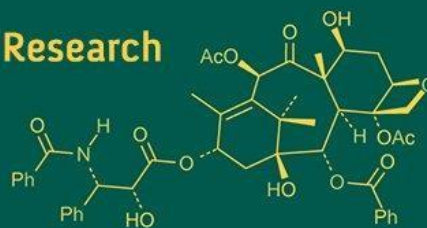
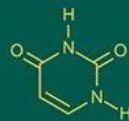
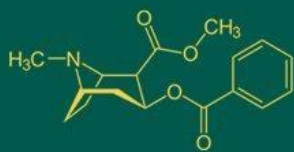


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Hypocholesterolemic and antioxidative effects of whole and skimmed goat milk in cholesterol-fed Wistar albino rats

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Abstract

This study evaluated the anti-hypercholesterolemic and antioxidative effects of whole goat milk (GM) and goat skim milk (GSM) in cholesterol-enriched diet (CED) induced hypercholesterolemic albino Wistar rats. Rats were divided into four groups (n=6): standard diet (SD), CED, CED+GM (25 ml/rat/day), and CED+GSM (25 ml/rat/day). Plasma lipid profile, atherogenic index (AI), faecal cholesterol excretion, hepatic lipid deposition, TBARS levels, liver index, and histomorphology were assessed after 30 days. CED significantly increased plasma total cholesterol, LDL-C, AI, hepatic cholesterol deposition, and oxidative stress markers. Supplementation with GM and GSM significantly reduced plasma TC, LDL-C, and AI, improved HDL-C levels, decreased TBARS in plasma and liver, and attenuated hepatic morphological alterations. GM demonstrated superior improvement in HDL-C and triglyceride reduction, whereas GSM showed moderate lipid-lowering effects. Faecal cholesterol excretion was not significantly elevated in GM and GSM groups compared to CED, suggesting hepatic modulation as the primary mechanism. These findings indicate that goat milk exerts protective effects against diet-induced hypercholesterolemia through lipid normalization and antioxidative mechanisms.

Keywords: Goat milk, hypercholesterolemia, Wistar rats, lipid profile, oxidative stress, atherogenic index

Introduction

Hypercholesterolaemia is a primary biochemical risk factor associated with the development of cardiovascular diseases (CVDs) (Nelson, 2012). Elevated levels of plasma total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) are strongly linked to atherogenesis, particularly when accompanied by oxidative stress-mediated lipid peroxidation (Fitzpatrick *et al.*, 2009) [4]. The oxidative modification of LDL particles enhances their uptake by macrophages, leading to foam cell formation and plaque development. From a food chemistry perspective, these processes are closely related to lipid oxidation reactions, free radical generation, and disruption of endogenous antioxidant defense systems. Therefore, the identification of food-derived components capable of modulating cholesterol metabolism and inhibiting oxidative reactions is of significant scientific and technological interest. Milk represents a complex biological matrix composed of lipids, proteins, carbohydrates, minerals, and minor bioactive constituents. Beyond its nutritional value, milk is increasingly studied as a source of functional ingredients with potential cardioprotective properties (Korhonen and Pihlanto, 2006) [13]. Among dairy species, goat milk has gained attention due to its distinct physicochemical and compositional characteristics (Kalyan *et al.*, 2021; Sansi *et al.*, 2026) [12, 18]. Goat milk fat is characterized by smaller globule size and a higher proportion of short- and medium-chain fatty acids (C6-C12), which exhibit rapid digestibility and preferential hepatic oxidation. Its phospholipid and unsaturated fatty acid fractions may contribute to improved lipid metabolism and membrane functionality. Additionally, goat milk generally contains lower cholesterol content compared with bovine milk, further enhancing its nutritional relevance in hyperlipidaemic conditions (Kalyan *et al.*, 2021) [12].

The protein fraction of goat milk, predominantly composed of caseins (α_1 -, α_2 -, β -, and κ -casein) and whey proteins (β -lactoglobulin, α -lactalbumin, serum albumin, and

immunoglobulins), serves as an important precursor for the generation of bioactive peptides upon enzymatic hydrolysis (Ahmed *et al.*, 2015; Zhu *et al.*, 2022) ^[1, 25]. During gastrointestinal digestion or controlled enzymatic processing, these proteins release peptides exhibiting antioxidative and angiotensin-converting enzyme activities, contributing to the emerging functional value of goat milk (Sansi *et al.*, 2023) ^[18]. At the molecular level, antioxidant peptides exert their activity through hydrogen atom donation, single-electron transfer mechanisms, and transition metal ion chelation, thereby inhibiting lipid peroxidation chain reactions. Structural attributes such as amino acid composition, sequence, hydrophobicity, and molecular weight significantly influence these bioactivities, with specific residues enhancing free-radical scavenging and redox modulation (Parcheta *et al.*, 2021; Zhu *et al.*, 2022) ^[17, 10]. Thus, goat milk proteins represent a promising substrate for generating functional peptides with redox-modulating and potential hypocholesterolaemic effects. Previous investigations from our laboratory demonstrated the antioxidative capacity of goat milk, its casein fractions, and enzymatically generated hydrolysates using established radical scavenging assays, including DPPH, ABTS, and lipid peroxidation inhibition systems. Furthermore, individual fractions of goat milk casein, whey proteins, and milk fat were evaluated in cholesterol-fed experimental models, where significant improvements in serum lipid profiles and oxidative stress markers were observed. These findings highlight the biochemical potential of isolated milk components to modulate lipid metabolism and oxidative pathways.

However, although fractionation studies provide mechanistic insight into the functional properties of individual components, milk is consumed as a whole system in which complex physicochemical interactions occur between lipids, proteins, and minor constituents. The presence of milk fat globule membrane (MFGM) components, phospholipids, and protein-lipid interactions may influence cholesterol absorption, micelle formation, and oxidative stability. Conversely, goat skim milk permits evaluation of the non-fat matrix, particularly protein-derived bioactive compounds, independent of the lipid fraction. To date, a systematic comparison of whole goat milk and goat skim milk in the context of diet-induced hypercholesterolaemia remains limited, particularly from a food chemistry standpoint. Therefore, the present study was designed to comparatively evaluate the hypocholesterolaemic and antioxidative effects of whole goat milk (GM) and goat skim milk (GSM) in CED-fed albino Wistar rats. Emphasis was placed on correlating compositional attributes with functional outcomes, including serum lipid profile (TC, TG, HDL-C, LDL-C, VLDL-C), atherogenic index, hepatic lipid deposition, fecal cholesterol excretion, and oxidative stability markers in plasma and liver tissues. By examining the integrated and fraction-specific contributions of goat milk constituents, this study aims to provide a mechanistic understanding of the role of goat milk as a functional dietary matrix in the management of hypercholesterolaemia.

Materials and Methods

Materials

Total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), were estimated using commercial diagnostic kits procured from Recombigen Laboratories Pvt. Ltd., Delhi, India. Thiobarbituric acid

(TBA), 1,1,3,3-tetraethoxypropane (TEP), cholesterol powder ($\geq 99\%$ purity), and other analytical reagents were obtained from standard suppliers. All chemicals used were of analytical and tissue culture grade.

Procurement and processing of goat milk (Isolation of skim milk from whole goat milk)

Fresh goat milk (Alpine $\times$ Beetal crossbred) was procured from the Livestock Research Centre, ICAR-National Dairy Research Institute (ICAR-NDRI), Karnal, India, collected aseptically, immediately chilled (4°C), and transported to the laboratory. The pooled milk was analyzed for pH, titratable acidity, fat, and protein using standard AOAC methods. Goat skim milk (GSM) was prepared by centrifugation at $5000 \times g$ for 20 min at 4°C , followed by removal of the cream layer; the centrifugation step was repeated three times to ensure efficient skimming. The fat content of the skim milk was verified using the Gerber method. Both whole goat milk (GM) and GSM were pasteurized (63°C for 30 min; LTLT), rapidly cooled, stored at 4°C , and used within 24 h. Milk was administered daily to rats at a dose of 25 mL/rat by oral gavage throughout the experimental period.

Animals and experimental design

Healthy Male Wistar albino rats (7-8 weeks old; ~ 150 g) were obtained from the Small Animal House, ICAR-National Dairy Research Institute (ICAR-NDRI), Karnal, Haryana, India. Animals were housed in polycarbonate cages under controlled environmental conditions ($24 \pm 1^{\circ}\text{C}$; 12 h light/12 h dark cycle) with free access to water. All experimental procedures were approved by the Institutional Animal Ethics Committee of ICAR-NDRI and conducted in compliance with CPCSEA guidelines. After a 1-week acclimatization period on a standard laboratory diet, twenty-four rats were randomly assigned to four groups ($n = 6$ per group) and fed for 30 days as follows: (i) standard diet (SD); (ii) cholesterol-enriched diet (CED); (iii) CED supplemented with whole goat milk (CED+GM; 25 mL/rat/day); and (iv) CED supplemented with goat skim milk (CED+GSM; 25 mL/rat/day). Diets were formulated according to AOAC (1990) recommendations, and their composition is presented in Table 1. Hypercholesterolemia was induced through dietary incorporation of cholesterol in the CED. Rats were provided 15 g of the respective diet per day, and feed intake was monitored daily by measuring residual feed. Mean daily caloric intake per rat was calculated from feed consumption and the metabolizable energy values of the diets.

Body weight, liver index, sample collection, and gross liver morphology

Body weight of each rat was recorded at the beginning of the experiment and subsequently at weekly intervals throughout the 30-day feeding period using a calibrated digital balance. Final body weight was recorded prior to sacrifice and used for calculation of the liver index. At the end of the 30-day experimental period, rats were fasted overnight and anesthetized prior to sample collection. Blood samples were obtained via the retro-orbital plexus into EDTA-coated tubes and centrifuged at 3500 rpm for 10 min at 4°C to separate plasma, which was stored at -20°C until analysis. Following blood collection, animals were sacrificed and the livers were carefully excised, rinsed with ice-cold normal saline to remove residual blood, blotted dry,

and weighed using a calibrated analytical balance (Adam HCB 3001, U.K.). The liver index was calculated as the ratio of liver weight to final body weight and expressed as a percentage (GAO *et al.*, 2005) [7]. Gross morphological examination of the liver was performed immediately after excision. Livers were visually inspected for changes in size, color, surface characteristics, and overall appearance. Representative photographs from each experimental group were captured to document diet-induced alterations and the effects of goat milk supplementation on hepatic morphology.

Estimation of plasma lipid profile

Plasma total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) were determined by enzymatic colorimetric assays using commercial diagnostic kits (Recombigen Laboratories Pvt. Ltd., Delhi, India) in accordance with the manufacturer's protocols. Very low-density lipoprotein cholesterol (VLDL-C) was estimated as TG/5. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation: $LDL-C = TC - (HDL-C + VLDL-C)$. The atherogenic index (AI) was calculated using Friedewald's equation (Friedewald, Levy, & Fredrickson, 1972) [6]; AI is the ratio of LDL-C to HDL-C computed as the ratio of LDL-C to HDL-C.

Estimation of hepatic cholesterol and triglycerides and faecal cholesterol

Approximately 250 mg of liver tissue or faecal sample from each rat was homogenized separately in 5 mL of chloroform:methanol (2:1, v/v) and maintained at 4 °C for 24 h, following the method of Folch, Lees, and Sloane-Stanley (1957). The homogenate was centrifuged at $950 \times g$ for 10 min, and the supernatant was collected. Subsequently, 1 mL of 0.9% NaCl solution was added, mixed thoroughly by vortexing, and centrifuged again to facilitate phase separation. The lower organic phase was carefully transferred into a glass test tube and evaporated to dryness. The dried lipid extract was reconstituted in 1 mL of methanol, and cholesterol and triglyceride concentrations were quantified using commercial assay kits (Recombigen Laboratories Pvt. Ltd.) according to the manufacturer's instructions.

Assessment of oxidative stress (TBARS)

Liver tissue homogenates (10% w/v) were prepared in ice-cold phosphate buffer (50 mM, pH 7.4) and centrifuged at $700 \times g$ for 10 min. The resulting supernatant was collected and used for thiobarbituric acid reactive substances (TBARS) analysis. Lipid peroxidation levels in plasma and liver samples were quantified according to the method described by Niehaus and Samuelsson (1968). This assay is based on the reaction of malondialdehyde (MDA) and other TBARS with thiobarbituric acid (TBA) under acidic conditions, forming a pink chromogen. The intensity of the developed color was measured spectrophotometrically at 535 nm, and the results were expressed as TBARS concentration. Which is based on the reactivity of malondialdehyde and other TBARS with thiobarbituric acid (TBA) in acidic conditions to generate a pink coloured compound, the absorbance of which is measured at 535 nm.

Statistical analysis

All experiments were conducted using six rats per group. Data were expressed as mean $\pm$ SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test using PRISM 8 and SPSS software. Differences were considered statistically significant at $p < 0.05$.

Results

Proximate composition of goat milk and goat skim milk

Male Wistar rats are a widely recognized model for diet-induced hypercholesterolemia due to their susceptibility to dietary cholesterol and associated alterations in lipid metabolism (González-Peña *et al.*, 2014) [8]. This study evaluated the hypocholesterolemic and antioxidative effects of whole goat milk (GM) and goat skim milk (GSM) in rats fed a cholesterol-enriched diet (CED) for 30 days. Analysis of milk composition revealed notable differences between GM and GSM. Whole goat milk had a pH of 6.60, acidity of 0.189%, and contained 4.61 ± 0.49 g/100 mL fat, 4.09 ± 0.55 g/100 mL protein, 4.69 ± 0.36 g/100 mL lactose, 0.73 ± 0.03 g/100 mL ash, and 14.00 ± 0.74 g/100 mL solids-not-fat (SNF). Skimming significantly reduced the fat content in GSM to 0.30 ± 0.05 g/100 mL, while protein (4.07 ± 0.52 g/100 mL), lactose (4.65 ± 0.34 g/100 mL), ash (0.72 ± 0.03 g/100 mL), and SNF (13.90 ± 0.68 g/100 mL) remained largely unchanged. The pH and acidity of GSM were 6.68 and 0.188%, respectively. This reduction in fat provides an opportunity to investigate whether the hypocholesterolemic effects of goat milk are primarily mediated by its lipid fraction or by non-lipid bioactive components such as proteins and peptides.

Effect on body weight and liver index

The final body weights of rats after 30 days of dietary intervention are presented in Figure 1a. No significant difference ($p > 0.05$) was observed in body weight among the SD (0.0292 ± 0.0009 kg), CED (0.0417 ± 0.0033 kg), CED+GSM (0.0418 ± 0.0040 kg), and CED+GM (0.0333 ± 0.0021 kg) groups at the end of the experimental period. Feeding of the cholesterol-enriched diet (CED) did not significantly alter body weight compared to the standard diet (SD), indicating that dietary cholesterol supplementation induced hypercholesterolemia without affecting overall growth performance. Similarly, supplementation with whole goat milk (CED+GM) or goat skim milk (CED+GSM) did not significantly influence body weight relative to the CED group ($p > 0.05$).

The liver index (liver weight/body weight ratio), however, showed significant alterations among groups (Figure 1b). Rats fed the CED exhibited a significant increase in liver index compared to the SD group (0.0450 ± 0.0032 vs. 0.0290 ± 0.0006 , $p < 0.05$), indicating hepatic enlargement. The increase in liver index was primarily attributable to increased liver weight rather than changes in body weight, suggesting cholesterol-induced hepatic lipid accumulation. These findings are consistent with the well-established effect of dietary cholesterol in promoting hepatic steatosis and liver hypertrophy. Notably, supplementation with whole goat milk (CED+GM) significantly attenuated the elevation in liver index compared with the CED group (0.0339 ± 0.0021 , $p < 0.05$). The liver index in the CED+GM group approached values comparable to the SD group,

indicating a protective effect against cholesterol-induced hepatic enlargement. In contrast, supplementation with goat skim milk (CED+GSM) did not produce a statistically significant reduction in liver index compared to the CED group (0.0416 ± 0.0037 , $p > 0.05$).

The differential effect observed between whole goat milk and goat skim milk suggests that components present in the fat fraction of goat milk may contribute to modulation of hepatic lipid metabolism (Kalyan *et al.*, 2018) [11]. Goat milk fat contains medium-chain fatty acids and bioactive lipids that are more readily oxidized and less likely to accumulate in hepatic tissue, potentially reducing lipid deposition additionally, synergistic interactions between milk fat and protein fractions may enhance lipid clearance and improve hepatic lipid homeostasis.

Effect on plasma lipid profile (TC, LDL-C, HDL-C, TG, VLDL-C, and atherogenic index)

The effect of whole goat milk (GM) and goat skim milk (GSM) supplementation on plasma lipid profile in cholesterol-fed rats is presented in Table 2. Feeding of the cholesterol-enriched diet (CED) for 30 days successfully induced hypercholesterolemia, with plasma total cholesterol (TC) increasing from 58.3 ± 6.4 mg/dL in SD rats to 164.4 ± 23.1 mg/dL in CED-fed rats ($p > 0.05$). Supplementation with whole goat milk significantly reduced plasma TC to 77.9 ± 12.6 mg/dL, whereas GSM lowered it to 95.3 ± 10.2 mg/dL ($p < 0.05$), indicating that both preparations attenuate diet-induced hypercholesterolemia, with GM showing superior efficacy (Table 2).

A similar trend was observed in plasma LDL-cholesterol (LDL-C), a major atherogenic lipoprotein fraction. CED feeding caused a marked increase in LDL-C from 16.5 ± 10.1 mg/dL in SD to 134.5 ± 25.8 mg/dL in CED rats. Supplementation with GM restored LDL-C to near SD levels (27.1 ± 13.5 mg/dL), whereas GSM produced a moderate reduction (55.6 ± 7.6 mg/dL) ($p > 0.05$) (Table 2). In contrast, HDL-Cholesterol (HDL-C) decreased in CED-fed rats (11.9 ± 4.0 mg/dL) relative to SD (23.5 ± 3.3 mg/dL), indicating impairment of reverse cholesterol transport under hypercholesterolemic conditions. Whole goat milk supplementation significantly increased HDL-C to 43.3 ± 1.2 mg/dL, restoring or slightly exceeding SD values, while GSM improved HDL-C moderately (17.3 ± 1.3 mg/dL) ($p > 0.05$).

Plasma triglyceride (TG) levels were not significantly altered by CED feeding compared with the standard diet (SD) group. Similarly, CED supplementation with goat milk whey (GMW), goat skim milk (GSM), and goat milk fat (GMF) did not produce significant changes in TG levels relative to SD. However, significant reductions in plasma TG levels were observed in the CED+GM and CED+GMC groups. The TG concentration decreased to 42.31 ± 3.390 mg/dL in the CED+GM group and 65.71 ± 4.500 mg/dL in the CED+GMC group, compared with 77.03 ± 7.702 mg/dL in the CED group and 79.39 ± 3.688 mg/dL in the SD group (Table 2). These findings indicate a marked triglyceride-lowering effect of whole goat milk and skimmed goat milk supplementation under cholesterol-enriched conditions.

VLDL-C values, calculated as one-fifth of TG concentrations, reflected a similar trend, with the highest levels observed in the CED group (average 15.41 ± 0.48 mg/dL), moderate reduction in GSM-fed rats (average 12.57 ± 0.57 mg/dL), and the lowest levels in GM-

supplemented rats (average 8.92 ± 0.50 mg/dL) (Table 2). These data indicate that whole goat milk effectively attenuates atherogenic lipoprotein levels and may promote improved lipid metabolism.

The atherogenic index (AI), a key predictor of cardiovascular risk calculated as LDL-C/HDL-C, was markedly elevated in CED-fed rats, with an average value of 7.37 ± 1.13 compared to 0.55 ± 0.17 in Control rats. Supplementation with GSM significantly reduced AI to an average of 3.57 ± 0.42 , while GM restored AI closer to normal levels (average 0.77 ± 0.28), comparable to Control (Table 2). These findings demonstrate that whole goat milk supplementation substantially mitigates CED-induced atherogenic risk, likely through synergistic effects of milk proteins and milk fat fractions that enhance lipoprotein clearance and modulate triglyceride metabolism.

Faecal cholesterol excretion and hepatic lipid deposition

Faecal cholesterol excretion in rats fed the standard diet (SD) ranged between 1.44 and 1.93 mg/g (Figure 2a). Feeding the cholesterol-enriched diet (CED) for 30 days did not produce a significant change in faecal cholesterol levels relative to the Control group, indicating that dietary cholesterol loading alone did not enhance intestinal cholesterol elimination. Supplementation with goat milk fractions showed differential effects. In the CED+GSM group, faecal cholesterol levels remained comparable to the CED group (average 3.09 ± 0.28 mg/g), indicating that skim milk exerted limited influence on cholesterol disposal through the faecal route. In contrast, whole goat milk (GM) supplementation significantly increased faecal cholesterol excretion to an average of 4.99 ± 1.12 mg/g, reflecting an approximate 2.5-fold enhancement relative to the CED group (average 1.93 ± 0.28 mg/g). The increased faecal cholesterol in GM-fed rats suggests reduced intestinal cholesterol absorption and/or enhanced biliary cholesterol secretion, which may contribute to the improved plasma lipid profile and lower atherogenic index observed in this group.

Hepatic cholesterol content in SD-fed rats ranged between 1.316 and 1.924 mg/g liver (Figure 2b). Feeding CED for 30 days significantly increased hepatic cholesterol deposition to 3.316-3.962 mg/g ($p > 0.05$), confirming effective induction of hepatic cholesterol accumulation under hypercholesterolemic conditions. Supplementation with goat skim milk (GSM) resulted in moderate hepatic cholesterol levels (2.608-2.988 mg/g), significantly lower than CED but still elevated compared with SD. Whole goat milk (GM) supplementation effectively normalized hepatic cholesterol content (1.797-2.287 mg/g), comparable to SD values (Figure 2b), suggesting improved hepatic cholesterol regulation.

Hepatic triglyceride (TG) concentrations ranged from 3.775 to 4.603 mg/g in the SD group and 4.04-5.298 mg/g in the CED group (Figure 2c). CED feeding did not produce a substantial or consistent increase in hepatic TG levels, indicating that cholesterol enrichment primarily affected hepatic cholesterol rather than triglyceride deposition. Supplementation with GSM and GM moderately decreased hepatic TG levels, with GM-fed rats showing values of 3.179-3.576 mg/g and GSM-fed rats 1.954-3.874 mg/g. Collectively, whole goat milk was more effective than skim milk in enhancing faecal cholesterol excretion and

preventing hepatic cholesterol accumulation, while both preparations exerted modest effects on hepatic triglyceride content under cholesterol-enriched dietary conditions.

TBARS in plasma and liver

The effect of goat milk supplementation on lipid peroxidation was evaluated by measuring thiobarbituric acid reactive substances (TBARS) in plasma and liver after 30 days of dietary intervention (Figure 2d,e). Feeding a cholesterol-enriched diet (CED) significantly increased plasma TBARS levels, with values ranging from 14.615 to 18.098 nmol/mL, compared with 11.218-12.115 nmol/mL in the standard diet (SD) group ($p > 0.05$). This indicates that hypercholesterolemia induced by CED enhances systemic oxidative stress. Hepatic TBARS levels were also elevated in CED-fed rats (11.346-31.154 nmol/g tissue) relative to SD (12.5-15.962 nmol/g), although the increase was more variable and did not reach statistical significance, suggesting tissue-specific differences in lipid peroxidation response to dietary cholesterol.

Supplementation with whole goat milk (GM) reduced plasma TBARS to 13.91-15.278 nmol/mL, while goat skim milk (GSM) decreased levels to 13.098-14.402 nmol/mL (Figure 2d). In the liver, GM supplementation resulted in TBARS values ranging from 3.168 to 48.649 nmol/g, whereas GSM-fed rats exhibited levels between 55.405 and 63.514 nmol/g (Figure 2e).

Effect on gross liver morphology

Gross examination of excised liver tissues after 30 days of dietary intervention revealed marked visual differences among the experimental groups (Figure 3a-d). Livers from the standard diet (SD) group appeared smooth, dark reddish-brown, and uniform in texture, indicating normal hepatic morphology (Figure 3a). In contrast, rats fed the cholesterol-enriched diet (CED) exhibited visibly enlarged livers with a comparatively paler appearance and rounded margins, suggestive of lipid accumulation and hepatic enlargement. The surface appeared less compact and more turgid relative to SD controls (Figure 3b).

Supplementation with goat skim milk (CED+GSM) showed partial improvement in gross morphology. Although mild enlargement was still observable, liver color and surface texture were comparatively improved relative to the CED group (Figure 3c). Notably, rats supplemented with whole goat milk (CED+GM) demonstrated a marked restoration of normal liver appearance. The livers were comparatively smaller, darker in color, and more similar to those of the SD group, indicating attenuation of cholesterol-induced hepatic alterations (Figure 3d). Although microscopic histopathological evaluation was not conducted, the gross morphological observations are consistent with the biochemical findings, including reductions in hepatic cholesterol deposition and oxidative stress markers. These results suggest that whole goat milk confers protective effects against diet-induced hepatic enlargement and morphological alterations.

Discussion

Diet-induced hypercholesterolemia is characterized by elevated circulating LDL-C, hepatic cholesterol deposition, impaired reverse cholesterol transport, and increased oxidative stress. In the present study, a cholesterol-enriched diet (CED) successfully induced dyslipidemia, as reflected

by increased plasma TC, LDL-C, VLDL-C, and atherogenic index (AI), along with hepatic cholesterol accumulation and elevated TBARS. Supplementation with whole goat milk (GM) markedly attenuated these alterations, whereas goat skim milk (GSM) exerted comparatively moderate effects, indicating a significant contribution of the milk fat fraction to metabolic regulation.

The hypocholesterolemic effect of GM may be attributed to its distinctive lipid composition. Goat milk fat is enriched in medium-chain fatty acids (MCFAs), including caproic (C6:0), caprylic (C8:0), and capric (C10:0) acids, which are rapidly absorbed via the portal vein and preferentially oxidized in hepatic mitochondria rather than incorporated into very-low-density lipoproteins (VLDL) (Chilliard *et al.*, 2004; Singh *et al.*, 2017) [2, 20]. Enhanced β -oxidation reduces substrate availability for triglyceride synthesis and VLDL assembly, thereby lowering circulating atherogenic lipoproteins. Recent lipidomic investigations have further demonstrated that modulation of dairy lipid fractions alters hepatic lipid metabolism and mitochondrial oxidative capacity. In particular, dietary interventions influencing milk lipid profiles were shown to impact systemic lipid handling and metabolic signaling in goat and rodent models, supporting the mechanistic plausibility of enhanced lipid oxidation observed.

Beyond fatty acids, the milk fat globule membrane (MFGM) and associated polar lipids likely contribute to reduced intestinal cholesterol absorption. Dairy phospholipids and sphingomyelin interfere with micellar cholesterol solubilization and may modulate Niemann-Pick C1-like 1 (NPC1L1) transporter activity, thereby enhancing fecal sterol excretion (Venkat, 2024) [21]. The significant increase in fecal cholesterol in GM-supplemented rats aligns with this mechanism. Contemporary analyses of milk polar lipids emphasize their capacity to modulate cholesterol transport, intestinal lipid digestion, and cardiometabolic risk markers (Venkat, 2024) [21]. Experimental evidence also indicates that dairy lipid components improve cholesterol clearance and reduce circulating atherogenic lipoproteins in animal models. These effects are consistent with the normalization of hepatic cholesterol levels and liver index observed in the present study.

The attenuation of hepatic enlargement in GM-supplemented rats suggests improved hepatic cholesterol handling. Excess dietary cholesterol activates sterol regulatory element-binding proteins (SREBPs), promoting lipogenesis and suppressing cholesterol efflux. Emerging data demonstrate that dairy bioactives influence hepatic lipid metabolic pathways and bile acid-related signaling, thereby modulating systemic lipid balance (Yin *et al.*, 2025; Zhang *et al.*, 2024) [23, 10]. Restoration of hepatic cholesterol toward control values in the GM group may therefore reflect coordinated regulation of synthesis, oxidation, and excretion pathways.

Although GSM improved lipid parameters moderately, its effects were inferior to whole milk, suggesting that milk proteins alone provide partial benefit. Goat milk proteins and their digestion-derived peptides have been shown to exert metabolic regulatory effects, including modulation of hepatic lipid pathways (Kalyan *et al.*, 2018; Shao *et al.*, 2025) [11, 19]. However, increasing attention has been directed toward the “dairy matrix effect,” wherein structural interactions among lipids, proteins, and micronutrients produce metabolic outcomes distinct from isolated fractions.

The superior performance of GM supports the concept that the intact lipid-protein matrix enhances functional efficacy beyond that of skimmed preparations. Hypercholesterolemia is strongly associated with oxidative stress due to excessive reactive oxygen species (ROS) generation during lipid overload. Elevated plasma TBARS in CED-fed rats confirm enhanced lipid peroxidation. GM supplementation significantly reduced TBARS levels, indicating attenuation of systemic oxidative stress. Goat milk contains antioxidative peptides, fat-soluble vitamins, and bioactive lipid components capable of stabilizing cellular membranes and reducing oxidative damage (Shao *et al.*, 2025; Ediriweera, 2025) [19, 3]. Recent experimental reports demonstrate that goat milk and related dairy lipid fractions improve oxidative status and metabolic resilience in animal models by reducing lipid peroxidation and modulating antioxidant defense systems (Zhang *et al.*, 2024) [10]. These findings are consistent with the antioxidative protection observed in the present study. Importantly, the hypocholesterolemic effects occurred independently of significant changes in body weight, indicating that lipid modulation was not secondary to

reduced caloric intake. Instead, improvements appear to result from targeted biochemical regulation of cholesterol absorption, hepatic lipid metabolism, and oxidative balance. The substantial reduction in AI following GM supplementation underscores its cardioprotective relevance, as AI integrates both LDL-C elevation and HDL-C suppression. Collectively, these findings demonstrate that whole goat milk exerts integrated hypolipidemic and antioxidative effects through complementary mechanisms: (i) enhanced β -oxidation of MCFAs, (ii) reduced intestinal cholesterol absorption via dairy polar lipids, (iii) increased fecal sterol excretion, (iv) attenuation of hepatic cholesterol accumulation, and (v) suppression of lipid peroxidation. While skim milk confers partial benefits attributable to protein-derived bioactivity, the intact lipid-protein matrix of whole goat milk appears essential for maximal metabolic efficacy. These results are consistent with contemporary evidence supporting whole dairy matrices as modulators of cardiometabolic health and position goat milk as a promising functional dietary intervention for managing diet- induced hypercholesterolemia.

Table 1: Diet Composition

Component (g/100g ration)	Diet			
	SD	CED	ED+GSM	CED+GM
Starch	61.20	59.33	59.33	59.33
Casein	12.00	12.00	12.00	12.00
Sucrose	10.00	10.00	10.00	10.00
GSM			25mL/rat/day	
GM				25mL/rat/day
Soybean oil	7.00	7.00		
Cellulose	5.00	5.00	5.00	5.00
Vitamin mixture	1.00	1.00	1.00	1.00
Mineral mixture	3.50	3.50	3.50	3.50
Methionine	0.30	0.30	0.30	0.30
Cholesterol	--	1.50	1.50	1.50
Sodium cholate	--	0.38	0.38	0.38

Table 2: Effect of Whole and Skimmed Goat Milk on Serum Lipid Profile in Male Wistar Rats

Groups	SD	CED	CED+GM	CED+GSM
TC (mg/dl)	53.8±3.49 ^a	153.7±10.01 ^c	82.43±5.00 ^b	90.6±4.44 ^b
LDL-C (mg/dl)	11.69±4.39 ^a	125.3±9.96 ^d	31.61±4.25 ^b	61.11±4.53 ^c
HDL-C (mg/dl)	26.29±3.54 ^c	13.00±1.42 ^a	42.36±0.66 ^d	16.93±0.63 ^b
TGs(mg/dl)	79.39±3.68 ^d	77.03±7.70 ^c	42.31±3.39 ^a	63.01±3.81 ^{ab}
VLDL-C(mg/dl)	15.88±0.73 ^a	77.03±1.54 ^c	42.31±0.67 ^b	63.01±0.76 ^{bc}
Atherogenic Index (AI)	0.54±0.20 ^a	11.94±3.08 ^c	1.51±0.19 ^a	3.63±0.26 ^b

Different lowercase letters within a row indicate significant difference $p > 0.05$, Duncan’s test). Data expressed as mean±SEM, $n = 6$

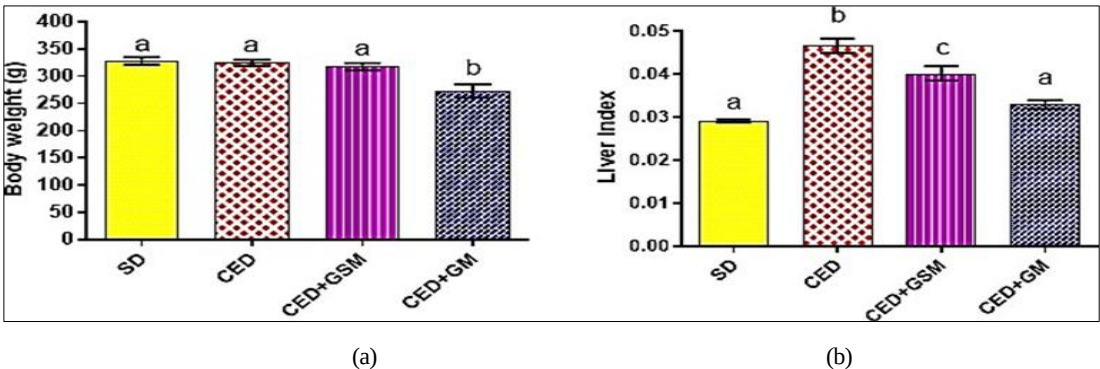


Fig 1: (a) Effect of dietary treatments on body weight; (b) Effect of dietary treatments on liver index in experimental rats. SD, standard diet; CED, cholesterol-enriched diet; CED+GM, cholesterol-enriched diet supplemented with whole goat milk; CED+GSM, cholesterol-enriched diet supplemented with skimmed goat milk. Values are expressed as mean±SEM ($n = 6$). Differences were considered statistically significant at $p < 0.05$.

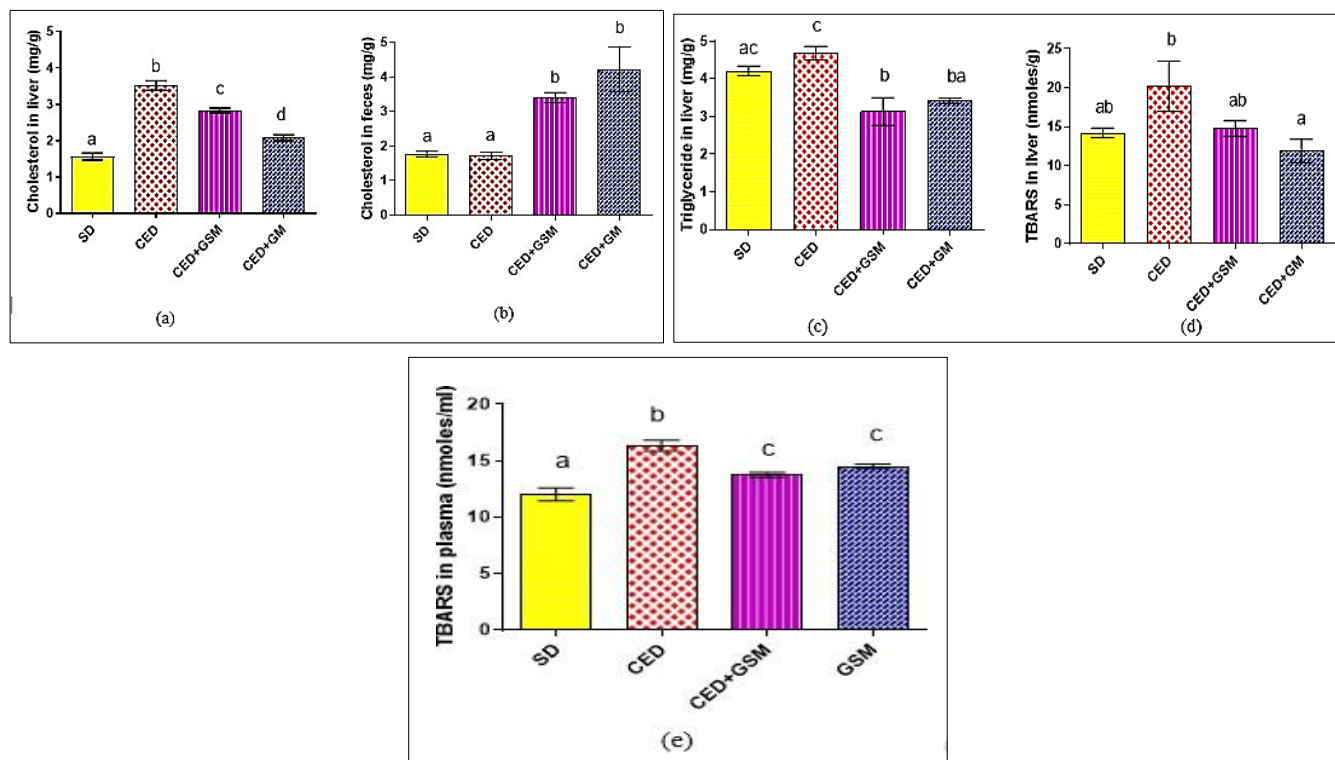


Figure 2: Effect of dietary treatments on hepatic lipid profile and oxidative stress markers in experimental rats. (a) Hepatic total cholesterol; (b) Cholesterol in faeces; (c) Hepatic triglycerides; (d) Hepatic thiobarbituric acid reactive substances (TBARS); (e) Plasma TBARS levels. SD, standard diet; CED, cholesterol-enriched diet; CED+GSM, cholesterol-enriched diet supplemented with whole goat milk; CED+GM, cholesterol-enriched diet supplemented with skimmed goat milk. Values are expressed as mean ± SEM (n = 6). Bars bearing different superscript letters differ significantly ($p > 0.05$).

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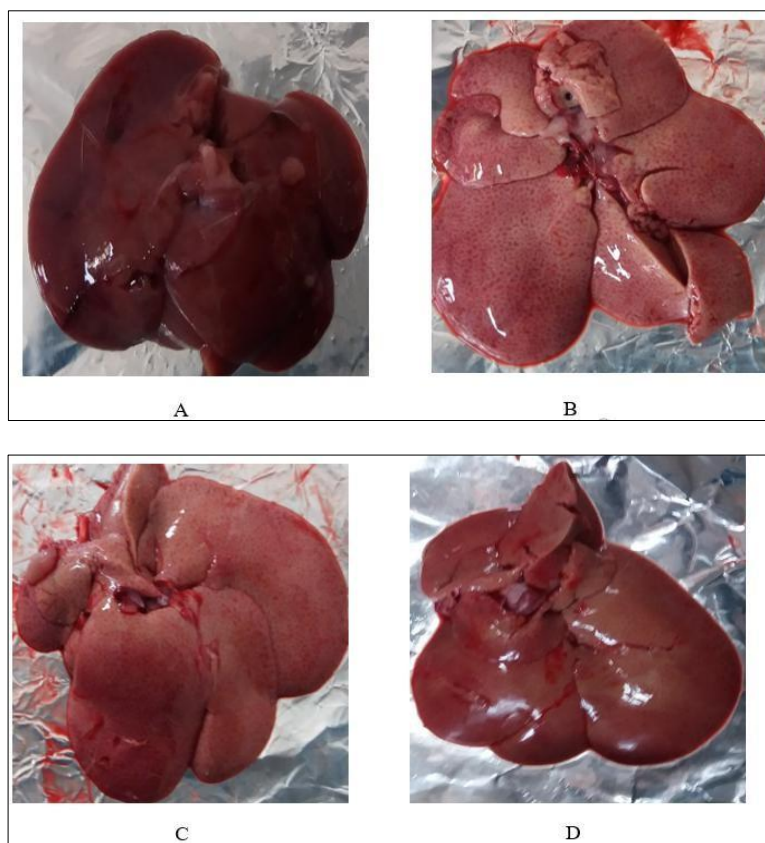


Fig 3: Gross liver morphology after 30 days of dietary intervention. (a) Normal liver appearance (b) CED: Enlarged, pale liver indicating lipid accumulation, (c) CED+GSM: Partial morphological improvement and., (d) CED+GM: Marked restoration toward normal liver morphology.

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Notes

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Abbreviations Used

AI - Atherogenic Index
AOAC - Association of Official Analytical Chemists
CED - Cholesterol-Enriched Diet
CPCSEA - Committee for the Purpose of Control and Supervision of Experiments on Animals
CVDs - Cardiovascular Diseases
DPPH - 2,2-Diphenyl-1-picrylhydrazyl
EDTA - Ethylenediaminetetraacetic Acid
GM - Whole Goat Milk
GSM - Goat Skim Milk
HDL-C - High-Density Lipoprotein Cholesterol
LDL-C - Low-Density Lipoprotein Cholesterol
LTLT - Low-Temperature Long-Time (Pasteurization)
MCFAs - Medium-Chain Fatty Acids
MDA - Malondialdehyde
MFGM - Milk Fat Globule Membrane
NPC1L1 - Niemann-Pick C1-Like 1
ROS - Reactive Oxygen Species
SD - Standard Diet
SEM - Standard Error of the Mean
SREBPs - Sterol Regulatory Element-Binding Proteins
SNF - Solids-Not-Fat
TBA - Thiobarbituric Acid
TBARS - Thiobarbituric Acid Reactive Substances
TC - Total Cholesterol
TEP - 1,1,3,3-Tetraethoxypropane
TG - Triglycerides
VLDL - Very-Low-Density Lipoprotein
VLDL-C - Very-Low-Density Lipoprotein Cholesterol

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