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ORIGINAL ARTICLE

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Effects of Fenugreek and Atorvastatin on Atherosclerotic Biomarkers in Rats Fed a Modified High-Ghee Diet

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ABSTRACT

Background: Diets rich in saturated fats, including ghee, may promote dyslipidemia, atherogenesis, and cardiovascular injury. This study evaluated the effects of fenugreek, atorvastatin (ATO), and their combination on lipid, inflammatory, oxidative stress, cardiac, and histopathological markers in rats fed a modified high-ghee diet (HGD).

Methods: In this experimental study, 35 adult male Wistar rats were randomly assigned to five groups (n = 7): a normal-diet control group, an untreated HGD group, an HGD plus fenugreek seed powder group (500 mg/kg/day), an HGD *plus* ATO (30 mg/kg/day) group, and an HGD *plus* combined fenugreek and ATO group. The HGD was administered for 12 weeks, and treatments were given orally from weeks 9 to 12 while HGD feeding continued. At week 12, body weight, relative heart weight, serum lipid profile, atherogenic index of plasma (AIP), creatine kinase-MB (CK-MB), C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), cardiac malondialdehyde (MDA), reduced glutathione (GSH), and cardiac histopathology were assessed. Data were analyzed using analysis of variance (ANOVA), followed by Tukey's post hoc test.

Results: Compared with the normal-diet control, HGD feeding significantly increased mean body weight, serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein-cholesterol (LDL-C), AIP, CK-MB, CRP, IL-6, TNF-α, and cardiac MDA, and significantly decreased high-density lipoprotein cholesterol (HDL-C) and cardiac GSH. However, relative heart weight was not significantly changed. Compared with untreated HGD-fed rats, fenugreek significantly reduced the mean levels of body weight, TG, LDL-C, AIP, CK-MB, CRP, IL-6, TNF-α, and cardiac MDA and increased HDL-C and cardiac GSH, but did not significantly change the mean level of TC. ATO significantly reduced mean body weight and cardiac MDA. Combined fenugreek and ATO treatment significantly improved TG, LDL-C, HDL-C, AIP, CRP, IL-6, TNF-α, and cardiac MDA. Fenugreek and ATO improved HGD-associated histopathological alterations.

Conclusion: An HGD induces dyslipidemia, inflammation, oxidative stress, and early cardiac alterations. Fenugreek improves lipid, inflammatory, antioxidant, and histopathological outcomes, while combination therapy with ATO provides broader cardiometabolic benefits. Fenugreek may therefore serve as a promising adjunct to conventional lipid-lowering therapy, although further standardized and long-term studies are required.

Keywords: Fenugreek ▪ Atorvastatin ▪ Atherosclerosis ▪ High-ghee diet ▪ Rat model

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1. Introduction

Cardiovascular diseases (CVDs) are a leading cause of mortality in developed and developing countries.[1] Atherosclerosis is a major underlying cause of CVD and is characterized by the accumulation of lipids, inflammatory changes, and plaque development within arterial walls, which may ultimately result in heart attacks and strokes. Food-related factors, especially eating a high-fat diet, are important in the onset and development of atherosclerosis. Ghee, also known locally in Yemen as samna, is commonly used in food preparation. A higher risk of dyslipidemia and atherosclerosis has been linked to the consumption of diets high in saturated fatty acids (SFAs), such as ghee.[2]

Fenugreek (*Trigonella foenum-graecum* L.) is widely cultivated and consumed in Southern Europe and Asia. The entire plant is utilized as a beneficial nutrient-dense vegetable because it is high in vitamins, minerals, and protein.[3] Fenugreek is a bioactive ingredient found in it, including as fiber and saponins, have been shown to have anti-atherosclerotic and hypocholesterolemia effects. These components may lower the risk of atherosclerosis by improving lipid metabolism, enhancing bile acid excretion, and decreasing the absorption of cholesterol.[4]

Atorvastatin (ATO), a commonly prescribed statin medication, is renowned for its strong lipid-lowering effects and ability to inhibit the progression of atherosclerosis. ATO inhibits the production of cholesterol and promotes the clearance of LDL from the circulation by the enzyme HMG-CoA reductase.[5]

An extensive amount of research in humans and animal models has shown that ATO is effective in lowering the risk of atherosclerotic plaque formation and improving lipid profiles. It is critical to look at the effects of fenugreek and ATO in relation to a high-ghee diet (HGD) because of their potential benefits in modifying lipid profiles and preventing or reducing atherosclerosis.

2. Methods

2.1. Experimental animals

Thirty-five adult male albino Wistar rats, aged 10–12 weeks and weighing 180 ± 20 g, were obtained from the Animal House of the University of Science and Technology. The rats were acclimatized to laboratory conditions for one week before the start of the experiment. Seven rats were housed per cage under a 12-hour light/dark cycle, with free access to standard diet and water before dietary intervention. Rats were weighed and marked for identification.

2.2. Preparation of HGD and fenugreek seed powder

The ghee was purchased from Taiz city, Yemen. It was traditionally prepared from cow's milk, with a small amount of corn flour added during heating until the mixture boiled and became golden yellow. The HGD was prepared at the Animal Care Facility of the University of Science and Technology according to previously described recommendations.[6] It contained 25% ghee, 44% carbohydrate, 17% protein, 13% fiber, and 1% ash.

Fenugreek seeds were purchased from a local market in Sana'a, Yemen, and identified by the Botany Department of Sana'a University. The seeds were cleaned with distilled water, dried, ground into a fine powder, and weighed according to the required dose of 500 mg/kg body weight.[7] The powder was then dissolved in purified water before oral administration. Qualitative phytochemical screening of the aqueous fenugreek seed extract was performed to identify the presence of major secondary metabolites using standard chemical tests. Alkaloids were detected by treating 2 mL of the extract with a few drops of 1% HCl followed by Wagner's reagent, producing a reddish-brown color.[8] Tannins were as-

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sessed by adding 1–3 drops of 1% ferric chloride to 2 mL of extract, with a blue-black color indicating a positive reaction.[9] Saponins were identified by mixing 6 mL of extract with 6 mL of distilled water and shaking vigorously until a stable persistent foam or emulsion formed.[8] Flavonoids were tested by adding dilute ammonia solution followed by concentrated sulfuric acid to 5 mL of extract, producing a transient yellow color.[9] Glycosides were detected by mixing 1 mL of extract with glacial acetic acid, ferric chloride, and concentrated sulfuric acid, resulting in a reddish-brown color.[8] Phenols were identified by adding diluted iodine solution to 1 mL of extract, producing a temporary red color.[8] Proteins were tested by adding concentrated nitric acid to 2 mL of extract, producing a yellow color.[8] Carbohydrates were detected by mixing 1 mL of extract with Benedict's solution followed by concentrated sulfuric acid, resulting in ring formation.[8]

2.3. Experimental design

The rats were randomly allocated to five groups of seven animals each. Group 1 (normal-diet control) was fed a regular diet and orally administered 2 mL of distilled water per day; Group 2 (untreated HGD control) was fed with HGD for 12 weeks and orally administered 2 mL of distilled water per day; Group 3 received HGD *plus* fenugreek seed powder orally at 500 mg/kg/day from week 9 to week 12; Group 4 received HGD *plus* ATO at 30 mg/kg/day from week 9 to week 12; and Group 5 received HGD diet for 12 weeks and both fenugreek seed powder and ATO at the same doses from week 9 to week 12. Treatment was administered for four weeks while high-ghee feeding continued.[10,11]

2.4. Measurement of body weight and relative heart weight

Body weight was measured before the intervention and at predetermined intervals throughout the experiment. At the end of the experiment, the heart was excised, cleaned, and weighed. Relative heart weight was calculated using the following formula:[12]

$$\text{Relative heart weight (\%)} = \text{heart weight/final body weight} \times 100$$

2.5. Collection of blood and cardiac tissue

At the end of week 12, the animals were anesthetized with diethyl ether and euthanized. Blood samples were collected by cardiac puncture, allowed to clot, and centrifuged at 3000 rounds per minute for 20 minutes to separate the serum.

The hearts were excised, washed with physiological saline, and weighed. Each heart was divided into two parts. One part was fixed in 10% neutral buffered formalin for histopathological examination, whereas the second part was preserved in phosphate-buffered saline at –80°C for oxidative stress analyses.

2.6. Measurement of serum lipid profile

Serum total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) were measured in mg/dL using commercially available kits (Química Clínica Aplicada, S.A., Amposta, Spain) using Rayto RT-9200 semi-automatic chemistry analyzer (Rayto Life and Analytical Sciences Co., Ltd., Shenzhen, China). Low-density lipoprotein cholesterol (LDL-C) was estimated using the Friedewald equation,[13] as follows:

$$\text{LDL-C} = \text{TC} - \text{HDL-C} - \text{TG}/5$$

2.7. Calculation of atherogenic index of plasma

The atherogenic index of plasma (AIP) was calculated using the following formula:[14]

$$\text{AIP} = \log_{10}(\text{TG/HDL-C}).$$

2.8. Measurement of cardiac and inflammatory biomarkers

Serum creatine kinase-MB (CK-MB) was measured in U/L with a commercial kit (Química Clínica Aplicada, S.A., Amposta, Spain) using Rayto RT-9200 semi-

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automatic chemistry analyzer (Rayto Life and Analytical Sciences Co., Ltd., Shenzhen, China).

C-reactive protein (CRP) was measured using a latex agglutination test (Fortress Diagnostics Ltd., Antrim, UK). Serum interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) concentrations were quantified with enzyme-linked immunosorbent assay (ELISA) kits supplied by Bioassay Technology Laboratory (BT LAB; Shanghai Korain Biotech Co., Ltd., Shanghai, China), according to the manufacturer's instructions, using a readwell TOUCH automatic ELISA plate analyzer (Robonik India Pvt. Ltd., Navi Mumbai, India).

2.9. Measurement of cardiac oxidative stress biomarkers

Cardiac tissue was homogenized in phosphate-buffered saline at a concentration of 0.1 g tissue per milliliter using an OMNI 125 handheld homogenizer (OMNI International, Kennesaw, GA, USA). The homogenate was centrifuged at 4000 × g for five minutes at 4 °C. The resulting supernatant was used to determine malondialdehyde (MDA) as a marker of lipid peroxidation and reduced glutathione (GSH) as an indicator of endogenous antioxidant capacity with commercial kits (Biodiagnostic, Dokki, Giza, Egypt) using Rayto RT-9200 semi-automatic chemistry analyzer (Rayto Life and Analytical Sciences Co., Ltd., Shenzhen, China).

2.10. Histopathological examination

Formalin-fixed cardiac tissues were dehydrated through graded concentrations of ethanol, cleared, and embedded in paraffin. Approximately 5-µm thick sections were prepared using a microtome, stained with hematoxylin and eosin (H&E), and examined using an Olympus BX53 digital microscope (Olympus Corporation, Tokyo, Japan) to assess morphological abnormalities.

2.11. Data analysis

Data were analyzed using GraphPad Prism version 10.6 (GraphPad Software, Boston, MA, USA). Data were expressed as the mean ± standard deviation (SD). Comparisons of means among multiple groups were conducted using one-way or two-way analysis of variance (ANOVA), followed by Tukey's multiple-comparisons post hoc test. Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. Qualitative phytochemical constituents of the aqueous extract of fenugreek seeds

Table 1 shows the presence of alkaloids, flavonoids, phenols, carbohydrates, proteins, saponins, and tannins, while glycosides were absent.

Table 1: Phytochemical constituents of the aqueous extract of fenugreek seeds

Constituent	Qualitative result
Alkaloids	Positive (+)
Glycosides	Negative
Flavonoids	Positive (++)
Phenols	Positive (+)
Carbohydrates	Positive (+)
Proteins	Positive (+)
Saponins	Positive (+)
Tannins	Positive (+)

3.2. Effect of fenugreek and ATO on body weight and relative heart weight in HGD-fed rats

Figure 1a shows that rats in the HGD group exhibited a significantly higher mean body weight than those in the control group ($P < 0.001$). Treatment with fenugreek ($P = 0.010$) or ATO ($P = 0.001$) significantly reduced the mean body weight compared with the HGD group. Figure 1b shows that the HGD group had a modest but statistically non-significant increase in the mean relative heart weight compared with the control group. Administration of fenugreek, ATO, or their combination produced non-significant reductions in relative heart weight compared with the HGD group.

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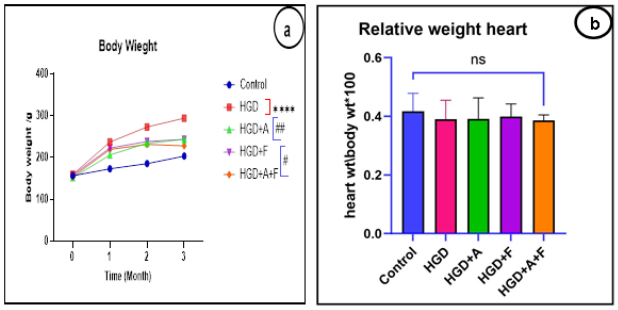

Figure 1: Effects of fenugreek seed powder (F) and ATO (A) on body weight and relative heart weight in HGD-fed rats

3.3. Effects of fenugreek and/or ATO on the lipid profile and AIP of HGD-fed rats

The effects of fenugreek and/or ATO on serum TC, TG, LDL-C, HDL-C, and AIP are presented in Figure 2a–e. Compared with the control group, rats fed the HGD exhibited significant increases in the mean levels of TC, TG, LDL-C, and AIP (all $P < 0.001$), together with a significant reduction in the mean level of HDL-C ($P = 0.001$). Compared with the HGD group, treatment with fenugreek alone or in combination with ATO did not significantly alter mean TC levels. However, both treatments significantly reduced the mean levels of TG, LDL-C, and AIP and significantly increased the mean levels of HDL-C (all $P < 0.001$).

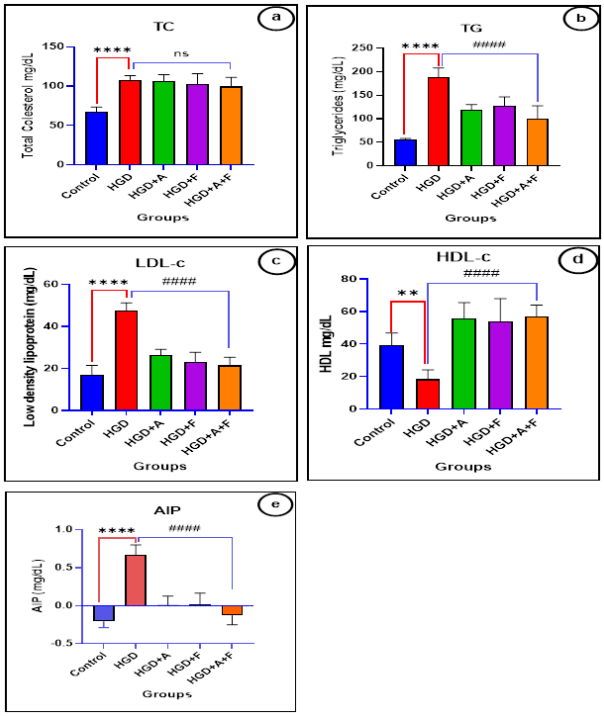

Figure 2: Effects of fenugreek seed powder (F) and ATO (A) on the lipid profile and AIP in HGD-fed rats

3.4. Effect of fenugreek and/or ATO on CK-MB of HGD-fed rats

Figure 3 shows that the HGD group exhibited a marked increase in the mean activity of CK-MB compared with the control group ($P < 0.001$). Fenugreek treatment significantly reduced mean CK-MB activity relative to the HGD group ($P < 0.001$). In contrast, ATO alone and the combined fenugreek-ATO treatment produced non-significant reductions in the mean CK-MB activity compared with the HGD group.

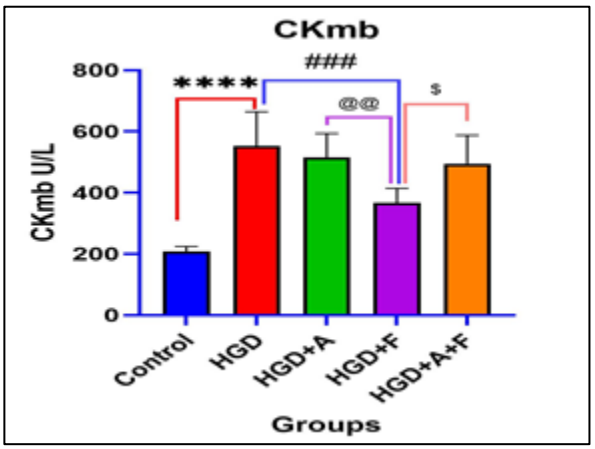

Figure 3: Effects of fenugreek seed powder (F) and ATO (A) on CK-MB in HGD-fed rats

3.5. Effects of fenugreek and ATO on cardiac MDA and GSH levels

Figure 4a shows that HGD-fed rats exhibited a significant increase in the mean cardiac MDA compared with the control group ($P = 0.020$). Treatment with fenugreek ($P = 0.010$), ATO ($P = 0.001$), and their com-

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bination (P <0.001) significantly reduced the mean levels of cardiac MDA relative to the HGD group.

Figure 4b shows that the mean level of cardiac GSH was significantly lower in the HGD group than in the control group (P <0.001). Fenugreek treatment significantly increased the mean level of cardiac GSH compared with the HGD group (P = 0.001), whereas ATO alone and the combined fenugreek-ATO treatment produced non-significant increases in cardiac GSH levels.

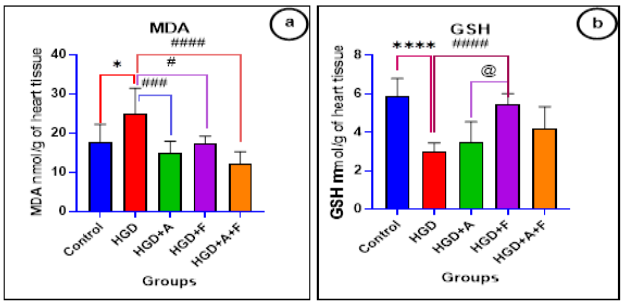

Figure 4: Effects of fenugreek seed powder (F) and ATO (A) on cardiac MDA and GSH levels in HGD-fed rats

3.6. Effects of fenugreek and ATO on CRP, IL-6 and TNF-α levels

Figure 5a–c shows that HGD-fed rats exhibited significant increases in the mean levels of CRP, IL-6, and TNF-α compared with the control group. Treatment with fenugreek alone or in combination with ATO significantly reduced the mean levels of CRP, IL-6, and TNF-α relative to the HGD group, with P = 0.010, P = 0.001, and P <0.001, respectively.

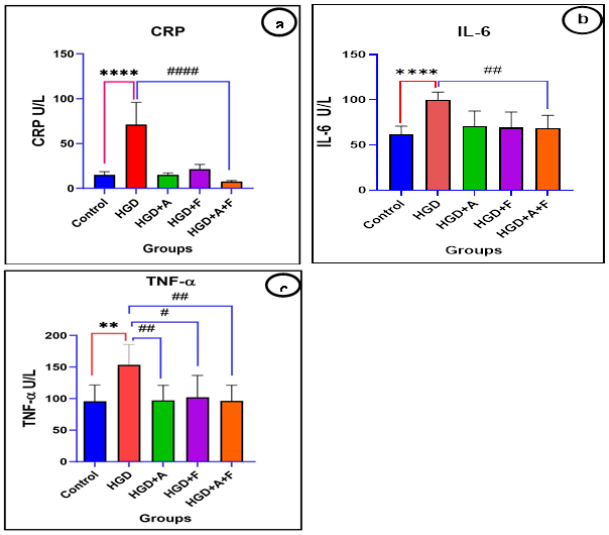

Figure 5: Effects of fenugreek seed powder (F) and ATO (A) on CRP, IL-6 and TNF-α levels in HGD-fed rats

3.7. Histopathological findings

Figure 6a–j illustrates the histopathological effects of fenugreek and ATO on cardiac tissue in rats fed an HGD, as assessed by H&E staining. The control group (a & b) showed preserved myocardial architecture, with no evidence of lipid deposition or vascular wall thickening. In contrast, the HGD group (c–f) exhibited marked histopathological alterations, including perivascular lipid accumulation, vacuolar changes in the aortic tissue, and enlargement of adipocytes surrounding the coronary artery, findings consistent with early atherogenic injury and metabolic stress. These changes were accompanied by elevated MDA levels, reflecting enhanced lipid peroxidation and oxidative damage. Treatment with ATO (g & h) or fenugreek (i & j) substantially improved myocardial architecture and reduced lipid accumulation compared with the HGD group.

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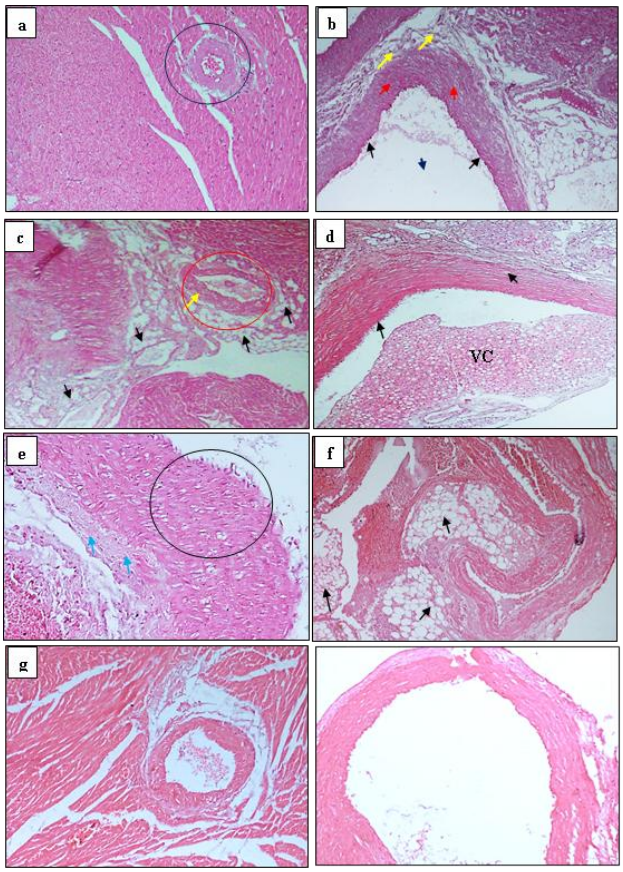

Figure 6: Representative H&E-stained photomicrographs of cardiac and vascular tissues in experimental groups compared with controls.

The control group (a & b) showed preserved vascular architecture, with an intact and continuous tunica intima and no evidence of endothelial disruption or detachment (black circle and black arrows). The tunica media exhibited regularly arranged smooth muscle fibers without hypertrophy or degeneration (red arrows), whereas the adventitia showed no inflammatory infiltration or fibrosis (yellow arrows). No lipid deposition, fatty streak formation, or vascular wall thickening was observed (×200). Rats fed the HGD (c–f) for 12 weeks exhibited marked histopathological alterations: (c) A cross-section of a cardiac blood vessel (red circle) showed prominent perivascular lipid accumulation, represented by multiple clear vacuolated spaces surrounding the vessel (black arrows), together with lipid deposition within the vascular wall (yellow arrows), indicating abnormal lipid infiltration (×100); (d) The aortic section showed multiple vacuoles, likely corresponding to lipid droplets removed during tissue processing (VC) with most nuclei remaining centrally located, although some appeared mildly hypertrophic (black arrow) (×200); (e) The perivascular matrix appeared less compact than that of the control group, although the vascular lumen remained free of intraluminal lipid deposition (black circle) (×200); (f) Enlarged adipocytes surrounding the coronary artery (black arrow) contributed to increased perivascular lipid accumulation and distortion of the adjacent tissue architecture (×200). These changes and metabolic stress were accompanied by elevated cardiac MDA levels, indicating enhanced lipid peroxidation and oxidative stress. Sections from rats treated with the HGD *plus* ATO (g & h) showed preserved vascular architecture, with no evident lipid deposition, fatty streaks, or vascular wall thickening, closely resembling the control group (×200). Sections from rats treated with the HGD *plus* fenugreek (i & j) showed largely preserved myocardial architecture and reduced lipid accumulation and fatty streak formation compared with the untreated high-ghee-diet group (×200 and ×100, respectively).

4. Discussion

The prevention and management of atherosclerotic CVD largely depend on controlling modifiable risk factors, particularly hyperlipidemia. Ghee, a form of clarified butter commonly consumed in Yemen and other regions, was used in the present study to induce hyperlipidemia in male rats because of its high SFAs and cholesterol content. In addition, repeated or prolonged heating of ghee may produce cholesterol oxidation products known as oxysterols. These compounds have been reported to constitute approximately 17.6% of the total sterol content of heated ghee and may contribute to atherogenesis independently of unoxidized cholesterol.[16] Oxidized ghee may contain several oxysterols, including 7-ketocholesterol, 20α-hydroxycholesterol, and 3-ketocholesterol.[17]

In the present study, HGD feeding markedly increased body weight compared with that in the normal-diet control group. This finding is consistent with evidence that diets rich in SFAs promote weight gain, adiposity, and metabolic dysfunction.[2,18] Similarly, a previous study in Wistar rats demonstrated that a diet containing 31% ghee significantly increased final body weight compared with other dietary interventions.[16] In contrast, treatment with fenugreek, ATO, or their combination significantly attenuated HGD-associated weight gain. The weight-modulating effect of fenugreek may be partly attributable to its fiber and galacto-oligosaccharide content, which may promote satiety, delay gastric emptying, reduce carbohydrate absorption, and consequently decrease overall energy intake.[19,20] These findings are consistent with those of a previous experimental study of diet-induced dyslipidemia.[10]

In the present study, no significant difference in the heart-weight-to-body-weight ratio was observed among the experimental groups, suggesting that the dietary and treatment interventions did not produce

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measurable cardiac hypertrophy during the study period. This interpretation is consistent with the use of the heart-weight-to-body-weight ratio as an indicator of cardiac hypertrophy.[21] Nevertheless, relative organ weight alone may not be sufficiently sensitive to detect early structural or functional cardiac abnormalities.

The HGD induced a pronounced dyslipidemic profile characterized by increased TC, TG, and LDL-C, together with reduced HDL-C. These findings support the reported association between excessive ghee consumption and cardiovascular risk. A previous study in male rabbits reported that ghee consumption increased TC and LDL-C by 8.43% and 10.8%, respectively.[21] Ghee has been reported to contain approximately 65% SFAs and 33% monounsaturated fatty acids.[22] Excessive ghee consumption may therefore contribute to an increased risk of CVD.[23] However, the high concentration of ghee used in the present study, representing 25% of the diet, substantially exceeded the proportion typically consumed as part of a human diet. Accordingly, these findings should be interpreted within the context of an experimental model of severe dietary lipid exposure and should not be directly extrapolated to usual human ghee consumption.

Fenugreek treatment significantly reduced TG and LDL-C while restoring HDL-C toward the control level. The hypolipidemic effects of fenugreek may be mediated by several bioactive constituents. Its soluble fiber and saponins may reduce intestinal cholesterol absorption, promote fecal bile-acid excretion, and modulate hepatic 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity.[19] The TG-lowering effect may also be associated with pectin-mediated bile-acid binding. In addition, diosgenin and other steroidal saponins may modulate lipid metabolism by inhibiting lipogenic enzymes and enhancing lipid catabolism.[24]

The antioxidant constituents of fenugreek may also inhibit LDL oxidation, thereby reducing its atherogenic potential.[25] The observed increase in HDL-C may be related to enhanced lecithin–cholesterol acyltransferase activity, which facilitates the incorporation of cholesterol into HDL particles and supports reverse cholesterol transport from peripheral tissues to the liver for excretion.[26] Furthermore, fenugreek's antioxidant properties may protect HDL particles from oxidative modification and preserve their functional activity.[24]

In the present study, ATO alone did not significantly improve all measured lipid variables or the AIP. Its lipid-lowering activity is principally mediated through inhibition of hepatic HMG-CoA reductase, which decreases endogenous cholesterol synthesis and upregulates hepatic LDL receptors, thereby enhancing receptor-mediated clearance of circulating LDL-C.[27] ATO may also modestly increase HDL-C by promoting reverse cholesterol transport and enhancing HDL-particle maturation.[5]

The AIP reflects the balance between circulating TG and HDL-C and has been proposed as a useful indicator of atherogenic risk and cardiovascular events.[27] In the present study, the index was markedly elevated in the HGD group and substantially reduced by fenugreek and combined fenugreek-ATO treatment, with the combined treatment producing the most favorable numerical response. This improvement may be related to the combined lipid-modulating effects of fenugreek and ATO, including reductions in TG and LDL-C and an increase in HDL-C, thereby decreasing the overall atherogenic potential of the lipid profile.[28] However, because a formal statistical interaction analysis was not performed, the findings cannot confirm a synergistic interaction between fenugreek and ATO.

The increase in serum CK-MB in the HGD group suggested myocardial or muscular stress associated

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with prolonged high-ghee feeding. Histopathological examination supported this interpretation by demonstrating perivascular lipid accumulation, multiple clear vacuolated spaces surrounding blood vessels, enlarged perivascular adipocytes, and a less compact perivascular matrix than that observed in the control sections. Although the vascular lumina remained free of clearly identifiable intraluminal lipid deposits, the perivascular lipid infiltration suggested early atherogenic changes and metabolic stress within the cardiac tissue. Fenugreek treatment significantly reduced CK-MB, suggesting a possible protective effect against HGD-associated cardiac injury.

The potential cardioprotective effect of fenugreek may be attributed to its antioxidant and anti-inflammatory properties, which may attenuate oxidative stress and inflammation-induced myocardial damage.[29] Its apparent association with reduced lipid-related morphological changes in cardiac tissue may provide additional protection against injury.[24] Nevertheless, CK-MB is not completely cardiac-specific, particularly in rodents. Future studies should therefore include more specific cardiac biomarkers, such as cardiac troponins, together with functional assessments such as echocardiography or electrocardiography. MDA was also significantly elevated in the HGD group. This increase was accompanied by histopathological evidence of lipid-associated morphological alterations and metabolic stress, suggesting that excessive dietary lipid exposure and lipid peroxidation may have contributed to oxidative injury in cardiac tissue.

The generation of reactive oxygen species under hyperlipidemic conditions can initiate lipid peroxidation and damage cellular membranes. Hyperlipidemic states are also associated with alterations in membrane structure and mitochondrial function, which may facilitate electron leakage from the mitochondrial respiratory chain or activate nicotinamide adenine dinucleotide phosphate (NADPH) oxidase.[30]

All treatment interventions significantly reduced cardiac MDA compared with untreated HGD-fed rats. The MDA-lowering effect of fenugreek may be attributed to its content of polyphenols and flavonoids, which scavenge free radicals, inhibit lipid peroxidation, and protect cellular membranes from oxidative damage.[31] Similarly, Bafadam et al.[29] demonstrated reduced cardiac lipid peroxidation, reflected by lower MDA concentrations, in rats treated with fenugreek seed preparations.

GSH is an essential component of endogenous antioxidant defense that protects cells against reactive oxygen species and various toxic compounds. In the present study, cardiac GSH concentrations were significantly reduced by HGD feeding but were significantly increased by fenugreek treatment. Fenugreek's enhancement of GSH may be related to its antioxidant constituents, which may upregulate antioxidant enzymes, promote GSH synthesis, and strengthen cellular defenses against oxidative stress.[10] Consistent with these findings, Arshadi et al.[32] reported that fenugreek enhanced cardiac antioxidant defenses, including GSH, catalase, and superoxide dismutase. The antioxidant activity of fenugreek seed extract is mainly attributed to its biologically active flavonoids and polyphenols. Isovitexin and vitexin have been identified among its principal antioxidant flavones.[33]

In the present study, CRP was elevated in the HGD group, together with increased concentrations of IL-6 and TNF-α. These changes reflect the heightened systemic inflammatory response associated with diet-induced dyslipidemia and atherogenesis. Both fenugreek and ATO, administered individually or in combination, markedly reduced these inflammatory biomarkers. The anti-inflammatory effects of fenugreek may be mediated by flavonoids, alkaloids,

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saponins, and other bioactive compounds that may inhibit pro-inflammatory cytokine production and modulate signaling pathways such as nuclear factor κB.[28] These findings are supported by a 2024 review by Alu'datt et al.,[24] which highlighted the potential role of fenugreek in attenuating inflammatory responses.

Fenugreek may also modulate the intestinal microbiota and improve intestinal barrier function, thereby reducing systemic inflammation; however, this proposed mechanism requires further investigation.[34] ATO may reduce CRP and other inflammatory mediators through pleiotropic effects that extend beyond its lipid-lowering activity. These effects include suppression of inflammatory cytokine production, improvement of endothelial function, inhibition of inflammatory cell activation, and stabilization of atherosclerotic lesions.[35,36]

The combined administration of fenugreek and ATO produced the greatest numerical reductions in several inflammatory biomarkers and shifted several lipid parameters toward control values. These findings suggest a potential complementary effect between the two interventions. These findings are consistent with the growing interest in the adjunctive use of nutraceuticals alongside conventional pharmacotherapy to improve cardiovascular profiles.[25]

This study was limited by its small sample size, inclusion of male rats only, use of single doses, and short treatment duration. The HGD may not reflect typical human intake, and the fenugreek preparation was not chemically standardized. Molecular mechanisms, specific cardiac biomarkers, functional cardiac assessments, and quantitative, blinded histopathological scoring were not evaluated. Moreover, the findings do not establish pharmacological synergy because no formal interaction analysis was performed. Larger, adequately powered studies using factorial statistical analyses, multiple dose levels, and longer treatment periods are needed to determine whether combined fenugreek and ATO treatment provides additive or synergistic benefits.

5. Conclusion

An HGD induces dyslipidemia, systemic inflammation, oxidative stress, and early cardiac tissue alterations. Fenugreek improves body weight, lipid profile, AIP, inflammatory biomarkers, antioxidant status, and cardiac histopathology, indicating potential hypolipidemic, anti-inflammatory, antioxidant, and cardioprotective effects. ATO primarily improves lipid abnormalities and inflammatory responses, while combined fenugreek and ATO treatment produces broader improvements across several cardiometabolic markers. These results identify fenugreek as a promising adjunct to conventional lipid-lowering therapy and support further studies using longer treatment periods, multiple dose levels, both sexes, standardized fenugreek preparations, and mechanistic and clinical assessments.

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Ethical approval

The study proposal was reviewed and approved by the Research Ethics Committee of the University of Science and Technology in Sana'a, Yemen (Ethical clearance No.: 1445/0034/UREC/UST). All procedures were conducted in accordance with institutional guidelines for the ethical care and use of laboratory animals.

Conflict of interest

The authors declare no conflict of interest associated with this article.

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None.

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