RESEARCH ARTICLE

Evaluation of the Antihyperlipidemic Effect of Encapsulated Octacosanol for Lipid-Lowering Effect Against High-Fat Diet-Induced Hyperlipidaemia in Experimental Rats

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ABSTRACT

Background: Elevated blood lipid levels, or hyperlipidemia, are the main risk factors for cardiovascular illnesses and are frequently brought on by high-fat diets.

Objectives: In this work, high-fat diet-induced hyperlipidemia in experimental rats is used to examine the antihyperlipidemic efficacy of a test formulation containing 1-octacosanol.

Method: A healthy female Wistar rat was utilized for investigation, separated into four groups: normal, disease, standard control, and test group, receiving the 1-octacosanol formulation.

Results: Hyperlipidemia was induced in the rats through a high-fat diet over 28 days, followed by a 28-day treatment period. Various parameters, including lipid profile (LDL, TC, TG, VLDL, HDL), body weight and antioxidant properties (MDA, SOD, GSH), were evaluated. The results showed that the 1-octacosanol formulation significantly reduced body weight and improved lipid profiles by decreasing levels of TC, TG, LDL and VLDL while increasing HDL levels. Additionally, the formulation exhibited notable antioxidant properties, contributing to its lipid-lowering effects.

Statistical analysis using ANOVA and multiple comparison tests confirmed the significance of these findings, underscoring the therapeutic potential of 1-octacosanol in managing hyperlipidemia and mitigating associated cardiovascular risks.

Conclusion: The test formulation of 1-octacosanol demonstrated a significant antihyperlipidemic effect in rats with high-fat diet-induced hyperlipidemia. The treatment effectively reduced serum levels of total cholesterol, triglycerides, LDL and VLDL while increasing HDL cholesterol levels. These marks suggest that 1-octacosanol has the potential as a therapeutic agent for managing hyperlipidemia, a key risk factor for cardiovascular diseases.

Keywords: 1-Octacosanol, Hyperlipidemia, Wistar rats, Cardiovascular risks, Lipid profile, Antioxidant properties.

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INTRODUCTION

A medical condition known as hyperlipidemia is defined by decreased levels of high-density lipoprotein (HDL) and increased levels of one or more plasma lipids, such as cholesterol esters, cholesterol, triglycerides, phospholipids, and/or plasma lipoproteins, such as low-density lipoprotein (LDL).[1]

One of the major hazard factors for cardiovascular disorders is hyperlipidemia.[2] Hyperlipidemia, or high cholesterol in the body, is a popular term. The body produces the waxy molecule known as cholesterol, which is also obtained through diet. The liver is in charge of regulating the blood's cholesterol levels. About 80% of cholesterol is produced by the liver, with the remaining 20% coming from diets high in meat, fish, and eggs.[3]

The production of bile acids and steroid hormones requires cholesterol as a necessary component.

All of the cholesterol in our body comes from two separate sources: either particular foods contain cholesterol, or our cells can generate it from scratch.[4]

The disorder of lipid metabolism known as hyperlipidemia is brought on by a rise in plasma concentration of various lipid and lipoprotein fractions, such as VLDL, TC, TG, LDL, and HDL, which raises the risk of various problems.[5]

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Lipoproteins are big, globular particles with polar covering made of phospholipids devoid of apoproteins and cholesterol, the oily core of nonpolar lipids (cholesteryl esters of triglycerides).[6]

They are categorized into:[7]

- Low-density lipoprotein (LDL)
- Chylomicrons (mainly triglycerides)
- Very low-density lipoprotein (VLDL)
- High-density lipoprotein (HDL)

Because of its increased ratio of cholesterol to protein, LDL is regarded as "bad" cholesterol (over 160 mg/dl), and its excess level may raise the risk of CVS illnesses. Plaque accumulation or deposits on the arterial walls, which causes the artery to constrict and reduces blood flow throughout the body.

Since HDL (greater than 60 mg/dl) may be protective against CVS disorders, stroke, and other conditions, it is regarded as "good" cholesterol. Compared to LDL, VLDL has significantly less protein and are linked to plaque buildup. TG are essentially those fat types that have higher cholesterol levels together with low HDL and high LDL levels.[8], Figures 1 and 2.

Lipid Metabolic Pathway

- Exogenous.
- Endogenous.
- Reverse cholesterol transport and HDL metabolism[9]

Excess cholesterol is transported along this channel from the peripheral to the liver, where it is excreted in the bile. Newly formed HDLs are produced by the gut and liver. Mature HDLs are formed when lecithin-cholesterol acyltransferase (LCAT) esterifies free cholesterol that is obtained from macrophages and further peripheral cells. Scavenger receptor class B (SR-BI) allows the liver to specifically absorb HDL cholesterol. As an alternative, chylomicrons and VLDLs can absorb cholesteryl ester by means of the CETP, which is present in HDLs.[10]

Atherosclerotic cardiovascular disease is caused by plaque formation and accumulation in the vasculature as a result of disorders of lipid metabolism that raise cholesterol levels in the body.[11]

Enzymes connected to the metabolism of fats:[12]

- Lipoprotein lipase (LPL)

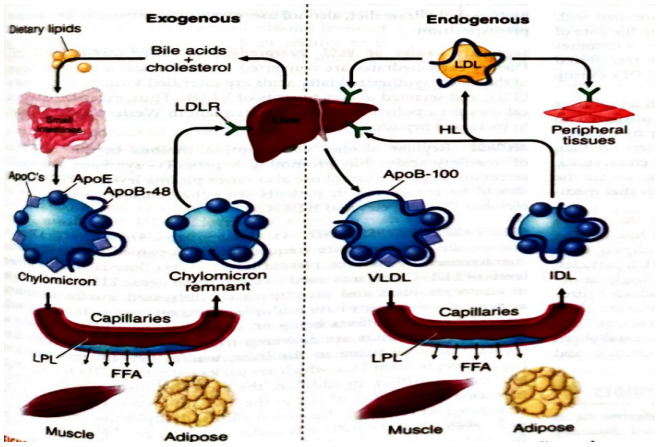


Figure 1: a) Exogenous pathway transports dietary lipids to the liver and periphery b) Endogenous pathway transports hepatic lipids to the periphery

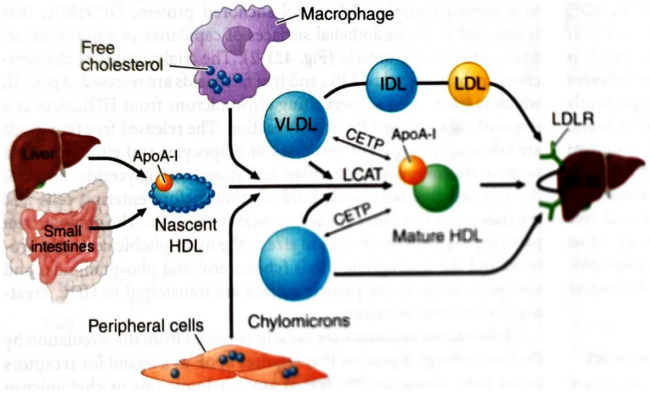


Figure 2: Reverse cholesterol transport and HDL metabolism

- Lecithin cholesterol acyl transferase (LCAT)
- Hepatic lipase (HL)
- Acyl Co-A transferase (ACAT)
- Cholesteryl ester transfer protein (CETP)

Current Medications[13,14]

In the current allopathy medicine system following categories of drugs are presented in Table 1:

Action of 1-octacosanol[15]

The primary ingredient in policosanol is 1-octacosanol. Octacosanol and policosanol influence lipid metabolism, limit fat storage and enhance cholesterol metabolism. By altering the expression of genes associated with lipid metabolism, such as AMPK, PPAR, SREBP1, SIRT1, ACC, and FASN, octacosanol may suppress these gene expressions and potentially have a significant effect on decreasing lipid levels. An enzyme called AMPK, or 5' adenosine monophosphate-activated protein kinase, is concerned with the absorption and oxidation of fatty acids as part of cellular energy regulation. It is a key regulator of energy metabolism that has the ability to decrease ATP-consuming activities while increasing ATP regeneration. Numerous biological processes, including the pathological processes of hyperlipidemia, obesity and diabetes, are mediated by AMPK. (PPARs) Peroxisome proliferator-activated receptors are implicated in lipid metabolism. Sterol regulatory element-binding proteins aid in increasing the synthesis of the enzymes necessary for the manufacture of sterols. By inhibiting SREBP-1 activation, AMPK can lower the transcriptional expressions of enzymes linked to TG production and fatty acid metabolism. Acetyl-CoA carboxylase (ACC), is an enzyme that supplies malonyl-CoA substrate needed for fatty acid production. Fatty acid synthase, or FASN, is a multienzyme protein that facilitates the production of fatty acids. AMPK can be activated by SIRT1 or sirutin-1, which can lessen fat buildup in liver tissues. ACC inhibition, SREBP activation, and AMPK activation improve hyperlipidemia treatment. By a number of mechanisms, octacosanol may help reduce hyperlipidemia and the lipid-lowering effect.[16]

A method of extracting and purifying 1-octacosanol from sugarcane raw, also referred to as press mud, using SCFE and novel ideas of solid-solid and liquid-liquid extraction,

Table 1: List of drugs on the basis of mechanism of action

Category	*Drugs*	*MOA*	*Side effects*
HMG CoA reductase inhibitors	Lovastatin Rosuvastatin Pravastatin Atorvastatin Simvastatin	Inhibit the HMG CoA reductase enzyme (key enzyme) for the synthesis of cholesterol. (Rate limiting step)	Headache, fatigue, Muscle or joints pain Myopathy, Rhabdomyolysis
Fibrates (Fibric acid derivatives) (LPL activators)	Gemfibrozil Bezafibrate Fenofibrate	Activation of lipoprotein lipase (LPL) enzyme, release of fatty acid from adipose tissues.	Myopathy, arrhythmia skin rashes and gallstones.
Bile acid sequestrants	Cholestyramine Colestipol	LDL receptors on hepatocytes, Bile acid absorption, hepatic conversion of cholesterol to bile acids.	GI disturbances – bloating and constipation, nausea, flatulence
Sterol absorption inhibitors	Ezetimibe	Prevent the intestinal absorption of cholesterol by targeting the Niemann pick C1-like1 protein.	Headache, abdominal pain and diarrhea

which increases satisfaction and decreases impurity and is an additional benefit during the purification stage; additionally, the use of green solvent rather than any carcinogenic solvent, which shortens processing times, increases content and is environmentally friendly.[17]

MATERIAL AND METHODS

1- Octacosanol formulation was a gift sample from Ennature Biopharma Ltd., Dehradun,[18] and atorvastatin was procured from Zydus Pvt. Ltd., Gujarat.

Female Albino Wistar rats (200–250 g) were selected as the experimental subjects for this study. Every experiment was carried out in compliance with the policies and guidelines established by the Department of Pharmacology's Institutional Animal Ethics Committee, Parul Institute of Pharmacy and Research and approved by the committee for control and supervision of experiments on animals.

Housing and Feeding Condition

Rats will be housed in polypropylene cages at 22 ± 3°C/30 to 70%RH, Light cycle: 12 hr light/12 hr dark. The housing of animals should be done 7 days prior to the test. Feeding can be done using conventional laboratory meals and an unlimited supply of water.

Experimental Design

Female Albino Wistar rats were obtained and acclimatized for one week and fed to a normal diet. Following the acclimation phase, the animals were weighed and split into 4 groups (n = 6/group). All groups are given a high-fat diet for 28 days in order to induce hyperlipidemia, with the exception of a control group. After the completion of the induction period, blood samples were obtained via the retro-orbital route for lipid profile assessment. Based on their lipid levels, animals were distributed into control groups:

Group 1: Normal: Laboratory rat pellet diet *ad libitum*
Group 2: Disease: High-fat diet by oral route
Group 3: Standard: High-fat diet +Atorvastatin (10mg/kg) by oral route
Group 4: Treatment group: High-fat diet +1-Octacosanol Formulation by oral route.

For a total 56 days, every animal group received the previously mentioned therapy. Days 0,28 & 56 were designated for lipid profile assessment.

The blood samples were together by retro-orbital puncture, and serum was split for 20 minutes at 4300 rpm in order to conduct biochemical assays for Cholesterol, LDL, HDL, and triglyceride. Several oxidative indicators, such as GSH, MDA and SOD, were performed using liver tissue homogenate, which was preserved in 10% formalin for the purposes of histological examination.

Evaluation Parameters

Food intake and body weight

Every animal's daily food intake was meticulously recorded, and every animal's body weight was determined once a week using an accurate weighing scale.

Biochemical estimation

The animal blood samples were together via retro-orbital puncture & serum was divided for 15 minutes at 4300 rpm for biochemical tests of Cholesterol, LDL, HDL, and TG levels by their particular colorimetric kits and a UV-visible spectrophotometer.

Cholesterol Level Estimation

Principle

Hydrogen peroxide is formed when cholesterol oxidase oxidizes cholesterol, which is produced by hydrolyzing cholesterol esters. In the presence of phenol & peroxide, 4-aminoantipyrine & hydrogen peroxide combine to generate the indicator quinone imine. The amount of cholesterol present in the sample determines the quantity of quinoneimine (red) dye is absorbed (Table 2).

Reagents

Reagent I: Cholesterol
Cholesterol standard: 200 mg/dL

Calculation

$$\text{Cholesterol mg/dL} = \frac{\text{Abs. of Sample (AT)}}{\text{Abs. of Standard (AS)}} \times \text{Standard Conc. (200)}$$

Low-density lipoprotein (LDL) Estimation

Principle

A consistent way to measure LDL-C levels in serum or plasma is the LDL Direct Cholesterol test. The process uses two reagents and is dependent on a special detergent's characteristics. Reagent 1 is the detergent that alone solubilizes non-LDL lipoprotein particles. In a reaction devoid of color formation, cholesterol esterase & cholesterol oxidase metabolize the liberated cholesterol. Reagent 2 is the second detergent that solubilizes the leftover LDL particles & chromogenic coupler facilitates the creation of color. When the coupler is present, the enzyme's interaction with LDL-C yields a color that corresponds to the quantity of LDL present in the sample (Table 3).

Reagent 1: Enzyme Reagent
Reagent 2: Developer reagent
Calibrator: (Lyophilized) Human serum

Calculation

$$\text{LDL Chol (mg/dL)} = \frac{\text{(A2-A1) of Unknown}}{\text{(A2-A1) of calibrator}} \text{ X Calibrator value}$$

High-density lipoprotein (HDL) estimation

Principle

The presence of magnesium ion phosphor tungstate precipitates the LDL & VLDL lipoproteins from serum. The HDL-containing cleared supernatant, which is obtained by centrifugation, is used to measure HDL cholesterol levels (Table 4).

Reagent

Reagent: HDL-C Precipitating reagent
HDL cholesterol standard: 50 mg/dL

Calculation

$$\text{HDL Chol (mg/dL)} = \frac{\text{(Abs) of sample}}{\text{(Abs) of standard}} \text{ X standard value x 2}$$

Where 2 is the serum dilution factor.

Triglycerides Estimation

Principle

Triglycerides are quantified through an enzymatic hydrolysis reaction involving lipases. The formation of quinonemine indicator from H_2O_2, 4-aminophenazone, and 4-chlorophenol is catalyzed by peroxidase (Table 5).

Table 2: Procedure

Aspirate the volume of serum and reagent as given in the table below	
Test sample	10 µL
Standard sample	10 µL
Reagent sample	1000 µL
Combination & incubate for 5 to 15 minute at 37°C. Stabilized for 30 minutes at RT.	
Quantify the absorbance of the sample (AT) & standard (AS) against reagent blank at 505 nm wavelength.	

Reagent

Reagent l: Triglycerides reagent
Triglycerides Standard: 200 mg/dL

Calculation

$$\text{Triglycerides mg/dL} = \frac{\text{Abs. of Sample (AT)}}{\text{Abs. of Standard (AS)}} \text{ x Standard Value (200mg/dL)}$$

Tissue Preparation and Storage

Following removal, the liver was gently blotted on ash-free filter paper & cleansed of tissue debris using phosphate-buffered saline. Unless stated otherwise, liver tissues were homogenized using a 1:3 w/v solution of tris-HCl buffer. Clear supernatants were obtained by centrifuging the crude homogenates for 15 minutes at 8000 rpm & 4°C. Subsequently, aliquots were stored at 20°C till additional analysis.

Assessment of Lipid Peroxidation

Principle

The Ohkawa method was applied to determine the lipid peroxidation amount in liver tissue, expressed in terms of MDA. Lipid peroxidation induced by ROS is acknowledged as a harmful process implicated in various acute & chronic diseases. Currently, the thiobarbituric acid assay (TBA test) is the most widely used method for detecting lipid peroxidation products (Table 6). It is predicated on the formation of a red adduct via the interaction of TBA with MDA, a by-product of lipid peroxidation (Table 7). This test also relies on a condensation reaction between one MDA molecule and two TBA molecules, with temperature, pH, and TBA concentration influencing the reaction's rate.

Assessment of reduced glutathione (GSH)

Ellman method was used to determine the GSH level

Table 3: Procedure

Aspirate the volume of serum and working reagent as given in the table below	
Reagent 1	750 µL
Calibrator/Sample	10 µL
Mix & incubate for 5 minutes at 37°C and immediately add reagent 2	
Reagent 2	250 µL
After 5 minutes at 37°C, read the absorbance (Ac) for the calibrator and absorbance (As) for the sample.	

Table 4: Procedure

Aspirate the volume of serum and reagent as given in the table.	
Blank	1000 µL
Sample/Standard	50 µL
Reagent	1000 µL
Mix & incubate for 5 minutes at 37°C.	
Read the sample and standard absorbance at 505 nm against the reagent blank.	

Table 5: Procedure

Aspirate the volume of serum and reagent as given in the table.	
Test sample	10 µL
Standard sample	10 µL
Reagent	1000 µL
Mix & incubate for 05 minutes at 37°C	
Measure the absorbance at 505 nm	

Principle

Glutathione is a naturally occurring antioxidant found in almost all cells in the body. It helps the body detoxify medications and xenobiotics and is also used as a supplement to relieve cancer-related cachexia (Table 8). Glutathione is a tripeptide that is made up of cysteine, glutamic acid, and glycine. It is essential for the regulation of detoxification, signaling, and other cellular functions. Glutathione disulfide (GSSG) is a glutathione oxidized form of glutathione that is converted to GSH when NADPH is present. Glutathione reductase (GR) converts glutathione to GSH. Glutathione peroxidase (GP) is the enzyme that converts hydrogen peroxide into water.

Assessment of superoxide dismutase (SOD)

Misra and Fridovich's method calculated SOD enzymatic action.

Principle

This test is based on the theory that O2 can react with NBT to cause the yellow tetrazolium in the gel to precipitate as blue. In SOD-active areas, achromatic bands develop & compete for O2 with NBT (Table 9).

Additionally, it is based on the generation of Superoxide anion radicals by xanthine oxidase and xanthine, which quantitatively react with 2-(4-iodophenyl) - 3 - (4-nitrophenol) to produce red formazan dye. 5- Tetrazolium chloride phenyl. SOD obstructs the process by turning the superoxide radical into oxygen (Table 10).

Histopathological Analysis

Upon completion of the study, the animals were sacrificed to obtain the liver. The organ was rinsed with a pH 7 buffer solution and then preserved in a 10% buffered formalin solution for 24 hours. Following this period, the organs were sent to Sakshi Laboratories, Vadodara, for histopathological evaluation.

Table 6: Reagents

S. No	*Name*	*Concentration*	*Preparation*
1.	Sodium Dodecyl Sulphate	8.1%	8.1 g SDS in 100 mL water
2.	Acetic acid (3.5 pH)	20%	20 mL Acetic acid in 100 mL water
3.	Aqueous solution of TBA	0.8%	0.8 g TBA in 100 mL water
4.	n-Butano and pyridine fusion	15:1 v/v	90 mL n-butanol + 6 mL pyridine

Table 7: Procedure

Blank	*Test*
0.2 mL of 8.1% SDS	0.2 mL of 8.1% SDS
1.5 mL of 20% acetic acid (pH 3.5)	1.5 mL of 20% Acetic acid (pH 3.5)
1.5 mL of 0.8% TBA	1.5 mL of 0.8% TBA
0.2 mL Distilled water	0.2 mL Crude liver homogenate
Raised volume up to 4 mL	Raised volume up to 4 mL
The mixture is heated for 1-hour at 95°C, resulting in a pink coloration. It is then allowed to cool to room temperature.	
A mixture of 5 mL of n-butanol and pyridine (15:1 v/v) was added to solution & vortexed.	
Centrifuged for 10 minutes at 8000 rpm	
Absorbance of organic layer was calculated at 532 nm	

Statistical Test

For all statistical studies, GraphPad Prism 9.0.0 was used. One-way ANOVA was used to calculate the data, and then Bonferroni Multiple Comparison Test was performed. The mean ± SEM was used to express the results. A *p-value* < 0.05 was used to conclude statistical significance.

RESULTS AND DISCUSSION

Body Weight

Comparing the DC group to Normal, a substantial rise in body weight was observed in Table 11.

When comparing DC group to the test group (1-octacosanol 500 mg/kg) and the standard group (Atorvastatin 10 mg/kg), a substantial drop in body weight was seen.

These findings imply that there was a significant effect on body weight from both the usual dose and treatment doses Figure 3.

All standards are communicated as Mean ± SEM (n = 6) by ANOVA using multiple comparison test.

Effect of treatment on Total Cholesterol (TC) level

The DC group showed a considerable increase in TC level when compared to the normal group (p <0.01) (Table 12).

There is a significant decrease in TC was seen when comparing the standard group (Atorvastatin 10 mg/kg), test group (1-octacosanol 500 mg/kg) with DC group (p <0.001) and (p <0.001) respectively, Figure 4.

These outcomes propose standard dose and treatment doses had a considerable impact on TC levels.

All numbers are referred as mean ± SEM (n = 6). Statistical was executed by ANOVA followed by multiple comparison test to compare each graph to Group 2 (Disease Control). Significance levels were denoted as ns (no significance), * (p <0.05), ** (p <0.01) and *** (p < 0.001).

Every number is referred to as mean ± SEM (n = 6). Experimental control groups were as follows: 1 (Normal), 2 (Disease), 3 (Standard) and group (Test).

Table 8: Reagents

S. No.	*Name*	*Concentration*	*Preparation*
1.	Phosphate buffer	pH 8	Ice-cold phosphate buffer
2.	Trichloroacetic acid (TCA)	10%	10 g TCA in 100 mL water
3.	Sodium Citrate	1%	1 g sodium citrate in 100ml water
4.	Dithiobis nitro benzoic acid (DTNB)	-	40 mg in 100 mL of 1% Sodium citrate and coat with aluminum foil.
Blank		Test	
-----		Take homogenate	
-----		Add 0.1 mL of 10% TCA	
Centrifuged for 10 minutes at 3000 rpm at 25°C			
-----		1-mL of supernatant	
2 ml of Phosphate buffer		1-mL of phosphate buffeL	
1-mL of DTNB reagent			
Mix it well and vortexed it thoroughly for 1 minute			
Incubate it for 5 minutes at RT			
Absorbance was measured at 412 nm			

Table 9: Reagents

Name	*Concentration*	*Preparation*
EDTA	0.0001M	9.3 g in 250 mL water
Carbonate buffer	pH 9.7	8.4 g NaHCO3 + 10.6 g Na_2CO3 in H2O
Epinephrine	0.003M	50 mg/100 mL in 2 pH HCL Cover it with aluminum foil

Table 10: Procedure

Blank	*Test*
100 µL D.W.	100 µL homogenate
0.15 mL ice-cold chloroform & 0.75 mL ethanol and centrifuge at 3000 rpm at 25°C for 10 minutes.	
0.5 mL supernatant + 0.5 mL EDTA + 1-mL carbonate buffer + 0.5 mL epinephrine	
Absorbance was measured against a blank at 30-second intervals over a duration of 3 minutes at 480 nm.	

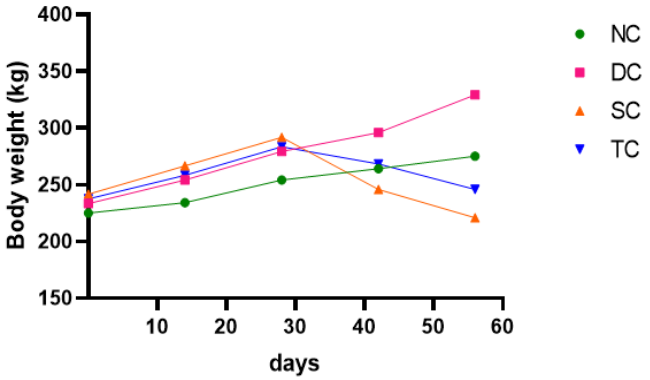


Figure 3: Result of treatment on body weight

Effect of treatment on Triglycerides (TG) level

The DC group presented an important increase in TG level when related to a normal control group (p <0.01) (Table 13). There is a significant decrease in TG was seen when comparing the standard group (Atorvastatin 10 mg/kg), and test group (1-octacosanol 500 mg/kg) with DC group (p < 0.01) and (p <0.001) respectively, Figure 5.

These outcomes recommend the standard dose and treatment doses had a considerable impact on TG levels.

All numbers are uttered as mean ± SEM (n = 6). Statistical was made by ANOVA followed by Multiple comparison test to compare each graph to Group 2 (Disease Control). Significance levels were denoted as NS (no significance), * (p < 0.05), ** (p <0.01) and *** (p <0.001).

Every value are communicated as mean ± SEM (n = 6). The experimental groups were as follows: 1 (Normal), 2 (Disease), 3 (Standard) and group (Test control).

Effect of treatment on Low-density lipoproteins (LDL)

The DC showed a significant increase in LDL level when associated to a normal control group (p <0.01) (Table 14).

There is a significant decrease in LDL was seen when comparing the standard group (Atorvastatin 10 mg/kg), and test group (1-octacosanol 500 mg/kg) with DC group (p < 0.01) and (p < 0.001), respectively, Figure 6.

These results advise that the standard dose and treatment doses had a considerable impact on LDL levels.

Every number are stated as mean ± SEM (n = 6). Statistical was executed by ANOVA followed by Multiple comparison tests to compare each graph to group 2 (Disease Control). Significance stages were denoted as NS (no significance), * (p < 0.05), ** (p < 0.01) and *** (p < 0.001).

Entire values are conveyed as mean ± SEM (n = 6). Experimental control groups were as follows: 1 (Normal), 2 (Disease), 3 (Standard) and group (Test).

Table 11: Mean value of body weight at Day 0, 28 & 56

Group	*Treatment*	*Body weight Mean ± SEM Day 0*	*Body weight Mean ± SEM Day 28*	*Body weight Mean ± SEM Day 56*
Group 1	Laboratory rat pellet diet	225 ± 6.45	254 ± 4.16	275 ± 6.45
Group 2	High fat diet (HFD)	233 ± 5.27	279 ± 4.16	329 ± 7.68
Group 3	HFD + Atorvastatin 10mg/kg	241 ± 5.27	291 ± 5.27	220 ± 7.68
Group 4	HFD+ 1-Octacosanol 500mg/kg	237 ± 5.59	283 ± 5.27	245 ± 7.68

Each value are uttered as mean ± SEM (n = 6). The experimental control groups were as follows: 1 (Normal), 2 (Disease), 3 (Standard) and group (Test).

Table 12: Mean value of TC at day 0, 28 & 56

Group	*Treatment*	*TC Mean ± SEM Day 0*	*TC Mean ± SEM Day 28*	*TC Mean ± SEM Day 56*
Group 1	Laboratory rat pellet diet	85.83 ± 4.94	84.33 ± 4.63	86.0 ± 4.42
Group 2	High-fat diet (HFD)	88.83 ± 3.80	245.6 ± 8.17	268.0 ± 7.32
Group 3	HFD + Atorvastatin 10 mg/kg	89.33 ± 3.32	249.6 ± 7.64	181.1 ± 5.15
Group 4	HFD+ 1-Octacosanol 500 mg/kg	87.50 ± 2.46	248.6 ± 5.63	149.8 ± 5.90

Table 13: Mean values of TG levels at Day 0,28 & 56

Group	*Treatment*	*TG Mean ± SEM Day 0*	*TG Mean ± SEM Day 28*	*TG Mean ± SEM Day 56*
Group 1	Laboratory rat pellet diet	87.50 ± 4.02	85.66 ± 4.97	88.00 ± 3.78
Group 2	High-fat diet (HFD)	88.66 ± 3.87	216.5 ± 7.53	237.1 ± 1.07
Group 3	HFD + Atorvastatin 10 mg/kg	87.16 ± 3.42	220.5 ± 6.17	157.6 ± 3.39
Group 4	HFD + 1-Octacosanol 500 mg/kg	85.00 ± 4.69	221.0 ± 6.42	119.8 ± 2.83

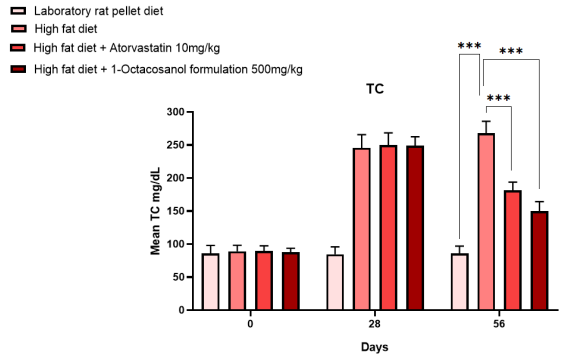


Fig. 4: Effect of treatment on Total Cholesterol

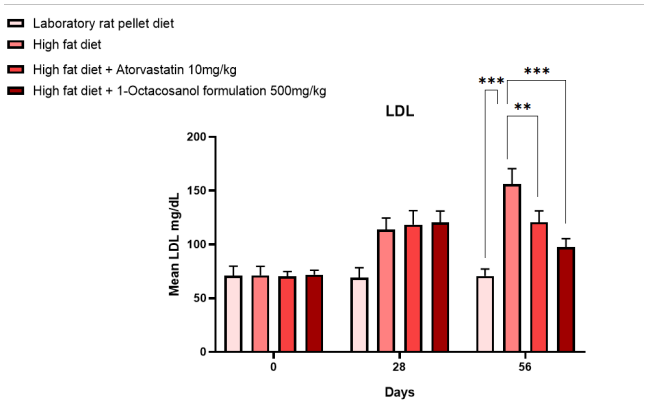


Figure 6: Effect of treatment on LDL levels

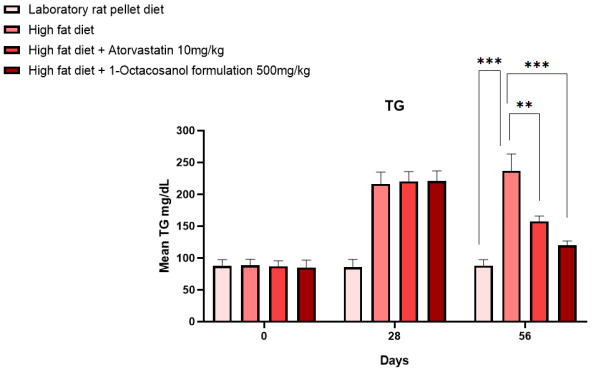


Fig. 5: Effect of treatment on TG levels

Effect of Treatment on Very low-density lipoproteins (VLDL)

The DC presented a considerable increase in VLDL level when evaluated to a normal control group (p <0.01) (Table 15).

There is a significant decrease in VLDL was seen when comparing the standard group (Atorvastatin 10 mg/kg), test group (1-octacosanol 500 mg/kg) with DC group (p < 0.01) and (p < 0.001) respectively, Figure 7.

These outcomes suggest standard dose and treatment doses had a considerable impact on VLDL levels.

Every number is uttered as mean ± SEM (n = 6). Statistical was used by ANOVA followed by Multiple comparison tests to

Table 14: Mean value of LDL levels at Day 0, 28 & 56

Group	*Treatment*	*LDL Mean ± SEM Day 0*	*LDL Mean ± SEM Day 28*	*LDL Mean ± SEM Day 56*
Group 1	Laboratory rat pellet diet	70.83 ± 3.63	69.16 ± 3.70	70.50 ± 2.66
Group 2	High-fat diet (HFD)	71.16 ± 3.44	113.6 ± 4.41	156.0 ± 5.87
Group 3	HFD + Atorvastatin 10mg/kg	70.16 ± 1.85	118.3 ± 5.32	120.6 ± 4.29
Group 4	HFD+ 1-Octacosanol 500mg/kg	71.83 ± 1.70	120.3 ± 4.37	97.33 ± 3.28

Table 15: Mean value of VLDL levels at Day 0,28 & 56

Group	Treatment	VLDL Mean ± SEM Day 0	VLDL Mean ± SEM Day 28	VLDL Mean ± SEM Day 56
Group 1	Laboratory rat pellet diet	17.50 ± 0.80	17.13 ± 0.99	17.60 ± 0.75
Group 2	High-fat diet (HFD)	17.73 ± 0.77	43.30 ± 1.50	47.43 ± 2.14
Group 3	HFD + Atorvastatin 10 mg/kg	17.43 ± 0.68	44.10 ± 1.23	31.53 ± 0.67
Group 4	HFD+ 1-Octacosanol 500 mg/kg	17.11 ± 0.90	44.20 ± 1.28	23.96 ± 0.56

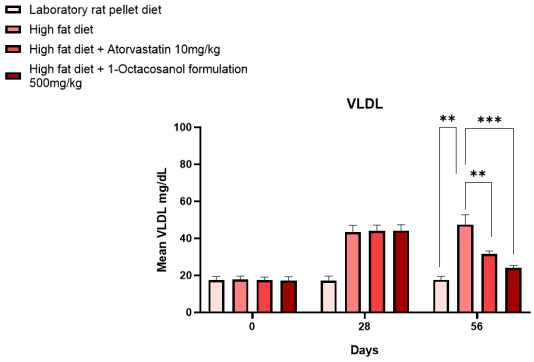


Figure 7: Effect of treatment on VLDL

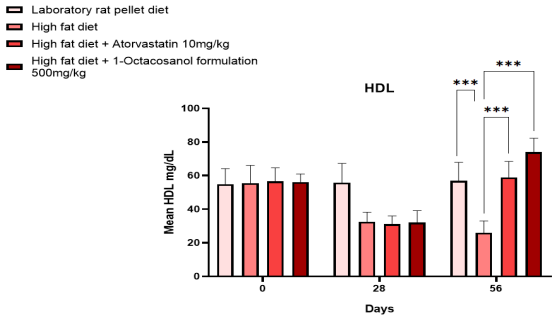


Figure 8: Effect of treatment on HDL levels

compare each graph to Group 2 (Disease Control). Significance levels were denoted as NS (no significance), * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$).

All numbers are expressed as mean ± SEM (n = 6). The experimental groups were as follows: 1 (Normal), 2 (Disease), 3 (Standard) and group (Test Control).

Effect of Treatment on High-density lipoproteins (HDL)

The DC showed a noteworthy decrease in HDL level when balanced with a normal control group ($p < 0.01$).

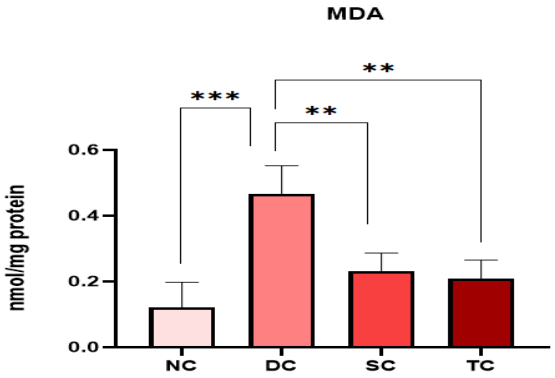


Fig. 9: Effect of treatment on MDA level

A significant increase in HDL was seen when comparing the standard group (Atorvastatin 10 mg/kg) (Table 16), test group (1-octacosanol 500 mg/kg) with the DC group ($p < 0.001$) and ($p < 0.001$), respectively, Figure 8.

These outcomes recommended standard dose and treatment doses had a considerable impact on HDL levels.

All numbers are uttered as mean ± SEM (n = 6). Statistical was achieved by ANOVA followed by Multiple comparison tests to compare each graph to group 2 (Disease Control). Significance levels were denoted as ns (no significance), * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$).

Every number is articulated as mean ± SEM (n = 6). Experimental Control groups were as follows: 1 (Normal), 2 (Disease), 3 (Standard), and group (Test Control).

MDA level (Malondialdehyde)

The disease control group (DC) displayed a considerable increase in MDA level when associated to the normal group ($p < 0.001$) (Table 17).

There is a significant decrease in MDA was seen when comparing the standard group (Atorvastatin 10 mg/kg), test

Table 16: Mean values of HDL at Day 0, 28 & 56

Group	*Treatment*	*HDL Mean ± SEM Day 0*	*HDL Mean ± SEM Day 28*	*HDL Mean ± SEM Day 56*
Group 1	Laboratory rat pellet diet	54.96 ± 3.76	55.83 ± 4.67	56.83 ± 4.51
Group 2	High-fat diet (HFD)	55.50 ± 4.32	32.50 ± 2.34	26.00 ± 2.85
Group 3	HFD + Atorvastatin 10 mg/kg	56.66 ± 3.24	31.16 ± 1.93	59.00 ± 3.84
Group 4	HFD+ 1-Octacosanol 500 mg/kg	56.16 ± 1.95	32.00 ± 2.92	74.00 ± 3.38

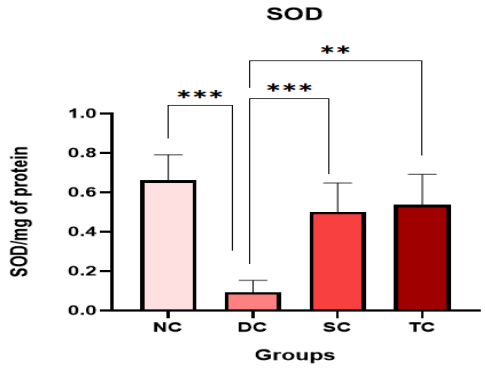


Fig. 10: Outcome of treatment on SOD levels

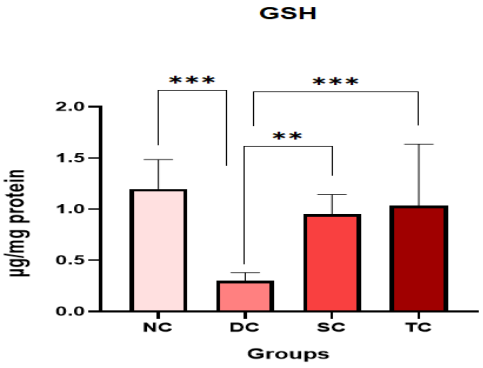


Fig. 11: Result of treatment on GSH levels

group (1-octacosanol 500 mg/kg) with DC group ($p < 0.01$) and ($p < 0.01$) respectively, Figure 9.

These outcomes propose standard dose and treatment doses had a considerable impact on MDA levels.

All numbers are articulated as mean ± SEM (n = 6). The experimental groups were as follows: 1 (Normal), 2 (Disease), 3 (Standard) and group (Test Control). Statistical was done by ANOVA followed by Multiple comparison tests to compare each graph to group 2 (Disease Control). Significance levels were denoted as ns (no significance), *($p < 0.05$), **($p < 0.01$) and ***($p < 0.001$).

Every number are stated as mean ± SEM (n = 6). Experimental groups were as follows: 1 (Normal), 2 (Disease), 3 (Standard) and group (Test control).

SOD level (Superoxide dismutase)

The DC showed a significant decrease in SOD stage when related to a normal control group ($p < 0.001$).

There is significant increase in SOD was seen when comparing the standard group (Atorvastatin 10 mg/kg)

Table 17: MDA levels at Day 56

Group	*Treatment*	*MDA Mean ± SEM Day 56*
Group 1	Laboratory rat pellet diet	0.1216 ± 0.076
Group 2	High-fat diet (HFD)	0.4665 ± 0.856
Group 3	HFD + Atorvastatin 10 mg/kg	0.2325 ± 0.053
Group 4	HFD+ 1-Octacosanol 500 mg/kg	0.2093 ± 0.055

Table 18: SOD levels at day 56

Group	*Treatment*	*SOD Mean ± SEM Day 56*
Group 1	Laboratory rat pellet diet	0.662 ± 0.128
Group 2	High-fat diet (HFD)	0.093 ± 0.059
Group 3	HFD + Atorvastatin 10 mg/kg	0.501 ± 0.145
Group 4	HFD+ 1-Octacosanol 500 mg/kg	0.536 ± 0.155

(Table 18), test group (1-octacosanol 500 mg/kg) with DC group ($p < 0.001$) and ($p < 0.01$) respectively, Figure 10.

These outcomes advise standard dose and treatment doses had a considerable impact on SOD levels.

Every number is stated as mean ± SEM (n = 6). Experimental groups were as follows: 1 (Normal control), 2 (Disease), 3 (Standard) and group (Test). Statistical was achieved by ANOVA using multiple comparison tests to evaluate each graph to group 2 (Disease Control). Significance stages were denoted as ns (no significance), * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$).

All standards are uttered as mean ± SEM (n = 6). The experimental groups were as follows: 1 (Normal), 2 (Disease), 3 (Standard) and group (Test Control).

GSH level (Glutathione)

The DC showed a significant decrease in the GSH stage when associated to the normal group ($p < 0.001$).

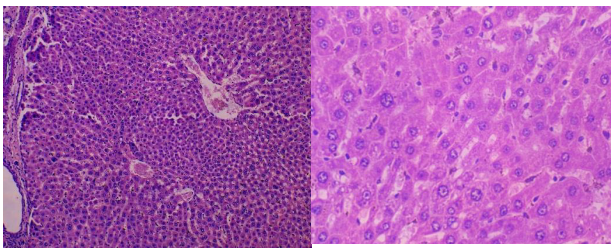
There is a significant decrease in GSH was seen when comparing the standard group (Atorvastatin 10 mg/kg) (Table 19), test group (1-octacosanol 500 mg/kg) with DC group ($p < 0.01$) and ($p < 0.01$) respectively, Figure 11.

These results suggest the standard dose and treatment doses had a considerable impact on GSH levels.

All numbers are communicated as mean±SEM (n = 6). Experimental groups were as follows: 1 (Normal), 2 (Disease), 3 (Standard) and group (Test Control). Statistical was achieved

Table 19: GSH levels at Day 56

Group	*Treatment*	*GSH Mean ± SEM Day 56*
Group 1	Laboratory rat pellet diet	1.197 ± 0.286
Group 2	High-fat diet (HFD)	0.299 ± 0.081
Group 3	HFD + Atorvastatin 10 mg/kg	0.949 ± 0.193
Group 4	HFD+ 1-Octacosanol 500 mg/kg	1.034 ± 0.599



A B

Figure 12: High-power microphotographs of rat liver show lipid infiltration compared test group; B with disease group A.

by ANOVA followed by multiple comparison test to compare each graph to group 2 (Disease control). Significance points were signified as NS (no significance), * (p <0.05), ** (p < 0.01) and *** (p < 0.001).

Every number are expressed as mean ±SEM (n = 6). Experimental groups were as follows: 1 (Normal), 2 (Disease), 3 (Standard) and group (Test).

Histology Analysis of the Liver

Histopathological examinations of organs from both the disease control and test groups revealed notable findings. Specifically, liver samples of the disease control group exhibited lipid infiltration. (Figure A) Rats receiving a high-fat diet (DC) displayed a trend towards a higher proportion of hepatic fat cells associated with the normal control (NC) group.

In comparison to DC, the test group (TC) exhibited a notable reduction in the quantity of lipid droplets. (Figure B) These findings propose that 1-octacosanol formulation has the potential to ameliorate pathological alterations in the livers of model rats, decrease hepatic fat accumulation, and mitigate liver damage tempted by a high-fat diet, Figure 12.

DISCUSSION

Elevated lipid levels in the bloodstream indicate hyperlipidemia, which significantly raises the possibility of cardiovascular illnesses. A high-fat diet is known to cause an elevated amount of triglycerides, cholesterol, and lipoproteins, all of which can contribute to this pathological state. This research aimed to replicate the human situation of diet-induced lipid abnormalities in rats by inducing hyperlipidemia through a high-fat diet. In an effort to evaluate the 1-octacosanol's therapeutic potential, a test formulation was given to these hyperlipidemic rats with the goal of determining whether this could reduce the diet-induced elevation of lipid levels.

Subsequently, an *in-vivo* study was conducted to corroborate the thesis findings. Healthy female Wistar rats were employed for this investigation. The experimental design comprised four groups: 1 (Normal), 2 (Disease), 3 (Standard), and 4 (Test Group), which received the 1-octacosanol formulation. Hyperlipidemia was induced using a high-fat diet. Following a 28-day induction period, the treatment was administered for an additional 28 days.

Upon completion of the study, a comprehensive evaluation of various parameters was conducted. These included body weight and lipid profile metrics such as LDL, TC, TG, VLDL, and HDL. In addition, antioxidant properties were assessed, including malondialdehyde (MDA), superoxide dismutase (SOD), and GSH. Since oxidative stress is recognized to be a key factor in the pathophysiology of hyperlipidemia, the possible contribution of antioxidant capabilities to the lipid-lowering actions of 1-octacosanol was also taken into consideration.

Upon the conclusion of the experiments, the results demonstrated that supplementation with the 1-octacosanol formulation led to a reduction in body weight and improvements in the lipid profile, evidenced by decreases in levels of TG, TC, LDL, and VLDL, together with an increase in HDL. The formulation also exhibited notable antioxidant properties, which could further aid in the management of hyperlipidemia. All values were stated as mean ± SEM (n = 6). Statistical was made using ANOVA, using a multiple comparison test to associate each group with Group 2 (Disease Control). Significance stages were shown as follows: NS (no significance), * (p <0.05), ** (p < 0.01), and *** (p < 0.001).

In conclusion, the *in-vivo* study's results support the 1-octacosanol formulation's therapeutic potential for the treatment of hyperlipidemia. Its noteworthy antioxidant and antihyperlipidemic characteristics highlight its potential effectiveness in treating hyperlipidemic disorders.

CONCLUSION

In conclusion, the test formulation of 1-octacosanol demonstrated a significant antihyperlipidemic effect in rats with high-fat diet-induced hyperlipidemia. The treatment effectively reduced the serum stage of total cholesterol, LDL, triglycerides, cholesterol and VLDL cholesterol while increasing HDL cholesterol levels. Results suggest that 1-octacosanol has potential as a therapeutic agent for managing hyperlipidemia, a key risk factor for cardiovascular diseases. Though, advance studies are needed to know the precise mechanisms of action, optimal dosage, and long-term effects of 1-octacosanol. Additionally, clinical trials are necessary to evaluate its safety and efficacy in human subjects before it can be considered for clinical use

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