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*Research Article*

# The Effect of Javanese Chili (*Piper retrofractum*) and Lemon (*Citrus limon*) Formula on Total Cholesterol Levels in Male White Rats

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## Abstract

Hypercholesterolemia, defined as blood cholesterol levels exceeding 200 mg/dL, is a significant risk factor for cardiovascular disease. Traditional Indonesian medicinal plants, such as Javanese chili (*Piper retrofractum*) and lemon (*Citrus limon*), have long been recognized for their potential lipid-lowering properties. This study aimed to scientifically evaluate the hypocholesterolemic effect of a combined *P. retrofractum* and *C. limon* extract in a hypercholesterolemic rat model. Thirty male Sprague-Dawley rats were randomly assigned to seven groups: a normal control, a negative control (Na-CMC), a positive control (simvastatin 0.018 mg/200 g BW), a scientific herbal formula (0.18 g/200 g BW), and three treatment groups receiving *P. retrofractum* and *C. limon* in ratios of 1:1, 1:2, and 2:1. Hypercholesterolemia was induced using a cholesterol-cholate-thiouracil (CCT) mixture alongside a high-fat diet. Total cholesterol levels were quantified using the Cholesterol Oxidase-Peroxidase Aminoantipyrine (CHOD-PAP) method. After 21 days of treatment, all *P. retrofractum* and *C. limon*-treated groups demonstrated a statistically significant reduction in total cholesterol compared to the negative control ($p < 0.05$). Notably, the 1:1 and 1:2 ratios of *P. retrofractum* to *C. limon* yielded the most substantial cholesterol reductions (44% and 43%, respectively), comparable to the scientific herbal formula and superior to simvastatin (30%). In contrast, the 2:1 ratio showed a comparatively lower reduction (38%), suggesting a potential inhibitory effect of excessive *P. retrofractum* on *C. limon*'s lipid-lowering properties. These findings collectively highlight the promising potential of optimized *P. retrofractum* and *C. limon* combinations, particularly at 1:1 and 1:2 ratios, as effective natural interventions for managing hypercholesterolemia.

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## INTRODUCTION

Hypercholesterolemia, defined as blood cholesterol levels exceeding 200 mg/dL, is a significant public health concern. In Indonesia, the prevalence of this condition affects 21.2% of individuals over the age of 15, with urban populations experiencing a slightly higher rate of 22.1% due to increasingly consumptive lifestyles and the rising popularity of high-cholesterol fast food. Elevated cholesterol levels are a primary risk factor for the development of coronary heart disease[1,2]. While standard pharmacological treatments, such as statins, effectively inhibit cholesterol biosynthesis[3], there is growing interest in natural alternatives. The Center for Research and Development of Medicinal Plants and Traditional Medicines (B2P2TOOT) has contributed to this trend by developing scientific herbal medicines that patients are increasingly using to manage cholesterol levels[4].

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Javanese chili (*Piper retrofractum*) is a plant known for its diverse pharmacological activities, including antihyperlipidemic, antioxidant, and anti-obesity properties. Studies have identified several bioactive compounds in *P. retrofractum*, such as piperine, palmitic acid, tetrahydropiperic acid, alkaloids, triterpenoids, and glycosides[5]. Similarly, lemon (*Citrus limon*) has been widely utilized for its beneficial biological effects, including anti-obesity, antioxidant, and anti-inflammatory activities, with a proven impact on the cardiovascular system[6].

Previous studies support the cholesterol-lowering potential of these plants individually. Pratiwi *et al*.[7] demonstrated that a 95% ethanol extract of *P. retrofractum* reduced total cholesterol and triglyceride levels in high-fat diet-fed animals. Similarly, Rana *et al*.[8] observed that a combination of *C. limon* and coriander significantly lowered total cholesterol in rats. The therapeutic synergy of polyherbal formulations is well-documented, often leading to enhanced effectiveness compared to single-herb treatments. Building on this principle and the individual benefits of each plant, this study aims to investigate the potential synergistic antihypercholesterolemic effects of a combined *P. retrofractum* and *C. limon* extract therapy in a white rat model.

## MATERIALS AND METHODS

### *Materials*

This study utilized a range of materials, including *P. retrofractum* fruits, *C. limon* fruits, and distilled water. The primary chemical reagents and drugs included simvastatin (Nova®), a high-cholesterol feed, and a cholesterol-cholate-thiouracil (CCT) induction mixture consisting of cholic acid (Tokyo Chemical Industry Co., Ltd), pure cholesterol (Nippon Fine Chemical Co., Ltd), and propylthiouracil (PTU; OGB Dexa®). For cholesterol analysis, a commercial cholesterol reagent (Glory®) was used. In addition, the Scientific Herbal formula (B2P2TOOT) was employed. Male Wistar rats served as the animal model for the *in vivo* experiments. For phytochemical screening, the following reagents were used: hot water, $FeCl_3$, concentrated HCl, 10% NaCl, Dragendorff reagent, Liebermann-Burchard reagent, and Mayer's reagent. The tools and equipment employed in this research included various laboratory instruments to facilitate sample preparation, analysis, and animal care. These included alcohol swabs, a brown glass bottle, a porcelain cup, Eppendorf tubes, a freeze-dryer, animal cages, a Kurs apparatus, a microplate reader (Azure Ao Absorbance), an oven, blood tubes, a thermometer, an analytical balance, and an animal scale.

### *Methods*

*Preparation of simplicia*

One kilogram of *P. retrofractum* fruit was collected from the Indonesian Center for Industrial Crops and Biotechnology Research (Balittro), Bogor, Indonesia. The plant was authenticated by the Herbarium Bogoriense, National Research and Innovation Agency (BRIN), Cibinong, West Java, and a voucher specimen number B-871/II.6.2/IR.01.02/5/2023 was deposited. The fruit was cleaned under running water to remove any dirt or debris. To facilitate the drying process, the fruits were cut into smaller pieces before being pulverized with a blender[9].

Two kilograms of *C. limon* fruit were also obtained from Balittro, Bogor, and authenticated by the Herbarium Bogoriense, BRIN, Cibinong, West Java, with a voucher specimen number B-872/II.6.2/IR.01.02/5/2023. The fruits were wet-sorted, rinsed with running water, and cut into two halves. The fruits were then juiced, and the resulting mass was freeze-dried at -48°C for three days to produce a powder[10].

*Piper retrofractum* and *C. limon* powders was subjected to a maceration and boiling extraction method. The powder was wrapped in a batiste cloth and boiled in 100 mL of distilled water (1:10 w/v) for 15 minutes at 90°C with intermittent stirring. The resulting extract was then filtered and subsequently freeze-dried at -48°C for three days to yield a fine powder.

*Phytochemical screening*

*Flavonoids*: The presence of flavonoids was determined using Mg powder and HCl test, a common method for the qualitative identification of these compounds. This test relies on the reduction of the flavonoid ring structure. Specifically, the addition of Mg powder forms bonds with the carbonyl groups within the flavonoid backbone. Subsequently, the addition of HCl facilitates the formation of colored flavylium salts. A positive result is indicated by a distinct color change to an orange-red hue, which confirms the presence of flavonoid compounds in the sample[11].

*Alkaloids*: The presence of alkaloids was qualitatively determined using established precipitation tests. A 0.5 g portion of the dried sample was accurately weighed and subjected to sequential testing with three distinct alkaloid-detecting reagents. The Dragendorff reagent was utilized, with the formation of an orange-yellow precipitate indicating a positive result. Similarly, the Mayer's reagent was employed, and a positive reaction was confirmed by the appearance of a white precipitate. Finally, the Bouchardat reagent was added, with the formation of a blackish-brown precipitate signifying the presence of alkaloids[12].

*Tannins*: The sample was placed in a test tube, and 10 mL of hot distilled water was added. The mixture was shaken vigorously to ensure proper extraction. Subsequently, 20 mL of a 10% NaCl solution was added, and the solution was filtered. The resulting filtrate was then treated with a few drops of 1% $FeCl_3$ solution. A positive result, indicating the presence of tannin compounds, was identified by the immediate formation of a dark blue or black color upon the addition of the $FeCl_3$ solution[13].

*Steroids and triterpenoids*: The presence of steroids and triterpenoids in the sample was determined using the Liebermann-Burchard test[14]. Briefly, a small portion of the sample was dissolved in chloroform. Subsequently, the Liebermann-Burchard reagent was carefully added to the solution. The development of a brownish-red color was interpreted as a positive indication for the presence of steroids, whereas a brown-purple color was considered indicative of triterpenoids.

*Saponins*: For the detection of saponins, a sample of 0.5 g was mixed with 10 mL of hot distilled water in a test tube. The mixture was then shaken vigorously for 10 minutes, and the formation of a stable foam was observed. The presence of saponins was confirmed if the foam persisted after the addition of a few drops of 2 N HCl[15].

*Preparation of scientific herbal formula*

The high-cholesterol herbal mixture was prepared following the protocol established by B2P2TOOT[16]. The composition of the mixture included a precise combination of seven different plant materials: 1 g of Chinese teak (*Tectona grandis*) leaves, 6 g of Dutch teak (*Guazuma ulmifolia*) leaves, 6 g of tempuyung (*Sonchus arvensis*) herbs, 5 g of green tea (*Camellia sinensis*) herbs, 5 g of temulawak (*Curcuma xanthorrhiza*) rhizomes, 4 g of turmeric (*Curcuma longa*) rhizomes, and 3 g of meniran (*Phyllanthus niruri*) herbs. All herbs were meticulously combined and then boiled with 1 liter of deionized water in a covered stainless-steel pot. The mixture was simmered over low heat for 15 minutes to ensure proper extraction of bioactive compounds. After boiling, the mixture was allowed to cool to room temperature before being filtered using a non-metallic filter to remove all solid residues. The resulting liquid extract was subsequently freeze-dried to obtain a concentrated powder for the study.

*Preparation of high-fat diet*

To prepare the high-fat diet, a specific formulation was created to induce hyperlipidemia in the animal model. The diet consisted of 5% wheat flour, 5% cooking oil, 10% margarine, 10% beef tallow, 15% egg yolk, 25% commercial feed (Pokphan 551), and 30% white rice[17]. All ingredients were thoroughly mixed to form a homogenous dough. This mixture was then shaped into pellets or blocks, which were subsequently dried in an oven to a consistent texture and moisture content, ensuring stable composition and palatability throughout the study period.

*Preparation of cholesterol-cholelate-thiourase inductor*

The atherogenic diet was prepared by incorporating a specific lipid-rich mixture into the standard rodent chow to induce hypercholesterolemia. This custom diet, used as a control CCT inductor, was formulated by mixing pure cholesterol at a concentration of 200 mg/kgBW and PTU at 12.5 mg/kgBW. Additionally, 0.1% cholic acid was added, based on the assumption of an average daily feed intake of 15 g per 200 gBW of rat[18].

*Preparation of animals*

Male Sprague Dawley rats, aged 2-3 months and weighing 200-250 g, were used for all experiments. A total of 35 animals were randomly allocated into seven distinct treatment groups, as detailed in **Table I**. All animal procedures were conducted in strict accordance with ethical guidelines for the care and use of laboratory animals. This study protocol, with a commitment to minimizing animal distress and ensuring their well-being, was officially approved by the Ethics Committee for Research and Animal Experimentation at Universitas Pakuan under protocol number 30/KEPHP-UNPAK/10-2023.

To establish a sustained hypercholesterolemic state, all groups except the standard control received a high-fat diet (15 g/rat/day) alongside a CCT mixture (1 mL/200 gBW). This induction regimen was maintained for 28 consecutive days.

Following this period, test treatments were administered daily for an additional 21 days. The high-fat diet and CCT mixture were continued concurrently with the test treatments to ensure the hypercholesterolemic condition persisted throughout the entire 21-day therapeutic evaluation phase.

**Table I.** Treatment groups.

| Groups | Samples |
|---|---|
| Normal control (Na-CMC vehicle) | Na-CMC vehicle |
| Negative (Induction + Na-CMC vehicle) | Induction – Na-CMC vehicle |
| Positive (Simvastatin 0.018 mg/200 gBW) | Induction – simvastatin 0.018 mg/200 gBW |
| Dose 1 *Piper retrofractum* : *Citrus limon* (1:1) | Induction – *Piper retrofractum* 8 mg/200 gBW – *Citrus limon* 14 mg/200 gBW |
| Dose 2 *Piper retrofractum* : *Citrus limon* (1:2) | Induction – *Piper retrofractum* 8 mg/200 gBW – *Citrus limon* 28 mg/200 gBW |
| Dose 3 *Piper retrofractum* : *Citrus limon* (2:1) | Induction – *Piper retrofractum* 16 mg/200 gBW – *Citrus limon* 14 mg/200 gBW |
| Scientific Herbal Formula (B2P2TOOT) | Induction – references herbal medicine 0.18 g/200 gBW |

*Total cholesterol level measurement*

Total cholesterol levels were quantified using the Cholesterol Oxidase Peroxidase Aminoantypirin (CHOD-PAP) method as previously described[18,19]. This enzymatic colorimetric assay operates on the principle of a multi-step reaction. First, cholesterol esters are hydrolyzed by cholesterol esterase, and the resulting free cholesterol is then oxidized by cholesterol oxidase. This oxidation produces hydrogen peroxide, which subsequently reacts with 4-aminoantipyrin and phenol in the presence of peroxidase to form a colored quinone imine dye. The intensity of this colored product is directly proportional to the total cholesterol concentration and is measured spectrophotometrically.

For the assay, blood samples (0.5 mL) were collected from the tail vein of each subject following an 18-hour fasting period. The blood was centrifuged at 3000 rpm for 15 minutes to obtain serum. A 5 µL aliquot of the resulting serum was mixed with 1000 µL of a commercial cholesterol reagent. The mixture was then incubated at room temperature (20-25°C) for 10 minutes, and the absorbance was measured at 500 nm using a microplate reader. Total cholesterol measurements were performed at critical time points: on day 0 (before induction), day 28 (during the induction phase), and on days 35 and 49 (after 21 days of oral treatment). This structured approach allowed for a comprehensive evaluation of cholesterol levels throughout the experimental period.

*Macroscopic observation of organs*

To assess the overall morphological changes induced by the treatment, a comprehensive macroscopic examination of the vital organs was performed. Immediately following euthanasia, the heart, liver, and kidneys were carefully excised and visually inspected. Key parameters, including the organ's color, texture, and weight, were meticulously recorded.

***Data analysis***

Data were analyzed using ANOVA to assess overall differences between the experimental groups. To evaluate the effect of alloxan-induced diabetes, a paired samples T-test was used to compare the data from before and after induction in each group. When a significant difference was identified by the ANOVA, LSD post-hoc test was employed to pinpoint the specific differences in the effects between the various treatment groups. All statistical analyses were conducted using a significance level of $p < 0.05$.

## RESULTS AND DISCUSSION

Phytochemical screening of the plant extracts revealed a diverse array of bioactive compounds. The ethanolic extract of *P. retrofractum* was found to contain flavonoids, alkaloids, tannins, saponins, and steroids. A similar analysis of the *C. limon* extract also confirmed the presence of several key phytochemical classes, including flavonoids, alkaloids, saponins, steroids, and triterpenoids. The presence of these compounds is noteworthy as they are well-documented for their various pharmacological properties, which may contribute to the observed biological activity of the extracts. For instance, flavonoids and tannins are known for their antioxidant and anti-inflammatory effects, while alkaloids often possess potent antimicrobial or cytotoxic properties. A comprehensive overview of these findings, including the specific results for each plant extract, is detailed in **Table II**.

**Table II.** Phytochemical screening results.

| Phytochemicals | *Piper retrofractum* | *Citrus limon* |
|---|---|---|
| Flavonoids | + | + |
| Alkaloids: | | |
| Dragendorff | - | + |
| Mayer's | + | + |
| Bouchardat | - | + |
| Tannins | + | - |
| Saponins | + | + |
| Steroids | + | + |
| Triterpenoids | - | + |

Note: "+" = detected; "–" = not detected

The hypercholesterolemia model was successfully established in the experimental animals. As detailed in **Table III**, total cholesterol levels in the induced groups showed a significant increase ($p < 0.05$), ranging from 32.01% to 61.33% post-induction. This represents a more than two-fold elevation compared to the normal control group, which exhibited only a 12.97% rise. These findings confirm that the administration of a high-fat diet (15 g/rat/day) in conjunction with the CCT mixture (1 mL/200 gBW) for 28 consecutive days effectively induced a state of hypercholesterolemia.

These results are consistent with previous studies on hypercholesterolemia induction. For instance, Kurniati *et al*.[18] reported that a similar CCT induction vehicle, consisting of PTU (12.5 mg/kgBW), pure cholesterol (200 mg/kgBW), and cholic acid (0.1%), led to a gradual increase in total cholesterol levels, reaching 190 mg/dL after 2.5 months and up to 390 mg/dL after five months. Their study also demonstrated a successful induction by observing a significant decrease in HDL levels from 16.17 mg/dL in the normal group to 7.90 mg/dL. The alignment of our findings with such established methods validates the effectiveness of our induction protocol in creating a reliable animal model for studying hypercholesterolemia.

**Table III.** Average results of total cholesterol levels during induction.

| Groups | Average total cholesterol level ± SD | | Percentage increase (%) |
|---|---|---|---|
| | Before induction | After induction | |
| Normal control (Na-CMC vehicle) | 106.04 ± 19.1 | 119.79 ± 42.34 | 12.97 |
| Negative (Induction + Na-CMC vehicle) | 147.65 ± 34.3 | 199.84 ± 112.59 | 35.35 |
| Positive (Simvastatin 0.018 mg/200 gBW) | 141.43 ± 58.6 | 194.42 ± 101.02 | 37.46 |
| Dose 1 *Piper retrofractum* : *Citrus limon* (1:1) | 140.07 ± 15.13 | 185.38 ± 9.38 | 32.35 |
| Dose 2 *Piper retrofractum* : *Citrus limon* (1:2) | 143.5 ± 26.56 | 189.44 ± 64.79 | 32.01 |
| Dose 3 *Piper retrofractum* : *Citrus limon* (2:1) | 137.438 ± 36.53 | 202.3 ± 62.02 | 47.2 |
| Scientific Herbal Formula (B2P2TOOT) | 121.586 ± 7.44 | 196.16 ± 91.29 | 61.33 |

The study commenced on Day 29, following a period of induction, with treatments administered daily until Day 49. Cholesterol levels were measured on Day 35 and again on Day 49 to assess the dynamic effects of the interventions. A total of 35 male Wistar rats were divided into seven distinct groups, each comprising five animals. All groups, with the exception of the normal control, were subjected to induction with a high-fat diet. The normal group served as a crucial baseline for comparison, receiving only a Na-CMC to establish the physiological norm and validate the induction success. In contrast, the negative control group received the high-fat diet and the Na-CMC vehicle, representing the untreated hypercholesterolemic state. The positive control group was treated with simvastatin (20 mg/kg), a well-established standard for cholesterol reduction. The remaining three groups received a combination of *P. retrofractum* (40 mg/kg) and *C. limon* (70 mg/kg) in varying ratios (1:1, 1:2, and 2:1), while a separate group received a standardized scientific herbal formula (10 g/kg). Each animal received a consistent daily dose of 1 mL of its respective treatment suspension.

Dynamic changes in mean total cholesterol levels (mg/dL) across all treatment groups on Day 35 and Day 49 are presented in **Table IV**. On Day 35, the negative control (169.69 ± 20.04 mg/dL), *P. retrofractum* and *C. limon* dose 1 (1:1) (189.60 ± 12.89 mg/dL), and the scientific herbal formula (174.48 ± 27.08 mg/dL) all exhibited cholesterol levels that were statistically similar to the untreated hypercholesterolemic group, indicating a lack of significant cholesterol-lowering effect at this early stage ($p > 0.05$). This observation is not uncommon with natural products, as their therapeutic effects often require a longer duration for compound accumulation and subsequent action. Conversely, the simvastatin-treated positive control group demonstrated a significant reduction ($p < 0.05$), with cholesterol levels dropping to 99.97 ± 19.86 mg/dL, a value comparable to the normal control group (111.55 ± 15.99 mg/dL).

**Table IV.** Average total cholesterol levels after treatment.

| Groups | Average total cholesterol level ± SD | |
|---|---|---|
| | Day 35 | Day 49 |
| Normal control (Na-CMC vehicle) | 111.55 ± 15.99$^{abc}$ | 103.08 ± 4.92$^{a}$ |
| Negative (Induction + Na-CMC vehicle) | 169.69 ± 20.04$^{d}$ | 155.08 ± 36.58$^{cd}$ |
| Positive (Simvastatin 0.018 mg/200 gBW) | 99.97 ± 19.86$^{a}$ | 84.15 ± 23.78$^{a}$ |
| Dose 1 *Piper retrofractum* : *Citrus limon* (1:1) | 189.6 ± 12.89$^{d}$ | 107.37 ± 16.27$^{ab}$ |
| Dose 2 *Piper retrofractum* : *Citrus limon* (1:2) | 147.96 ± 45.1$^{bcd}$ | 81.01 ± 22.26$^{a}$ |
| Dose 3 *Piper retrofractum* : *Citrus limon* (2:1) | 153.93 ± 36.12$^{cd}$ | 98.06 ± 22.37$^{a}$ |
| Scientific Herbal Formula (B2P2TOOT) | 174.48 ± 27.08$^{d}$ | 96.78 ± 8.51$^{a}$ |

Note: Numbers followed by the same superscript letter in both rows and columns have the same effect.

A remarkable shift in efficacy was noted by Day 49. At this point, all treatment groups—including the simvastatin-positive control, the three *P. retrofractum* and *C. limon* groups (doses 1, 2, and 3), and the scientific herbal formula group—showed a significant and sustained reduction in cholesterol levels, with values ranging from 81.01 to 107.3 mg/dL. These values were statistically different from the negative control ($p < 0.05$) and were comparable to the levels observed in the normal control group. These findings conclusively demonstrate that all tested interventions were effective in reducing hypercholesterolemia to within a normal physiological range. The percentage reduction in cholesterol levels across all groups is visually represented in **Figure 1**, further supporting the efficacy of the herbal interventions.

**Figure 1** visually represents the percentage of total cholesterol reduction across the various treatment groups. The results show that the combination of *P. retrofractum* and *C. limon* yielded the most significant cholesterol reductions, particularly at a 1:1 and a 1:2 ratio. These formulations led to substantial decreases of 44% and 43%, respectively, which are comparable to the 43% reduction observed in the scientific herbal formula group and notably surpass the 30% reduction achieved with simvastatin. In contrast, the negative control group demonstrated only a minimal 7% decrease, underscoring the severity of sustained hypercholesterolemia without intervention. As anticipated, the normal control group maintained stable cholesterol levels with a minimal 2% reduction.

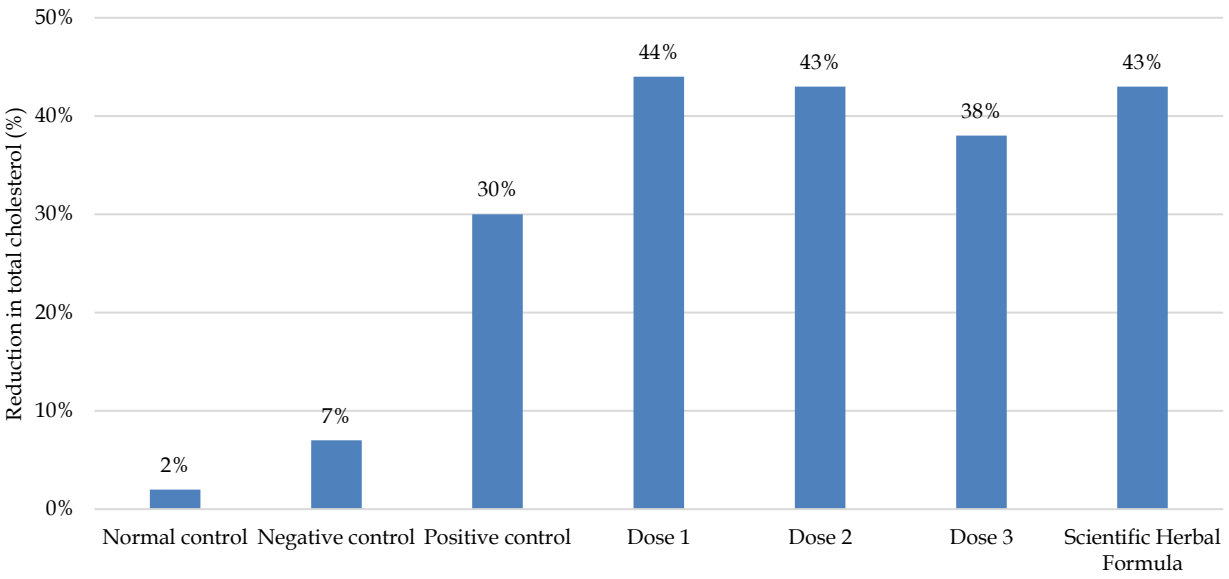


**Figure 1.** Total cholesterol level reduction.

The superior efficacy of the 1:1 and 1:2 ratios compared to the 2:1 ratio suggests that the therapeutic outcome is highly dependent on the precise proportion of each component. This observation supports the hypothesis that a synergistic interaction between the bioactive compounds of *P. retrofractum* and *C. limon* is at play. It appears that a higher proportion of *P. retrofractum*, as seen in the 2:1 ratio, may have a masking or antagonistic effect on the lipid-lowering properties of *C. limon*, thereby diminishing the overall therapeutic benefit[20]. These findings are consistent with established principles of multi-herbal formulation, which emphasize that meticulous optimization of ingredient ratios is crucial for maximizing therapeutic efficacy[21].

The results align with previous research on similar herbal interventions. A study by Zulkarnain *et al*.[22] demonstrated that a scientific herbal medicine administered to patients with mild hypercholesterolemia for 28 days showed no adverse effects on kidney, liver, or blood function. In another rat study, this herbal medicine exhibited a synergistic mechanism of action to suppress appetite, shorten intestinal food transit time, inhibit pancreatic lipase, and increase body metabolism[23]. It also acts by inhibiting key enzymes in fat cell formation, such as acetyl CoA carboxylase and HMG-CoA reductase, while simultaneously increasing fat elimination via bile acids[24]. Furthermore, it enhances the expression of cholesterol 7α-hydroxylase, heme oxygenase 1, and low-density lipoprotein (LDL) receptors, and activates the enzyme lecithin-cholesterol acyltransferase (LCAT)[25]. While generally safe, some minor side effects were reported, including increased frequency of bowel movements and heartburn[26].

## CONCLUSION

This study demonstrated that a combined formulation of *P. retrofractum* and *C. limon* possesses significant cholesterol-lowering properties. The results indicate that specific ratios, particularly the 1:1 and 1:2 combinations, are most effective. The therapeutic efficacy of these formulations is comparable to that of conventional pharmaceutical agents, suggesting their potential as a viable complementary therapy for managing hypercholesterolemia. Future development of herbal medicines should be guided by a comprehensive understanding of pharmacodynamics to maximize their clinical potential and ensure optimal patient outcomes.

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## AUTHORS' CONTRIBUTION

**Conceptualization**: Lusi Agus Setiani, Nina Herlina
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## DATA AVAILABILITY

None.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this study.

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