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Original Article

Camel Milk Supplemented with Fenugreek Seeds Improve Lipid Profile, Atherogenic Index and Antioxidative Status in Animal Model of Cardiovascular Disorders

Sami A. Althwab[1], Thekra A. Alharby[1], Waleed Al Abdulmonem[2,*], Khalid H. Musa[1], Zafar Rasheed[2], Abdullah S. M. Aljohani[3], Mona S. Almujaydil[1], Bashayr Y. Alghonaim[1], Nasser A. Alharbi[1], Ala Al Rajabi[4], Muhammad Ismail Khan[5], Nelson Fernández[6], Essam M. Hamad[1,7]

[1]Department of Food Science and Human Nutrition, College of Agriculture and Food, Qassim University, Buraidah, SAUDI ARABIA.
[2]Department of Pathology, College of Medicine, Qassim University, Buraidah, SAUDI ARABIA.
[3]Department of Medical Biosciences, College of Veterinary Medicine, Qassim University, Buraydah, SAUDI ARABIA.
[4]Department of Human Nutrition, College of Health Science, Qatar University, Doha, QATAR.
[5]Faculty of Medicine, School of Public Health, University of Queensland, Brisbane, AUSTRALIA.
[6]School of Life Sciences, University of Essex, Colchester, UNITED KINGDOM .
[7]Department of Dairy Science, Faculty of Agriculture, Cairo University, EGYPT.

ABSTRACT

Background: Despite of advancement in the technology and an improvement in handing of patients with atherosclerosis and Cardiovascular Diseases (CVDs) but the mortality associated with them remains to be highest in all over the globe. The Atherogenic Index of Plasma (AIP) has now been considered to be a best indicator for the analysis of atherosclerosis and CVDs. This study was undertaken to investigate the potential of Fermented camel Milk (FM) and Fenugreek seed powder (FG) on the animal model of CVDs. **Materials and Methods:** The animal models of atherosclerosis and CVDs were made by feeding of rats with high fat and cholesterol diets and the effect of FM supplemented with FG either alone or in a combination (FGFM) were tested on the lipid profile and AIP by Total Antioxidant Capacity (TAC), Malondialdehyde (MDA), Glutathione Peroxidase (GPx) activity, Total Cholesterol (TC) and small dense Low-Density Lipoprotein (sd-LDL). **Results:** The data revealed that FM, FG and their combination potentially increased in TAC level ($p<0.05$) and decreased in lipid peroxidation via reduction in the serum concentrations of MDA. Whereas, experimental rats on FM were fully restored GPx activity. Moreover, FM and FG and their combination lowered TC and sd-LDL concentrations significantly ($p<0.05$). **Conclusion:** The novel findings of this study clearly indicated that the FG, FM and FGFM have potential in preventing the onset of atherosclerosis and CVDs by reducing the levels of sd-LDL and improving lipids profile, AIP and antioxidant capacity.

Keywords: Cardiovascular disorders, Camel milk, Fenugreek, Lipid profile, High fat diet.

Correspondence:
Dr. Waleed Al Abdulmonem
Department of Pathology, College of Medicine, Qassim University, Buraidah, SAUDI ARABIA.
Email: dr.waleedmonem@qu.edu.sa

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INTRODUCTION

Cardiovascular Diseases (CVDs) are the fundamental reason for death in all over the globe and hyperlipidemia has now been considered a leading risk factor for their onset including atherosclerosis.[1] An elevation of total Triglyceride (TG) in plasma is associated with small dense Low-Density Lipoprotein (sd-LDL) and also with Very Low-Density Lipoprotein (VLDL) and have been considered as important emerging risk factors for the onset of CVDs.[2] Atherogenic Index of Plasma (AIP) is defined as a log (TG/ HDL), which has now been well considered a strong predictor of the risk of atherosclerosis and CVDs.[3] In addition, the measurement of different forms of apolipoproteins also considered as a biomarker for the prediction of onset of CVDs.[4] Other than these, antioxidant therapy has also gained attention for the reduction of chances of the onset of CVD as more than eighty diseases are linked with the excess production of free radicals such as atherosclerosis and under such circumstances, antioxidant therapy plays the utmost importance.[5] In the last two decades, several reports showed that consumption of Camel Milk (CM) has been numerous health benefits because of high contents of bioactive constituents.[6-8] The consumption of CM has also reported to reduce the levels of LDL and to maintain lipid profile due to the high content of L-carnitine in CM.[9,10] Studies also concluded that CM consumption also lower TG levels due to the high content of insulin-like protein and height quantity of zinc.[11,12] On the other hand, the fenugreek plant

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and its active biomolecular constituents have been found to have several anti-disease activities such as anti-atherosclerotic, cardioprotective, anti-inflammatory, blood cholesterol lowering, and lipid-lowering effects.[13-15] Recently, fenugreek seed extract has been reported to have a detoxifying potential against free radicals and now has been considered as an antioxidant agent because of high polyphenolic ingredients such as flavonoids and phenols.[15-18] In this study. we hypothesized that camel milk supplemented with fenugreek seeds improve lipid profile, atherogenic index and antioxidative status in animal model of cardiovascular disorders. To test this hypothesis, the potential of fermented camel milk and fenugreek seed powder were tested on an animal of cardiovascular disorders. The data revealed that camel milk with fenugreek seeds significantly improved lipid profile and also significantly improved the total antioxidant status in animal model of cardiovascular disorders. These findings are novel and have not been investigated before.

MATERIALS AND METHODS

Collection and preparation camel milk and fehugreek seeds

Camel Milk (CM) used in this study was collected from the experimental station of animal production at Qassim University. The composition of CM was measured using a Funke Gerber Lactostar milk analyzer (Waring Laboratory Products, Canada). The CM was heated in a water bath at 85°C for 5 min, cooled to approximately 43°C, inoculated with a starter culture (3% w/v, YF-L811), and incubated at 43°C until the milk pH-value reached 4.6. The final fermented milk then stored at -18°C, and freeze-dried using a Martin Christ freeze-dryer (GmbH, Germany). Whereas, fenugreek seeds were purchased from the local market of Qassim region of Saudi Arabia and were ground in a Snijders Scientific Tilburg Mill (Model: 8010E, Holland), sieved (60 mesh sieve) and the homogeneous powder was prepared.

Animals model of cardiovascular disorders

Male Wistar rats (5 to 7 weeks old, the body weight of 150 to 200 g) were housed in laboratory polypropylene rats' cages (4 animals per cage/2 cages per group) under standard laboratory conditions of 22-23°C, 12 hr light/dark cycle, and under relative humidity of 50% (±5) in the experimental animal house at the Department of Food Science and Human Nutrition, College of Agriculture and Vet. Med., Qassim University. Animal model of CVD was prepared as described by previously.[18] Briefly, the High-Fat-Cholesterol (HFC) diet was prepared using a standard basal diet composition in accordance with the published guidelines as described previously.[19-21] The HFC diets were formulated to contain 1% cholesterol and represent 20% and 45% of energy from protein and fat, respectively plus casein and coconut oil were also added. Forty rats were divided into five groups (n=8 for each group) as follows: NC: Normal Control group (rats were fed on a standard basal diet). PC: Positive Control group (rats were fed on an HFC diet). FM: Rats were fed on an HFC diet containing 4% (w/w) lyophilized fermented camel milk. FG: Rats were fed on an HFC diet containing 10% (w/w) fenugreek seed powder. FGFM: Rats were fed on an HFC diet containing 10% (w/w) fenugreek seed powder + 4% (w/w) lyophilized fermented camel milk. All experimental diets (PC, FM, FG, FMFG) were formulated to contain the same amount of carbohydrates, fats and proteins. All groups were fed on experimental diets for seven successive weeks. The dose of fenugreek (1 g/kg of body weight) was chosen according to Mowl *et al.* (2009). The dose of fermented camel milk (4% in diet) was selected depending on a previous report (Yahya *et al.*, 2018). Each animal's body weight was recorded at the beginning of the experimental period and at seven days intervals. Initial and final body weights were recorded, and body weight gain was calculated. At the end of the experiment and after overnight fasting (12 hr), rats were killed by decapitation; blood samples were collected in plane tubes and centrifuged at 3000 rpm to harvest the serum stored at (–80°C) for biochemical analysis.

Lipid profile analysis

Lipid profile analysis was performed on the basis of estimation of cholesterol, triglyceride and HDL as described previously[22-24] and the concentration of LDL was calculated using the formula described by the Friedewald *et al.*[25]

Determination of small dense LDL particles, malondialdehyde, total antioxidant capacity

The amount of small and dense LDL particles was calculated as described previously.[26] The serum level of Malondialdehyde (MDA) was estimated as previously described.[27] The total antioxidant capacity in the serum samples was determined as described previously.[28]

Atherogenic index of plasma estimation

Atherogenic Index of Plasma (AIP) was estimated using a logarithm ratio TG to HDL, log (TG/HDL) as described previously.[29]

Total phenolic compound, and DPPH and ABTS-radical scavenging activities

Total Phenolic Compound (TPC) content of fenugreek was determined by using the Folin-Ciocalteau method and the antioxidant activity of fermented camel milk and fenugreek seed powder was determined colorimetrically by using DPPH (2,2- diphenylpicrylhy-drazyl) and ABTS (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) radical scavenging activity as described previously.[30]

Statistical analysis

The data were analyzed using one-way Analysis of Variance (ANOVA) followed by Tukey's multiple comparisons using SPSS

statistical package software (Version 22.0, IBM, NY, USA). The data were presented as mean (± SD) and $p<0.05$ was considered as statistically significant.

RESULTS

Analysis of raw materials showed that fenugreek possessed high phenolic content (73.29±10.6 mg/g; GAE). In addition, both fenugreek and fermented camel milk showed high DPPH (88.10±1.82% and 67.01±0.86%) and ABTS (84.47±0.44% and 39.51±1.25%) radical scavenging activity, respectively. Growth parameters of experimental groups are shown in Table 1. It is obvious that all experimental groups have similar initial body weight ($p>0.05$). Feeding rats on experimental diets did not result in any significant differences in final body weight or body weight gain ($p>0.05$). There were significant effects ($p<0.05$) on rats' liver weight, liver index, and kidney's index of the experimental groups (Table 1). However, there is no significant effect of diets on kidneys' weight. Significant differences ($p<0.05$) were found between the PC and NC groups regarding liver weight and liver index. Also, no significant difference ($p>0.05$) in the kidneys index was observe between PC and NC. There were significant differences ($p<0.05$) in liver weight among experimental groups and PC. The lowest liver weight is recorded for the FGFM group (20.76% less than the PC, $p<0.05$). FG and PC groups showed the highest liver index (2.772 and 3.12%, respectively). While, FGFM treatment showed the highest kidney's index (0.66%). The results also showed that no significant differences ($p>0.05$) in liver Index and kidney index between the experimental groups and NC (Table 1). Oxidative stress parameters in rats fed on high-fat-cholesterol diet containing fenugreek seed powder, fermented camel milk and their combination are shown in Table 2. It can be noticed that feeding rats on HFC diet significantly affected the Glutathione Per Oxidase (GPx), Superoxide Dismutase (SOD) total Antioxidant Capacity (TAC), and Malondialdehyde (MDA). The PC group exhibited a significant elevation ($p<0.05$) in MDA and a significant reduction ($p<0.05$) of the serum level of GPx, SOD, and TAC when compared to the NC group (Table 2). FM diet increased GPx activity by 234.12% compared to PC, while the other experimental diets (FG and FGFM) showed GPx activities similar to that of PC ($p>0.05$). On the contrary, feeding rats on FGFM or FG showed a significant reduction ($p<0.05$) in the activity of SOD compared to the PC, while no significant effect was observed between PC and FM groups. FG, FGFM and FM diets significantly increased ($p<0.05$) TAC levels by (55.56, 67.28, 84.26%, respectively) compared to PC group. In addition, FG and FM either alone or in combination resulted in a significant reduction ($p<0.05$) in MDA levels compared to the PC group (40.72, 45.48, 61.24% respectively).

Lipid profile and lipoproteins data are presented in Table 3 and Figures 1 and 2. Feeding rats on experimental groups affect significantly ($p<0.05$) Total Cholesterol (TC), Triglycerides (TG), Low Density Lipoprotein cholesterol (LDL), and sd-LDL levels with no effect ($p>0.05$) on High Density Lipoprotein cholesterol (HDL). In PC group, there was a significant elevation in TC, TG, LDL, and sd-LDL levels compared to the NC group (Table 3, and Figure 1). FM, FG or their combination reduced TC and sd-LDL significantly compared to the PC ($p<0.05$). The FM group recorded the lowest TC (37.81% less than that of the PC, $p<0.05$). While FGFM treatment showed the lowest sd-LDL (54.34% less than that of the PC, $p<0.05$). Regarding TG, only FM that showed levels similar to that of NC ($p>0.05$), while FG and FGFM did not significantly reduced TG levels compared with PC group. Both FM, FGFM significantly reduced ($p<0.05$) LDL levels (by 53.01 and 56.44%, respectively) compared to PC. On the other hand, there was a significant difference between groups in AIP, Apo A1 and Apo B (Table 3). The PC group exhibited a significant elevation ($p<0.05$) of AIP and Apo B and a significant reduction ($p<0.05$) of Apo A1 level compared to the NC group. FG and FGFM groups reduced AIP significantly compared to the PC group, while there is no significant difference ($p>0.05$) between the FM and PC groups (Figure 2). FM or FG showed significant increase ($p<0.05$) in the levels of Apo A1 compared to the PC. The highest Apo A1 is recorded for the FM and FG groups (41.37 and 48.98% more than the PC, respectively, $p<0.05$). While the addition of FG and FM reduced Apo B significantly compared to the PC (55.58 and 55.67%, respectively). No significant

Table 1: Some growth parameters of rats fed on high-fat-cholesterol diet containing fenugreek seeds powder, fermented camel milk and their combination.

Groups	IBW (g)	FBW (g)	BWG (g)	Liver Wt. (g)	Liver Index*	Kidneys' Wt. (g)	Kidneys Index*
NC	286±21^{a}	347±37^{a}	67±38^{a}	9.66±1.35^{b}	2.70±0. 21^{b}	2.09±0.23^{a}	0.60±0.07^{ab}
PC	287±21^{a}	372±30^{a}	78±31^{a}	11.80±0.47^{a}	3.12±0.04^{a}	2.07±0.22^{a}	0.57±0.03^{b}
FM	277±18^{a}	339±19^{a}	58±21^{a}	9.36±0.81^{b}	2.69±0.26^{b}	2.07±0.12^{a}	0.61±0.04^{ab}
FG	278±17^{a}	357±36^{a}	79±50^{a}	10.07±1.15^{b}	2.77±0.27^{ab}	2.27±0.29^{a}	0.62±0.05^{ab}
FGFM	278±17^{a}	337±39^{a}	69±39	9.35±0.74^{b}	2.74±0.15^{b}	2.26±0.23^{a}	0.66±0.04^{a}

Data are mean ± SD (n=8 per group).NC = Normal control, PC = Positive control, FM = Fermented camel milk, FG = Fenugreek seed powder, FGFM = Fermented camel milk supplemented with fenugreek seed powder. IBW = Initial body weight, FBW = Final body weight, BWG = Body weight Gain.* % of final body weight.Means in the same column with different letters differ significantly at $p<0.05$.

Table 2: Oxidative stress parameters in rats fed on high -fat -cholesterol diet containing fenugreek seed powder, fermented camel milk and their combination.

Groups	GPx (mU/dL)	SOD (ng/mL)	TAC (mM/L)	MDA (nmol/mL)
NC	27.56±12.15^{a}	2.60±0.17^{a}	6.34±0.39^{a}	2.15±0.91^{b}
PC	7.62±2.37^{b}	2.29±0.22^{b}	3.24±0.39^{b}	6.09±0.51^{a}
FM	25.46±7.9^{a}	2.15±0.24^{bc}	5.97±1.03^{a}	3.32±1.24^{b}
FG	10.32±4.92^{b}	1.89±0.11^{c}	5.04±0.73^{a}	3.61±1.59^{b}
FGFM	8.66±5.18^{b}	1.995±0.20^{c}	5.42±1.06^{a}	2.36±0.39^{b}

Data are mean ± SD (n=8 per group).NC = Normal control, PC = Positive control, FM = Fermented camel milk, FG = Fenugreek seed powder, FGFM = Fermented camel milk supplemented with fenugreek seed powder.GPx = Glutathione Peroxidase, SOD = Superoxide dismutase, TAC = Total antioxidant capacity, MDA = Malondialdehyde.Means in the same column with different letters differ significantly at p<0.05.

Table 3: Serum Lipid profile and apolipoproteins (A1 and B) of rats fed on high -fat -cholesterol diet containing fenugreek seed powder, fermented camel milk and their combination.

Groups	TC (mg/dL)	HDL (mg/d)	TG (mg/dL)	LDL (mg/dL)	Apo A1 (ug/dL)	Apo B (mg/dL)
NC	160.20±19.33^{b}	45.89±23.19^{a}	195.76±7.91^{c}	85.48±12.20^{b}	8.85±0.25^{a}	68.93±4.31^{b}
PC	252.53±18.64^{a}	24.39±10.56^{a}	228.72±16.48^{a}	194.3±24.45^{a}	5.39±0.79^{b}	89.83±8.36^{a}
FM	157.05±20.42^{b}	30.90±1.90^{a}	203.53±12.41^{bc}	91.33±36.23^{b}	7.62±0.76^{a}	39.82±10.42^{c}
FG	197.15±36.40^{b}	46.64±10.38^{a}	211.78±10.20^{abc}	129.7±58.52^{ab}	8.03±0.90^{a}	39.90±4.55^{c}
FGFM	162.59±31.31^{b}	41.76±9.44^{a}	224.33±2.71^{ab}	84.65±31.02^{b}	5.61±0.51^{b}	74.74±5.76^{ab}

Data are mean ± SD (n=8 per group).NC = Normal control, PC = Positive control, FM = Fermented camel milk, FG = Fenugreek seed powder, FGFM = Fermented camel milk supplemented with fenugreek seed powder.TC = Total Cholesterol, HDL = High-Density Lipoprotein, TG = Total Triglycerides, LDL = Low-Density Lipoprotein, sd-LDL = Small Dense Low-density Lipoprotein, AIP = Atherogenic Index of Plasma.Means in the same column with different letters differ significantly at p<0.05.

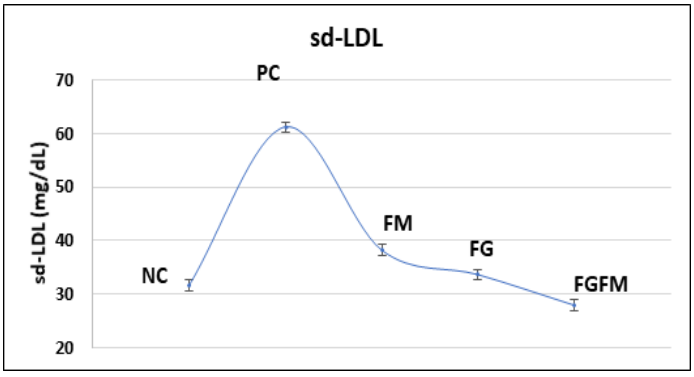

Figure 1: Small Dense Low-Density Lipoprotein (sd-LDL) of rats fed on high -fat -cholesterol diet containing fenugreek seed powder, fermented camel milk and their combination. NC = Normal control, PC = Positive control, FM = Fermented skim camel milk, FG = Fenugreek seed powder, FGFM = Fermented skim camel milk supplemented with fenugreek seed powder.

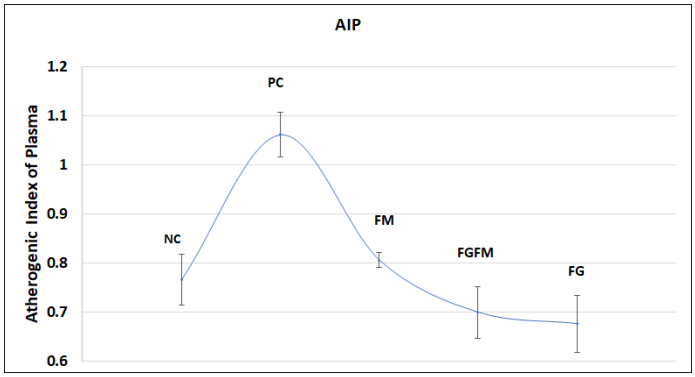

Figure 2: Atherogenic Index of Plasma (AIP) of rats fed on High-Fat-Cholesterol Diet containing fenugreek seed powder, fermented camel milk and their combination. NC = Normal control, PC = Positive control, FM = Fermented skim camel milk, FG = Fenugreek seed powder, FGFM = Fermented skim camel milk supplemented with fenugreek seed powder.

differences (p>0.05) were observed between the FGFM and PC groups in Apo A1 and Apo B.

DISCUSSION

This study investigated the potential of camel milk supplemented with fenugreek seed on atherogenicity in animal model of cardiovascular disorders. The findings indicated that camel milk with fenugreek seeds significantly improved lipid profile and

also significantly improved the total antioxidant status in animal model of cardiovascular disorders. The DPPH and ABTS assays showed that both fenugreek seed powder and fermented camel milk possessed high radicals scavenging activities. These findings fully supported the view that the fenugreek seeds contain high content of phenols and flavonoids.[31] In addition, camel milk previously showed scavenging activity due to the presence of bioactive peptides among its protein sequence.[32] One of the acknowledged processes by which antioxidants prevent lipid oxidation is via free radical scavenging and phenolic compounds are known for their capacity to trap free radicals and thus delay lipid auto-oxidation.[33] The obtained results showed no significant effect on the Rats' body weights, except for FM group that showed the lowest body weight gain, which has also been supported by previous findings.[34] The possible mechanism of this effect on the body weight is the pancreatic lipase inhibition due to the presence of bioactive peptides from FM proteins.[35] Raised liver weight and Liver Index in rats given a high fat-cholesterol diet could be attributable to cholesterol and TG accumulation in their livers due to increased TG synthesis and/or reduced TG secretion during the treatment period.[36] Moreover, the reduction in liver weights and Liver Index of rats fed FM could be due to the cholesterol lowering effect of lactic acid bacteria during fermentation.[37] The obtained results showed that the HFC diet reduced TAC, GPx, and SOD, as well as an increase in MDA activity in rats, indicating a state of oxidative stress. When rats were fed on FM, GPx activity was completely restored. These results are in agreement with Meena *et al.* as they reported the ability of camel milk to improve GPx activity rats.[37] Moreover, camel milk showed high content of tocopherol and ascorbic acid and antioxidant trace elements, which are key components of GPx and SOD, respectively.[38] On the contrary to our findings, Meena *et al.* reported that camel milk enhanced SOD activity.[37] This inconsistency in results might be due to the difference in the forms of camel milk used as non-fermented.[38] Our findings contradict those of Shamim *et al.*, who found that ten days of FG therapy significantly restored the antioxidant enzyme SOD in hyperlipidemic animals.[39] These differences might be due to differences between our study and the other studies in terms of the duration of study execution. In the present study, both FM, FG, or their combination reduced lipid peroxidation, as evidenced by lower MDA levels in the serum. The natural antioxidant content and free radical scavenging properties of FM and FG may account for this lipid peroxidation-reducing effect. These findings have also been supported by other studies reported that CM and FG significantly improved lipid profile.[6,40-42] The obtained data observed that both FM and FG, as well as their combination, significantly reduced TC and sd-LDL levels. These findings have also in accordance with other reports showed that camel milk reduced the TC, TG, LDL, and VLDL levels.[6,40] FM in the present study lowered serum levels of cholesterol and TG. It might be that FM affected TG and cholesterol uptake in the gastrointestinal tract, affect LDL cholesterol via the LDL receptor or impact lipid metabolism.[41] Furthermore, the obtained results showed a hypolipidaemic impact of FG. This effect may be attributed to the fiber fraction of fenugreek seeds that might show a hypocholesterolemic impact, its ability to increase fecal excretion of bile acids, and its hepatic-cholesterol-inhibitory activity through short-chain fatty acids produced by bacterial fermentation of soluble dietary fibers in the lower parts of the large intestine.[43] In this study, FM and FG significantly reduced Apo B levels and significantly ($p<0.05$) increased serum Apo A1 levels in rats. In addition, AIP is a powerful indicator of atherosclerosis and coronary heart disease risk.[44] The serum AIP levels of the rats in the FG and FGFM groups were significantly lower while the FM group reduced AIP levels with no significant differences. These findings are consistent with other published reports showed that the treatment with *Trigonella foenum-graecum* seeds produced a significant reduction in the atherogenic index and apo-B levels which might be a better predictor for improvement in coronary heart disease risk.[45] These studies further support that the combination of camel milk with fenugreek seed has potential to reduce the risk of onset of cardiovascular disorders. In conclusion, the present study explored the effect of fermented camel milk and fenugreek seed powder either alone or in combination on atherogenicity in rats fed on high-fat-cholesterol diet in rats. Outcomes of these findings concluded that came milk or fenugreek seeds either alone or in combination might be an option to be used as an alternative medicine in prevention of the onset of coronary heart diseases.

CONCLUSION

This is the first study to the best of our knowledge that demonstrated the potential of fermented camel milk, fenugreek seed powder, and their combination reduce the risk of onset of atherosclerosis and cardiovascular disorders. These substances, whether used individually or together, show reduction in levels of small dense Low-Density Lipoprotein, and improving overall lipid profile. They also reducing the atherogenic index of plasma, and boosting antioxidant capacity.

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CONFLICT OF INTEREST

The authors confirmed that they have no competing interests to declare.

ABBREVIATIONS

CVDs: Cardiovascular diseases; **AIP:** Atherogenic index of plasma; **FM:** Fermented camel milk; **FG:** Fenugreek seed powder; **FGFM:** Combination of fermented camel milk and fenugreek seed powder; **TAC:** Total antioxidant capacity; **MDA:** Malondialdehyde; **GPx:** Glutathione peroxidase; **TC:** Total cholesterol; **sd-LDL:** Small dense low-density lipoprotein.

SUMMARY

Although advancements in technology and patient care for atherosclerosis and cardiovascular diseases have been improved but the global mortality rates remain high. The atherogenic index of plasma has emerged as a crucial indicator for assessing these conditions. This study determined the therapeutic potential of fermented camel milk and fenugreek seed powder on an animal model of cardiovascular disorder. Rats were fed high-fat, high-cholesterol diets to induce atherosclerosis and cardiovascular diseases and the impact of fermented camel milk and fenugreek seed powder and their combination on lipid profiles and AIP was examined. The findings showed that fermented camel milk, fenugreek seed powder and their combination increased total antioxidant capacity and decreased malondialdehyde levels, indicating reduced lipid peroxidation. The tested compounds also restored glutathione peroxidase activity, and all treatments significantly lowered total cholesterol and small dense Low-Density Lipoprotein levels. These findings suggest that fermented camel milk and fenugreek seed powder have potential in preventing atherosclerosis and cardiovascular diseases by improving lipid profiles and antioxidant capacity.

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