

Impact of Black Pepper (*Piper nigrum*) Powder on Lipid Profile Parameters Among Subjects with Non-Alcoholic Fatty Liver Disease (NAFLD)

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Abstract. Non-alcoholic fatty liver disease (NAFLD) is closely associated with dyslipidemia, a critical risk factor for cardiovascular diseases and metabolic disorders. This study examines the effects of supplementation of black pepper (*Piper nigrum*) which contains piperine a bioactive compound as its prominent constituent, on lipid profile parameters in NAFLD patients over 12 weeks. A randomized, double-blind, placebo-controlled trial was conducted with 170 clinically and radiologically diagnosed NAFLD patients. Participants were randomly allocated into a piperine supplementation group (n=85) and a placebo group (n=85). Lipid profile parameters, including total cholesterol, triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL), were measured at baseline and post-intervention using enzymatic colorimetric analysis. The piperine group exhibited a significant reduction in total cholesterol, triglycerides, and LDL, along with a marked increase in HDL levels, whereas the placebo group showed no significant changes. Percent Changes in Lipid Profile (Piperine Group): Total Cholesterol ↓15.13%, Triglycerides ↓44.94%, LDL ↓21.58%, HDL ↑3.51%. The findings suggest that piperine supplementation could be an effective adjunct in managing lipid profiles in NAFLD patients, potentially reducing dyslipidemia-related complications. Further studies are required to assess long-term safety and underlying mechanisms.

KEY WORDS: Dyslipidemia, LDL, Triglycerides, Piperine, Non-alcoholic steatohepatitis (NASH)

1. INTRODUCTION

Black pepper was originally solely grown in the western coastal regions of India, and its production extended to most tropical countries. People often use black pepper, also called the "*King of Spices*," to enhance the taste of other ingredients or to enhance food flavor when used alone. Known for its powerful aroma, the perennial woody climber *Piper nigrum* may reach a height of 50 to 60 cm [1]. Piperine is the primary oleoresin present in black pepper. While piperine was first observed in the 1800s, its chemical makeup was fully unrevealed between 1882 and 1894 [2]. Since then, many studies have been conducted on black pepper, which has piperine, as one of its primary components. Also, many more comparable alkaloids, such as piperlylin A, piperlylin B, piperanine, piperettine, and pipericine, have been present in black pepper; however, these counterparts of piperine are less pungent. Apart from the alkaloids of the piperine group, the following alkaloids were also observed: Sarmentine, Sarmentosine, Retrofractamide. A, Sarmentine B, and Tricholine [4]. Notably, the bioactivities of piperine are diverse and extensive, including anti-inflammatory, antioxidative, antibacterial, and anticancer effects. Studies on rats have shown that piperine may help alleviate dyslipidemia, potentially via activating the melanocortin-4 receptor in the central nervous system. The pharmacologic characteristics of piperine may make it suitable for clinical trial evaluation to determine its effectiveness against long-term inflammatory disorders [5].

Besides, nearly 25% of people worldwide have non-alcoholic fatty liver disease (NAFLD), making it the most prevalent chronic liver illness in the last 40 years [9]. The liver has extraordinary resilience since it can self-repair and regenerate after significant cellular damage. NAFLD is a liver condition linked to metabolic syndrome, hypertension, insulin resistance, obesity, type 2 diabetes mellitus (T2DM), and hyperlipidemia. It is a key mechanism of disordered fat metabolism. The cause of this abnormal fat metabolism is often diet-induced insulin resistance, which is typically brought on by years of consuming an excessive amount of unhealthy food.

Nutritional factors, behavior, diabetes, and metabolic syndrome are closely related to NAFLD. In India, the prevalence of NAFLD varies from 9% to 42%, with a median of 20% worldwide [6]. The liver tissue harmed by NAFLD may start to mend if factors encouraging cellular damage are taken care of, halting the disease's development into cirrhosis or liver cancer.

Even in specific instances with evident inflammation and fibrosis (scarring), timely intervention with dietary and lifestyle modifications and nutritional assistance may reduce or reverse the buildup of fat and liver damage. When hepatocyte damage occurs, the inflammation and fibrosis coexist with lipid buildup, which is indicative of a more severe type

of non-alcoholic steatohepatitis (NASH). NASH, with increasing fibrosis, raises the chance of primary liver cancer (hepatocellular carcinoma) and may ultimately result in cirrhosis and liver failure. Researchers are interested in piperine because of its beneficial impacts on hepatic lipid metabolism and NAFLD [8].

Moreover, several studies have revealed the impact of black pepper (*Piper nigrum*) on NAFLD. Also, researchers are studying to determine if piperine may reduce the onset and severity of NAFLD. New drug development strategies could enhance piperine's bioavailability and bioactivity, especially when paired with other bioactive substances or food additives. When tested on individuals with NAFLD, the effects of black pepper (*Piper nigrum*) powder on liver function have shown mixed results. Earlier research revealed the effects of piperine alone and with other drugs and plant substances on chronic diseases [9]. Another study addressed the blood-liver enzymes, lipid profiles, and glycemic indices of NAFLD patients with a combination of curcumin and piperine to enhance curcumin bioavailability and reduce the severity of NAFLD. A recent study covered patients with moderate to high degrees of hepatic steatosis and NAFLD to determine the effects of curcumin and piperine on cardiometabolic risk factors and hepatic steatosis and fibrosis. Another recent study found that the compound piperine found in black pepper (*Piper nigrum* L.) improved the lipid profiles of overweight and obese individuals by decreasing total cholesterol and triglycerides and raising low-density lipoprotein (LDL). To add to the existing literature, the current study aimed to evaluate the impact of black pepper (*Piper nigrum*) on lipid profile parameters in subjects with NAFLD.

2. MATERIALS AND METHODS

2.1 Study Design and Participants

A randomized, double-blind, placebo-controlled trial design was employed to assess the effects of piperine supplementation on lipid profile parameters in subjects with NAFLD at Yathart Hospital, Greater Noida. It is also used to ensure the reliability and accuracy of the findings. The inclusion criteria were adults aged 18–65 with confirmed NAFLD (Grade 2) and a BMI between 25 and 40 kg/m². Also, those who were not engaged in other clinical trials. The exclusion criteria comprised of i) subjects having a history of alcohol misuse, viral hepatitis, secondary causes of fatty liver disease, or other chronic liver illnesses to remove confounding variables, ii) those with significant renal or cardiovascular conditions, iii) those on medications known to influence liver function, iv) pregnant or breastfeeding women, and v) those with allergies to black pepper. The sample size was calculated at a confidence interval of 95% with a power of 80% and an effect size of 0.30 for total cholesterol using GPower software (version 3.1.9.7). Concerning the estimated 10% attrition of subjects, this study considered 170 as the sample size. Using the selection criteria, 170 subjects diagnosed with NAFLD based on clinical and imaging criteria

were recruited for this study. Those were randomly assigned to two groups, the piperine supplementation group (n=85), and the placebo group (n=85), using simple random sampling

2.2. Intervention

Subjects in the piperine group received a daily dose of 20 mg black pepper powder (standardized to 95% purity), while the placebo group received an identical capsule containing inert substances. Both interventions were administered orally for a duration of 12 weeks. Those were instructed to continue their regular diet and exercise routines throughout the study to minimize external influences on lipid profile changes. During the study duration, no subjects were dropped and no adverse events were observed.

2.3. Data collection and outcome measurements

Lipid profile parameters, including total cholesterol, triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL), were evaluated at baseline (pre-intervention) and after 12 weeks (post-intervention). Those parameters were examined using enzymatic colorimetric means in a certified laboratory.

2.4. Statistical Analysis

Statistical analyses were performed using SPSS software (version 29.0). The mean values for lipid profile parameters were calculated for both groups using descriptive statistics. The paired t-tests were applied to compare pre-and post-intervention values within each group. The unpaired t-tests were employed to compare variations between the piperine and placebo groups. Statistical significance was set at $p < 0.05$.

2.5. Ethical Considerations

This study was approved by the institutional ethics committee, adhering to the Declaration of Helsinki guidelines. All subjects were informed about the study objectives, potential risks, and benefits, and participation was voluntary. Written informed consent was obtained from all subjects prior to the commencement of the study. (SU/RES/SAHS/N&D/2021212365/710) from Sharda University, Greater Noida ethical approval was obtained).

3. RESULT AND DISCUSSION

Table 1 describes the demographic characteristics of the subjects. Further, the comparative effects of piperine supplementation and placebo on lipid profile parameters, including total cholesterol, LDL, and HDL were illustrated in Table 2. Regarding total cholesterol, the piperine group showed a statistically significant reduction from a mean pre-intervention value of 211.21 mg/dL to a post-intervention value of 179.26 mg/dL ($p < 0.05$). In contrast, the placebo group exhibited no significant change between pre-and post-

intervention, with the mean total cholesterol levels remaining broadly consistent (212.80 mg/dL versus 211.21 mg/dL; $p > 0.05$). There was a significant reduction of triglycerides in the piperine group, i.e., from 219.40 mg/dL (pre-intervention) to 120.82 mg/dL (post-intervention) ($p < 0.05$). In placebo group, no significant change was observed between pre- and post-intervention meaning triglycerides values (222.40 mg/dL versus 219.31 mg/dL; $p > 0.05$). Similarly, LDL levels in the piperine group significantly decreased from 115.14 mg/dL (pre-intervention) to 90.31 mg/dL (post-intervention) ($p < 0.05$). However, the placebo group showed no significant change between pre- and post-intervention mean LDL values (116.14 mg/dL versus 115.14 mg/dL; $p > 0.05$). Regarding HDL levels, the piperine group demonstrated a significant improvement in the mean values, increasing from 41.00 mg/dL (pre-intervention) to 42.44 mg/dL (post-intervention) ($p < 0.05$). Conversely, the placebo group presented no significant change between pre- and post-intervention means HDL values (42.44 mg/dL versus 43.21 mg/dL; $p > 0.05$) (Table 2). These findings underscore the potential efficacy of pipeline in improving metabolic health in NAFLD subjects.

Table 1: Demographics characteristics of the subjects

Variables	Piperine group Mean±SD	Placebo group Mean±SD
Age (in years)	41.26±7.65	42.18±7.32
Gender	Male	102 (60)
	Female	68 (40)
Body mass index (BMI)	26.19±3.22	26.54±2.95
Height	1.64±0.07	1.64±0.06
Weight	69.91±4.99	71.34±4.76
Waist ratio	1.14±0.07	1.15±0.06

Table 2: Paired ‘t’ test comparing the effect of Piperine and Placebo on Lipid Profile Parameters

Parameters	Intervention stage	Piperine		Placebo	
		Mean±SD	‘t’ value (p-value)	Mean±SD	‘t’ value (p-value)
Total Cholesterol	Pre-intervention	211.21±8.19	27.249 (0.000)	212.80±7.84	1.253 (0.096)
	Post-intervention	179.26±5.92		211.21±8.19	
Triglycerides	Pre-intervention	219.40±41.16	19.029 (0.00)	222.40±41.47	1.435 (0.246)
	Post-intervention	120.82±17.72		219.31±41.17	
Low-Density Lipoprotein (LDL)	Pre-intervention	115.14±6.89	20.459 (0.000)	116.14±6.89	0.730 (0.101)
	Post-intervention	90.31±7.77		115.14±6.89	
High-Density Lipoprotein (HDL)	Pre-intervention	41.00±4.36	7.301 (0.000)	42.44±2.53	0.554 (0.124)
	Post-intervention	42.44±2.53		43.21±2.39	

*Significant at 0.05

Table 3: Unpaired ‘t’ test comparing the Impact of Piperine and Placebo on Lipid Profile Parameters

Parameters	Pre-Intervention			Post-Intervention		
	Piperine	Placebo	‘t’ value (p-value)	Piperine	Placebo	‘t’ value (p-value)
	Mean±SD	Mean±SD		Mean±SD	Mean±SD	
Total Cholesterol	211.21±8.19	212.80±7.84	2.443 (0.124)	179.26±5.92	211.21±8.19	36.930 (0.000)
Triglycerides	219.40±41.16	222.40±41.47	2.914 (0.832)	120.82±17.72	219.31±41.17	26.726 (0.000)
Low-Density Lipoprotein (LDL)	115.14±6.89	116.14±6.89	1.776 (0.382)	90.31±7.77	115.14±6.89	31.329 (0.000)
High-Density Lipoprotein (HDL)	41.00±4.36	42.44±2.53	2.651 (0.080)	42.44±2.53	43.21±2.39	4.534 (0.000)

*Significant at 0.05 level

At the post-intervention stage, there was a significant difference between the mean total cholesterol, triglycerides, LDL, and HDL values of both groups ($p < 0.05$). The piperine group showed a significant reduction in total cholesterol, triglycerides, and LDL values and an improvement in the HDL values compared to the placebo group (Table 3).

The study outcomes provide strong evidence regarding the metabolic benefits of piperine supplementation in lipid profile parameters among patients with NAFLD. Following 12 weeks of intervention, the piperine group showed a significant reduction in total cholesterol, triglycerides, and LDL values and a marked improvement in HDL values. However, the placebo group failed to show significant changes in observed lipid profile parameters. While comparing the post-intervention means, the piperine group showed a significant reduction in the total cholesterol, triglycerides, and LDL values and an improvement in the HDL values compared to the placebo group. On the other hand, a recent study observed that 12 weeks of piperine intake significantly reduced the total cholesterol, triglycerides, and LDL levels and improved the HDL levels in patients with NAFLD and early cirrhosis. After the intervention duration, there was a significant reduction of cholesterol, triglycerides, and LDL levels in the piperine group than in the placebo group. Nevertheless, those two groups had no significant difference in HDL levels. Previous studies observed that curcumin and piperine use for 12 weeks significantly reduced total cholesterol, triglycerides, and LDL levels within and between the groups than the placebo group; however, it failed to significantly change HDL levels within and between the groups. These studies explored the combined effect of curcumin and piperine on the lipid profile of NAFLD cases.

However, the current research has only revealed the impact of piperine on the lipid profile, which can be compared with curcumin or other substances in the future. Further, it presented a lack of significant changes in the placebo group that underscores the specificity and efficacy of the piperine intervention.

In this study, the observed effect of piperine supplementation on total cholesterol, triglycerides, LDL, and HDL levels in patients with NAFLD might be due to its lipid lessening effect and anti-obesity activity with no variation in appetite, which was observed in high-fat diet rats. The piperine decreases liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and enhances lipid and glucose metabolism in humans with NAFLD. Also, it alleviates dyslipidemia [10] and decrease the mass of visceral compared to subcutaneous adipose tissue in mice. Furthermore, the piperine has thyrogenic activity, moderating apolipoprotein measures and insulin resistance in rats. It also impedes the lipoprotein and lipid

buildup by pointedly controlling the enzymes related to lipid metabolism, such as Lecithin-cholesterol acyltransferase (LCAT) and Lipoprotein lipase (LPL) [12]. The piperine intake was observed to lessen the risk of atherosclerosis evidently through its antiatherogenic and hypolipidemic effects in high-fat fed rats. Besides, piperine might modulate lipid metabolism via its anti-inflammatory and antioxidant activities. It also interferes with cholesterol biosynthesis and lipid oxidation pathways to contribute to lipid-lowering effects. Moreover, the considerable increase in HDL levels indicates that piperine may enhance reverse cholesterol transport, an essential mechanism for lowering cardiovascular risk in patients with metabolic syndrome and liver diseases such as NAFLD [13, 14].

This study is limited to a smaller sample size and focused on assessing the lipid profile parameters of individuals with NAFLD. Future studies can be conducted with a larger sample size to aid the generalization of the findings. Also, further research can be carried out to assess the effect of piperine supplementation on other biochemical variables such as AST, ALT, fasting blood sugar, hemoglobin A1c, homeostatic model assessment, gamma-glutamyl transferase [15]. Notably, additional studies are needed to be conducted to assess the safety and efficacy of long-term use of piperine supplementation, especially in different populations at varying stages of liver disease and metabolic dysregulation.

4. CONCLUSION

Piperine supplementation resulted in considerable lipid profile changes in individuals with NAFLD. It effectively reduces total cholesterol, triglycerides, and LDL and increases HDL levels considerably, proving it is a possible therapeutic intervention for dyslipidemia. However, the placebo group did not show any significant improvement since the group received an identical capsule containing inert substances. These findings confirmed that piperine is a promising adjunctive therapy in the management of dyslipidemia in patients with NAFLD, complementing lifestyle and medical interventions. However, long-term efficacy, safety, and mechanisms of action need to be explored among diverse populations and clinical settings. A holistic approach with supplementation of piperine, coupled with weight management strategies, could offer optimal outcomes in improving metabolic health and preventing further progression of NAFLD.

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