

In Vivo Evaluation of *Pithecellobium dulce* Leaves Anti-bacterial and Antihyperlipidaemic Activities

Raphael Dzinyela (✉ dzinyelar@gmail.com)

Nanjing Forestry University <https://orcid.org/0000-0003-4869-6337>

Abdul Razak Alhassan

Nanjing Forestry University

Delight Hwarari

Nanjing Forestry University

Vincent Kuetsidzo

University for Development Studies

Abdul-Nasir Abdul-Baasit

Mugla Sitki Kocman University: Mugla Sitki Kocman Universitesi

Kwadwo Nketia Opoku

Nanjing Forestry University

Peter Suglo

Nanjing Forestry University

Emmanuel Kwesi Asomaning

University for Development Studies

Ali Movahedi

Nanjing Forestry University

Research Article

Keywords: Anti-bacterial, Antihyperlipidaemic, *Pithecellobium dulce*, *Salmonella*, *Escherichia coli*

Posted Date: October 17th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-2164511/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

The natural origin and reduced side effects of medicinal plants have recently enhanced their popularity in both developed and developing nations. *Pithecellobium dulce* (*P. dulce*), an evergreen blooming spiky plant belonging to the *Fabaceae* family, with a number of therapeutic uses. *P. dulce* has medicinal qualities, such as antidiabetic, abortifacient, locomotor, anticonvulsant, antiulcer, and antioxidative ones. Unknown is *P. dulce's* ability to treat rats with hyperlipidaemia brought on by dexamethasone. In this article, we demonstrate how ethanolic leaf extract contains phytonutrients, has antibacterial action against *Salmonella* species, and has antihyperlipidemic activity against rats that have been given dexamethasone to cause hyperlipidaemia. Leaf extracts from *P. dulce* contain phytonutrients like phenols, flavonoids, and phytosterols. With increasing doses of the extract combined with the usual reference medicine atorvastatin, the levels of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol were significantly decreased (VLDL-C). When compared to the antihyperlipidemic control, the extract was found to have dramatically enhanced HDL-C (70.78%), significantly decreased TC (47.03%), TG (47.51%), LDL-C (41.69%), and VLDL-C (48.00%). In contrast to *Escherichia coli* ATCC25922, which had the lowest inhibition zones, the 50mg/ml extract on *Salmonella* ATCC14028 had the largest inhibition zone on all examined organisms. *P. dulce* has demonstrated phytonutrients, antibacterial effectiveness, and antihyperlipidemic action in ethanolic leaf extract. Our research thus supports the use of *P. dulce* as a potential candidate in the search for naturally occurring antibacterial and antihyperlipidemic chemicals.

Introduction

A condition known as hyperlipidaemia, which is marked by a significant increase in any one or more of the serum lipids (total cholesterol, LDL-C, very low-density lipoprotein cholesterol, VLDL-C, and triglycerides, TG), may develop as a result of an excessive intake of aberrant forms of lipids (Dzinyela et al. 2021). Hyperlipidaemia is a contributor to diabetes mellitus type II and cardiovascular disease. All racial and ethnic groups' most common causes of heart disease and death have been linked to hyperlipidemia and atherosclerosis (Kumar et al. 2014; Dzinyela et al. 2021). Hyperlipidaemia, on the other hand, is brought on by high blood lipid levels. Hyperlipidaemia can be divided into two types, acquired or secondary hyperlipidaemia, which develops from other disorders including diabetes, persistent drinking, use of oral contraceptives, beta-blockers, diuretics, etc., and familial or primary hyperlipidaemia, which results from genetic changes (Kumar et al. 2014). According to Ochani and D'Mello (Ochani and D'Mello 2009), changes in the serum lipid and subsequently lipoprotein profile lead to hyperlipidaemia because of elevated levels of TC, LDL-C, VLDL-C, and TG, which are accompanied by a concurrent decline in high-density lipoprotein-cholesterol (HDL-C) in the blood circulation. The generation of oxygen free radicals by polymorph nuclear leukocytes and monocytes is indirectly stimulated by hyperlipidemia. It is one of the top five global causes of death (Oriakhi and Uadia 2020). There are several efficient antihyperlipidemic synthetic medications, but none of them works for all types of hyperlipidemic illnesses, and they are all linked to side effects. For this, it is necessary to employ organic remedies made

from medicinal plants, which have a wealth of phytochemical components that have long served as the primary source of therapeutic agents (Oriakhi and Uadia 2020). Herbal remedies are superior to synthetic pharmaceuticals in terms of efficiency, safety, price, accessibility in local markets, and improved patient tolerance (Sushma et al. 2013).

Pithecellobium dulce (*P. dulce*) is a perennial flowering spiny tree in the *Fabaceae* plant family. It is frequently referred to as "Manila tamarind" and can reach a height of 18 to 20 m. It is mainly grown on Indian plains and found in Mexico and America (Preethi and Saral 2014). In Latin, the species name is "*dulce*," which also means delicious in reference to the eatable component of the pod. In Greek, the generic name is "*Pithekos*," which means an ape, and "*lobos*," which means a pod (Nadkarni 1996). One of the species in this genus belongs to the "*Mimosoideae*" subfamily (Trease and Evans 1989). According to reports, *P. dulce* has medicinal qualities such as antidiabetic, abortifacient, locomotor, free radical scavenging, protease inhibitor, anti-inflammatory, anticonvulsant, antiulcer, antivenom, and oxidative (Kulkarni and Jamakhandi 2018; Megala and Geetha 2010; Selvakumar et al. 2019; Srinivas, Geeta, and Shashikumar 2018).

Tannins and oils can be found in *P. dulce*. The leaves produce quercetin, kaempferol, dulcitol, and afzelins, while the seeds generate steroids, saponins, lipids, phospholipids, glycosides, and glycolipids (Preethi and Saral 2014; Shweta, Mehta, and MP 2013). In this investigation, ethanolic extracts of *P. dulce* were used to test their ability to reduce serum cholesterol levels in animal models. The phytochemical components of the *P. dulce* leaf extract was evaluated, the anti-bacterial potential was confirmed, the anti-hyperlipidaemic effect of the extract was compared to that of standard synthetic drugs, and hypolipidemic rats' body weight and lipid profile were compared before and after *P. dulce* leaf extract administration.

Materials And Methods

Equipment and Apparatus used:

Volumetric flasks, syringes, round-bottomed flasks, heparinized test tubes, mortar and pestle, filter paper, gauze, beaker, measuring cylinder, stirrer, funnel, OHAUS electronic balance, EYELA pre-freezer and rotary evaporator, NORD refrigerator, Heto power dry freeze dryer, ROYAL- MEK UK centrifuge, Uri semi-automated chemistry analyzer (URIT-810 chemical analyzer).

Reagents and Chemicals used:

Acetic anhydride, bench ammonium solution, distilled water, dexamethasone sodium phosphate, ferric chloride, magnesium ribbon, hydrochloric acid, sulphuric acid, Fehling's solution A and B, chloroform, Chem lab absolute ethanol 100 %, Lipitor atorvastatin calcium tablets, and acetone. All reagents were of laboratory grade.

Animals used:

Adults Wistar rats weighing between 170g and 260g were employed in the investigation. The rats were kept in typical lab lighting, temperature, and humidity settings while being acclimated to the environment. Standard pellets, rat food, and water were provided to the animals as food ad libitum during the trial.

The Centre for Plant Medicine Research's Institutional Ethics Committee for Animal Research gave the study its prior permission. The animals' housing, treatment, and management complied with the standards set forth by the Committee for the Purpose of Control and Supervision Experiments in Animals.

Sampling of Plant material and Identification

In Ghana's Ashanti area, near Ahinsan- Kumasi, fresh *P. dulce* leaves were gathered. The Centre for Plant Medicine Research verified the authenticity of the leaves (CPMR). The leaves were sorted to eliminate damaged leaves and debris, carefully washed under running water, and then allowed to air dry for 72 hours at room temperature (30°C–31°C) with a relative humidity of 20%–30%. Using a Tai Cheung stainless grain mill, the dried leaves and grass were ground into a fine powder for two minutes before being collected in a sterilized airtight container and kept chilled at 5°C.

Preparation of Leaf extract for phytochemical and anti-hyperlipidaemic analysis

P. dulce leaves were collected, cleaned, and dried in the shade. The dry sample was ground into fine particles and sieved. A cold maceration method utilizing 70% ethanol and 30% distilled water was used to extract about 300g of the powdered material. After 72 hours, the sample was filtered, and a rotary evaporator was used to concentrate the hydroethanolic extract. Using the Heto power dry freeze drier, the concentrated sample was then pre-frozen and freeze-dried to produce a solid sample (Sikarwar and Patil 2012).

Qualitative Phytochemical Analysis

Through qualitative analysis, the phytochemicals present in the ethanolic leaf extract of *P. dulce* were identified. Saponins, reducing sugars, phenolics, polyuronides, cyanogenic glycosides, alkaloids, anthracynosides, flavonoids, triterpenes, and phytosterol are among the phytochemicals that are evaluated.

2.6.1. Test for Saponins

A test tube containing 2ml of *P. dulce* leaf extract was filled with roughly 5ml of distilled water. The mixture was well shaken before being watched for 15 minutes. Saponins produced froth (foam), which was a promising sign.

2.6.2. Test for Reducing Sugars

Fehling's solutions A and B, which had just been made, were added to the extract and heated for 15 minutes. Reducing sugars were found when a brick-red precipitate was formed.

2.6.3. Test for Phenolics

The extract has ferric chloride (FeCl_3) added to it. The presence of phenolics was indicated by blue-black or dark green colouring.

2.6.4. Test for Polyuronides

The extract was mixed with acetone. The development of precipitate that adhered to the test tube walls indicated the existence of polyuronides.

2.6.5. Test for Cyanogenic Glycoside

The extract was heated with chloroform added, and the vapour was then released onto picric paper. Picric paper's pink hue indicated the presence of cyanogenic glycoside.

2.6.6. Test for Alkaloids

The material in the separatory funnel was added chloroform after the extract had been basified with NH_4OH . Mayer's reagent was then added once a fine, agitated chloroform layer had been formed and liquefied with 2N HCl. Alkaloids were identified when a precipitate turned yellow or milky white.

2.6.7. Flavonoids

Ethyl ether extract evaporated to dryness with methanol (divided into two for control, add magnesium ribbon followed by concentrated HCl). The formation of orange or red coloration revealed the presence of flavonoids.

2.6.7. Anthracynosides

Ethyl ether extract was treated with ammonium solution (Borntrager's test). Anthracynosides were present as evidenced by the development of red coloring.

2.6.8. Triterpenes

Extract of ethyl ether was dried by evaporation, then chloroform was added (divided into two for control). The addition of concentrated H_2SO_4 was followed by the addition of acetic anhydride. Triterpenes might be seen as violet or brownish-red rings that formed.

2.6.9. Phytosterols

Extract of ethyl ether was dried by evaporation, then chloroform was added (divided into two for control). The addition of acetic anhydride was followed by the addition of concentrated H_2SO_4 . Phytosterols were present as indicated by the creation of a green color ring.

Acute Toxicity Test

To ascertain the extract's fatal dose, this test was conducted. For this investigation, nine rats were employed. After giving the rats doses of *P. dulce* leaf extract of 1000, 3000, and 5000 mgkg⁻¹, they were monitored for 72 hours. None of the animals passed away or manifested any unusual symptoms after 72 hours. To represent high and low doses of the extract for ingestion, portions of 1/10th and 1/20th of the 5000 mgkg⁻¹ were taken.

Design of the Experiment

Five groups of six rats each were formed by randomly selecting the rats. Blood was drawn from each rat and its body weight was recorded for baseline analysis. For 8 days, Group 1 received saline as usual (standard control). For 8 days, Group 2 received dexamethasone solely by intra peritoneal injection (negative control). Dexamethasone and the normal atorvastatin dosage of 10 mg/kg were given to group 3 animals for 8 days (positive control). Dexamethasone and *P. dulce* leaf extracts were fed to Group 4 and Group 5 rats at doses of 250 mgkg⁻¹ and 500 mgkg⁻¹, respectively, for 8 days (Table 1). The entire lipid profile, including the TC, TG, HDL, LDL, and VLDL, as well as the effect of the extract on the hyperlipidaemic rats, were determined after 8 days when the final body weights were taken.

Table 1: Groups of Experimental Rats

DETAILS	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
GROUP	NORMAL	NEGATIVE CONTROL	POSITIVE CONTROL	TEST GROUP I	TEST GROUP II
TITLE	CONTROL				
NUMBER OF ANIMALS	6	6	6	6	6
TREATMENT	Normal saline	10 mgkg ⁻¹ per day of dexamethasone for 8 days	10 mgkg ⁻¹ per day of Atorvastatin along with dexamethasone treatment	<i>P. dulce</i> ethanolic extract (250 mgkg ⁻¹ per day) with dexamethasone treatment	<i>P. dulce</i> ethanolic extract (500 mgkg ⁻¹ per day) with dexamethasone treatment

Body Weight measurement, Blood collection, and Lipid profiling

Through tail bleeding, blood was obtained from the rats after 8 days. The drawn blood was maintained in heparinized vacuum tubes, where it was allowed to clot for two hours before being centrifuged at 3000 rpm for five minutes and having the serum removed for testing. Using a Uri semi-automated chemistry

analyzer, the total cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein, and very-low-density lipoprotein were measured (URIT-810 chemical analyzer).

Extraction of *P. dulce* for anti-bacterial test

Aseptic procedures were followed during the extraction. In a conical flask, 50 g of powdered samples were weighed. The samples were then added to 500 ml of methanol and thoroughly mixed. After 72 hours, the combination underwent intermittent swelling. A sterilized conical flask was used to collect the extract with the use of a sterile muslin towel. The extract was then kept on a rotating drum evaporator to evaporate the methanol, and the volume was measured for the stock solution using a sterile measuring cylinder. Furthermore, the stock solution was used to create a number of concentrations, including 6.25, 12.5, 25, and 50.

Obtaining and Culturing of Bacteria Strains

The Kwame Nkrumah University of Science and Technology's microbiology department provided the bacterium strains for this study.

To create the pure culture for the anti-bacterial test, confirmed bacteria strains were sub-cultured on a nutrient agar plate. The inoculation plates were kept at a temperature of 37°C overnight for 10 to 24 hours.

Anti-bacterial Test for *P. dulce* Leaves against the Bacteria Strains

Two milliliters of saline solution were put into each sterile test tube while maintaining aseptic conditions. To create a homogenous mixture, clean colonies from the nutrient agar plates were scooped with a sterilized inoculating loop and rubbed against the test tube wall until they totally dissolved. To assess for turbidity, the test tubes holding the combination were compared using a 0.5 MacFarland densitometer. After dipping sterilized cotton swab sticks into the liquid, they were evenly distributed on Mueller-Hinton agar substrate.

After that, holes were drilled in the Mueller-Hinton agar plates that had been inoculated using a 6 mm cork borer and labeled appropriately at different concentrations (50, 25, 12.5, and 6.25) with a negative control (methanol) and a positive control (ciprofloxacin, imipenem, ceftriaxone, and ceftazidime). For 24 to 48 hours, the infected plates were incubated at 37°C. Triplicates of each experiment were used in each.

Statistical analysis

The findings were expressed as Mean SEM. One-way analysis of variance (ANOVA) and Neuman-test Keul's were used for the statistical analysis of all five groups and the anti-bacterial test, with a P-value of 0.05 considered to be statistically significant. Table and graph pad Prism version 5.00 for Windows was used to manage the data.

Results

Yield of Plant material

A mass of 44.5g of crude extract was obtained when 300 grams of the powdered material were macerated in 2100 ml of ethanol and 900 ml of distilled water, yielding a yield of 14.83%.

Phytochemical Screening

The phytochemical study of *P. dulce* leaf extract revealed the presence of phytonutrients, excluding alkaloids, including flavonoids, phytosterols, saponins, reducing sugars, etc (Table 2).

Table 2. Phytochemical Screening of Ethanolic Leaf Extract of *P. dulce*

Phytoconstituents	Ethanolic extract of <i>P. dulce</i> leaves
Saponins	+
Flavonoids	+
Phenolic compounds	+
Alkaloids	+
Cyanogenic glycosides	-
Anthracenosides	+
Phytosteroids	+
Reducing sugars	+
Polyuronides	+
Triterpenes	-

KEY: (+) =present, (-) =absent

Acute Oral Toxicity Studies

The three distinct doses of the extract were administered to the nine rats, who were divided into three groups, but after 72 hours none of them displayed any indications of discomfort, coma, or death. The rats were re-observed for the remaining eight days, and they continued to act normally in response to food and drink and exhibited no signs of diarrhoea. Since the rats were anticipated at 5000 mgkg-1, *P. dulce's* LD50 is more significant than 5000 mgkg-1. To represent high and low doses of the extract for ingestion, portions of 1/10th and 1/20th of the 5000 mgkg-1 were taken.

Effect of *P. dulce* Leaf Extract on Biochemical Parameters in Serum

Tables 3 and 4 show how *P. dulce* leaf extract affected the lipid profile of Wister rats that had been exposed to 10 mgkg⁻¹ dexamethasone.

Table 3. Baseline Lipid Profile

GROUP	TREATMENT	TC mmolL ⁻¹	TG	HDL-C mmolL ⁻¹	LDL-C mmolL ⁻¹	VLDL-C
			mmolL ⁻¹			mmolL ⁻¹
A	Normal control	3.108 ±0.175	1.845 ±0.097	1.067 ±0.117	1.442 ±0.236	1.393 ±0.255
B	Negative control	3.528 ±0.146	1.947 ±0.038	1.060 ±0.121	1.217 ±0.084	1.530 ±0.162
	Dexamethasone (10 mgkg ⁻¹)					
C	Positive control	3.118 ±0.290	1.857 ±0.095	1.253 ±0.144	1.550 ±0.235	1.628 ±0.147
	Atorvastatin					
	(10 mgkg ⁻¹)					
D	<i>P.dulce</i>	3.100 ±0.167	2.010 ±0.039	1.043 ±0.112	1.557 ±0.171	1.617 ±0.191
	(250 mgkg ⁻¹)					
E	<i>P. dulce</i>	3.270 ±0.225	1.930 ±0.070	1.197 ±0.017	1.480 ±0.212	1.525 ±0.220
	(500 mgkg ⁻¹)					

Table 4. Lipid Profile after Termination

GROUP	TREATMENT	TC mmolL ⁻¹	TG mmolL ⁻¹	HDL-C mmolL ⁻¹	LDL-C mmolL ⁻¹	VLDL-C mmolL ⁻¹
A	Normal control	2.673 ±0.120***	1.763 ±0.111***	1.360 ±0.054*	1.225 ±0.101**	1.357 ±0.248*
B	Negative control Dexamethasone(10 mgkg ⁻¹)	6.217 ±0.171	4.485 ±0.200	0.365 ±0.086	4.722 ±0.169	3.223 ±0.060
C	Positive control Atorvastatin (10 mgkg ⁻¹)	1.273 ±0.031*** (59.17 %) ↓	0.762 ±0.127*** (58.96 %) ↓	2.027 ±0.091*** (61.45 %) ↑	0.605 ±0.071*** (74.83 %) ↓	0.560 ±0.077*** (65.60 %) ↓
D	<i>P. dulce</i> (250 mgkg ⁻¹)	2.320 ±0.049 *** (25.16 %) ↓	1.592 ±0.066*** (20.79 %) ↓	1.413 ±0.156*** (35.47 %) ↑	1.273 ±0.107*** (18.24 %) ↓	1.183 ±0.065*** (26.84 %) ↓
E	<i>P. dulce</i> (500 mgkg ⁻¹)	1.732 ±0.057*** (47.03 %) ↓	1.013 ±0.087*** (47.51 %) ↓	2.128 ±0.043*** (77.78 %) ↑	0.863 ±0.079*** (41.69 %) ↓	0.793 ±0.046*** (48.00 %) ↓

Values represent mean ± S. E. M. (n=4), Stars represent significant differences: *** P< 0.001, ** P< 0.01, * P< 0.05, Values in parentheses indicates percentage increase or decrease in the respective serum level, ↑ denotes increase, ↓ denotes decrease.

Effect of ethanolic extract of *P. dulce* on Serum Total Cholesterol (TC)

Animals in Group 2 were solely given dexamethasone during the experiment. After 8 days, there was a noticeable increase in TC level compared to the normal control (Group 1). The positive control animals (Group 3) that received atorvastatin and dexamethasone treatment showed a statistically significant reduction (p<0.05) in TC level. When compared to the healthy control (NC) rats, Test Group 4 and Group 5 animals received dexamethasone and ethanolic leaf extract at doses of 250 mgkg⁻¹ and 500 mgkg⁻¹, respectively, and after day 8 showed a substantial (p<0.05) decrease in TC level (Figure 1).

Effect of ethanolic extract of *P. dulce* on Triglyceride (TG)

After 8 days, the dexamethasone-treated rats in the Group 2 negative control group had significantly higher triglycerides than the Group 2 normal control group (Group 1). When dexamethasone and atorvastatin were administered to the positive control rats (Group 3), triglyceride levels were shown to significantly decrease ($p<0.05$). When compared to the normal control (NC) rats, Test Group 4 and Group 5 animals treated with dexamethasone and ethanolic leaf extract in doses of 250 mg/kg-1 and 500 mg/kg-1, respectively, showed a significant ($p<0.05$) reduction in triglycerides level after day 8 (Figure 2).

Effect of ethanol extract of *P. dulce* on High Density Lipoprotein-cholesterol (HDL-C)

After 8 days, the dexamethasone-treated negative control animals (Group 2) only displayed a significant ($p<0.05$) reduction in HDL-C levels in comparison to the healthy control group (Group 1). The positive control animals (Group 3) that received atorvastatin and dexamethasone treatment showed a statistically significant rise ($p<0.05$) in HDL-C levels. When compared to the normal control (NC) rats, Test Group 4 and Group 5 animals treated with dexamethasone and ethanolic leaf extract in doses of 250 mg/kg-1 and 500 mg/kg-1, respectively, showed a significant ($p<0.05$) elevation in HDL-C level after day 8 (Figure 3).

Effect of ethanolic extract of *P. dulce* on Low Density Lipoprotein (LDL-C)

After 8 days, the dexamethasone-treated negative control animals (Group 2) only significantly ($p<0.05$) increased their LDL-C levels in comparison to the untreated control animals (Group 1). When dexamethasone and atorvastatin were administered to the positive control mice (Group 3), LDL-C levels were decreased ($p<0.05$). When compared to the normal control (NC) rats, Test Group 4 and Group 5 animals treated with dexamethasone and ethanolic leaf extract in doses of 250 mg/kg-1 and 500 mg/kg-1, respectively, revealed a significant ($p<0.05$) reduction in LDL-C level after day 8 (Figure 4).

Effect of ethanolic extract of *P. dulce* on Very Low-Density Lipoprotein (VLDL-C)

When compared to the regular control animals (Group 1), the negative control animals (Group 2) treated with dexamethasone only manifested a significant ($p<0.05$) rise in VLDL-C level after 8 days (Group 1). When dexamethasone and atorvastatin were administered to the positive control animals (Group 3), the VLDL-C level significantly decreased ($p<0.05$). When compared to the normal control (NC) rats, Test Group 4 and Group 5 animals treated with dexamethasone and ethanolic leaf extract in doses of 250 mg/kg-1 and 500 mg/kg-1, respectively, revealed a substantial ($p<0.05$) reduction in VLDL-C level after day 8 (Figure 5).

Body weight measurement

All rats' body weights were noted both before and after the experiment. The outcomes are displayed in Table 5.

Table 5. Body Weight Measurement Before and After the Experimental Period

GROUP	TREATMENT/DOSE	DAY 0	DAY 8
A	Normal control	220.667 ±8.678	246.333 ±13.144
B	Negative control Dexamethasone (10 mgkg ⁻¹)	226.000 ±4.344	169.500 ±6.125
C	Positive control Atorvastatin (10 mgkg ⁻¹)	218.333 ±6.070	187.667 ±8.007
D	<i>P. dulce</i> (250 mgkg ⁻¹)	226.167 ±7.761	185.500 ±2.884
E	<i>P. dulce</i> (500 mgkg ⁻¹)	216.833 ±10.190	177.667 ±6.576

Values are represented in mean ± S. E. M. (n=4)

Table 6. Shows *P. dulce* extract against bacteria strains

Treatment (mg/ml)	Pathogens		
	Inhibition zone diameter (mm)		
<i>P. dulce</i> Extract	<i>Escherichia coli</i> ATCC14028	<i>Salmonella</i> ATCC25922	<i>Salmonella</i> spp.
50	6.000 ^a	12.000 ^b	9.667 ^a
25	7.333 ^a	9.000 ^{ab}	9.333 ^a
12.5	6.333 ^a	7.333 ^{ab}	8.667 ^a
6.25	6.667 ^a	8.667 ^{ab}	8.667 ^a
Control(methanol)	6.000 ^a	6.000 ^a	7.000 ^a
CV	13.7	21.2	18.8
LSD	1.694	3.322	2.971
F.pr	0.236	0.024	0.374

Means that do not share a same letter are significantly difference; at p value <0.01.

Table 7. *P. dulce* shows antibiotics against bacteria strains

Pathogens	Antibiotics			
	Ciprofloxacin	Imipenem	Ceftriaxone	Ceftazidime
<i>Salmonella ATCC14028</i>	30	26	13	9
<i>Escherichia coli ATCC25922</i>	25	32	12	6
<i>Salmonella spp.</i>	30	27	30	30

Discussion

1. Anti-hyperlipidaemic activity

Cardiovascular diseases are one of the leading global causes of death, and hyperlipidaemia is connected with them (Jain et al. 2007). Numerous studies have shown that decreasing serum lipid levels to desired levels in various animals and interventional studies have shown a decrease in cardiovascular disease cases and fatalities. According to reports, a low risk of ischemic heart disease results from an increase in HDL cholesterol and a decrease in total cholesterol, LDL cholesterol, and triglycerides (Sivaelango et al. 2012). A synthetic glucocorticoid steroid that raises serum lipid levels is dexamethasone sodium phosphate. This is accomplished by elevating hepatic lipogenesis and triacylglycerol production by boosting the primary enzymes in fatty acid biosynthesis in the liver (Kumar 2016). Dexamethasone sodium phosphate was administered continuously by intraperitoneal injection for 8 days, and it was shown that this caused an increase in the important blood lipids TC, TG, LDL-C, and VLDL-C (Tables 3 and 4), as well as a decrease in HDL-C. Treatment with the standard reference medicine, atorvastatin, and the ethanolic leaf extract at increasing doses successfully stopped the rise in the different lipid levels; consequently, it dramatically reduced levels of TC, TG, LDL-C, and VLDL-C (Tables 3 and 4).

A statin called atorvastatin is frequently used to treat hyperlipidaemia. By blocking HMG-CoA reductase (3-hydroxy-3-methylglutaryl-CoA), a crucial enzyme in cholesterol synthesis, statins are known to reduce cholesterol production (Rohilla et al. 2012). In addition, statins work better at reducing LDL-C (Srinivasa et al. 2011). When hyperlipidaemic rats were treated with ethanolic leaf extract at various doses compared to the control, several significant differences were seen. Figures 1 through 4 depict the effects of treating hyperlipidaemic rats with ethanolic leaf extract at various doses (250 mgkg⁻¹ and 500 mgkg⁻¹) on blood TC, TG, LDL-C, and VLDL-C levels as well as the corresponding increases in serum HDL-C levels (Fig. 5). The ethanolic leaf extract of *P. dulce* was found to be effective in treating hyperlipidaemic situations by significantly lowering serum lipid levels and increasing HDL-C levels when compared to the normal control group. Higher than the 250 mgkg⁻¹ treatments is the decrease in serum lipid profile in the ethanolic extract leaf extract therapy (Table 4). An optimal antihyperlipidemic medicine enhances HDL-C while lowers LDL-C, and the administration of ethanolic leaf extract did not change this (Srinivasa et al. 2011). According to biochemical estimates, ethanolic leaf extract (500 mgkg⁻¹) markedly decreased atherogenic LDL-C and VLDL-C and raised the amount of "good" cholesterol HDL-C.

The efficacy of *P. dulce*'s ethanolic leaf extract to decrease cholesterol may be due to enhanced excretion of bile acids and cholesterol through faecal sterol excretion. The mobilization of cholesterol from auxiliary cells to the liver through Lecithin Cholesterol Acyltransferase (LCAT), which is believed to be the probable mechanism of the extract, may result in an increase in HDL-C (Khanna, Rizvi, and Chander 2002). The creation of HDL-C, the transesterification of cholesterol, and the sequestration of cholesterol from cell membranes into HDL-C are all made possible by the LCAT enzyme. In dexamethasone-induced hyperlipidaemia, enzyme activity tends to decline (Zulet et al. 1999). Saponins, phytosterols, and phenolic components are present in *P. dulce* leaf. Numerous plant species contain saponins, surface-active glycosides, in large quantities (Dobiášová and Frohlich 2001).

According to numerous research, saponins from various plant extracts lower serum cholesterol levels (Latha et al. 2011). The development of large mixed micelles as a result of saponins' interaction with bile acids, which increases cholesterol excretion, may explain their likely cholesterol-lowering effects. This increases liver metabolism of cholesterol, which lowers blood levels (Francis et al. 2002). Another significant phytochemical involved in a variety of biological processes is phenolic compounds. The hydroxyl groups in phenol provide it exceptional free radical scavenging abilities. Due to its high phenolic content, *P. dulce* leaf is a powerful antioxidant (Dzinyela, Abdul-Basit, and Alhassan 2021). Numerous research conducted on various plants have shown a link between the presence of phenolic chemicals and a drop in blood cholesterol levels (Afonso et al. 2013).

This beneficial effect on cholesterol levels may be brought about by an increase in the hepatic tissues' antioxidant capacity, which in turn causes oxidative stress and lipid peroxidation to decline. Similarly, phytosterols play a critical role in the majority of biological processes. In the small intestine, phytosterols compete with cholesterol for its uptake, preventing cholesterol from being taken into the bloodstream. Therefore, saponins, phytosterols, and phenolic contents may have a complementary or separate role in the potential antihyperlipidemic activity of ethanolic leaf extract of *P. dulce*. The study's findings were comparable to those of prior studies (Shweta, Mehta, and MP 2013; Mule et al. 2016; Sundarajan et al. 2010). These results imply that the ethanolic leaf extract of *P. dulce* affects cholesterol production and metabolism in a beneficial way. Additionally, *P. dulce* has the ability to rectify lipid imbalances, which makes it a useful cardio protective agent.

On three strains, the extract concentrations (50, 25, 12.5, 6.25, and control) exhibit antibacterial activity (Table 6). *E. coli* reported the lower zones of inhibitions for the various treatments from 6.0001.5 to 7.3332.75, according to Table 6.0. The research also showed that *P. dulce* leaves generally had lower zones of inhibition than the tested conventional antibiotics. According to Bayiti et al. (2004), the effectiveness of extracts exhibiting anti-bacterial activity occurs when the inhibitory diameter zone measured is more significant than 10 mm or above. The effectiveness of *P. dulce* as a potential antibiotic substitute may not be fully inferred statistically from the aforementioned data. However, there is a substantial difference between the two strains (*E. coli* ATCC25922 and *Salmonella* ATCC14028) in their zone of inhibition, with the exception of the control and the unidentified strain (*Salmonella* spp) ($p < 0.01$).

The findings (Table 7) showed which organisms were examined for antibiotic susceptibility. All of the studied bacteria were more sensitive to Ciprofloxacin and Imipenem.

In contrast to the work of *P. dulce* pods by Pradeepa et al. (2014) which gives more active secondary metabolites, the low anti-bacterial activity of *P. dulce* leaves could be attributed to low presence constituents of secondary metabolites such as flavonoids, tannins, saponins, etc. responsible for inhibitory activity on the microbial strains. Therefore, due to their larger concentration of anti-bacterial pharmacological compounds, the *P. dulce* pods' inhibitory activity may be preferable to that of the *P. dulce* leaves.

Conclusion

This current study was meticulously carried out using the suppressive model. The study's findings showed that in dexamethasone-induced hyperlipidaemic rats, the ethanolic leaf extract of *P. dulce* dramatically increased HDL-C concentration while lowering serum lipid levels (TC, TG, LDL, and VLDL). One reasonable assumption would be that *P. dulce* possesses antihyperlipidemic properties. This treatment has the potential to be effective and affordable. Additionally, the antibacterial test showed that *P. dulce* has the ability to stop the growth of various bacteria, including *Salmonella* species. This offers yet another option for the future management of *Salmonella* infection.

To assess the quantity of phytochemical components in the leaves that may contribute to its anti-hyperlipidemic property, quantitative and qualitative phytochemical study of *P. dulce* leaves should be carried out. Further studies should look into the anti-hyperlipidaemic curative and preventative properties of *P. dulce* leaves and other plant parts.

Declarations

Funding

This study was supported by Nanjing Forestry University (No. 163108059).

Author contribution

Raphael Dzinyela prepared the original draft. Abdul Razak Alhassan, Vincent Kuetsidzo, and Abdul-Nasir Abdul-Baasit performed conceptualization, methodology, and laboratory investigations. Ali Movahedi reviewed and edited the final manuscript. Ali Movahedi supervised and funded this project.

Conflict of Interest Statement

All the authors do not have any possible conflicts of interest.

Acknowledgments

The authors are grateful to Dr. Olga Quarsie, Mr. Stephen Antwi, and the entire institute of Centre for Plant Medicine Research Akuapim-Mampong for their guidance and support.

References

- Afonso, Milessa S, Ana Mara de O Silva, Eliane BT Carvalho, Diogo P Rivelli, Sílvia BM Barros, Marcelo M Rogero, Ana Maria Lottenberg, Rosângela P Torres, and Jorge Mancini-Filho. 2013. 'Phenolic compounds from Rosemary (*Rosmarinus officinalis* L.) attenuate oxidative stress and reduce blood cholesterol concentrations in diet-induced hypercholesterolemic rats', *Nutrition & metabolism*, 10: 1-9.
- Biyiti, LF, DJL Meko'o, V Tamz, and PH Amvam Zollo. 2004. 'Research of the antibacterial activity of four Cameroonian medicinal plants', *Pharmacopoeia and African Traditional Medicine*, 13: 11-20.
- Dobiášová, Milada, and Jiri Frohlich. 2001. 'The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apob-lipoprotein-depleted plasma (FERHDL)', *Clinical biochemistry*, 34: 583-88.
- Dzinyela, Raphael, Abdul-Nasir Abdul-Baasit, and Abdul Razak Alhassan. 2021. 'Evaluation of the Antioxidant Activity of Crude Whole Plant Methanolic Extract of *Oxalis stricta* Linn', *American Journal of Applied Chemistry*, 9: 43-48.
- Dzinyela, Raphael, Emmanuel Kwesi Asomaning, Abdul-Nasir Abdul-Baasit, Abdul Razak Alhassan, and Ali Movahedi. 2021. 'An in vivo Evaluation of Antihyperlipidaemic Activity of Ethanolic Extract of *Amaranthus spinosus* Leaves on Dexamethasone Induced Hyperlipidaemic Rats', *Biochemistry and Molecular Biology*, 6: 25-34.
- Francis, George, Zohar Kerem, Harinder PS Makkar, and Klaus Becker. 2002. 'The biological action of saponins in animal systems: a review', *British journal of Nutrition*, 88: 587-605.
- Jain, Kishor S, MK Kathiravan, Rahul S Somani, and Chamanlal J Shishoo. 2007. 'The biology and chemistry of hyperlipidemia', *Bioorg Med Chem*, 15: 4674-99.
- Khanna, AK, F Rizvi, and R Chander. 2002. 'Lipid lowering activity of *Phyllanthus niruri* in hyperlipemic rats', *Journal of ethnopharmacology*, 82: 19-22.
- Kulkarni, Kaushik V, and Varsha R Jamakhandi. 2018. 'Medicinal uses of *Pithecellobium dulce* and its health benefits', *Journal of Pharmacognosy and Phytochemistry*, 7: 700-04.
- Kumar, G Vijay. 2016. 'Antihyperlipidemic Activity of Leaf Extracts of *Leucas aspera* Linn. against Dexamethasone-induced Hyperlipidemia in Rats', *Asian Journal of Pharmaceutics (AJP): Free full text articles from Asian J Pharm*, 10: 408-13.
- Kumar, Rai Puneet, Shammy Jindal, Nitin Gupta, and Rinu Rana. 2014. 'An inside review of *Amaranthus spinosus* Linn: a potential medicinal plant of India', *International Journal of Research in Pharmacy and*

Chemistry, 4: 643-53.

Latha, B. P., T Vijaya, RM Reddy, M Ismail, and S Dattatreya Rao. 2011. 'Therapeutic efficacy of *Achyranthes aspera* saponin extract in high fat diet induced hyperlipidaemia in male wistar rats', *African Journal of Biotechnology*, 10: 17038-42.

Megala, J, and A Geetha. 2010. 'Free radical-scavenging and H⁺, K⁺-ATPase inhibition activities of *Pithecellobium dulce*', *Food chemistry*, 121: 1120-28.

Mule, VS, NS Naikwade, CS Magdum, and VA Jagtap. 2016. 'Antidiabetic activity of extracts of *Pithecellobium dulce* Benth leaves in alloxan induced diabetic rats', *International Journal of Pharmaceutical Sciences and Drug Research*, 8: 275-80.

Nadkarni, Krishnarao Mangeshrao. 1996. *[Indian materia medica]; Dr. KM Nadkarni's Indian materia medica: with Ayurvedic, Unani-Tibbi, Siddha, allopathic, homeopathic, naturopathic & home remedies, appendices & indexes. 1* (Popular Prakashan).

Ochani, Pooja C, and Priscilla D'Mello. 2009. 'Antioxidant and antihyperlipidemic activity of *Hibiscus sabdariffa* Linn. leaves and calyces extracts in rats', *Indian J Exp Biol*, 47: 276-82.

Oriakhi, Kelly, and Patrick Uadia. 2020. 'Hypolipidemic Activity of *Tetracarpidium conophorum* (African walnut) Seed Oil and Its Mechanism of Action', *Planta Medica International Open*, 7: 170-78.

Pradeepa, S, S Subramanian, and V Kaviyarasan. 2014. 'Evaluation of antimicrobial activity of *Pithecellobium dulce* pod pulp extract', *Asian Journal of Pharmaceutical and Clinical Research*, 7: 32-37.

Preethi, S, and AM Saral. 2014. 'GC-MS analysis of microwave assisted ethanolic extract of *Pithecellobium dulce*', *Malaya J Biosci*, 1: 242-47.

Rohilla, Ankur, Nidhi Dagar, Seema Rohilla, AMARJEET Dahiya, and Ashok Kushnoor. 2012. 'Hyperlipidemia-a deadly pathological condition', *International journal of current pharmaceutical research*, 4: 15-18.

Selvakumar, M, L Dinesh Kumar, A Velusamy, and A Ronaldo Anuf. 2019. 'Nutritional and therapeutic benefits of medicinal plant *Pithecellobium dulce* (Fabaceae): A review', *J Appl Pharmaceut Sci*, 9: 130-39.

Shweta, Sharma, BK Mehta, and Ujjain MP. 2013. 'A review on pharmacological activities of *Pithecellobium dulce* extract, and there effective doses', *J Med Pharm Allied Sci*, 5: 37-45.

Sikarwar, Mukesh S, and MB Patil. 2012. 'Antihyperlipidemic activity of *Salacia chinensis* root extracts in triton-induced and atherogenic diet-induced hyperlipidemic rats', *Indian journal of pharmacology*, 44: 88-92.

Sivaelango, G, P Senthil Kumaran, P Kumaravel, P Revathi, and A Jaswant. 2012. 'Antihyperlipidaemic activity of *Spermacoe hispida* ethanolic extract in Triton WR-1339 induced hyperlipidaemic rats', *Journal of Applied Pharmaceutical Science*, 2: 95-98.

Srinivas, G, HP Geeta, and JN Shashikumar. 2018. 'A review on *Pithecellobium dulce*: A potential medicinal tree', *IJCS*, 6: 540-44.

Srinivasa, RK, T Prasad, GP Mohanta, and PK Manna. 2011. 'An overview of statins as hypolipidemic drugs', *IJPSSDR*, 3: 178-83.

Sundarrajan, T, T Rajkumar, E Udhayakumar, and G Arunachalam. 2010. 'Hypolipidemic activity of *Pithecellobium dulce* Bench. in triton Wr-1339 induced hyperlipidemic rats', *Int J Chem Pharm Sci*, 1: 50-53.

Sushma, N, P Venkata Smitha, Y Venu Gopal, R Vinay, N Srinivasa Reddy, M Mohan, and B Raju. 2013. 'Antidiabetic, antihyperlipidemic and antioxidant activities of *buchanania lanzan* spreng methanol leaf extract in streptozotocin-induced types I and II diabetic rats', *Tropical Journal of Pharmaceutical Research*, 12: 221-26.

Trease, GE, and WC Evans. 1989. "Pharmacognsy. 11th edn. Brailliar Tiridel Can." In.: Macmillian publishers.

Zulet, M Angeles, Ana Barber, Henri Garcin, Paul Higuere, and José Alfredo Martinez. 1999. 'Alterations in carbohydrate and lipid metabolism induced by a diet rich in coconut oil and cholesterol in a rat model', *Journal of the American College of Nutrition*, 18: 36-42.

Figures

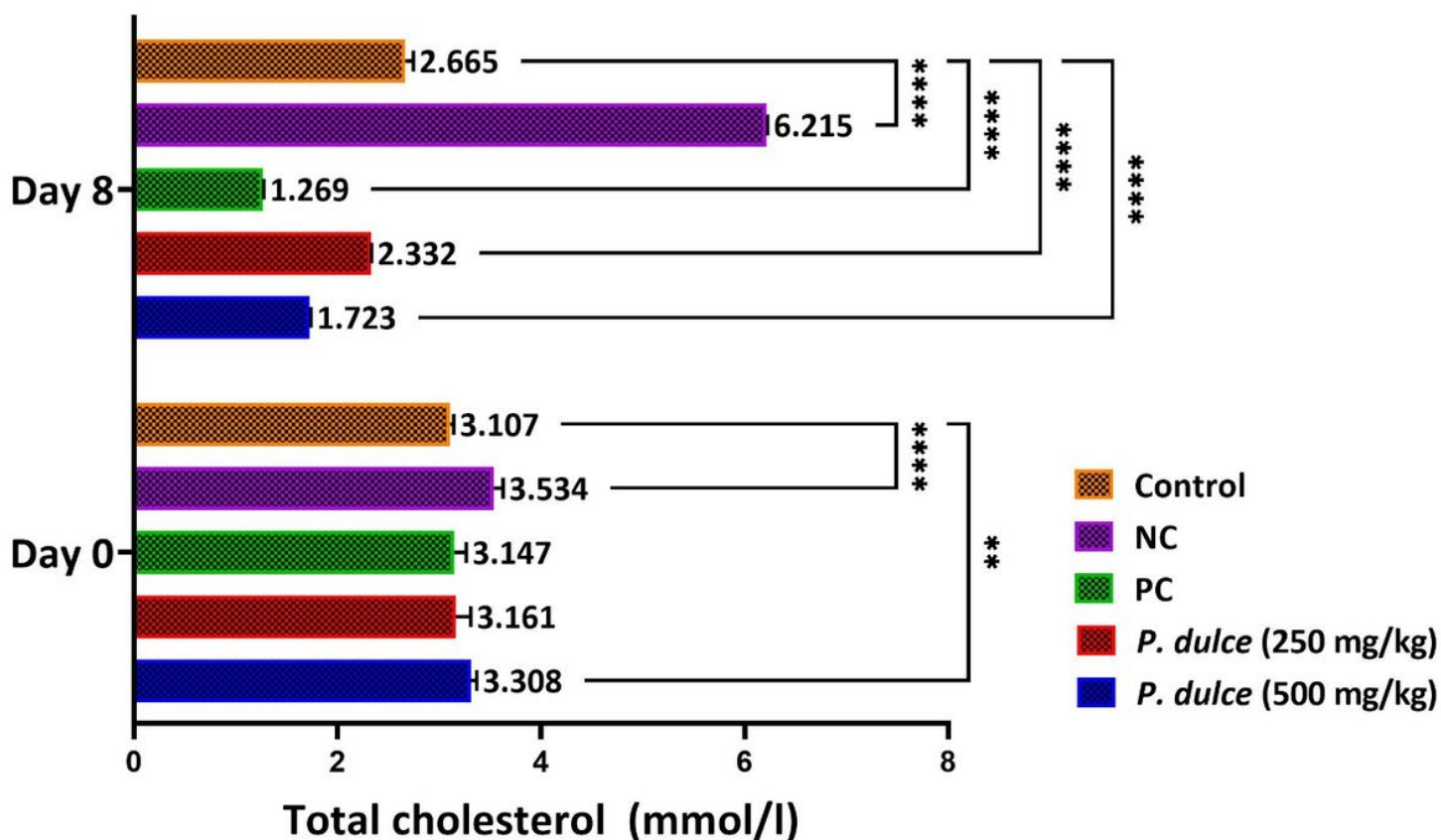


Figure 1

shows the impact of *P. dulce* on the levels of serum total cholesterol in hyperlipidemic rats induced by dexamethasone. Calculated total cholesterol levels (mmolL⁻¹) were compared to the control on several days. Each treatment has undergone four independent repeats (n=4). Dexamethasone- and atorvastatin-treated animals serve as the respective positive (PC) and negative (NC) controls. After day 8, animals given dosages of 250 mgkg⁻¹ and 500 mgkg⁻¹ of ethanolic leaf extract showed a significantly lower level of serum total cholesterol (TC) compared to the rats in the normal control (NC) group (p<0.05). Standard deviation (SD) is shown by error bars, while significant differences are indicated by stars: **** P< 0.0001, ** P< 0.01.

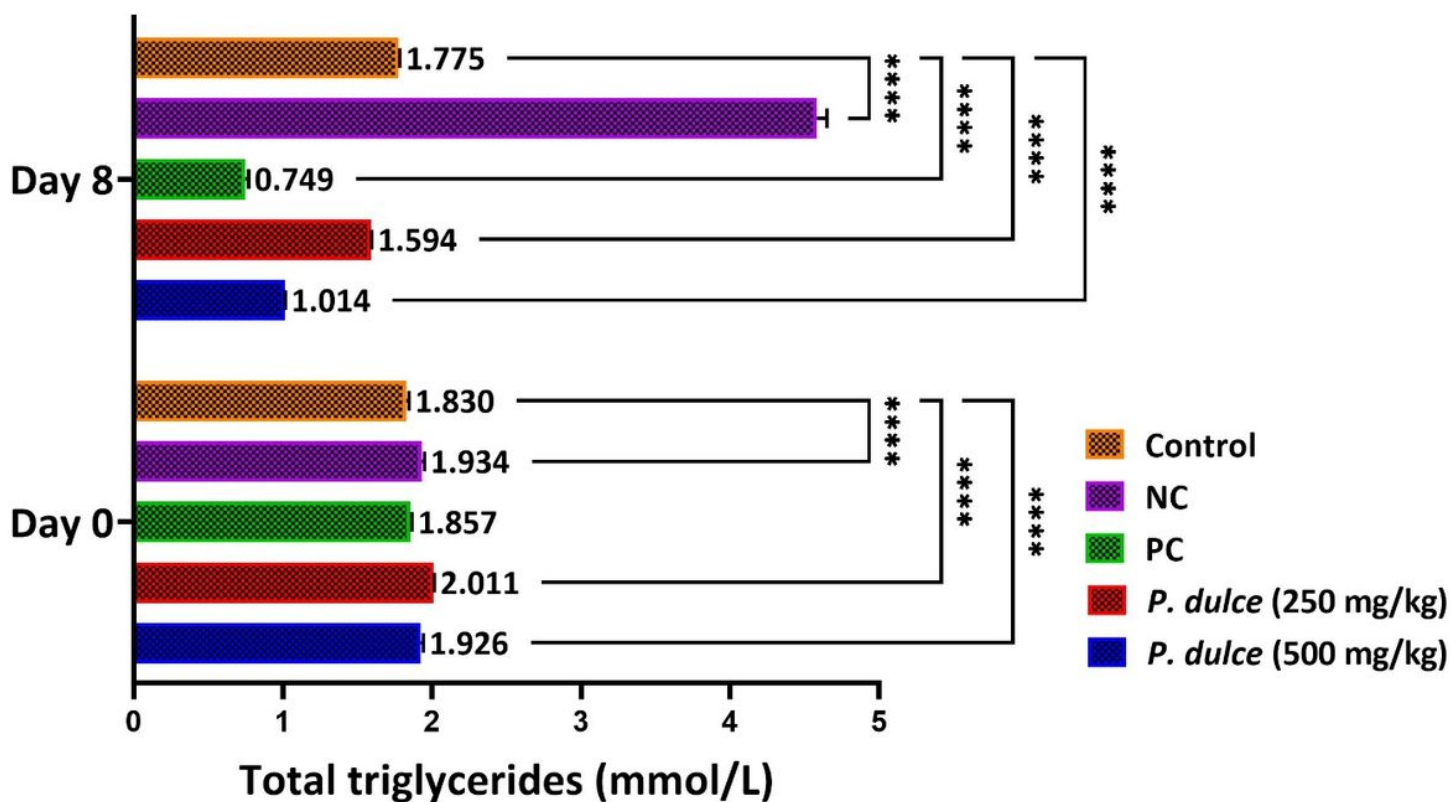


Figure 2

shows how *P. dulce* affected the serum total triglycerides in rats with hyperlipidemia brought on by dexamethasone. Calculated total triglyceride levels (mmolL⁻¹) were compared to the control on several days. Each treatment has undergone four independent repeats (n=4). Dexamethasone- and atorvastatin-treated animals serve as the respective positive (PC) and negative (NC) controls. After day 8, animals given dosages of 250 mgkg⁻¹ and 500 mgkg⁻¹ of ethanolic leaf extract showed a significantly lower level of serum total triglycerides (TG) compared to the rats in the normal control (NC) group (p<0.05). Standard deviation (SD) is shown by error bars, while significant differences are indicated by stars: **** P< 0.0001.

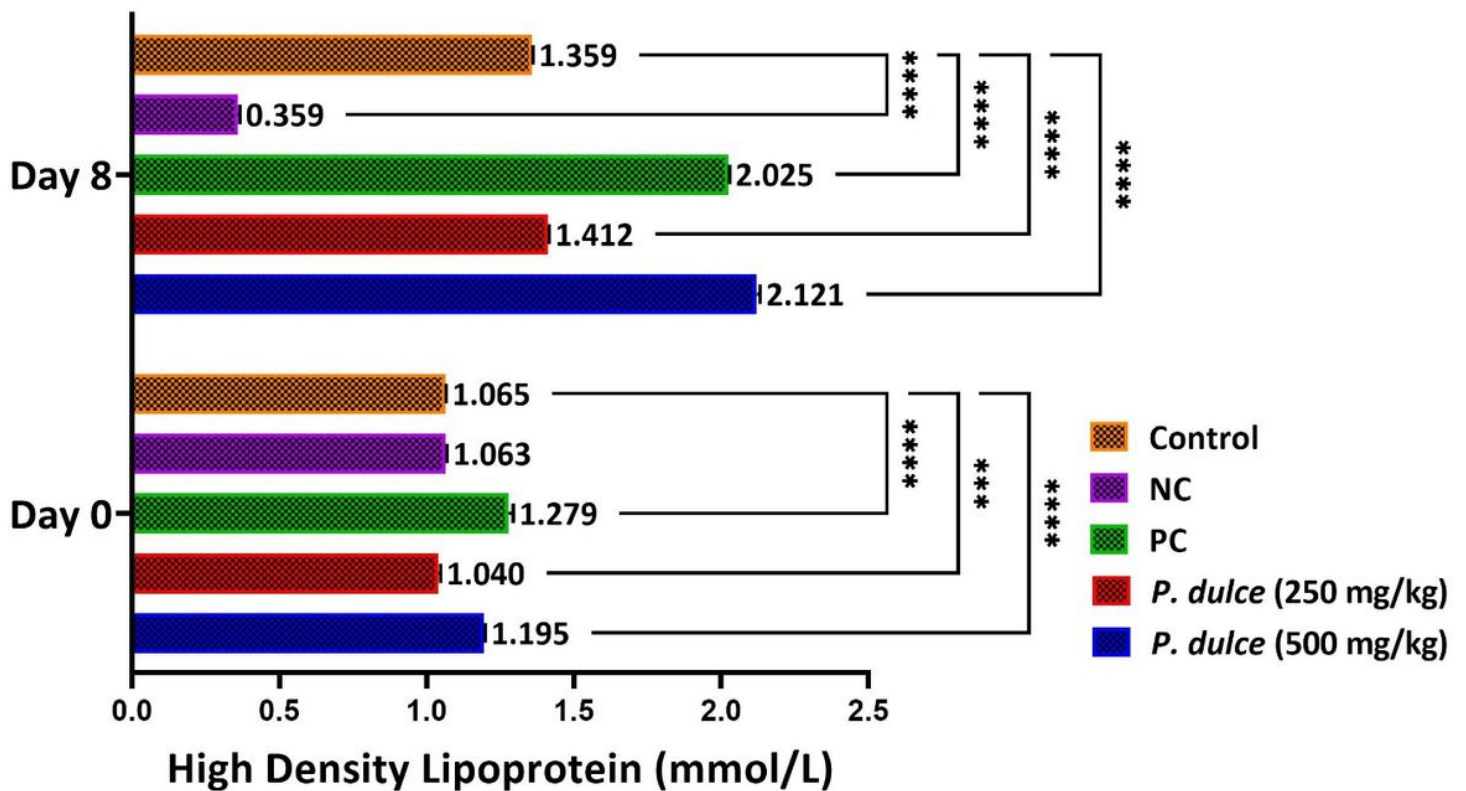


Figure 3

shows how *P. dulce* affected high density lipoprotein in rats with hyperlipidemia brought on by dexamethasone. Calculated high density lipoprotein values (mmolL⁻¹) were compared to the control on several days. Each treatment has undergone four independent repeats (n=4). Dexamethasone- and atorvastatin-treated animals serve as the respective positive (PC) and negative (NC) controls. After day 8, animals given doses of 250 mgkg⁻¹ and 500 mgkg⁻¹ of ethanolic leaf extract showed significantly higher levels of high-density lipoprotein (HDL-C) compared to the rats in the normal control (NC) group (p<0.05). Standard deviation (SD) is shown by error bars, while significant differences are indicated by stars: **** P< 0.0001, *** P< 0.001.

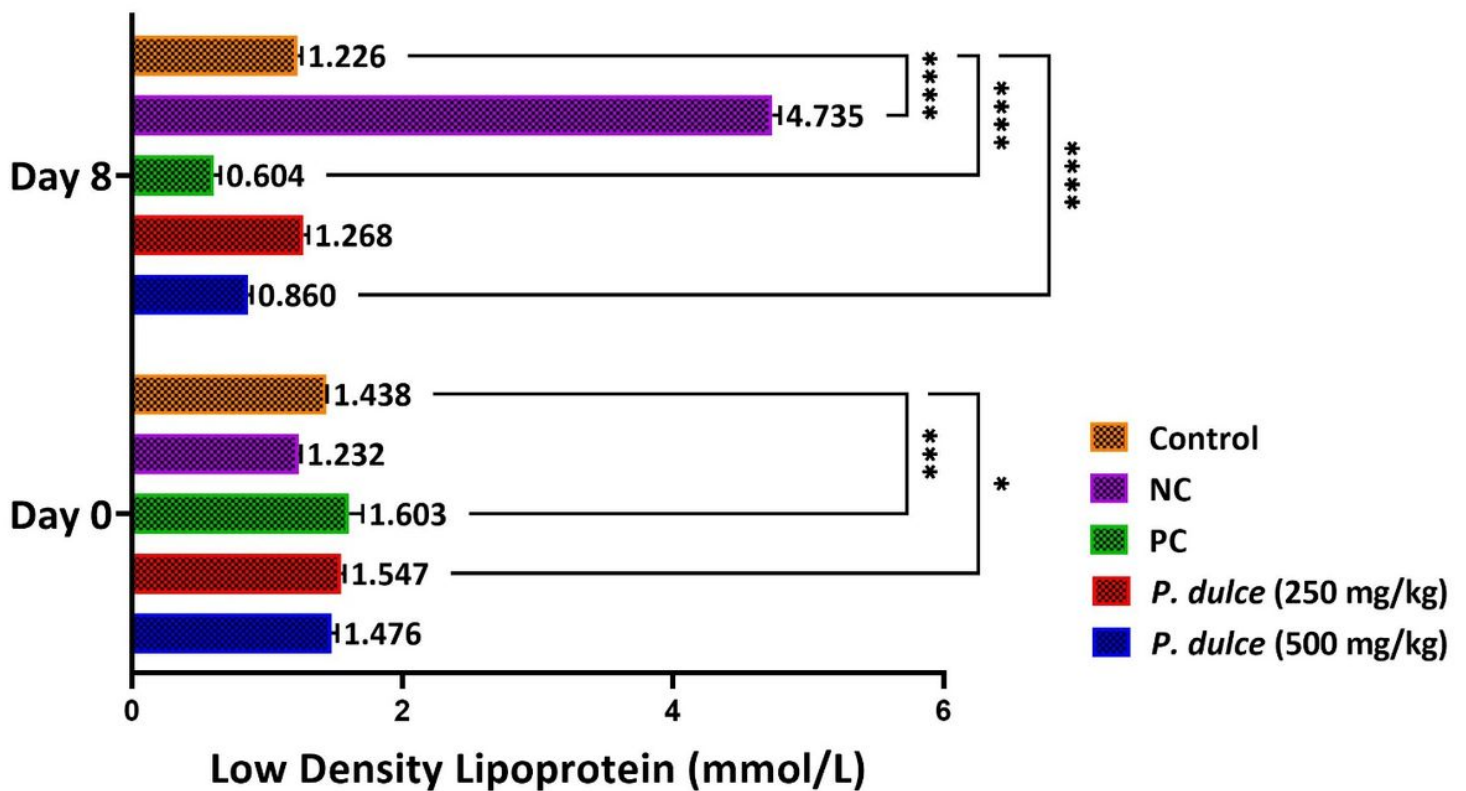


Figure 4

shows how *P. dulce* affected low-density lipoprotein in rats with hyperlipidemia brought on by dexamethasone. Calculated low density lipoprotein values (mmolL⁻¹) were compared to the control on several days. Each treatment has undergone four independent repeats (n=4). Dexamethasone- and atorvastatin-treated animals serve as the respective positive (PC) and negative (NC) controls. After day 8, animals given doses of 250 mgkg⁻¹ and 500 mgkg⁻¹ of ethanolic leaf extract showed a significantly lower level of low-density lipoprotein (LDL-C) compared to the normal control (NC) rat (p<0.05). Standard deviation (SD) is shown by error bars, while significant differences are indicated by stars: **** P< 0.0001, *** P< 0.001, * P< 0.05.

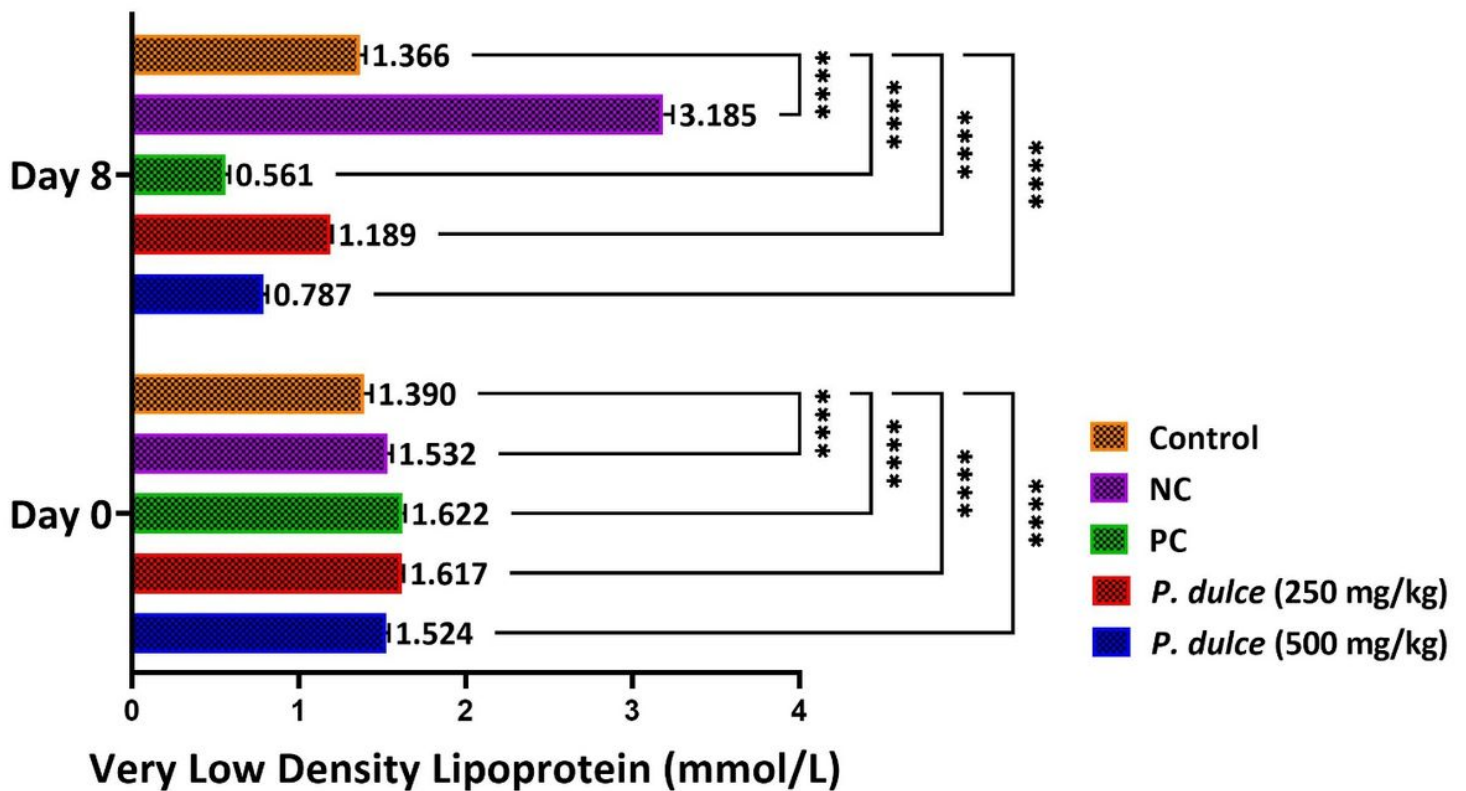


Figure 5

P. dulce's impact on very low-density lipoprotein in rats with hyperlipidemia brought on by dexamethasone. In comparison to the control, very low-density lipoprotein levels (mmolL⁻¹) were calculated throughout various days. Each treatment has undergone four independent repeats (n=4). Dexamethasone- and atorvastatin-treated animals serve as the respective positive (PC) and negative (NC) controls. After day 8, animals given doses of 250 mgkg⁻¹ and 500 mgkg⁻¹ of ethanolic leaf extract showed a significantly lower level of low-density lipoprotein (VLDL-C) than the normal control (NC) rat (p<0.05). Standard deviation (SD) is shown by error bars, while significant differences are indicated by stars: **** P< 0.0001.