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EFFECT OF PIPER LONGUM-BASED COOKIES ON LIPID PROFILES: A HUMAN INTERVENTION STUDY

Original Research

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ABSTRACT

Background: Hyperlipidemia is a metabolic disorder characterized by elevated lipid levels that increases the risk of cardiovascular disease. Its prevalence is rising due to sedentary lifestyles, higher fat intake, and obesity. Conventional pharmacological therapies often cause side effects, prompting interest in natural alternatives. *Piper longum*, widely recognized in traditional medicine, possesses antioxidant, anti-inflammatory, and hypolipidemic properties. The integration of this medicinal plant into food formulations represents a promising approach to improve patient compliance and provide safe, functional dietary interventions.

Objective: This study aimed to develop *Piper longum*-based cookies and evaluate their hypolipidemic effects in patients with hyperlipidemia.

Methods: A total of 60 hyperlipidemic patients, both male and female, aged 25–45 years with baseline cholesterol levels above 200 mg/dL and BMI greater than 24.4 kg/m², were enrolled through non-probability sampling. Participants were divided equally into three groups: control (cookies without *Piper longum*), treatment group T1 (100 g cookies containing 96 g flour and 4 g *Piper longum*, 2–3 biscuits), and treatment group T2 (100 g cookies containing 96 g flour and 4 g *Piper longum*, 4–6 biscuits). Fasting blood samples were collected at baseline (day 0), day 30, and day 60 to measure cholesterol, triglycerides, LDL, and HDL. Data were analyzed using repeated-measures ANOVA, with $p \leq 0.05$ considered significant.

Results: At day 60, total cholesterol decreased from 262.15±44.48 mg/dL to 229.60±24.79 mg/dL in T1 ($p=0.001$) and from 261.90±44.45 mg/dL to 189.85±6.62 mg/dL in T2 ($p<0.001$), while no significant change was seen in the control group ($p=0.327$). LDL levels fell markedly in T1 (182.05±32.06 to 118.10±23.96, $p<0.001$) and T2 (181.70±32.31 to 95.25±7.05, $p<0.001$), whereas control values remained stable ($p=0.843$). HDL increased significantly in both treatment groups (T1: 42.75±1.86 to 44.65±2.30, $p=0.001$; T2: 43.50±1.88 to 48.15±3.92, $p=0.001$) but not in controls ($p=0.054$). Triglycerides dropped from 253.55±87.42 to 179.85±79.14 mg/dL in T1 ($p=0.026$) and from 252.90±86.98 to 142.55±14.44 mg/dL in T2 ($p<0.001$), while controls showed no significant reduction ($p=0.132$).

Conclusion: The findings confirmed that *Piper longum*-based cookies significantly improved lipid parameters in hyperlipidemic patients, demonstrating reductions in cholesterol, triglycerides, and LDL, alongside increases in HDL. As a novel and patient-friendly dietary intervention, these cookies highlight the therapeutic potential of herbal formulations in hyperlipidemia management, though larger and longer-term trials are warranted to validate these outcomes.

Keywords: Cookies, HDL cholesterol, Herbal formulation, Hyperlipidemia, LDL cholesterol, Lipid profile, *Piper longum.*

INTRODUCTION

Hyperlipidaemia is a metabolic disorder characterized by elevated levels of plasma lipids, including triglycerides, cholesterol, phospholipids, and lipoproteins, which collectively disrupt the balance between low-density lipoprotein (LDL) and high-density lipoprotein (HDL) in the blood (1). This imbalance is a key risk factor for the development of cardiovascular diseases and other metabolic complications, making lipid regulation a critical component of preventive and therapeutic strategies. While pharmacological interventions remain essential, the persistent rise in hyperlipidaemia prevalence underscores the importance of exploring safer, complementary approaches to lipid management. Historically, medicinal plants have been widely used to address metabolic diseases, with evidence suggesting their potential to reduce lipid levels and improve cardiovascular outcomes (2-4). Among these, *Piper longum*, a member of the Piperaceae family, has attracted attention for its broad pharmacological properties, including antihyperlipidemic, anti-inflammatory, antioxidant, and cardioprotective effects. The plant contains a rich array of bioactive constituents, such as alkaloids, essential oils, quercetin, and piperine, which are believed to modulate lipid metabolism and mitigate oxidative stress (5,6). Preclinical and clinical studies have shown that *Piper longum* may lower triglycerides, total cholesterol, very low-density lipoprotein (VLDL), and LDL, while simultaneously improving HDL levels, thereby exerting a protective role against dyslipidemia and associated complications (7,8).

Conventional strategies for lipid control emphasize lifestyle modification, including dietary regulation, physical activity, and weight management, which have been shown to reduce LDL cholesterol by approximately 4%, lower triglycerides by 30%, and increase HDL by up to 14% in hyperlipidemic individuals (9,10). However, in today's obesogenic environment, where processed and calorie-dense foods are prevalent, adherence to lifestyle changes remains a challenge. Pharmacological treatments such as statins, orlistat, and lorcaserin are commonly employed but are often accompanied by adverse effects, highlighting the need for safer and more sustainable therapeutic options. Recent investigations into functional foods incorporating *Piper longum*, such as cookies enriched with its extracts, have demonstrated promising results in alleviating hyperlipidemic symptoms over a three-month period. These findings suggest the feasibility of integrating plant-based formulations into dietary interventions to provide both therapeutic benefits and patient acceptability. By combining traditional knowledge with modern therapeutic strategies, medicinal plants like *Piper longum* may represent a viable adjunct to conventional lipid-lowering approaches. The present study is designed to evaluate the antihyperlipidemic potential of *Piper longum*, with a specific focus on its incorporation into food-based formulations. The objective is to investigate its efficacy in reducing plasma lipid levels and to explore its role as a safe, complementary strategy for the prevention and management of hyperlipidaemia.

METHODS

The present study was designed as an effectiveness trial to evaluate the hypolipidemic potential of *Piper longum*-based cookies in patients with hyperlipidemia. A non-probability sampling technique was employed, and a total of 60 adult participants, both male and female, were recruited from private hospitals in Toba Tek Singh, Punjab, Pakistan. Eligible participants were between 25 and 45 years of age, had body mass index (BMI) values greater than 24.4 kg/m², and demonstrated total cholesterol levels above 200 mg/dL at baseline screening. Pregnant and lactating women, children, individuals younger than 25 or older than 45 years, and patients with normal cholesterol levels or BMI were excluded to minimize confounding variables and to maintain homogeneity within the study sample (11). Participants were randomized into three equal groups of 20. The control group (T0) received cookies prepared with 100 g of flour but without *Piper longum*. The first treatment group (T1) received cookies prepared with 96 g of flour and 4 g of *Piper longum*, corresponding to approximately 2–3 biscuits (100 g serving), while the second treatment group (T2) was provided cookies prepared with the same flour and *Piper longum* proportion but offered in a higher number of biscuits, 4–6 pieces (100 g serving). It is important to note a methodological inconsistency in the intervention design: both treatment groups contained the same total dose of *Piper longum* (4 g per 100 g cookies), differing only in the number of biscuits administered.

Cookies were prepared by mixing flour (96 g), baking powder, sugar, salt, vanilla essence, extra virgin olive oil, and one beaten egg with 4 g of ground *Piper longum*. The dough was rolled, shaped with cutters, and baked at 180 °C for approximately 5 minutes until

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the edges browned. Cookies were cooled, stored appropriately, and administered to participants as per group allocation. The preparation method was standardized across all groups to maintain consistency. Blood samples were obtained after an overnight fast at baseline (day 0), mid-intervention (day 30), and post-intervention (day 60). Biochemical assessment included serum lipid profile analysis, specifically measuring high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, and total cholesterol levels (12). Standardized laboratory protocols were followed to ensure accuracy of biochemical testing. Data were expressed as mean ± standard deviation (SD). Analysis of variance (ANOVA) was applied to compare lipid profile changes between groups, and p-values ≤0.05 were considered statistically significant. This approach allowed the assessment of differences in lipid modulation between control and treatment groups. Ethical approval for the study was obtained from the Research Ethics Committee of Minhaj University Lahore (Approval No. MUL/CRD/23/253), ensuring compliance with ethical standards for human research. Written informed consent was obtained from each participant prior to inclusion, and participants retained the right to withdraw at any stage without penalty. Confidentiality of data was maintained throughout the study period.

Table : Treatment Plan for Intervention

Groups	Intervention Details	Intervention plan	No. of Participants
T_0	Control group	Cookies without *Piper Longum* (100g flour)	20
T_1	Experimental group of pateints have low dose	96g flour 4g piper logum grounded (2-3) biscuits	20
T_2	Experimental group of patients have high dose	96g flour & 4g *Piper longum* (4-6) biscuits	20

RESULTS

Participant flow and baseline characteristics indicated balanced gender and age distributions across groups. Males comprised 70% in the control and low-dose groups and 80% in the high-dose group, whereas females comprised 30%, 30%, and 20%, respectively; the association between gender and study groups was not significant (χ^2=6.82, p=0.711). Age was similarly distributed across categories 25–29, 30–34, 35–39, and 40–44 years without significant differences between groups over the study time points (χ^2=4.63, p=0.592). Total cholesterol declined over time in the treatment groups but not in the control. Mean±SD values (mg/dL) for the control were 264.05±44.21 at Day 01, 257.70±45.38 at Day 30, and 277.60±37.80 at Day 60; change over time was not significant (repeated-measures ANOVA F=1.139, p=0.327). The low-dose group showed 262.15±44.48, 220.85±26.04, and 229.60±24.79 with a significant within-group effect (F=8.687, p=0.001). The high-dose group showed 261.90±44.45, 184.75±10.53, and 189.85±6.62 with a significant within-group effect (F=52.430, p<0.001). Pairwise comparisons at Day 30 demonstrated higher cholesterol in the control versus low dose (mean difference 41.30, p=0.001) and control versus high dose (32.55, p=0.008); low versus high dose did not differ (−8.75, p=0.681). At Day 60, the control remained higher than both low dose (77.15, p<0.001) and high dose (72.05, p<0.001), whereas low versus high dose did not differ (−5.10, p=0.818). Triglycerides demonstrated minimal change in the control and significant reductions in both treatment groups. Control means were 253.80±87.50, 254.15±88.53, and 209.65±56.55 with a non-significant time effect (F=2.10, p=0.132). Low-dose means were 253.55±87.42, 238.85±97.51, and 179.85±79.14 with a significant effect (F=3.90, p=0.026). High-dose means were 252.90±86.98, 141.10±9.28, and 142.55±14.44 with a highly significant effect (F=31.40, p<0.001). At Day 30, pairwise differences were non-significant for control versus low dose (14.70, p=0.859) and low versus high dose (59.00, p=0.096) but significant for control versus high dose (73.70, p=0.028). At Day 60, control values exceeded both low dose (111.80, p<0.001) and high dose (110.35, p<0.001), while low versus high dose did not differ (−1.45, p=0.996).

LDL cholesterol was stable in the control and decreased in the treatment arms. Control means were 182.40±32.23, 181.75±32.65, and 176.40±41.09 with a non-significant time effect (F=0.17, p=0.843). Low-dose means were 182.05±32.06, 127.00±25.86, and 118.10±23.96 with a significant effect (F=31.70, p<0.001). High-dose means were 181.70±32.31, 98.80±10.31, and 95.25±7.05 with a significant effect (F=119.66, p<0.001). At Day 30, LDL was higher in the control versus low dose (55.05, p<0.001) and control versus high dose (63.95, p<0.001), with no difference between low and high dose (8.90, p=0.566). At Day 60, the control again exceeded low dose (82.90, p<0.001) and high dose (86.45, p<0.001), whereas low and high dose did not differ (3.55, p=0.841). HDL cholesterol increased in both treatment groups and remained largely unchanged in the control. Control means were 42.45±1.73, 42.45±1.70, and

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41.05±2.44 with a borderline time effect (F=3.31, p=0.054). Low-dose means were 42.75±1.86, 44.90±1.59, and 44.65±2.30 with a significant effect (F=7.36, p=0.001). High-dose means were 43.50±1.88, 47.60±0.88, and 48.15±3.92 with a significant effect (F=19.66, p=0.001). Pairwise comparisons at Day 30 showed higher HDL in both low dose (mean difference from control −2.15, p=0.003) and high dose (−1.90, p=0.008) versus control, with no difference between the two treatment groups (0.25, p=0.913). At Day 60, HDL remained higher in low dose (−4.10, p=0.001) and high dose (−4.65, p=0.001) compared with control, with no difference between treatment groups (−0.55, p=0.777). Sensory testing employed a five-point hedonic scale to evaluate flavour, texture, colour, appearance, and overall acceptability; assessment forms were administered and scores averaged.

Table 1: Gender and Age of Sample Data

Gender	**T_0**	**T_1**	**T_2**	**χ^2**	**p-value**
				6.82	.711
Male	14(70%)	14(70%)	16(80%)		
Female	6(30%)	6(30%)	4(20%)		
Age (years)					
25-29	5(25%)	8(40%)	11(55%)	4.63	.592
30-34	5(25%)	3(15%)	3(15%)		
35-39	5(25%)	4(20%)	4(20%)		
40-44	5(25%)	5(25%)	2(10%)		

Table 2: Pairwise Comparisons of Cholesterol, Triglycerides, LDL, and HDL Across Study Groups Using Tukey Test

Parameter	**Day**	**(I) Groups**	**(J) Groups**	**Mean Diff (I-J)**	**Sig.**
Cholesterol	Day 30	T0	T1	41.300*	.001
		T0	T2	32.550*	.008
		T1	T2	-8.750	.681
	Day 60	T0	T1	77.150*	<.001
		T0	T2	72.050*	<.001
		T1	T2	-5.100	.818
Triglycerides	Day 30	T0	T1	14.700	.859
		T0	T2	73.700*	.028
		T1	T2	59.000	.096
	Day 60	T0	T1	111.800*	<.001
		T0	T2	110.350*	<.001
		T1	T2	-1.450	.996
LDL	Day 30	T0	T1	55.050*	.000
		T0	T2	63.950*	.000
		T1	T2	8.900	.566
	Day 60	T0	T1	82.900*	.000
		T0	T2	86.450*	.000
		T1	T2	3.550	.841
HDL	Day 30	T0	T1	-2.150*	.003
		T0	T2	-1.900*	.008
		T1	T2	0.250	.913
	Day 60	T0	T1	-4.100*	.001
		T0	T2	-4.650*	.001
		T1	T2	-0.550	.777

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Table 3: Means and SDs of Cholesterol on Day-01, Day-30 and Day-60 of treatments T_0, T_1 and T_2

	Cholesterol			Repeated Measure ANOVA	
	Day 01	Day 30	Day 60	F-value	P-value
T_0	264.05±44.21	257.7±45.38	277.6±37.8	1.139	0.327
T_1	262.15±44.48	220.85±26.04	229.6±24.79	8.687	0.001
T_2	261.9±44.45	184.75±10.53	189.85±6.62	52.430	<.001

Table 4: Means and SDs of Triglycerides on Day-01, Day-30 and Day-60 of Treatments T_0, T_1 and T_2

Treatments	Triglycerides			Repeated Measure ANOVA	
	Day 01	Day 30	Day 60	F-value	P-value
T_0	253.8±87.5	254.15±88.53	209.65±56.55	2.10	0.132
T_1	253.55±87.42	238.85±97.51	179.85±79.14	3.90	0.026
T_2	252.9±86.98	141.1±9.28	142.55±14.44	31.40	0.000

Table 5: Means and SDs of LDL on Day-01, Day-30 and Day-60 of treatments T_0, T_1 and T_2

Treatments	LDL			Repeated Measure ANOVA	
	Day 01	Day 30	Day 60	F-value	P-value
T_0	182.4±32.23	181.75±32.65	176.4±41.09	0.17	0.843
T_1	182.05±32.06	127±25.86	118.1±23.96	31.70	<.001
T_2	181.7±32.31	98.8±10.31	95.25±7.05	119.66	<.001

Table 6: Means and SDs of LDL on Day-01, Day-30 and Day-60 of treatments T_0, T_1 and T_2

Treatments	HDL			Repeated Measure ANOVA	
	Day 01	Day 30	Day 60	F-value	P-value
T_0	42.45±1.73	42.45±1.7	41.05±2.44	3.31	0.054
T_1	42.75±1.86	44.9±1.59	44.65±2.3	7.36	0.001
T_2	43.5±1.88	47.6±0.88	48.15±3.92	19.66	.001

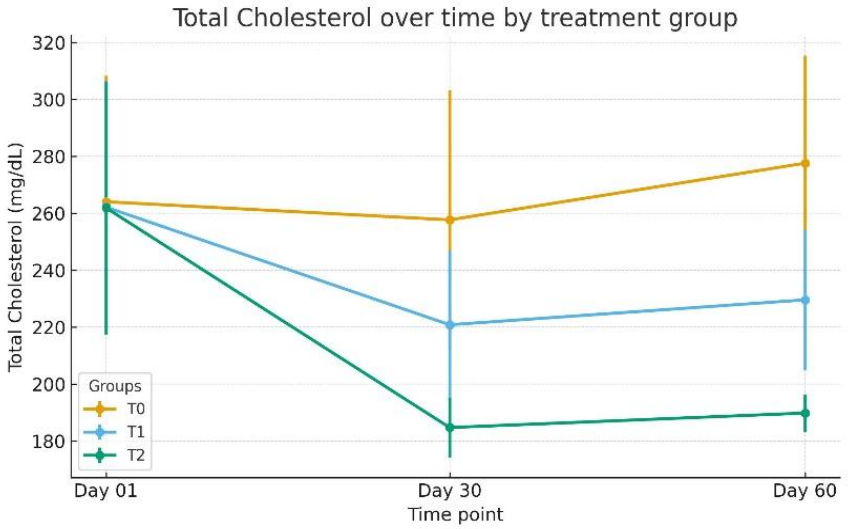

Figure 1 Total Cholesterol Over Time by Treatment Group

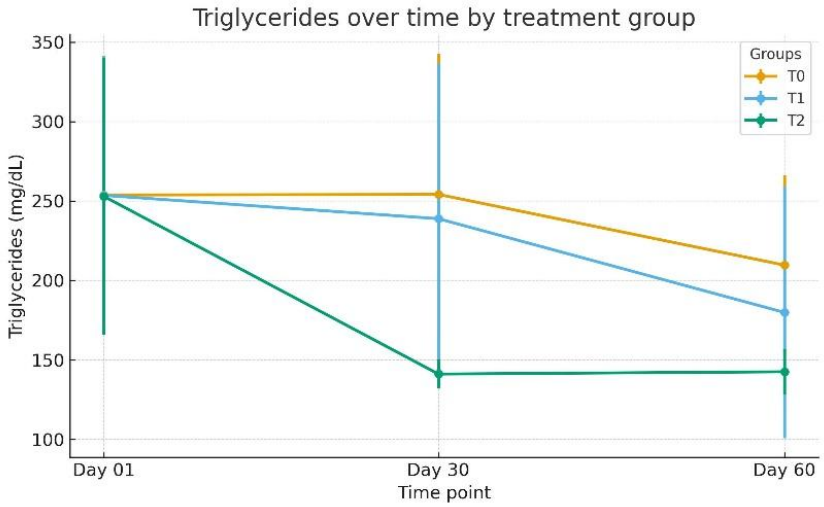

Figure 2 Triglycerides Over Time by treatment Group

DISCUSSION

The findings of this study demonstrated that *Piper longum*-based cookies produced significant improvements in lipid parameters, particularly LDL and HDL cholesterol, among hyperlipidemic patients. LDL cholesterol remained largely unchanged in the control group, while both treatment groups experienced highly significant reductions over the 60-day intervention. These outcomes align with previous research on the lipid-lowering potential of phytochemicals and alkaloids contained in medicinal plants, particularly piperine, which is known to modulate lipid metabolism and reduce circulating LDL concentrations (13,14). The significant differences observed between the control group and both treatment groups at Days 30 and 60 confirm the therapeutic potential of *Piper longum* in improving dyslipidemia. The absence of significant differences between the two treatment groups, however, highlights a design limitation, as both groups received an identical total dose of *Piper longum*, precluding a true dose–response assessment (15,16). The results for HDL cholesterol were equally noteworthy, with significant increases observed in both treatment groups compared with the control. This improvement in HDL levels is clinically important, as elevated HDL provides cardioprotective effects by enhancing reverse cholesterol transport and reducing atherogenic burden. Interestingly, significant differences were already apparent at Day 01 between the control and high-dose groups, suggesting possible baseline imbalances that were not fully reported. While the positive impact on HDL supports the cardioprotective role of *Piper longum*, the lack of baseline statistical comparisons for lipid parameters limited the certainty of attributing early differences entirely to the intervention (17-20). The study adds strength to the growing body of evidence that dietary incorporation of medicinal plants may serve as a safe and practical adjunct to conventional hyperlipidemia management. The use of a food-based delivery system, such as cookies, enhances patient acceptability and compliance compared with traditional herbal preparations. Moreover, the structured biochemical assessments and repeated measures design provided clear insight into longitudinal changes in lipid profiles. Ethical approval and informed consent procedures were appropriately observed, strengthening the integrity of the trial.

Nevertheless, several limitations must be acknowledged. The small sample size restricted the generalizability of the results and reduced the power to detect subtle differences between groups. Non-probability sampling introduced potential selection bias, while the short duration of the study limited conclusions regarding long-term sustainability of lipid improvements. Adherence to cookie consumption was not systematically measured, adverse events were not reported, and sensory evaluation results were described but not quantified, leaving important aspects of acceptability and safety unaddressed. Furthermore, the omission of VLDL and non-HDL cholesterol measurements restricted the ability to fully characterize the impact of *Piper longum* on atherogenic lipoprotein fractions. The identical dosing across the so-called “low” and “high” treatment groups was a key methodological flaw that weakened the

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interpretation of dose-dependent effects (21,22). Future research should address these limitations by incorporating larger, randomized controlled trials with stratified randomization to ensure balanced baseline characteristics. Detailed reporting of compliance, tolerability, and sensory outcomes should be included, alongside comprehensive lipid profiling that extends beyond LDL and HDL. Longer follow-up periods would help establish whether the improvements in lipid parameters are sustained and translate into meaningful reductions in cardiovascular outcomes. Standardization of the dosing protocol and exploration of varying concentrations of *Piper longum* would also allow for dose–response analysis, which is critical to inform clinical and dietary recommendations. Overall, the study supports the therapeutic potential of *Piper longum*-based dietary interventions for hyperlipidemia while also emphasizing the need for more rigorous and comprehensive investigations to establish efficacy, safety, and translational relevance in clinical practice.

CONCLUSION

The study concluded that *Piper longum*-based cookies hold promising therapeutic potential in the management of hyperlipidemia by effectively improving lipid profiles, particularly through reductions in cholesterol and LDL levels alongside favorable changes in triglycerides and HDL. As a food-based intervention, these cookies represent a practical, accessible, and patient-friendly approach that combines traditional medicinal knowledge with modern dietary strategies. The findings highlight the value of incorporating medicinal plants into everyday nutrition as a complementary method for controlling lipid disorders, offering a safe and convenient alternative to conventional therapies.

AUTHOR CONTRIBUTION

Author	**Contribution**
Ayesha Malik*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Kainat Noor	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Sadia Naeem	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Uswa Ahmad	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Sana Azhar	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published

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