EXLORING THE ANTI HYPERCHOLESTEROLEMIC POTENTIAL OF *MELIA AZEDARACH LINN*. LEAVES EXTRACT IN COMBINATION WITH RED YAST RICE (RYR)

Annu Thapliyal[1], Mrs. Priti Khanduri[*], Dr. Sanjay Singh[2]
[1]Research Scholar, Department of Pharmacology, Siddhartha Institute of pharmacy,
*Associate Professor Department of Pharmacy, Siddhartha Institute of pharmacy,
pritiajay.nautiyal@gmail.com
[2]Principal, Siddhartha Institute of pharmacy, Sahashtradhara Road Dehradun, Uttarakhand, India, 248001

Abstract

This experimental study was done to determine the Anti Hypercholesteremic outcome of leaves of *Melia azedarach* on hypercholesterolemic rats. In this study the animals were grouped in 5 different groups having 6 rats in every group. The Groups I, II, III, IV and V were the Normal Control, Disease control, Standard Control, Treatment group 1 and Treatment group 2 respectively. In Group I Standard Pellet Diet was given for 35 days. In group II as the rats were given High Fat Diet for 35 days. In group III the rats were given High Fat Diet same as disease group but they were also given the treatment i.e., Rosuvastatin with a dose of 5 mg/Kg/day for last 35 days. In IV group the rats were given High Fat Diet with Melia Azedarach extract lower dose ie, 300 mg/kg in combination with Red Yeast Rice in dose 1gm/Kg/day and in V group the rats were given High Fat Diet with Melia Azedarach extract higher dose i.e., 600 mg/kg in combination with Red Yeast Rice in dose 2gm/kg/day both for 35 days. The Anti Hypercholesteremic activity of Melia Azedarach was evaluated by the estimation Lipid Profile i.e. Total cholesterol (TC), Triglycerides (TG), Low-density lipoprotein cholesterol (LDL-C), High-density lipoprotein cholesterol (HDL-C) & Liver Function Tests I.e. Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) & Alkaline phosphatase (ALP). Histopathological examinations were also done of the liver of each group to see the possible changes in structure of the cells. It was noted that the ethanolic extract Melia Azedarach leaves shows Anti Hypercholesteremic activity. It is able in protecting the liver cells from the damage caused by higher fat levels in the body of rats and also able in reversing the changes which was caused by these agents.

Key words: - *Melia azedarach*, Anti Hypercholesteraemic, High Fat Diet, Cholesterol, Rosuvastatin, Red yeast rice, Total cholesterol, Liver function test, Histopathology.

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INTRODUCTION

Hypercholesterolemia is a biochemical condition known when the level of cholesterol increases in the body. It poses a significant risk for atherosclerosis and cardiovascular diseases (CVDs). The condition's prevalence has surged due to poor dietary choices, sedentary lifestyles, and obesity, raising public health concerns [1]. Cholesterol plays crucial roles in cell membranes and hormone production, and its homeostasis is regulated by synthesis, absorption, and excretion [2].

It can be classified as primary (genetic) or secondary (acquired), with the latter often linked to obesity, diabetes, and certain medications. Hypercholesterolemia's pathophysiology involves LDL cholesterol infiltrating the arterial wall, becoming oxidized, and instigating inflammation that leads to atherosclerotic plaque development, ultimately increasing the risk of blood flow obstruction and thrombotic events [3].

Hypercholesterolemia is a metabolic disorder marked by excessive cholesterol accumulation in the bloodstream, particularly LDL-C. It disrupts cholesterol homeostasis—balance between synthesis, absorption, utilization, and excretion, with the liver central to regulation. Increased synthesis occurs due to HMG-CoA reductase activity, enhancing VLDL production, while impaired LDL clearance results from decreased LDL receptor function or genetic factors [4]. Elevated LDL leads to its oxidation, vascular penetration, and endothelial dysfunction, promoting inflammation and foam cell formation, which contribute to atherosclerosis. Inflammation exacerbates plaque instability, possibly resulting in acute cardiovascular events through plaque rupture and thrombus formation [5].

Rosuvastatin is a powerful synthetic lipid-lowering agent in the statin class, known as HMG-CoA reductase inhibitors, primarily prescribed for hypercholesterolemia, mixed dyslipidaemia, and cardiovascular disease prevention. Its effectiveness in lowering cholesterol is attributed to its high potency and favourable pharmacokinetic profile [6].

Rosuvastatin is classified as a statin. Its water loving nature limits diffusion into cells, and decreases the after effects of other lipophilic statins. It selectively stops HMG-CoA reductase, interrupts cholesterol formation. It also helps in removing the low-density lipoproteins. Rosuvastatin's dose-dependent effects include a 45–63% reduction in LDL-C, a 10–35% [7]. Additionally, it has pleiotropic effects, such as improved endothelial function, reduced vascular inflammation, stabilized atherosclerotic plaques, and decreased oxidative stress [8].

Its oral rate and extend of absorption are around 20%, and it reaches to its peak concentration within 3 to 4 hours. It has about 88% plasma protein binding, undergoes minimal hepatic metabolism primarily via CYP2C9, its ½ life is 19 hours, and is predominantly excreted in feces [9].

Red Yeast Rice (RYR), derived from Monascus purpureus cultured on rice, is used for enhancing digestion and cardiovascular health. It is recognized for its ability to lower cholesterol, serving as a natural alternative or adjunct to statin therapy [10]. RYR contains bioactive compounds including monacolins (notably Monacolin K, similar to lovastatin), pigments, isoflavones, unsaturated fatty acids, and sterols, which collectively support lipid metabolism and cardiovascular health [11].

Monacolin K selectively stops HMG-CoA reductase, interruption of cholesterol formation and enhances LDL receptor upregulation, which led to the decrement of TC, LDL-C, and triglycerides & upgrading HDL-C [12]. Its pharmacological effects include lowering cholesterol levels, preventing atherosclerosis, improving endothelial function, and reducing vascular inflammation. Additionally, RYR may enhance liver lipid metabolism, exhibit mild hypotensive effects, and improve cardiovascular health in individuals with metabolic syndrome [13].

Melia azedarach Linn., known as Indian Lilac, Chinaberry, Persian Lilac, or White Cedar, is a deciduous tree from the family *Meliaceae*, reaching heights of 5–12 meters. It features pinnate, compound leaves with lilac or white flowers and produces yellow drupes [14]. This tree is heavily cultivated as an ornamental plant due to its attractive appearance and has significant medicinal uses, particularly in Asian countries, where its leaves, bark, seeds, and fruits are employed in various therapies [15].

Melia azedarach thrives across India in tropical and subtropical regions, found in states like UP, Punjab, Haryana, Rajasthan, MP, Chhattisgarh, Gujarat, Maharashtra, West Bengal, Bihar, Odisha, Karnataka, Tamil Nadu, Andhra Pradesh, and Kerala. It prefers sunny locations with well-drained soil and is commonly planted along roadsides and in gardens, valued for its hardiness and drought tolerance [16] [17].

This study examines the chemical compositions of *Melia azedarach Linn* (Chinaberry) leaf ethanolic extracts and their effects in combination with Red Yeast Rice against Hypercholesterolemia.

MATERIALS AND METHODS

Agents

Rosuvastatin, Red Yeast Rice extract, High-Fat/High-Cholesterol (HFC) diet, Standard Pellet (SP) diet was taken from the laboratory of institute.

Collection and authentication of Plant Material

The plant employed in this study was *Melia Azedarach Linn*. The plant was collected from Selaqui, Dehradun. After that the herbarium was prepared for the authentication procedure of plant because it is very important to authenticate the plant before the study. The plant was authenticated from Botanical Survey of India, Dehradun. The authentication certificate was provided and is preserved for the further use. Then leaves were dehydrated firstly in shade & then in the sunlight. Once the leaves were completely dry then they were triturated into a powder with the help of mortar pestle. Then it was run through sieve to get a smooth powder [18] [19].

Extract preparation

Ethanol was used as a solvent for the extraction process. Soxhlet Assembly was arranged and set up properly. Then plant material was taken in a ratio of 3:1 with the solvent and the process of extraction begun [20].

Around 40 -50 cycles were run to get a extracted solution and then the solvent was evaporated until the semi solid consistency of extract was formed. The extract was then kept aside till the further use [21] [22].

Determination of total flavonoid content:

Rutin was used as a reference to calculate the total flavonoid content. 10% (w/v) aluminum chloride (AlCl3), 5% (w/v) sodium nitrite (NaNO2), 1 M or 4% sodium hydroxide (NaOH), and methanol solutions were utilized. The absorbance was measured at various dilutions of standard and plant extract (10 to 100µg/ml) at 510nm. [23] [24] [25].

Determination of total phenolic content:

Gallic acid was used as a standard in the Folin-Ciocalteu colorimetric technique to quantify the total phenolic content. Folin-Ciocalteu reagent solutions (10%), (7.5%) Na2CO3, or sodium carbonate, was employed. The absorbance at 760 nm was measured using various dilutions of the standard and plant extract (10 to 100µg/ml). [23] [24] [25].

Oral toxicity test:

Studies on the acute oral toxicity of *Melia azedarach* leaf extract show minimal toxicity in test animals. Ethanolic leaf extracts given orally to rats at doses up to 600 mg/kg in pharmacological trials did not result in mortality or noticeable toxic effects during the study period, indicating an acceptable safety profile at therapeutic doses. It was previously done by *A. Srinivasa Rao et al.* [26]

Procurement of experimental Animals

The study was done on the male Wistar rats. The approval of animal study protocol was done through institute of pharmacy i.e., the "Siddhartha Institute of Pharmacy, Dehradun" and their committee is the IAEC (Institutional Animal Ethical Committee) that reviewed and approved the protocol and procedures employed in the experiment with Approval number SIP/IAEC/PCOL/03/2026.

The rats were acclimatized for 10 days prior to protocol. The relative humidity and temperature were according to the CPCSEA i.e., 55% ±5% and 25˚C ±1˚C respectively. The standard feed and water were given properly to all the animals. The age of the rats was six to eight weeks.

Induction of disease and treatment:

The animal was divided into 5 groups each group consist of 6 animals, before the treatment all the rats were given high cholesterol rich diet along with standard food pellets for about 3 weeks so that they may gain weight and induced with hyper cholesterol condition.
After 3 weeks the treatment was given to each group for 2 weeks .Group 1 was given no treatment and serves as control ,group 2 was diseased group and also given no treatment ,group 3 serves as standard and was given standard drug Rosuvastatin 5 mg/kg, group 4 was given *Melia azedarach* leave extract (300mg/kg)along with red yeast rice (1g/kg) which serves as treatment group 1 and lastly the group 5 was given *Melia azedarach* leaves extract (600mg/kg) along with red yeast rice (2g/kg)which was treatment group 2.

Study design

Total 30 male Albino Wistar rats were allocated in 5 groups as mentioned below in the table;

Group No.	**Group Name**	**Diet**	**Treatment**	**Dose**	**Duration**
Group I	Normal Control	Standard Pellet (SP) diet	Normal Saline (Oral)	--	35 days
Group II	Disease Cont	High-Fat/Hig	Normal Salin	--	35 days

	rol	h-Cholesterol (HFC) diet	e (Oral)		
Group III	Standard Control	High-Fat/High-Cholesterol (HFC) diet	Rosuvastatin (Oral)	5.0 mg/kg/day	35 days
Group IV	Treatment Group 1	High-Fat/High-Cholesterol (HFC) diet	*Melia azedarach* extract + Red Yeast Rice extract (oral)	300 mg/Kg/day + 1.0 g/kg/day	35 days
Group	Treatm	High-F	*Melia*	600 mg	35 da

V	ent Group 2	at/High-Cholesterol (HFC) diet	*azedarach* extract + Red Yeast Rice extract (oral)	/Kg/day + 2.0 g/kg/day	ys

Evaluation parameters

Physical evaluation- In physical evaluation the body weight and food water intake was assessed during the whole study.

Biochemical parameter-

Lipid profile- The total concentration of cholesterol found in the serum or plasma, including cholesterol found in very-low-density lipoproteins (VLDL), high-density lipoproteins (HDL), and low-density lipoproteins (LDL), is known as total cholesterol (TC). It is one of the main metrics in a lipid profile and is used to evaluate the risk of cardiovascular disease and lipid metabolism.

- Total cholesterol (TC)-
- Triglycerides (TG)
- Low- density lipoprotein cholesterol (LDL-C)
- High-density lipoprotein cholesterol (HDL-C)

Liver Function test -A set of biochemical blood tests called Liver Function Tests (LFTs) are used to evaluate the integrity and functioning state of the liver. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), bilirubin, albumin, and total protein are commonly measured. Hepatobiliary illnesses and liver diseases can be diagnosed, tracked, and evaluated with the aid of these tests.

- Aspartate aminotransferase (AST)
- Alanine aminotransferase (ALT)
- Alkaline phosphatase (ALP)

Oxidative stress- Oxidative stress refers to a state where the generation of reactive oxygen species (ROS) surpasses the ability of the body's antioxidant defence mechanisms. In hyperlipidaemic Wistar rats, increased plasma lipid levels facilitate lipid peroxidation and the overproduction of free radicals, resulting in oxidative harm to tissues including the liver, heart, kidneys, and blood vessels [27].

Histopathology-

To evaluate tissue damage brought on by hyper lipidemia, especially in the liver, aorta, heart, a nd kidneys, histopathological examination is fr equently carried out.

Lipid buildup, oxidative stress, and inflammati on are all brought on by hyperlipidemia, which causes distinctive microscopic changes [28].

RESULTS

1. **Evaluation of Total Flavonoid and Phenolic Content**

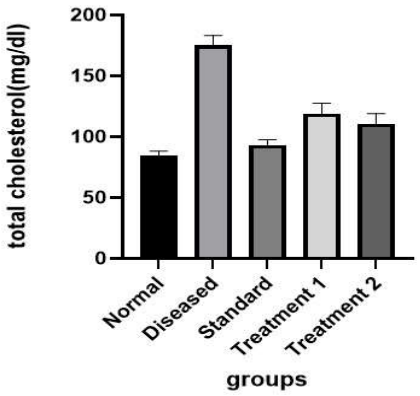
Its is very important to determine total phenolic content for the estimation of Anti-inflammatory & Anti-oxidant activity of the plant's active constituents. The total flavonoid and phenolic content were 42.6mg RE/g extract & 20.5GAE/g extract respectively.

2. **Physical evaluation** – during the protocol the body weight was measured in 5 days gap and it was observed that, body weight was increased before the treatment as they were given high cholesterol diet but after the treatment the weight get decrease and there were no significant changes in food and water intake.

3. Evaluation of Biochemical Parameters

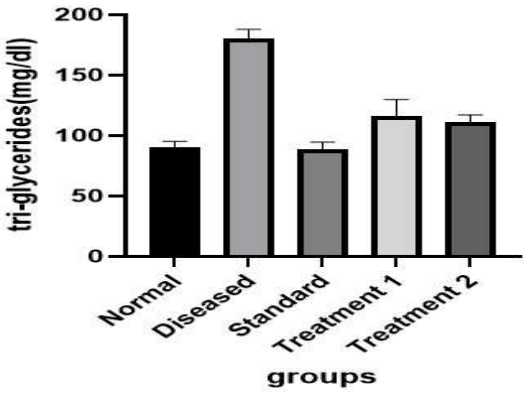
Evaluation of different parameters was done and the data is expressed as mean ± standard deviation (SD). Statistical comparisons between groups is performed using one-way ANOVA followed by Tukey's post hoc test. A p-value of <0.05 is considered statistically significant.

a. Total Cholesterol (TC)



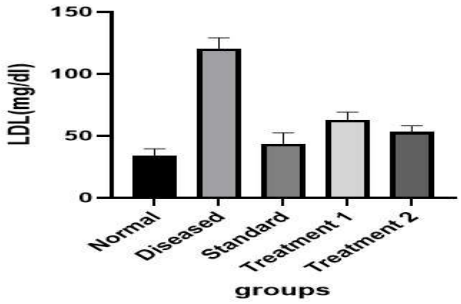
Graph a represents Total Cholesterol levels of each group...Normal group have normal range , Diseased group has increased level of TC, there is decrease of TC in standard group after treatment, In treatment group 1 there is slightly decrease in TC, but in the group 2 there is significant decrease in TC and is near about to the Standard drug.

b. Triglycerides (TG)



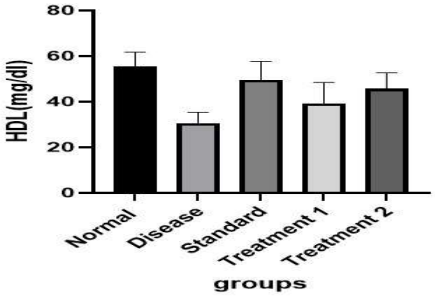
Graph b represents Triglycerides levels of each group...Normal group show TG level within the normal range, Diseased group show significant increase in TG level, there is decrease of TG in standard group after treatment, treatment group 1 there is slightly decrease in TG, but in the group 2 there is decrease in TG.

c. LDL-C



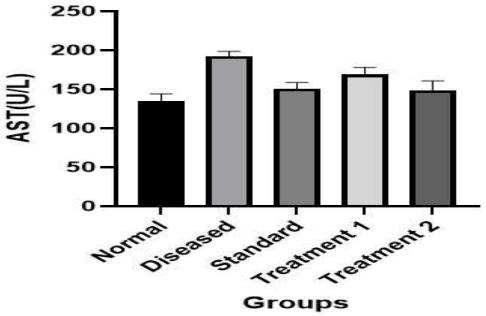
Graph c represents LDL-C level of each group...Normal group have normal range, Diseased group has increase in level of LDL-C there is reduction of LDL-C in standard group after treatment, in treatment group 1 there is decrease in LDL-C, but in the group 2 there is significant decrease in LDL-C.

d. HDL -C



Graph d represents HDL cholesterol levels of each group. The normal group shows higher HDL levels within the physiological range. The diseased group shows a significant decrease in HDL levels, indicating reduced protective cholesterol. The standard drug group shows an increase in HDL levels after treatment. In treatment group 1, there is a slight to moderate increase in HDL levels compared to the diseased group. In treatment group 2, there is a more significant increase in HDL levels, and the values are closer to the standard drug group,

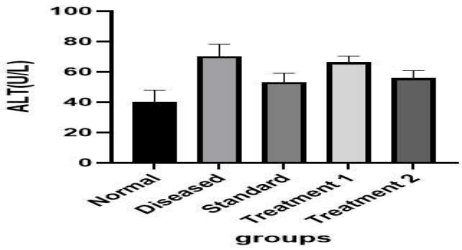
e. AST (SGOT)



Graph e represents AST levels of each group. The normal group shows

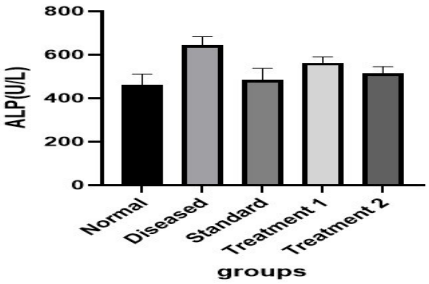
normal physiological range. The diseased group shows a significant increase. The standard drug group shows a decrease after treatment. In treatment group 1, there is a slight to moderate decrease compared to the diseased group. In treatment group 2, there is a more significant decrease and the values are closer to the standard drug group,

f.ALT (SGPT)



Graph f represents ALT levels of each group. The normal group shows normal range indicating healthy liver function. The diseased group shows a significant increase. The standard drug group shows a decrease after treatment. In treatment group 1, there is a slight to moderate decrease compared to the diseased group. In treatment group 2, there is a more significant decrease and the values are closer to the standard drug group,

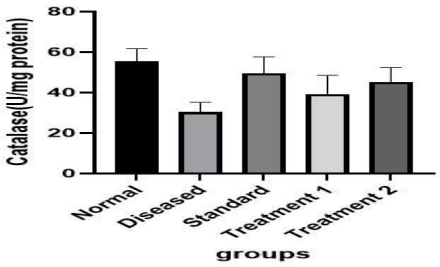
g. ALP



Graph g represents ALT levels of each group. The normal group shows normal range. The diseased group have increase. The standard drug group decrease after treatment. In treatment group 1, there is a slight to moderate decrease compared to the diseased group. In treatment group 2, there is a more significant decrease and the values are closer to the standard drug group,

4. Antioxidant Marker

a. Catalase



This graph represents Catalase assay of Liver. Normal group have value of Catalase in normal ranges, Diseased group have decreased Catalase due to Hyperlipidaemia, while Standard group, Treatment 1 and treatment 2 groups there is increase in Catalase activity.

5. Histopathology

Fig a: Normal Control Group

Fig b: Disease Group

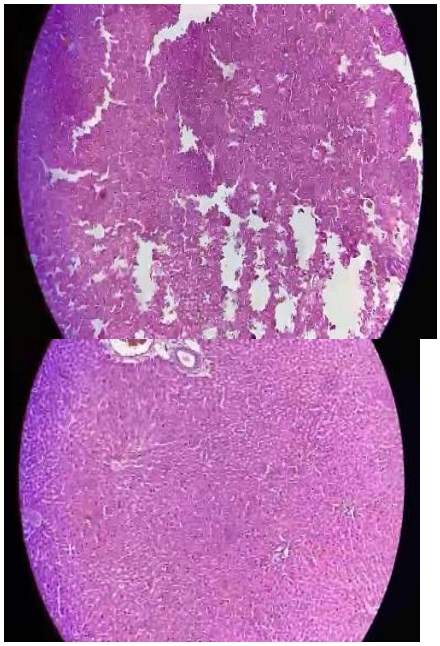


Fig c: Standard Group

Fig d: Treatment Group 1

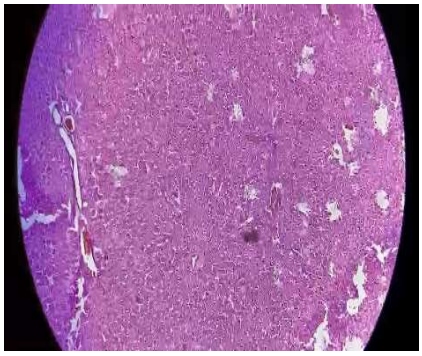
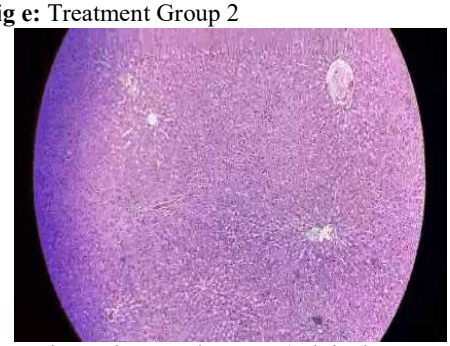


Fig e: Treatment Group 2



Figure, Photomicrograph (original magnification 10x) of histopathological studies of rat liver of different groups. The recovery order was like in the treatment group 2, then standard group, then treatment group 1. In disease group the liver cells condition was worst, accumulation of fat was there too much according to the other group because of the no treatment given. No changes were seen in the normal group.

DISCUSSION

This study was done on the topic "Anti hypercholesterolemic potential of *Melia azedarch Linn*. leaves extract in combination with Red Yeast Rice in a High Fat Diet induce hypercholesterolemia injury model" in which ethanolic extract of plant was used and the toxicity induction done by High Fat Diet in albino wistar rats.

The main goal was to know the cholesterol lowering effect of *Melia azedarch Linn*. leaves extract in combination with Red Yeast Rice in heavy fat diet. There was different conclusion from this study like: -

The study indicates that the combination of *Melia azedarach* Linn. leaf extract and red yeast rice exhibits significant anti-hypercholesterolemic activity in rats, as shown by reduced total cholesterol, LDL, and triglycerides, along with improved HDL levels. The effect is comparable to the standard drug rosuvastatin, suggesting strong lipid-lowering potential.

This activity may be due to the synergistic action of bioactive compounds, including monacolin K and plant phytoconstituents, which help regulate cholesterol synthesis and improve lipid metabolism. Overall, the combination shows promise as a natural alternative or adjunct therapy for hypercholesterolemia, though further studies are needed to confirm its safety and efficacy in humans.

FUTURE PERSPECTIVE

The promising Anti hypercholesterolemic effects observed with the combination of *Melia azedarach* Linn. leaf extract and red yeast rice highlight several important directions for future research. Detailed phytochemical investigations are needed to isolate and characterize the specific bioactive compounds responsible for the synergistic lipid-lowering activity. Understanding the precise molecular mechanisms, particularly their influence on cholesterol biosynthesis pathways and lipid metabolism, will further validate their therapeutic potential.

Long-term toxicity and safety studies should be conducted to ensure the absence of adverse effects with prolonged use. Additionally, dose optimization and pharmacokinetic studies are essential to establish effective and standardized formulations.

Future research should also focus on conducting well-designed clinical trials in humans to confirm efficacy and safety. The development of novel drug delivery systems or herbal formulations may enhance bioavailability and therapeutic outcomes.

Overall, this combination holds potential as a cost-effective, plant-based alternative or adjunct to conventional statin therapy, contributing to the development of safer and more accessible treatments for hypercholesterolemia.

Conflict of Interest: The authors affirm that the work presented in this paper was not influenced by any known conflicting financial interests or personal relationships.

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