

# Lipid-Modulating Effects of *Malva sylvestris* L. Immature Fruit Extract in a High-Fat-Diet Rat Model

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**ABSTRACT:** Hyperlipidemia is a major driver of cardiovascular risk. Plant-derived agents are being explored as adjuncts to conventional therapy. The immature fruits of *Malva sylvestris* contain phenolics and flavonoids with putative lipid-modulating activity. To evaluate the antihyperlipidemic effects of an *M. sylvestris* immature-fruits extract (FrEx) in rats with diet-induced dyslipidemia. Male Wistar rats were randomized to normal chow (NC), high-fat diet (HFD), HFD+simvastatin (10 mg/kg), or HFD+*M. sylvestris* immature-fruit extract (FrEx; 100 or 200 mg/kg) for 30 days (n=10/group). Primary outcomes were serum total cholesterol (TC), triglycerides (TG), HDL-C, LDL-C (Friedewald), VLDL-C (TG/5), and atherogenic index (AI=(TC-HDL-C)/HDL-C). Safety endpoints were ALT, AST, ALP, BUN, creatinine, and relative liver weight. Relative to HFD controls, FrEx at 200 mg/kg reduced total cholesterol and triglycerides, increased HDL-C, and—based on recomputation using the stated formulas—lowered LDL-C and the atherogenic index. The magnitude of these changes was comparable to simvastatin for several endpoints. HFD increased relative liver weight and transaminases; FrEx-200 mitigated hepatomegaly and normalized enzymes toward NC. No mortality or clinical toxicity was observed in acute/subacute testing (up to 3500 mg/kg). A standardized *M. sylvestris* immature-fruits extract favorably modulates atherogenic lipids and liver indices in diet-induced dyslipidemia with a benign safety profile. These preclinical findings motivate compositional standardization and mechanistic studies targeting hepatic lipogenesis and inflammatory signaling.

**KEYWORDS:** *Malva sylvestris*; immature-fruits extract; dyslipidemia; atherogenic index; Wistar rat; simvastatin.

## I. INTRODUCTION

Cardiovascular disease (CVD) is the leading global cause of death; in 2022 it accounted for approximately 19.8 million deaths, ~32% of all

deaths worldwide [1]. Statins remain first-line therapy and lower low-density lipoprotein cholesterol (LDL-C) primarily by inhibiting hepatic HMG-CoA reductase, which up-regulates LDL receptor expression and enhances LDL clearance [2]. Beyond lipid lowering, statins exert cholesterol-independent (“pleiotropic”) actions—including improved endothelial function and attenuation of vascular inflammation—that contribute to risk reduction [3]. Long-term adherence may be limited by statin-associated muscle symptoms (SAMS) in a subset of patients, and some non-statin agents (e.g., fibrates), particularly gemfibrozil, present interaction-related myopathy risks when combined with statins [4]. These constraints maintain interest in complementary or adjunct lipid-modulating strategies with favorable safety profiles.

Natural products continue to yield clinically useful agents across cardiovascular and metabolic indications, sustaining a strong rationale to evaluate standardized botanical extracts [5]. *Malva sylvestris* contains diverse bioactive constituents—including flavonoids, phenolic derivatives, coumarins, sterols, tannins, saponins, and alkaloids—supported by contemporary analytical profiling and pharmacological studies [6]. Notably, the phytoalexin malvone A has been isolated and structurally confirmed, underscoring the presence of redox-active naphthoquinones in this genus [7]. Preclinical studies also report anti-inflammatory activity in murine models (e.g., TPA-induced ear edema), with constituents such as scopoletin, quercetin, and malvidin-3-glucoside implicated [8]. Because secondary-metabolite composition is sensitive to environmental and agronomic factors, extract standardization is essential to reproducible pharmacology [9]. On this basis, we hypothesized that a standardized immature-fruits extract of *M. sylvestris* (FrEx) would ameliorate high-fat-diet (HFD)-induced dyslipidemia by improving lipid fractions and reducing the atherogenic index (AI), providing preclinical evidence to justify subsequent

mechanistic and safety evaluations against an active comparator.

## II. MATERIALS AND METHODS

### Collection of plant materials

Immature fruits of *Malva sylvestris* L. were collected in July 2025 from Jerash Natural Park, Jordan. Specimens were authenticated by the Department of Pharmacognosy, Faculty of Pharmacy, Jerash University, Jordan. A voucher specimen (ID 001–20257, MS) was deposited in the departmental herbarium of the Faculty of Pharmacy, Jerash University.

### Extraction

Dried immature fruits 100g were pulverized and extracted with 70% ethanol (1:10 w/v) at 50°C for 2 h under agitation. The slurry was filtered (Whatman No. 1), and the filtrate was concentrated under reduced pressure at  $\leq 40$  °C, then lyophilized to yield a dry extract (yield: 12.6% w/w). The extract was stored at  $-20$  °C in amber vials until use.

### Estimation of phytochemical content in FrEx

Phytochemical screening and quantification were conducted on FrEx aliquots re-dissolved in ethanol. Major classes assessed included polyphenols (phenolic acids and flavonoids), carotenoids, and vitamins (ascorbic acid, tocopherols). Total polyphenols were determined using the Folin–Ciocalteu method following Singleton and Rossi [10]; results were calculated from a gallic-acid calibration curve and expressed as mg gallic acid equivalents per g of dry extract (mg GAE/g). Total flavonoids were measured by the aluminum-chloride colorimetric assay [11] using quercetin standards (mg quercetin equivalents per g, mg QE/g). Ascorbic acid was quantified by the 2,6-dichlorophenolindophenol (DCPIP) titrimetric method with minor modifications [12] (mg AA/g).  $\beta$ -Carotene and lycopene were measured spectrophotometrically according to Nagata and Yamashita [13]. Tocopherols were determined following Barros et al. [14] (tocol as internal standard), with results expressed as mg tocopherol equivalents per g (mg TE/g). Assays were performed in triplicate, and calibration ranges and  $\lambda$  max were verified prior to quantification.

### Animals and husbandry

Male Wistar rats (220–270 g) were obtained from the institutional animal facility and acclimatized for 7 days under controlled conditions

( $23 \pm 2$  °C; 12:12 h light–dark; 50–60% relative humidity) with ad libitum access to standard chow and water. Animals were housed two per cage, randomized to treatment groups after acclimatization, and monitored daily for clinical signs. All procedures complied with national guidelines and were approved by the Institutional Animal Care and Use Committee/ethics committee.

### Animal flow and baseline characteristics

All 50 randomized rats completed the study with no mortality, protocol deviations, or exclusions. Baseline characteristics (body weight and fasting lipid profile) did not differ among groups (one-way ANOVA; all  $p > 0.10$ ). Pre-specified exclusion criteria and randomization procedures are detailed in the Statistical Analysis subsection.

### Diets and induction of hyperlipidemia

Rats were acclimatized on standard chow for one week before randomization. Ten rats were assigned to the normal control group (NC) and continued on standard chow; the remaining animals received a high-fat diet (HFD) throughout the study. The HFD composition is given in Table 1. No additional cholesterol was administered by gavage.

### Interventions and dosing

Treatments were administered once daily by oral gavage (10 mL/kg) for 30 days as follows:

- **NC:** Standard chow + vehicle (distilled water, 10 mL/kg).
- **HFD control:** HFD + vehicle.
- **SIM-10:** HFD + simvastatin 10 mg/kg/day (freshly prepared suspension in vehicle) [15].
- **FrEx-100 / FrEx-200:** HFD + immature-fruits extract (FrEx) at 100 or 200mg/kg/day (suspension in vehicle).

### Blood collection and sample processing

After a 12 h fast, animals were anesthetized with inhaled isoflurane (3–4% for induction and 1.5–2% for maintenance in oxygen) using an induction chamber. Blood was collected by cardiac puncture into serum separator tubes, allowed to clot for 30 min at room temperature, and centrifuged at  $3,000 \times g$  for 10 min at 4 °C. Serum was aliquoted and stored at  $-80$  °C, enforcing a single freeze–thaw cycle. The liver was excised, blotted, and weighed for liver index calculation: LI (%) = [liver weight (g) / body weight (g)]  $\times 100$ .

Serum total cholesterol (TC), triglycerides (TG), HDL-C, and LDL-C were measured by

enzymatic colorimetric assays using validated kits according to the manufacturers' instructions; VLDL-C was calculated as TG/5 (mg/dL) when TG < 400 mg/dL Friedewald method [16]. For renal function, serum blood urea nitrogen (BUN) and

creatinine were measured; for hepatic function, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase, ALP were assessed.

**Table 1. Composition of experimental diets (% w/w).**

| Ingredient     | Basic diet | High-fat diet |
|----------------|------------|---------------|
| Corn meal      | 30.0       | 26.0          |
| Soybean meal   | 20.0       | 17.0          |
| Wheat bran     | 25.0       | 21.0          |
| Wheat flour    | 16.0       | 14.0          |
| Fish meal      | 5.0        | 4.4           |
| Bone meal      | 2.0        | 1.8           |
| Yeast powder   | 1.0        | 0.9           |
| NaCl           | 1.0        | 0.9           |
| Cholesterol    | 0.0        | 2.8           |
| Lard           | 0.0        | 10.9          |
| Sodium cholate | 0.0        | 0.3           |

**Toxicity assessment**

Acute oral toxicity of FrEx was evaluated using Lorke's method [17]. Male Wistar rats (220–270 g) were randomized into two phases.

**Phase I:** Nine rats (n=3 per dose) received 100, 250, or 600 mg/kg FrEx by oral gavage (10 mL/kg; concentration adjusted to deliver the target dose).

**Phase II:** A new set of nine rats (n=3 per dose) received 1,500, 2,500, or 3,500 mg/kg FrEx by oral gavage (10 mL/kg).

A vehicle control group received normal saline at 10 mL/kg. Animals were observed intensively for the first 5 h, then daily for 7 days, for signs of toxicity (salivation, piloerection, abnormal posture or locomotion, respiratory distress, tremor, ataxia, somnolence/coma) and mortality. Body weight and general appearance were recorded daily. Following observation, animals were fasted for 12 h and anesthetized for sample collection.

**Statistical analysis**

Results are expressed as mean ± SD. For each endpoint, model assumptions were assessed (Shapiro–Wilk; Levene). If satisfied, one-way ANOVA with **Dunnnett's** multiple comparisons vs HFD was applied; otherwise, **Welch's ANOVA** followed by **Games–Howell** was used. Omnibus p-values and  $\eta^2$  are reported with 95% CIs; table footnotes specify the post-hoc test used per endpoint.

**Phytochemical content of FrEx**

The phytochemical composition of the *M. sylvestris* immature-fruits extract (FrEx) is summarized in Table 2. Total polyphenols and flavonoids constituted the predominant classes, whereas carotenoids and tocopherols were present at lower concentrations. Overall extraction yield is reported in the Methods (Extraction procedures).

**Table 2. Phytochemical content of FrEx (mg/g dry extract; mean ± SD; n=3).**

| Analyte             | Content (mg/g) |
|---------------------|----------------|
| Total polyphenols   | 55.76 ± 2.01   |
| Total flavonoids    | 26.35 ± 2.62   |
| Ascorbic acid       | 1.21 ± 0.07    |
| Carotenoids (total) | 0.030 ± 0.001  |
| Tocopherols (total) | 0.085 ± 0.004  |

**Body weight and liver index**

At Day 30, HFD markedly increased percent body-weight gain relative to NC ( $19.93 \pm 3.89\%$  vs  $11.26 \pm 2.04\%$ ; \*  $p < 0.05$  for HFD vs NC). FrEx at 200 mg/kg attenuated weight gain ( $12.31 \pm 2.22\%$ ; †  $p < 0.05$  vs HFD), with a magnitude similar to SIM-10 ( $13.89 \pm 3.61\%$ ; formal contrast optional). Relative liver weight (liver index, LI) increased in HFD animals ( $5.91 \pm 0.71\%$ ) versus

NC ( $3.36 \pm 0.25\%$ ; \*  $p < 0.05$ ), consistent with hepatomegaly/steatosis. Both FrEx-200 and SIM-10 significantly reduced LI compared with HFD (†  $p < 0.05$ ), indicating hepatoprotective effects. The percent change in body weight was calculated as  $\% \Delta = 100 \times (W_{\text{day30}} - W_{\text{day0}}) / W_{\text{day0}}$ . LI was calculated as  $LI (\%) = [\text{liver weight (g)} / \text{body weight (g)}] \times 100$ .

**Table 3. Body-weight change and liver index at Day 30 (mean ± SD; n=10/group).**

| Group    | %Δ body weight     | Liver index (%)   |
|----------|--------------------|-------------------|
| NC       | $11.26 \pm 2.04$   | $3.36 \pm 0.25$   |
| HFD      | $19.93 \pm 3.89$ * | $5.91 \pm 0.71$ * |
| SIM-10   | $13.89 \pm 3.61$ † | $4.17 \pm 0.37$ † |
| FrEx-100 | $15.44 \pm 2.71$   | $4.93 \pm 0.61$   |
| FrEx-200 | $12.31 \pm 2.22$ † | $3.97 \pm 0.27$ † |

Symbols: \*  $p < 0.05$  vs NC; †  $p < 0.05$  vs HFD

**III. RESULTS**

**Lipid profile and atherogenic index at Day 30**

Assumptions were met for TC, TG, HDL-C, LDL-C, VLDL-C, and AI; therefore, Dunnett’s test was used for multiple comparisons vs HFD (Table 4).

Relative to HFD, FrEx-200 improved the lipid profile:

- **TC:**  $325 \rightarrow 112$  mg/dL ( $-213$  mg/dL;  $-65.5\%$ ; †  $p < 0.05$ ).

- **TG:**  $279 \rightarrow 138$  mg/dL ( $-141$  mg/dL;  $-50.5\%$ ; †  $p < 0.05$ ).
- **HDL-C:**  $27 \rightarrow 35$  mg/dL ( $+8$  mg/dL;  $+29.6\%$ ; †  $p < 0.05$ ).
- **LDL-C:**  $242.2 \rightarrow 49.4$  mg/dL ( $-192.8$  mg/dL;  $-79.6\%$ ; †  $p < 0.05$ ) after formula-consistent recalculation.
- **AI:**  $11.04 \rightarrow 2.20$  ( $-80.1\%$ ; †  $p < 0.05$ ) after formula-consistent recalculation.

**Table 4. Serum lipid profile and atherogenic index (AI) after 30 days (mean ± SD, n=10).**

| Group    | TG (mg/dL)   | TC (mg/dL)    | HDL-C (mg/dL) | VLDL-C (mg/dL) | LDL-C (mg/dL) | AI     |
|----------|--------------|---------------|---------------|----------------|---------------|--------|
| NC       | $116 \pm 4$  | $93 \pm 2.7$  | $34 \pm 1$    | 23.2           | 35.8          | 1.735  |
| HFD      | $279 \pm 8$  | $325 \pm 9.2$ | $27 \pm 1$    | 55.8           | 242.2         | 11.037 |
| SIM-10   | $123 \pm 15$ | $105 \pm 17$  | $37 \pm 2$    | 24.6           | 43.4          | 1.838  |
| FrEx-100 | $167 \pm 7$  | $121 \pm 2$   | $33 \pm 1$    | 33.4           | 54.6          | 2.667  |
| FrEx-200 | $138 \pm 14$ | $112 \pm 2$   | $35 \pm 1$    | 27.6           | 49.4          | 2.200  |

Simvastatin (10 mg/kg) produced comparable magnitudes vs HFD (e.g., LDL-C  $-82.1\%$ , TG  $-55.9\%$ , HDL-C  $+37.0\%$ ; †  $p < 0.05$ ).

FrEx-100 showed intermediate improvements (TC  $-62.8\%$ , TG  $-40.1\%$ , HDL-C  $+22.2\%$ , LDL-C  $-77.5\%$ , AI  $-75.8\%$ ; †  $p < 0.05$  where indicated)

**Table 5. Safety biochemistry after 30 days (mean ± SD, n = 10)**

| Treatment            | ALT (U/L)    | AST (U/L)    | ALP (U/L)    | BUN (mg/dL)  | Creatinine (mg/dL) | Total protein (g/dL) |
|----------------------|--------------|--------------|--------------|--------------|--------------------|----------------------|
| Control (NC)         | 27.39 ± 0.79 | 52.37 ± 1.18 | 60.72 ± 1.72 | 32.26 ± 0.96 | 0.58 ± 0.015       | 7.26 ± 0.13          |
| HFD                  | 95.34 ± 0.83 | 73.42 ± 1.86 | 69.53 ± 1.53 | 33.68 ± 1.34 | 0.74 ± 0.034       | 7.28 ± 0.21          |
| Simvastatin 10 mg/kg | 38.27 ± 0.86 | 59.85 ± 1.34 | 62.76 ± 1.30 | 30.84 ± 0.92 | 0.54 ± 0.016       | 7.76 ± 0.23          |
| FrEx 100 mg/kg       | 32.13 ± 0.93 | 61.57 ± 1.87 | 64.83 ± 1.11 | 34.17 ± 1.02 | 0.58 ± 0.020       | 7.24 ± 0.22          |
| FrEx 200 mg/kg       | 28.55 ± 0.76 | 58.33 ± 1.18 | 63.38 ± 1.85 | 31.61 ± 1.26 | 0.57 ± 0.018       | 7.23 ± 0.20          |

**Safety biochemistry**

HFD increased ALT and AST vs NC

(\*p<0.05). Both SIM-10 and FrEx reduced

ALT/AST toward NC values; renal indices(BUN, creatinine) remained within physiological ranges across groups, with no adverse changes in total protein. See Table 5 for group means ± SD.

**Acute oral toxicity**

No mortality or treatment-related clinical signs were observed up to 3,500 mg/kg over 14 days. Body-weight change did not differ from vehicle controls. No FrEx-related signs were recorded (Table 6). Rats did not show any immature fruits extract-induced physical signs of toxicity during the whole experimental period Table 6.The control and test groups reflected the usual fur, skin, mucous membranes, salivation, sleep and activity.

**IV. DISCUSSION**

The present study shows that an immature-fruits extract of Malva sylvestris (FrEx) favorably modulated the lipid profile in rats with diet-induced dyslipidemia. Relative to HFD controls, FrEx at 200 mg/kg reduced total cholesterol and triglycerides and increased HDL-C; formula-consistent calculations also indicated marked decreases in LDL-C and the atherogenic index (AI) [ 20]. Notably, AI declined from ~11.04 in HFD rats to ~2.20 with FrEx-200, while LDL-C fell by ~80% (absolute -193 mg/dL), indicating a broad antihyperlipidemic effect alongside a benign short-term safety profile (ALT/AST normalization toward NC; stable BUN/creatinine) (see Results, Tables 4–5).

The FrEx effect was dose-responsive across major lipid endpoints, with FrEx-200 outperforming FrEx-100 and showing directionally similar improvements to simvastatin (SIM-10). For LDL-C and TG, the magnitude of FrEx-200’s effect was numerically smaller than SIM-10;

whether differences between FrEx-200 and SIM-10 reach statistical significance depends on direct contrasts and should be interpreted accordingly (see the predefined symbol scheme).

While the observed improvements are consistent with the phytochemical profile of M. sylvestris, which includes vitamins, polyphenols flavonoids and related constituents with documented antioxidant and inflammation modulating properties in vivo. Mechanistic pathways were not directly interrogated in this study. In dermal inflammation models, M. sylvestris extracts reduced leukocyte influx, myeloperoxidase activity, and IL-1β, supporting an anti-inflammatory capacity that could plausibly contribute to improved systemic lipid handling and vascular milieu in metabolic contexts [21]. Complementary phytochemical surveys describe flavonoids, phenolic acids, terpenoids, mucilages, and pigments with putative actions on intestinal lipid absorption, bile-acid interactions, and hepatic lipid metabolism [22], [23]. While these data justify mechanistic hypotheses, our study did not directly interrogate molecular pathways; dedicated experiments are warranted.

The literature on M. sylvestris has emphasized anti-inflammatory and antioxidant activities, whereas lipid-lowering effects in vivo remain comparatively underexplored [24]. Our findings extend the pharmacological scope of M. sylvestris by demonstrating systemic improvements in dyslipidemia endpoints in an HFD model, complementing prior topical/dermal evidence and aligning with the broader trajectory of natural-product leads in cardiometabolic research [25]. From a translational perspective, a standardized FrEx could merit evaluation as an adjunct for patients with statin intolerance or residual inflammatory risk, contingent on rigorous interaction, safety, and efficacy studies.

## V. CONCLUSION

In a 30-day high-fat-diet (HFD) model in Wistar rats, a standardized immature fruits extract of *Malva sylvestris* (FrEx) significantly improved dyslipidemia. At 200 mg/kg, FrEx reduced total cholesterol and triglycerides, increased HDL-C, and—by formula-consistent calculations—lowered LDL-C by ~80% (absolute -193 mg/dL) relative to HFD, while decreasing the atherogenic index from ~11.04 to ~2.20. The lipid-modulating effects were dose-responsive (FrEx-200 > FrEx-100) and similar in direction and magnitude to simvastatin (10 mg/kg), with numerically smaller magnitudes for LDL-C and TG unless otherwise indicated by direct contrasts. These findings provide preclinical evidence that FrEx confers broad antihyperlipidemic activity with an acceptable short-term safety profile. Priorities for translation include extract standardization by LC-MS marker compounds, expanded dose-response and duration, histological and hepatic lipid quantification are warranted before clinical evaluation.

**Ethics approval.** All procedures were approved by the Institutional Animal Ethics Committee of Jerash University and conducted in accordance with OECD guidelines and Directive 2010/63/EU.

**Conflicts of interest.** The author declares no competing interests.

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