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The Impact of Ghee on Lipid Profile, Liver Enzymes, and Glucose, Urea and Creatinine Levels in Healthy and Diabetic Rats

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ABSTRACT

Background: Diabetes Mellitus (DM) is a metabolic disorder that leads to an abnormal glucose level. The goal of this study was to examine the impact of ghee on lipid profile, liver enzymes, and glucose, urea and creatinine levels in diabetic and healthy rats.

Methods: Forty eight male Wistar rats were categorized into six equal groups including a diabetic control group, diabetic groups receiving 4 and 8 mg of ghee, a healthy control group, and healthy groups receiving 4 and 8 mg of ghee. Blood, liver, and pancreas samples were provided and investigated for these variables after three months of interventions.

Results: Ghee could significantly increase the glucose level in healthy rats (p=0.0001); but decreased the glucose level in diabetic rats (p=0.001 for 4 mg/kg and p=0.0001 for 8 mg/kg). Cholesterol level was significantly lower in diabetic rats treated with ghee (p=0.0001 for 4 mg/kg and p=0.048 for 8 mg/kg). Triglyceride level and liver enzymes exhibited a dose-dependent change and an enhanced kidney function in diabetic rats.

Conclusion: Ghee administration resulted in a reduction in blood glucose level, improvement in lipid profile, and an enhanced kidney function in diabetic rats. Notably, a significant difference was observed between the doses of 4 mg/kg and 8 mg/kg of ghee, with an increasing effect that was dose dependent. However, in healthy rats, ghee led to an elevation in blood sugar level in a dose-dependent manner.

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Introduction

Diabetes mellitus is a chronic metabolic disorder marked by persistent hyperglycemia due to impaired insulin secretion or resistance that can lead to a disruption in lipid profile, and carbohydrate and protein metabolism. Chronic hyperglycemia causes complications such as retinopathy, nephropathy, and vascular damage. Treatment focuses on lowering the blood glucose level and glycosylated hemoglobin to normal ranges (1-4). Diabetes can

result in an elevation in lipid profile, promote Low Density Lipoprotein Cholesterol (LDL-C) glycation and oxidation and accelerate atherosclerosis and vascular complications. Effective management of both diabetes and lipid profile is crucial to minimizing these risks (5-7). Diabetic dyslipidemia is characterized by an increase in LDL-C and triglyceride (TG) levels resulting from reduced lipoprotein lipase activity, while High Density Lipoprotein Cholesterol (HDL-C) level drops, that rises the risk of cardiovascular diseases (CVDs). Addressing these lipid abnormalities is essential in managing diabetic vascular complications (8-10).

The metabolic disorder in DM patients affects multiple organs, including the liver, which plays a vital role in the regulation of carbohydrates, lipids and the protein metabolism. This is reflected in an elevation in serum aminotransferase levels, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and γ-glutamyltransferase (GGT) (11-13).

Nutritional therapy is effective in managing diabetes, emphasizing the reduction in saturated fats and dietary cholesterol intake. Diabetic individuals are more sensitive to dietary cholesterol than the general population (14, 15). Ghee, also known as clarified butter has the origin from India and is derived from animal milk sources like cow's milk, buffalo's milk, or a combination of both (16, 17). Its key flavoring components include carbonyls, lactones, and free fatty acids (18).

Ghee is notable for its high content of short-chain and essential fatty acids, as well as fat-soluble vitamins A, D, E, and K, making it a valuable nutrient source, particularly for vitamin A. It contains approximately 59% saturated fatty acids and includes natural antioxidants, phospholipids, and protein residues that prevent rancidity (19, 20). Several studies suggest that ghee may effectively modify serum lipid profiles in metabolic syndrome by reducing LDL-C, increasing (HDL-C and decreasing TG levels. This evidence highlights the potential role of ghee in managing dyslipidemia associated with metabolic syndrome (21, 22).

Moreover, it was shown that ghee may contribute to a reduction in ALP level, which serve as a reliable indicator for predicting CVDs, stroke, and dyslipidemia. An elevated ALP level is associated with an increased risk of these conditions, suggesting that factors that can lower the ALP level may provide protective effects against CVDs. Thus, the potential of ghee to decrease ALP level may have significant implications for cardiovascular health (23-25). Additionally, another study indicated that ghee had a reducing effect on liver enzymes such as AST and ALT levels (26).

Several studies demonstrated that ghee had antidiabetic properties, and cow ghee specifically reduced the glucose concentration in diabetic patients. Its linolenic acid content stimulated the insulin release by binding to beta cells. Furthermore, it was shown that rice when prepared with ghee could release a minimal glucose during an *in vitro* digestion, suggesting that ghee may benefit in diabetes management (27, 28). It can therefore be assumed that ghee has favorable effects on diabetes and obesity-related CVDs. Hence, the focus of this study was to examine the impact of ghee on lipid profile, liver enzymes and glucose levels, as well as on urea and creatinine levels in both normal and diabetic rats.

Materials and Methods

Forty-eight male Wistar rats, each with an average weight of 250±20 grams were housed under controlled conditions at 25°C with consistent lighting and humidity. The rats had free access to water and a standard diet throughout the experiments. After initial weighing, they were randomly allocated into six groups of the healthy sham group, which received a placebo; the healthy ghee group, which received 4 mg/kg of ghee; the healthy ghee group, which received 8 mg/kg of ghee; the diabetic sham group, which received a placebo; the diabetic ghee group, which received 4 mg/kg of ghee; and the diabetic ghee group, which received 8 mg/kg of ghee. The rats were monitored over a period of three months. Based on similar studies, the administered amount of ghee served as a substitute for a portion of the daily fat intake in the rats' diet. The dose was calculated according to their daily caloric and fat intake requirements to ensure it did not increase their overall caloric intake.

To induce type 1 diabetes, a single intraperitoneal injection of streptozotocin (STZ) was administered at a dose of 60 mg/kg in 15 mL of solution per 250 g rat weight. Diabetes was confirmed by blood glucose level exceeding 300 mg/dL, measured 72 hours after the STZ injection. Following confirmation of hyperglycemia, blood samples (10 mL) were collected from the femoral vein of all rats 72 hours post-STZ injection. These samples were centrifuged and stored at -70°C. An additional 10 mL of femoral vein blood sample was collected 24 hours after the final dose of ghee administration, centrifuged, and stored at -70°C. Ghee was prepared by boiling sheep milk, adding a yogurt culture for fermentation, and churning the yogurt until fat globules formed on the surface. These globules were collected as animal butter, which was then heated and melted to produce the ghee used in the experiment.

Data collection was conducted using a laboratory checklist. Measurements included lipid profile, liver enzymes, and glucose, urea, and creatinine levels, all analyzed using the Pars Ezmon commercial kit (Tehran, Iran). Data were presented as mean±standard deviation. Statistical analyses were conducted using SPSS software (Version 20, Chicago, IL, USA). ANOVA and t-tests were employed to compare values within and between groups. A significance level of $p<0.05$ was set for all analyses.

Results

It was demonstrated that in the diabetic ghee group (4 mg/kg), glucose level significantly decreased when compared to the diabetic control group ($p=0.001$). Similarly, in the diabetic ghee group (8 mg/kg), glucose level significantly decreased in comparison to the diabetic control group ($p=0.001$). Furthermore, a significant reduction in glucose level was observed in the diabetic ghee group (8 mg/kg) when compared to the diabetic ghee group (4 mg/kg) ($p=0.0001$). In contrast, this study showed a significant increase in glucose level in the healthy ghee group (4 mg/kg) in comparison to the healthy control group ($p=0.0001$). Additionally, in the healthy ghee group (8 mg/kg), glucose level significantly increased when compared to the healthy control group ($p=0.003$). However, no significant difference was found in glucose level between the healthy ghee group (8 mg/kg) and the healthy ghee group (4 mg/kg) ($p=0.3$). Overall, the consumption of ghee at a dose of 8 mg/kg demonstrated the greatest effect in reducing blood glucose level among the diabetic animals.

In the diabetic ghee group (4 mg/kg), TG level significantly decreased when compared to the diabetic control group ($p=0.0001$). Conversely, in the diabetic ghee group (8 mg/kg), TG level significantly increased in comparison to the diabetic control group ($p=0.050$). Furthermore, a significant increase in TG level was noticed in the diabetic ghee group (8 mg/kg) when compared to the diabetic ghee group (4 mg/kg) ($p=0.001$). In healthy rats, a significant decrease in TG level was noted in the healthy ghee group (8 mg/kg) in comparison to the healthy ghee group (4 mg/kg) ($p=0.02$). However, no significant difference was observed between the healthy ghee group (4 mg/kg) and the healthy control group ($p=0.95$) or between the healthy ghee group (8 mg/kg) and the healthy control group ($p=0.06$). In summary, no significant changes in blood TG level was visible in healthy samples. However, in diabetic samples, ghee consumption at 4 mg/kg could decline the TG level, while 8 mg/kg resulted in an elevation.

In the diabetic ghee group (4 mg/kg), cholesterol level significantly decreased when compared to the diabetic control group ($p=0.0001$). Similarly, in the diabetic ghee group (8 mg/kg), the cholesterol level significantly decreased in compariaon to the diabetic control group ($p=0.048$). Furthermore, the diabetic ghee group (4 mg/kg) showed a significant decrease in cholesterol level when compared to the diabetic ghee group (8 mg/kg) ($p=0.02$). In the healthy ghee group (8 mg/kg), cholesterol level significantly increased in comparison to the healthy control group ($p=0.04$). However, no significant difference was found between the healthy ghee group (4 mg/kg) and the healthy control group ($p=0.3$) or between the healthy ghee group (8 mg/kg) and the healthy ghee group (4 mg/kg) ($p=0.5$).

In the diabetic ghee group (4 mg/kg), HDL level illustrated a significantly reduction in comparison to the diabetic control group ($p=0.025$). Additionally, HDL level was significantly lower in the diabetic ghee group (4 mg/kg) when compared to the diabetic ghee group (8 mg/kg) ($p=0.001$). However, no significant difference was exhibited between the diabetic ghee group (8 mg/kg) and the diabetic control group ($p=0.06$). Among healthy groups, differences in HDL level were not significant. In the diabetic ghee group (4 mg/kg) and the diabetic ghee group (8 mg/kg), LDL level significantly decreased in comparison to the diabetic control group ($p=0.0001$ for both). The diabetic ghee group (4 mg/kg) also revealed a significantly lower LDL level when compared to the diabetic ghee group (8 mg/kg) ($p=0.02$). However, there were no significant differences in LDL level among the healthy groups.

In the diabetic ghee groups (4 mg/kg and 8 mg/kg), AST level significantly increased in comparison to the diabetic control group ($p=0.015$ and $p=0.02$, respectively). However, no difference was observed between the diabetic ghee groups (4 mg/kg and 8 mg/kg) ($p=0.2$). In the healthy ghee group (8 mg/kg), AST level showed a significant decline when compared to both the healthy control group ($p=0.0001$) and the healthy ghee group (4 mg/kg) ($p=0.0001$). No significant difference was noticed between the healthy ghee group (4 mg/kg) and the healthy control group ($p=0.23$). In the diabetic ghee group (4 mg/kg), ALT level demonstrated a significant reduction in comparison to the diabetic control group ($p=0.0001$). ALT level was also significantly lower in the diabetic ghee group (8 mg/kg) whe compared to the diabetic ghee group (4 mg/kg) ($p=0.04$). However, no significant difference was noted between the diabetic ghee group (4 mg/kg) and the diabetic control group ($p=0.4$).

In the healthy ghee group (4 mg/kg), ALT level revealed a significant decrease in comparison to the healthy control group ($p=0.03$).

Table 1: Comparison of various variables between ghee consumed groups.

Variable		Healthy ghee group (4 mg/kg) vs. Healthy sham group		Healthy ghee group (8 mg/kg) vs. Healthy sham group		Healthy ghee group (4 mg/kg) vs. Healthy ghee group (8 mg/kg)		Diabetic ghee group (4 mg/kg) vs. Diabetic sham group		Diabetic ghee group (8 mg/kg) vs. Diabetic sham group		Diabetic ghee group (4 mg/kg) vs. Diabetic ghee group (8 mg/kg)	
		Healthy	Ghee (4 mg/kg)	Healthy	Ghee (8 mg/kg)	Ghee (4 mg/kg)	Ghee (8 mg/kg)	Healthy	Ghee (4 mg/kg)	Healthy	Ghee (8 mg/kg)	Ghee (4 mg/kg)	Ghee (8 mg/kg)
Glucose (mg/dl)	Mean	105	143	105	162	143.4	162.08	559.64	341.58	559.6	107.00	107.00	341.58
	SD	28.1	19.6	28.144	50.753	19.59	50.75	50.196	173.043	50.196	29.339	29.339	173.043
	P value	0.0001		0.003		0.3		0.001		0.001		0.0001	
Urea (mg/dl)	Mean	64	58	64	58	58.3571	58.3333	83.78	79.75	83.7857	76.6429	76.64	79.75
	SD	5.9	5.3	5.58	2.93	5.34	2.93	11.18	11.70	11.18501	10.38	10.38	11.70
	P value	0.01		0.006		0.99		0.038		0.09		0.5	
Creatinine (mg/dl)	Mean	0.58	0.63	0.6	0.6	0.6343	0.6192	0.59	0.71	0.5929	0.66	0.66	0.715
	SD	0.05	0.04	0.048	0.0744	0.04271	0.07440	0.0538	0.095	0.053	0.105	0.105	0.095
	P value	0.007		0.16		0.5		0.001		0.04		0.2	
Cholesterol (mg/dl)	Mean	60	65.5	60	68.5	65.500	68.500	81.57	61.33	42.35	46.92	72.71	61.33
	SD	10.40	15.38	10.40	8.52	15.36	8.52	10.65	11.93	5.37	6.88	11.88	11.93
	P value	0.3		0.04		0.5		0.0001		0.048		0.02	
Triglyceride (mg/dl)	Mean	109	108	109	82	107.85	82.16	173.57	80.83	173.57	271.42	271.42	80.83
	SD	41.9	32.4	41.92	15.94	32.36	15.94	53.35	20.03	53.35	163.82	163.82	20.03
	P value	0.95		0.06		0.02		0.0001		0.050		0.001	
High-density lipoprotein (mg/dl)	Mean	37	34	37	39.6	34.42	39.58	42.35	36.16	42.35	46.92	46.92	36.16
	SD	6.27	7.81	6.27	6.65	7.871	6.653	5.37	7.34	5.37	6.88	6.88	7.34
	P value	0.44		0.28		0.08		0.025		0.06		0.001	
Low-density lipoprotein (mg/dl)	Mean	2.6	3.6	2.6	3	3.64	1.780	7.14	3.25	7.14	2.28	2.28	3.25
	SD	0.99	1.78	0.996	0.738	3.0000	0.738	1.23	0.86	1.23	1.138	1.13	0.86
	P value	0.08		0.26		0.2		0.0001		0.0001		0.02	
Aspartate aminotransferase (mg/dl)	Mean	225	203	225	135.6	203.21	135.58	163.5	271.0	163.5	216.78	216.78	271.0
	SD	56.91	36.27	56.91	23.72	36.27	23.72	32.13	128.61	32.13	71.49	71.49	128.61
	P value	0.23		0.0001		0.0001		0.015		0.02		0.2	
Alanine aminotransferase (mg/dl)	Mean	92	77.6	92	83	77.571	82.833	125.57	146.75	125.57	88.92	88.92	146.75
	SD	18.13	13.04	18.13	5.2	13.048	5.236	19.92	82.40	19.92	16.63	16.53	82.40
	P value	0.03		0.12		0.2		0.4		0.0001		0.04	
Alkaline phosphatase (mg/dl)	Mean	637.5	715	637.5	632	716.07	632.416	3012.00	1374.25	3012	741.14	741.1429	1374.2500
	SD	140.07	162.70	140.07044	56.16446	162.70	56.164	361.56	553.24	361.56	120.80	120.80745	553.24565
	P value	0.21		0.09		0.09		0.0001		0.0001		0.002	

Significant differences were noted where $p<0.05$. SD: Standard deviation.

However, no significant difference was visible between the healthy ghee groups (4 mg/kg and 8 mg/kg) or between the healthy ghee group (8 mg/kg) and the healthy control group (p=0.12 and p=0.2, respectively). In both the diabetic ghee groups (4 mg/kg and 8 mg/kg), ALP level significantly decreased when compared to the diabetic control group (p=0.0001 for both). Additionally, ALP level was significantly lower in the diabetic ghee group (8 mg/kg) in comparison to the diabetic ghee group (4 mg/kg) (p=0.002). No significant differences were observed among the healthy groups.

In the diabetic ghee group (4 mg/kg), urea level significantly decreased when compared to the diabetic control group (p=0.038). No significant differences were observed between the diabetic ghee group (8 mg/kg) and the diabetic control group (p=0.09) or between the diabetic ghee groups (4 mg/kg and 8 mg/kg) (p=0.5). In healthy groups, urea level showed a significant decline in both the healthy ghee groups (4 mg/kg and 8 mg/kg) when compared to the healthy control group (p=0.01 and p=0.006, respectively). However, no significant difference was found between the two healthy ghee groups (p=0.99). In the diabetic ghee groups (4 mg/kg and 8 mg/kg), creatinine level exhibited a significant rise when compared to the diabetic control group (p=0.001 and p=0.04, respectively). However, no significant difference was observed between the diabetic ghee groups (4 mg/kg and 8 mg/kg) (p=0.2). In the healthy ghee group (4 mg/kg), the creatinine level significantly increased when compared to the healthy control group (p=0.007). However, no significant differences were observed between the healthy ghee group (8 mg/kg) and either the healthy control group (p=0.16) or the healthy ghee group (4 mg/kg) (p=0.5). The comparison of mean values and standard deviations across the groups was presented in Table 1.

Discussion

The impact of a specific diet on health status has been described before (29, 30). In this relation, dairy products have played an important role (31, 32). In our study, when comparing the healthy control group to the healthy group that received 4 mg/kg of ghee, there was a notable elevation in glucose and creatinine levels in the ghee-treated group. Additionally, a significant reduction in urea and ALT levels was observed in the same group. Comparing the healthy control group to the healthy group receiving 8 mg/kg of ghee, significant increases in glucose and cholesterol levels were noticed, along with a notable decrease in urea and AST levels. A clinical trial by Najafi *et al.* who investigated the effect of Kermanshahi animal oil on blood lipids in 25 healthy men during 30 days revealed increases in cholesterol (+0.52 mg/dL) and LDL (+3.17 mg/dL) levels, while TG level decreased by 5.08 mg/dL and HDL level by 0.64 mg/dL. However, these changes were statistically insignificant demonstrating that substituting 30 grams of Kermanshahi animal oil for other oils over a month did not significantly impact the serum lipid profile in healthy individuals (33).

Ahmadi *et al.* studied the effect of ghee on the lipid profile and memory in rats finding significant increases in HDL and cholesterol levels when compared to the control group (34). In contrast, Jafarnjad *et al.* compared cholesterol level in rural individuals who primarily consumed ghee with those consuming solid vegetable oil. It was shown that the total cholesterol level was significantly lower in ghee consumers (195.3±40.9 mg/dL) in comparison to the vegetable oil consumer group (232±7.6 mg/dL). Other fat indicators were 8-20% lower in the ghee group, suggesting that long-term ghee consumption may reduce blood lipid profile (35). Rahimi *et al.* conducted a study on 28 male rats treated with ghee, olive oil, and barley. Ghee and barley were associated with significantly lower serum cholesterol and LDL levels when compared to the controls, while HDL level slightly increased, while TG decreased in both groups. It was shown that ghee and olive oil could positively affect lipid profile and reduce atherosclerosis risk factors (36).

In contrast to these findings, another study illustrated that butter sourced from cows grazing on mountain pastures had no significant effect on blood lipid profile, lipoproteins, glucose, or insulin tolerance (37). In diabetic rats, significant and beneficial changes were observed in glucose level, renal function, and lipid profile in groups receiving 4 mg/kg of ghee. However, these benefits were accompanied by significant increases in creatinine and AST levels. Similarly, in diabetic rats treated with 8 mg/kg of ghee, improvements in glucose level, renal function, and lipid profile were noted, alongside with significant elevations in creatinine and AST levels. These findings suggest that higher doses of ghee (8 mg/kg) had more pronounced benefits for blood glucose, lipid profile, and liver function, though changes in kidney function were not significant.

Aldabbagh *et al.* compared the effect of sunflower oil and ghee on liver tissue and biochemical parameters. While sunflower oil increased the body weight, cholesterol level, and liver enzymes (ALT and AST), ghee could reduce

the liver enzymes and cholesterol level without affecting the body weight, demonstrating its potential health benefits (26). Moreover, it was shown that cow ghee had hepatoprotective effects (38). Our findings are in agreement with this study revealing that in healthy groups receiving 4 or 8 mg/kg of ghee, significant reduction in AST level was visible. Additionally, administration of 4 mg/kg of ghee significantly decreased the ALT level in both healthy groups.

The dose-dependent effects of ghee on TG level in diabetic rats indicate complex lipid metabolism interactions. At dose of 4 mg/kg, ghee declined the TG level, possibly through enhanced lipid metabolism or a reduced hepatic TG synthesis. However, at higher dose of 8 mg/kg, TG level increased, potentially due to lipid clearance saturation or enhanced lipogenesis. This paradoxical responses may also result from exacerbated insulin resistance or inflammatory states impairing the TG utilization (39). This study highlighted the positive effects of ghee on glucose level, lipid profile, and liver enzymes. However, its small sample size and short duration can limit the findings. Future research should consider larger sample sizes, longer durations, and additional biomarkers (e.g., HbA1c, postprandial glucose) to provide a comprehensive understanding of ghee's potential benefits.

Conclusion

Ghee significantly lowered the blood glucose level, improved the lipid profile, and enhanced the liver enzyme activity in diabetic rats, with more pronounced effects at doses of 4 and 8 mg/kg. However, in healthy rats, ghee increased the blood glucose level dose-dependently. Ghee's hepatoprotective properties and positive effect on lipid profile suggest its potential in treatment of hyperglycemia, hyperlipidemia, and liver enzyme disorders in diabetes. Further exploration of dose-dependent effects and related biomarkers would improve understanding of ghee's therapeutic potential.

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Authors' Contribution

All of the authors contribute in all sections.

Conflict of Interest

The authors declare no conflict of interest.

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