral administration of Fuji apple simplicia at doses of 150 mg/200 g BW/day (T1), 300 mg/200 g BW/day (T2), and 450 mg/200 g BW/day (T3).

The high-fat diet comprised standard pellet feed supplemented with additional lipid sources to achieve approximately 40% total energy from fat. On day 36, metabolic syndrome was chemically induced via intraperitoneal injection of streptozotocin at 45 mg/kg BW and nicotinamide at 110 mg/kg BW. Following a 72-hour stabilization period post-injection, simplicia administration commenced on day 39 and continued daily until day 67, delivered via oral gavage in the morning hours (08:00 to 10:00).

The Lee index, calculated as the cube root of body weight (g) divided by naso-anal length (cm) multiplied by 1000, served as an indicator of obesity status.

Biochemical analysis of total cholesterol

Serum total cholesterol concentrations were quantified at two critical time points: day 36 (post-induction baseline) and day 68 (post-treatment endpoint). At each time point, animals were fasted for 12 hours prior to blood collection. Blood samples were obtained from the retro-orbital venous plexus under general anesthesia induced by intraperitoneal injection of ketamine (80 mg/kg BW) combined with xylazine (10 mg/kg BW). Approximately 1.5 to 2.0 mL of whole blood was collected into sterile microcentrifuge tubes and allowed to clot at room temperature for 30 minutes. Serum samples were obtained by centrifugation at 2,500 to 4,000 rpm for 10 minutes [27] and immediately stored at 4 °C until

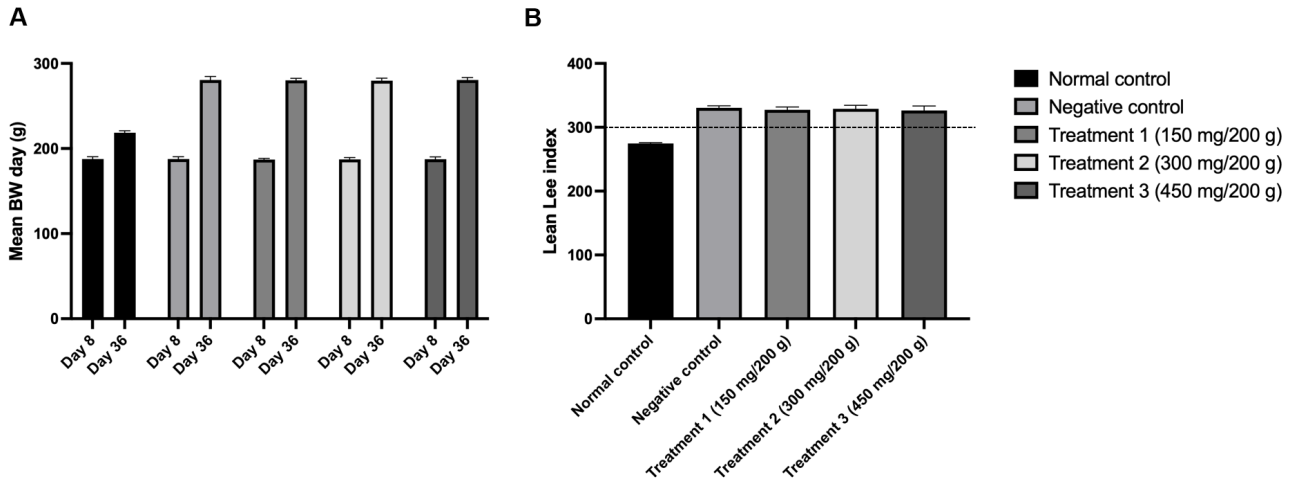

Figure 1. Validation of metabolic syndrome model. (A) Body weight changes from day 8 to day 36. (B) Lee index at day 36; dotted line indicates obesity threshold (Lee index = 300). Data are mean ± SD (n=6). ***p < 0.001 vs negative control.

Table 1. Baseline metabolic parameters

Group	Triglycerides (mg/dL)*	FPG day 39 (mg/dL)†	Total cholesterol (mg/dL)*
Normal control	69.85±2.25	69.42±2.69	86.49±2.56
Negative control	135.92±4.55	270.43±9.2	208.18±4.66
Treatment 1	131.92±2.31	270.93±3.84	210.53±2.72
Treatment 2	130.51±2.31	270.74±4.69	210.41±3.19
Treatment 3	131.57±1.75	270.24±1.24	212.39±2.47

Data are presented as mean ± SD. *Measured at day 36 (post-HFD, pre-treatment baseline). †Measured at day 39 (72 hours post-STZ-NA injection to confirm diabetes induction). FPG = fasting plasma glucose; TG = triglycerides; TC = total cholesterol. T1: 150 mg/200 g BW/day, T2: 300 mg/200 g BW/day, T3: 450 mg/200 g BW/day

analysis within 24 hours. Total cholesterol levels were determined using an enzymatic colorimetric method with the DiaSys Diagnostic Kit (Diagnostic Systems GmbH, Holzheim, Germany) according to the manufacturer's protocol. All measurements were performed in duplicate, and mean values were recorded for statistical analysis.

Statistical analysis

Sample size was determined based on previous similar studies, with n=6 per group providing adequate power. Data are expressed as mean ± standard deviation (SD). Normality was assessed using Shapiro-Wilk test, and homogeneity of variance using Levene's test. Total cholesterol data were analyzed using two-way mixed ANOVA with treatment group (between-subjects) and time (within-subjects) as factors. Following significant ANOVA results, one-way ANOVA was performed for each time point, with Tukey's HSD post hoc test for pairwise comparisons. Within-group changes were analyzed using paired t-tests. Statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Significance was set at $p<0.05$. Graphs were generated using GraphPad Prism version 10.0.

Results

Validation of metabolic syndrome model

The metabolic syndrome model was successfully established in groups K2–K5, as confirmed by anthropometric and metabolic parameters. Body weight analysis (Figure 1A) revealed substantial weight gain in induced groups (49.42–49.82% increase from day 8 to day 36) compared to negative control (16.43% increase). Lee index values (Figure 1B) for groups K2–K5 all exceeded the obesity threshold of 300 (range: 326.15–330.59), while

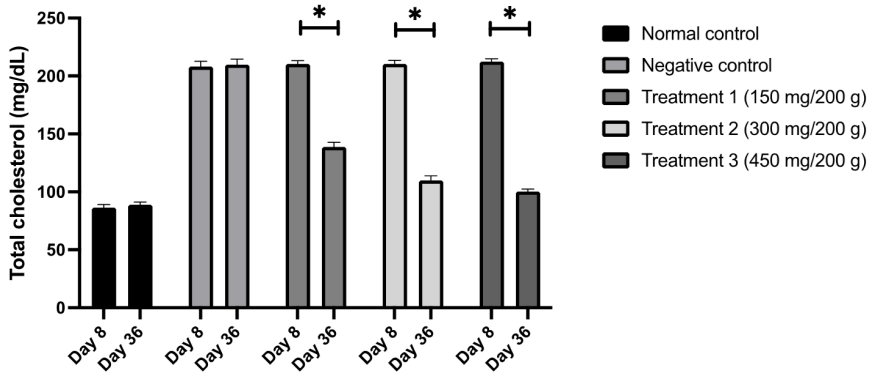

Figure 2. Dose-dependent effects of Fuji apple simplicia on total cholesterol. Total cholesterol at day 36 (baseline) and day 68 (post-treatment). Data are mean ± SD (n=6). *p < 0.001. Mixed ANOVA with Tukey's HSD

normal control remained in the normal range (274.77 ± 1.29).

Baseline metabolic parameters (Table 1) confirmed dyslipidemia and hyperglycemia in induced groups. Serum triglyceride concentrations in STZ-NA-induced groups (130.51–135.92 mg/dL) were approximately 2-fold higher than normal group (69.85 ± 2.25 mg/dL). Total cholesterol levels in induced groups (208.18–212.39 mg/dL) were 2.4–2.5-fold higher than normal group (86.49 ± 2.56 mg/dL). Fasting plasma glucose measured 72 hours post-STZ-NA injection demonstrated marked hyperglycemia in induced groups (270.24–270.93 mg/dL), representing 3.9-fold elevation compared to negative control (69.42 ± 2.69 mg/dL). The consistency of metabolic abnormalities—obesity, dyslipidemia, and hyperglycemia—confirmed successful metabolic syndrome induction.

Dose-dependent cholesterol-lowering effects of Fuji apple simplicial

Following 28 days of intervention (days 39–68), total cholesterol levels demonstrated differential responses across groups (Figure 2). Control groups K1 and K2 showed minimal changes, maintaining their respective baseline levels. In contrast, all treatment groups exhibited substantial dose-dependent reductions. Group K3 (150 mg/200 g BW/day) showed 34.09% reduction from baseline, K4 (300 mg/200 g BW/day) achieved 47.78% reduction, and K5 (450 mg/200 g BW/day) demonstrated the greatest effect with 52.75% reduction. Statistical analysis confirmed significant differences between all treatment groups and positive control ($p < 0.001$), with progressive dose-dependent effects among treatment groups.

Discussion

This study demonstrates that 28-day administration of Fuji apple (*Malus domestica*) simplicia produces significant, dose-dependent reductions in total cholesterol levels in a rat model of metabolic syndrome. Animals receiving simplicia at 150, 300, and 450 mg/200 g body weight per day exhibited mean cholesterol reductions of 71.77, 100.53, and 112.02 mg/dL, respectively, compared to untreated controls which maintained hypercholesterolemia throughout the intervention period.

These findings align with previous investigations demonstrating that apples and apple-derived products improve lipid profiles in high-fat diet models [28–31]. The dose-response relationship observed here corroborates dose-dependent patterns documented in earlier studies [28,30], suggesting that bioactive constituents exert proportionally greater effects at higher concentrations within the tested range. However, the modest incremental benefit between 300 and 450 mg/200 g body weight doses (11.49 mg/dL difference) suggests a potential saturation point may be approached at higher doses, warranting investigation in future studies.

The cholesterol-lowering effects are likely attributable to multiple bioactive compounds in

Fuji apple simplicia, particularly polyphenols, pectin, and phytosterols, acting through complementary mechanisms. Polyphenols modulate lipid metabolism through several well-characterized pathways. These compounds upregulate cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme catalyzing cholesterol conversion to bile acids, thereby enhancing cholesterol excretion through enterohepatic circulation [32]. Additionally, polyphenols activate AMP-activated protein kinase (AMPK), which phosphorylates and inactivates acetyl-CoA carboxylase (ACC) and suppresses sterol regulatory element-binding protein-1c (SREBP-1c) expression, collectively reducing endogenous lipid and cholesterol synthesis in hepatic tissue [33]. Polyphenols also reduce intestinal cholesterol absorption and increase fecal cholesterol excretion [34]. The pectin content may contribute through bile acid binding and gut microbiota modulation, both influencing cholesterol metabolism.

While these mechanisms are well-established for polyphenol-rich foods, we did not directly measure enzyme activities or expression levels in this study. Therefore, our mechanistic interpretation remains speculative and requires validation through targeted biochemical and molecular investigations.

The experimental model successfully recapitulated key features of human metabolic syndrome. The combination of high-fat diet and streptozotocin-nicotinamide injection induced obesity (Lee index >300), hyperglycemia, hypertriglyceridemia, and hypercholesterolemia in treatment groups, satisfying diagnostic criteria requiring at least three metabolic abnormalities [35].

High-fat diet promotes visceral adipose accumulation, induces insulin resistance through inflammatory signaling, and stimulates hepatic production of atherogenic lipoproteins [36–38]. The addition of streptozotocin-nicotinamide provides chemical induction that enhances metabolic dysfunction while maintaining partial β-cell function, mimicking gradual metabolic syndrome progression in humans. These findings are consistent with observations by Gunawan et al. [39] characterizing similar metabolic alterations in high-fat diet-fed animals.

The elevation in total cholesterol from 86.49 mg/dL in negative controls to values exceeding 200 mg/dL in metabolic syndrome groups represents characteristic dyslipidemic response to high-fat feeding [39–41]. This hypercholesterolemia results from disrupted cholesterol homeostasis, particularly impaired cholesterol excretion pathways and suppressed CYP7A1-mediated bile acid synthesis under conditions of dyslipidemia and insulin resistance [42].

This study has several limitations. First, biochemical assessment was limited to total cholesterol without lipoprotein fractionation (LDL, HDL, VLDL), preventing determination of effects on specific lipid fractions that have distinct cardiovascular implications. Second, hepatic enzyme activities (CYP7A1, AMPK, ACC) and liver histopathology were not evaluated, limiting mechanistic insight and precluding assessment of potential hepatotoxicity. Third, the 28-day intervention may not capture long-term efficacy, safety, or potential tolerance development.

The highest dose (450 mg/200 g body weight) was well-tolerated without observed toxicity, though comprehensive toxicological evaluation including liver and kidney function markers would be needed for clinical translation. Based on allometric scaling, this dose corresponds approximately to consuming one fresh Fuji apple daily in humans (assuming 12–15% dried yield from fresh fruit), suggesting practical feasibility for dietary intervention.

Future research should prioritize: (1) comprehensive lipoprotein profiling to determine whether reductions occur primarily in atherogenic LDL-C; (2) mechanistic studies directly measuring AMPK phosphorylation, CYP7A1 expression, and related pathway components; (3) well-designed human clinical trials to validate translational relevance and establish evidence-based therapeutic recommendations.

Conclusion

Fuji apple simplicia produces significant, dose-dependent reductions in total cholesterol levels in a rat model of metabolic syndrome, with reductions

of 34.09%, 47.78%, and 52.75% observed at doses of 150, 300, and 450 mg/200 g body weight/day, respectively. These findings provide preliminary evidence supporting the potential of Fuji apple simplicia as a natural adjuvant for lipid management in metabolic syndrome. However, comprehensive lipoprotein profiling, mechanistic validation, and controlled clinical trials are essential before clinical translation.

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Author contributions

HPA: Conceptualization, Formal analysis, Investigation, Data Curation, Writing – Original Draft. RPAP: Supervision, Methodology, Writing – Review & Editing. AS: Supervision, Methodology, Writing – Review & Editing. VIB.: Validation, Writing – Review & Editing.

Declaration of interest

The authors declare no conflict of interest.

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