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RESEARCH ARTICLE

SMART THERAPEUTIC ANTIDYSLIPIDEMIC NATURE OF POLICOSANOL IN MINIMAL DOSING LEVEL

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ABSTRACT

Policosanol is a mixture of higher aliphatic primary alcohol extracted from sugar cane wax with hypolipidemic activity. It protects cardiovascular morbidity. This study was conducted to determine Antihyperlipidemic activity of Policosanol in Albino Rats (Rattus norvegicus) at low dose levels. Hyperlipidemia was induced in rats (by Triton) then Antihyperlipidemic activity of Policosanol was observed. Animals were divided into 6 Groups, Vehicle Control (Hyperlipidemic animals treated with Distilled Water as Vehicle), Standard Simvastatin (10mg) Simlep-FC cipla, 1mg/kg, 5mg/kg, 10mg/kg, 20mg/kg Policosanol Test Groups and doses were given accordingly. *In-Vitro*, *In-Vivo* study was conducted on experimental animals and estimated Total Cholesterol and HDL Cholesterol Concentrations. Results were compared with that of Simvastatin. Policosanol showed significant reduction in Cholesterol level in Triton X-100 LR grade induced Hyperlipidemic Albino Rats and results were comparable to group of animals treated with Standard Simvastatin 10mg. It was concluded that Policosanol (1mg/kg) was optimum dose for Hypocholesteremic activity above or below which dose may cause variation in response. Results revealed that Policosanol showed significant reduction in Cholesterol level in triton induced Hyperlipidemia animals and results were comparable to the Standard Simvastatin.

INTRODUCTION

Hyperlipidemia is a medical condition characterized by an elevation of lipoproteins in the blood. It is dangerous as the extra cholesterol circulating in the blood stream forms the basis of plaque lining the arteries, which slows the blood flow through the arteries. Coronary artery disease can result in angina or a heart attack. Saturated carbon structure makes Policosanol hydrophobic in nature. Policosanol produces a dose-dependent and significant reduction of serum total cholesterol and LDL-C Concentrations. HDL-C values also increase in a dose-dependent manner. [1] Triglycerides also get significantly reduced. Policosanol's effect on cholesterol is through reduction in synthesis and degradation is rate-limiting step of cholesterol biosynthesis, enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase. Policosanol causes significant reduction of serum total cholesterol, LDL-C and Triglycerides. It is a safe lipid-lowering agent for managing of Hyperlipidemia. Policosanol has lipoprotein-lowering effects comparable with statins. [2] The current objective of the research investigation is to screen hypolipidemic potentiality of Policosanol at low dose in combination with Dextrose dispersion.

MATERIALS AND METHODS

Chemicals:

Policosanol sample was obtained from India Glycols Ltd. (division: Ennature Biopharma), Noida & Dehradun, India. Triton X-100 LR grade from Sigma Aldrich, cholesterol reagents, cholesterol standard, HDL precipitation reagent obtained from Bio lab diagnostics.1F.NO.20.Industy East Rost Road Iv. Science-Based Industrial Park, Hsinchu, Taiwan 300, Lipidometer, Easy Touch GCU Multi-Function Monitoring System, Bioptik Technology.

Research methodology *invitro* hypolipidemic activity of policosanol:

Principle:

Iodine forms a Pink colour chromophore with Cholesterol showing absorbance at λmax 520nm. Cholesterol solution was treated with predetermined concentration of Policosanol/ Simvastatin and subjected for incubation at 37°C, the reduction in Cholesterol was determined upon treatment with the various reagents followed by measurement of Absorbance at 520nm. Later on reduction in Cholesterol concentration was calculated,

% Reduction in Absorbance was compared with Standard. [3]

Procedure:

1mg/ml stock solution of policosanol was prepared using Chloroform as solvent and 1ml of Stock Solution diluted to 10ml. Aliquots of 0.1, 0.2, 0.4 and 0.5 ml taken and diluted to 10ml with Chloroform. To this 1.5ml iodine solution was added and 1ml of 0.1% w/v of cholesterol solution was added to all above dilutions. The solutions were kept in incubator at 37ºC for 1hr in order to develop a pink chromophore. Absorbance of solutions was measured using shimadzu UV 1800, using Chloroform and Iodine as Blank Solution. Similar procedure was applied for Simvastatin drug at concentrations of 0.01, 0.02, 0.04% Solutions.

The % reduction in cholesterol was calculated by using the below formulae:

$$\% \text{ Absorbance Reduction} = \frac{\text{Absorbance of Plain Cholesterol}}{\text{Absorbance of cholesterol treated with Policosanol/ Simvastatin}} \times 100$$

Cholesterol estimation using lipidometer: [4, 5]

PRINCIPLE:

The study was conducted by administering Policosanol as well as Simvastatin (standard drug) in dose line of 1mg/kg, 5mg/kg, 10mg/kg, and 20mg/kg daily dose. The treatment was continued for a period of fifteen days and later treatment was stopped after fifteen days. All experimental animals were subjected for measuring their cholesterol Concentration at 0, 1,4,8,12,15 days. In order to see the ability of therapeutic effect of the drug the cholesterol Concentration was checked on 45th day and results were registered and compared.

Procedure:

Blood samples were taken from Tail of Rats, measured Cholesterol Concentration using Lipidometer, This study trial was performed to corroborate the reported Anti-Hyperlipidemic activity of Policosanol. As per Gouni-Berthold I. etal. The test focused on the lipid profile of Policosanol which include total plasma cholesterol, HDL Concentrations. [6, 7]

***In-vivo* hypolipidemic screening of policosanol: Preliminary high throughput *in-vivo* anti-hyperlipidemic Screening model**

Principle:

Six male Albino Rats were used in the Study. Initially Hyperlipidemia was induced to all animals by administering Triton Solution at dose Concentration of 100mg/ml/kg body

weight. All animals were allowed free access of water and feed. After 48hrs of Triton administration, Blood Concentration was determined by using Cholesterol Strips of Lipidometer.

Procedure:

Estimation of cholesterol was done in all experimental animals divided into 6 groups of vehicle control intoxicant, standard simvastatin (10mg/kg), policosanol (1mg/kg, 5mg/kg, 10mg/kg & 20mg/kg) administering Policosanol orally. Blood cholesterol level was estimated regularly for a period of 15days. Then % reduction in cholesterol was graphically plotted by taking cholesterol concentration on Y-axis and number of days on X-axis. The treatment was withdrawn after 15days. All animals were allowed to free access of water and standard diet during study period. At the end of 45th Day, again % reduction of cholesterol was estimated using lipidometer and compared. [8]

***In vivo* cholesterol determination method by colourimetry** [9]

Principle:

Cholesterol oxidizes ferric ions to a Brown coloured complex in hot acidic medium which absorbs at 520nm.

Study protocol:

Study was approved by CPCSEA, letter no. 1156/AC/07/CPCSEA dated 13th February 2008 as per Institutional Animal Ethical Committee norms. Study was conducted on both males and females albino rats, weighed, Triton Solution injected Intraperitonially to all animals, and Policosanol was given orally to each animal and their Cholesterol Concentration was measured. Triton induced Hyperlipidemia: Triton shows Hyperlipidemic Action. It physically alters very low density lipoproteins rendering them refractive to the action of lipolytic enzymes of blood and tissue. After 72 hrs. of Triton dosing, Policosanol was given for 15 days orally. [10]

Preparation of solutions & dilutions of policosanol

Preparation of Policosanol Dispersion: 5 mg of Policosanol was weighed accurately and 50 mg of Dextrose was incorporated an triturated for 3 mins, to this 10 ml of water was added and subjected to sonication at 25 KHz for 3 cycles in order to get clear dispersion. This Policosanol dispersion was screened for Hypolipidemic activity in Hyperlipidemic experimental animals and the findings were compared with a group of

animals treated with 10 mg/kg and 20 mg/kg using non-ionic surfactant Tween 80 as dispersing agent in Distilled Water.

20mg Policosanol mixed with 50 mg dextrose as dispersing agent and triturated with 100ml Distilled Water. Transferred in volumetric flask, sonicated for 5 cycles at 25 KHz until drug dissolved homogeneously in solution. This approach was used in order to verify the impact of dextrose on dispersibility of Policosanol and its therapeutic efficacy. 0.1ml equivalent to 1mg/kg body weight was administered per orally to Group III animals. Similarly 100mg Policosanol mixed to get 0.5ml equivalent to 5mg/kg body weight administered per orally to Group IV animals. 1ml equivalent to 10mg/kg body weight was then administered per orally to Group V animals. 2ml equivalent to 10mg/kg body weight was then administered per orally to Group VI animals. Similarly 400mg Policosanol mixed with 0.2ml Tween 20 and 2ml equivalent to 20mg/kg body weight was administered per orally to Group I animals 10mg/kg Standard Simvastatin (10mg) Solution: 10 tablets of Hypolipidemic drug Simvastatin 10mg were crushed in pestle mortar, added 0.1ml Tween 20 and dissolved in 50ml of Distilled Water. Mixed to get homogeneous solution.

Sample collection procedure

Blood samples were collected by retro orbital method. Albino rats were anaesthetized with Chloroform. A sterile smooth capillary tube (to avoid periorbital infection and Potential long-term eye damage) was penetrated in retro-orbital plexus. Adequate haemostasis occurred after following the procedure. Collected Blood samples were centrifuged at 4000rpm for 20mins to separate the serum to be used in the Cholesterol determination procedures. A minimum of 10 days were allowed for tissue repair before repeating sampling from same orbit else the healing process may interfere with blood flow.

Preparation of blank, standard, test solutions:

In each of 3 test tubes, labeled as Blank, Standard and Test, 5ml of Cholesterol Reagent No. 1 was taken. 0.05ml (50µL) of Distilled Water was added to blank while 0.05ml (50µL) of Standard Reagent No.2 was added to Standard and 0.05ml (50µL) of Sample was added to Test solution. Mixed well for 20secs. Kept in boiling water bath immediately for exactly 90sec (1.5mins). Cooled immediately for 5 minutes under running tap water. Measured on U.V. Spectroscopy at 520 nm

Step i: for hdl cholesterol: (precipitation)

Serum…… 0.2ml

Hdl reagentno.3……. 0.2ml

Mixed well. Kept for 10mins and centrifuged. Separated clear supernatant and Estimated Cholesterol Concentration of the supernatant as per

Step ii : for hdl cholesterol

Preparation of blank, standard, test solutions for hdl cholesterol

Determination

In each of 3 test tubes, labeled as Blank, Standard and Test, 5ml of Cholesterol Reagent No. 1 was taken. 0.2ml of HDL Reagent No.3 was added to Blank and Standard test tubes while 0.2 ml of Supernatant from Step I was added to Test solution.0.02ml (20µl) of Cholesterol Standard was added to Standard Test Tube. Mixed well for 20secs.Kept in boiling water bath immediately for exactly 90sec (1.5mins). Cooled immediately for 5mins under running tap water. Measured U.V. Spectroscopy at 520-540 nm [5, 6]. Total Cholesterol and HDL was calculated.

RESULTS AND DISCUSSION

Novel *in-vitro* hypolipidemic activity of policosanol

Graph was plotted by taking % Reduction in Cholesterol Concentration on Y- axis and Concentration of Cholesterol and Simvastatin Solutions on X-axis. Novel In-vitro method results revealed that significant reduction of cholesterol concentration was observed in the Test Solution containing 10µg, 20µg and 40µg of Policosanol solution ranging from 60-80% reduction and results were comparable to standard Simvastatin. This clearly indicated that Policosanol and Simvastatin possessed significant anti-Hyperlipidemic activities. Policosanol and Simvastatin have ability to reduce Cholesterol level significantly.

Cholesterol estimation using lipidometer:

It was observed that significant reduction in the Cholesterol Levels was noticed during the study period in experimental animals treated with 5mg of Policosanol with dextrose as dispersant. The significant reduction may be due to enhanced bioavailability of Policosanol 1mg aqueous dispersion upon oral administration when compared to the animals treated with 10mg/kg Policosanol using Tween 80 as dispersing surfactant and Hyperlipidemic animals which were treated with distilled water as vehicle.

***In-vivo*hypolipidemic screening of policosanol:**

Novel high throughput *in-vivo* anti-hyperlipidemic screening model

The main significance in this method is using single animal, daily monitoring Reduction in Cholesterol Concentration can be screened. Thus, only single animal is used and same animal will be observed for monitoring Cholesterol Concentration for complete period of study from Day 1 to 15. The 45^{th} day also showed sustainability in maintaining the Cholesterol Concentration upon discontinuing the treatment in Albino Rats 4, 5 (5mg/kg, 10mg/kg Policosanol treated rats). During 15^{th}, 20^{th}, 45^{th} Days, Reduction in Cholesterol was observed. Albino rat 3 (1mg/kg) showed best results among other animals due to improved bioavailability of policosanol as the policosanol was administered using Dextrose solution. Policosanol showed a significant reduction in Cholesterol level.

***In vivo* cholesterol determination method by colourimetry**

Graph was plotted to compare % Reduction in Cholesterol Concentrations of HDL Cholesterol of Albino Rats of each study group. All the animals of Group II to Group VI showed a significant Cholesterol range. Animals were treated with Simvastatin, Policosanol 1mg/kg, 5mg/kg, 10 mg/kg, 20 mg/kg respectively.

Policosanol was screened for its Antihyperlipidemic activity in experimental animals in various doses of 1mg/kg, 10mg/kg, 20mg/kg, and vehicle control (Hyperlipidemic animals treated with Distilled Water as Vehicle) and compared its therapeutic pharmacological effect with Simvastatin. Berthold HK. Suggested that policosanol at doses of 5 to 40 mg/d has lipoprotein-lowering effects comparable with statins. [11]

Barbagallo CM. etal. revealed that Policosanol showed reduction in LDL cholesterol similar to that of statins (about 25%), and a 10% increase of HDL-C. [12]

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Tables

TABLE 1: TABLE DEPICTING DIFFERENT STUDY GROUPS,

S.NO.	GROUP NO.	GROUP NAME/ DOSING
1.	I	Vehicle Control (Distilled water)
2.	II	Standard Simvastatin (10mg)
3.	III	Test 1 (Policosanol 1mg/kg with 50 mg Dextrose dissolved in Distilled Water)
4.	IV	Test 2 (Policosanol 5 mg/kg with 50 mg Dextrose dissolved in Distilled Water)
5.	V	Test 3 (Policosanol 10 mg/kg with 0.1 ml Tween 20 dissolved in Distilled Water)
6.	VI	Test 4 (Policosanol 20 mg/kg with 0.1 ml Tween 20 dissolved in Distilled Water)

TABLE 2: % CHOLESTEROL CONCENTRATION AND % REDUCTION IN CHOLESTEROL CONCENTRATION OF SIMVASTATIN AND POLICOSANOL SOLUTIONS

Drugs	**Conc.**	**% Cholesterol Concentration**	**%Reduction in Cholesterol Concentration**
SIMVASTATIN	Cholesterol Conc.	100%±0.000	0%±0.000 (No change)
	20 µg/ml	36.7%±0.200***	63.3%±0.200***
	40 µg/ml	81.0%±0.153***	19%±0.153***
	100 µg/ml	21.8%±0.200***	78.2%±0.200***
POLICOSANOL	10 µg/ml	32.1%±0.015***, a3	67.9%±0.015***,a3
	40 µg/ml	22.1%±0.016***, a3	77.9%±0.016***,a3
*** p<0.001 as compared to blank cholesterol; a3<0.001 as compared to standard; One way Anova followed by Dunnet test			

TABLE 3: TABLE SHOWING CHOLESTEROL CONCENTRATION OF DIFFERENT GROUPS IN 0,4,8,12,15 DAY

DAYS	**CHOLESTEROL CONCENTRATION**					
	Group **I** (Vehicle Control Intoxicant)	GROUP **II** (Standard Simvastatin) (10mg)	GROUP **III** 1mg/kg)	GROUP **IV** (5mg/kg)	GROUP **V** (10mg/kg)	GROUP **VI**(20mg/kg)
0	120±4.1	110±22.4	105±5.6	120±5.8	113±10.2	116±8.8
4	290±5.8	160±9.3***	160±4.1***	188±23.7***	160±22.0***	160±30.9***
8	285±11.4	246±34.8	185±22.4***,a1	198±37.7**	237±32.0	246±48.6
12	270±22.8	160±38.0***	160±14.6***	160±11.4***	160±5.8***	160±38.2***
15	275±9.5	235±4.1	177±4.1***,a3	160±4.1***,a3	170±4.1***,a3	245±4.1***
*** p<0.001 as compared to Group 1 toxicant; a3<0.001 as compared to standard; One way Anova followed by Dunnet test						

TABLE 4: TOTAL CHOLESTEROL DETERMINATION BY *IN-VIVO* COLOURIMETRY METHOD

S.NO.	GROUP NO.	TOTAL CHOLESTEROL (mGs/dL)
1.	I (Vehicle Control Intoxicant) (Hyperlipidemic animals treated with Distilled Water as Vehicle)	**240±0.015**
2.	II (Std. Simvastatin)(10mg)	**200±0.015*****
3.	III (1mg/kg Policosanol)	**180.129±0.010**^{***,a3}
4.	IV (5mg/kg Policosanol)	**197.415±0.015**^{***,a3,b3}
5.	V (10mg/kg Policosanol)	**69.305±0.010**^{***,a3,b3,c3}
6.	VI(20mg/kg Policosanol)	**190.792±0.015**^{***,a3,b3,c3,d3}

TABLE 5: HDL CHOLESTEROL DETERMINATION BY *IN-VIVO* COLOURIMETRY METHOD

S.NO.	GROUP NO.	HDL CHOLESTEROL (mGs/dL)
1.	I (Vehicle Control) (Hyperlipidemic animals treated with Distilled Water as Vehicle)	**7.5±0.010**
2.	II (Std. Simvastatin) (10mg)	**40±0.153*****
3.	III (1mg/kg Policosanol)	**8.762±0.153**^{***,a3}
4.	IV (5mg/kg Policosanol)	**8.381±0.100**^{***,a3,b1}
5.	V (10mg/kg Policosanol)	**14.857±0.200**^{***,a3,b3,c3}
6.	VI(20mg/kg Policosanol)	**25.905±0.100**^{***,a3,b3,c3,d3}

*** p<0.01 and 0.001 respectively as compared to Vehicle Control (Hyperlipidemic animals treated with Distilled Water as Vehicle)
^{a3} p< 0.05 and 0.001 respectively as compared to Standard
$^{b1, b3}$ p<0.05 and 0.001 respectively as compared to Policosanol 1mg/kg
^{c3} p<0.05 and 0.001 respectively as compared to Policosanol 5mg/kg
^{d3} p< 0.001 as compared to Policosanol 10mg/kg

One way anova followed by tukey test

FIGURES:

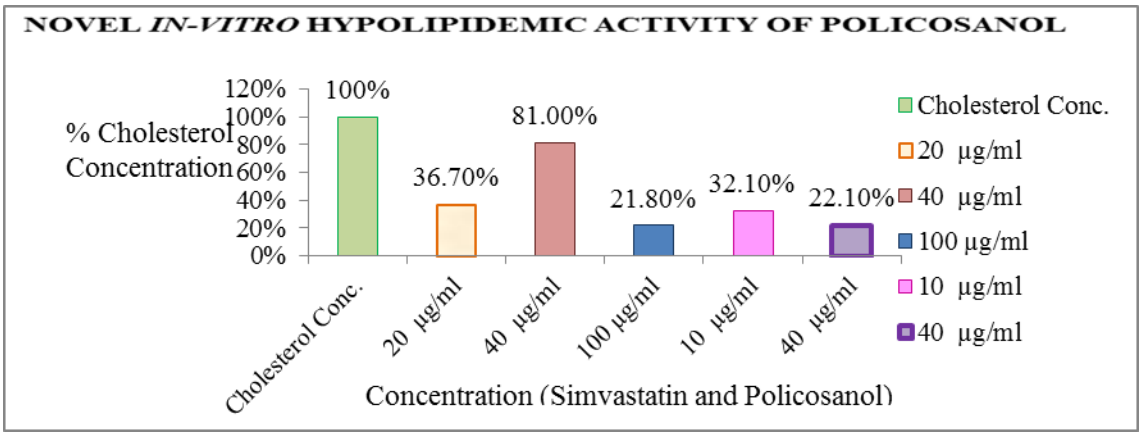


figure 1: graph between % cholesterol concentration v/s simvastatin, policosanol concentration

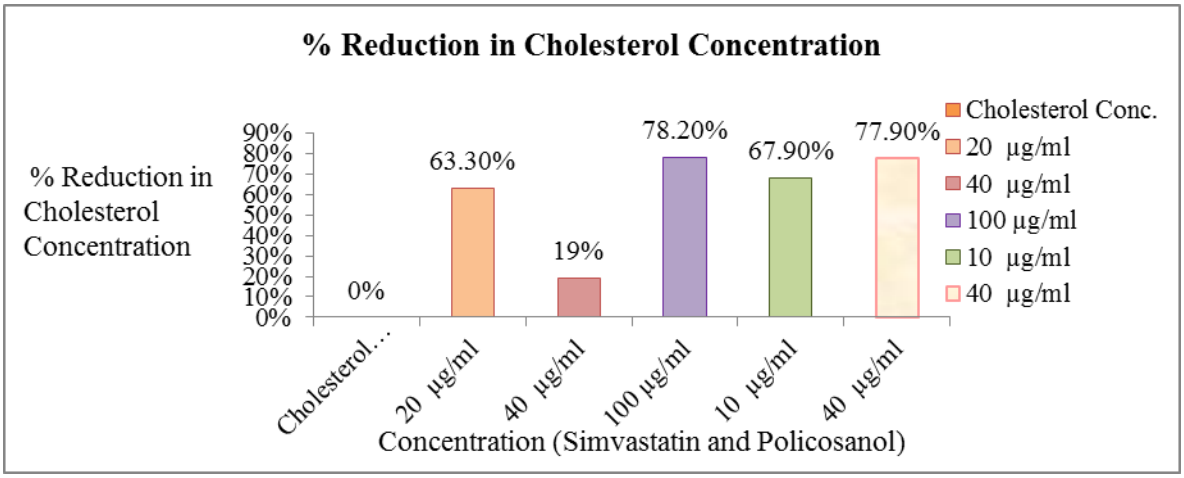


Figure 2: graph between % reduction in cholesterol concentration v/s concentration (simvastatin and policosanol)

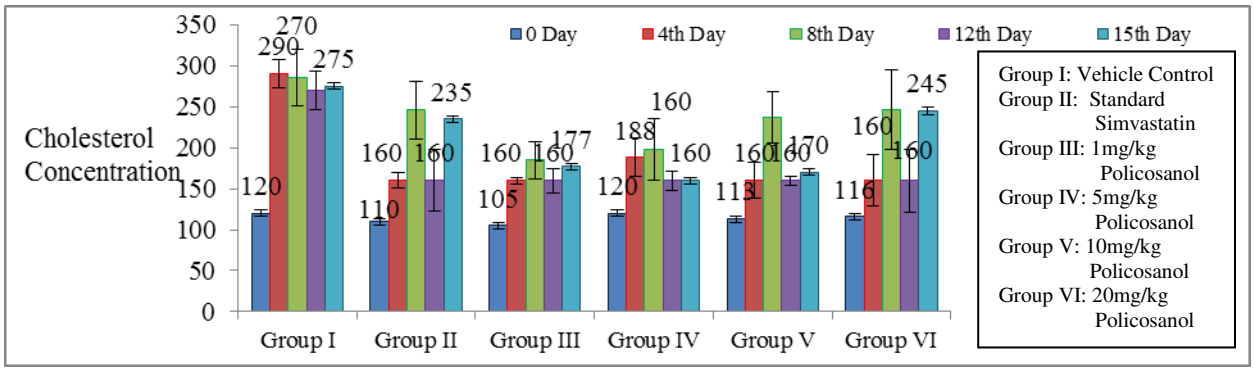
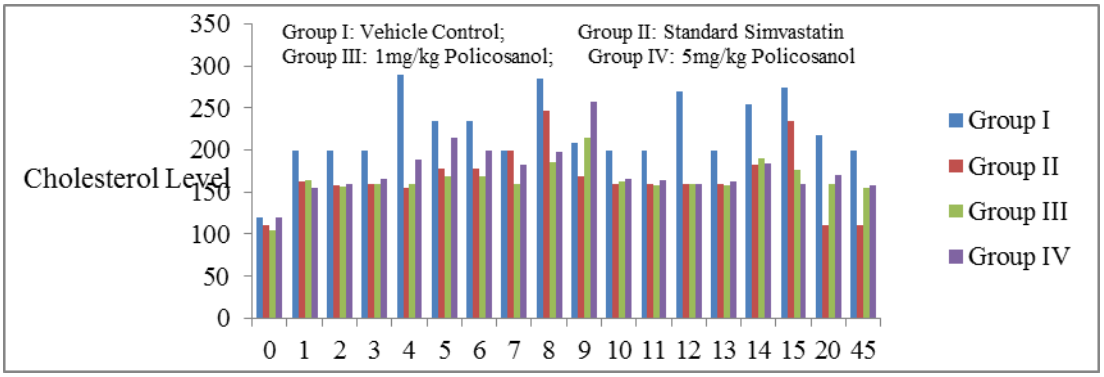


Figure 3: graph between cholesterol determination v/s days of groups i-vi



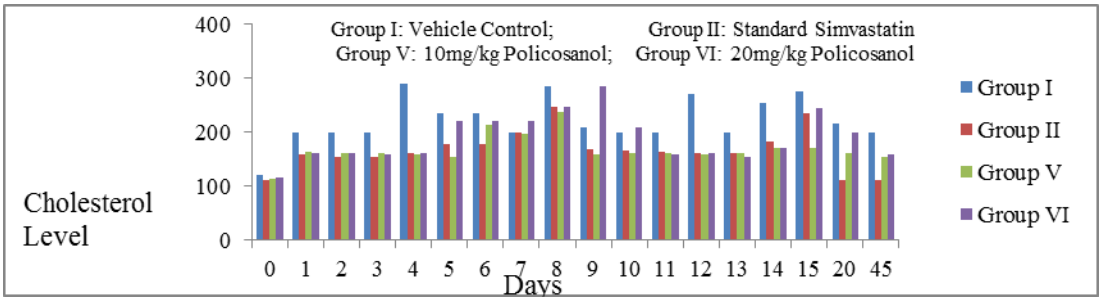


FIGURE 4(i) & (ii): GRAPHS BETWEEN CHOLESTEROL CONCENTRATION v/s NO. OF DAYS FOR *IN-VIVO* CHOLESTEROL DETERMINATION

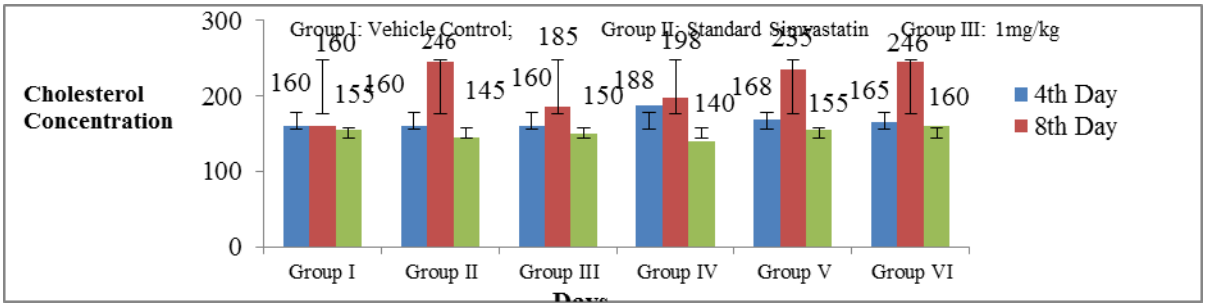


FIGURE 5: GRAPH BETWEEN CHOLESTEROL CONCENTRATIONS OF GROUPS v/s DAYS (4^{TH}, 8^{TH}, 12^{TH} DAY)

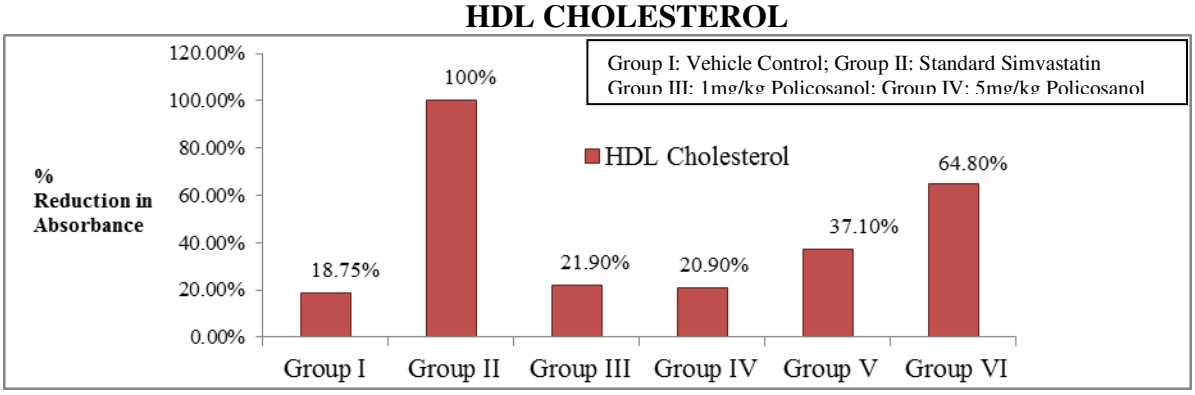


Figure 6: graph depicting hdl cholesterol

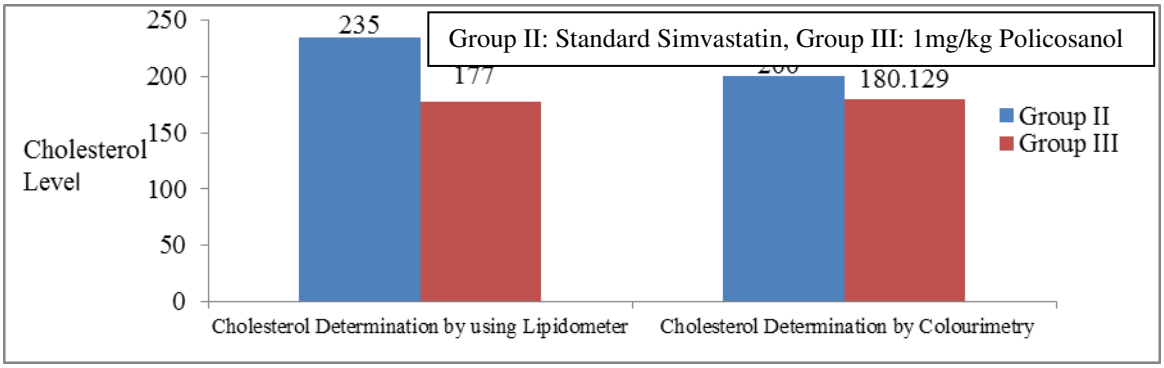


Figure 7: graph between cholesterol determination using lipidometerv/s colourimetry method of group ii and group iii

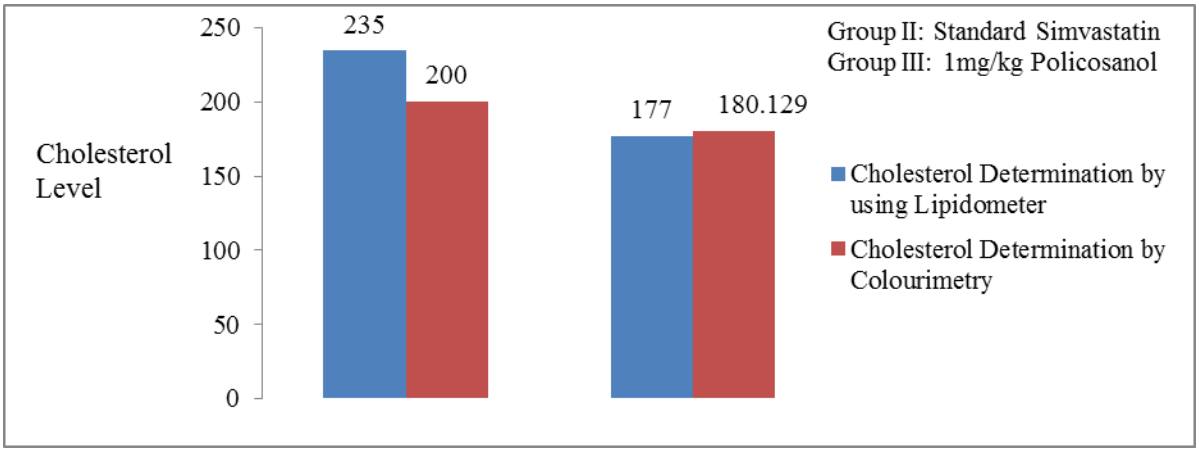


Figure 8: graph between cholesterol determination using lipidometer v/s colourimetry method of group ii and group iii

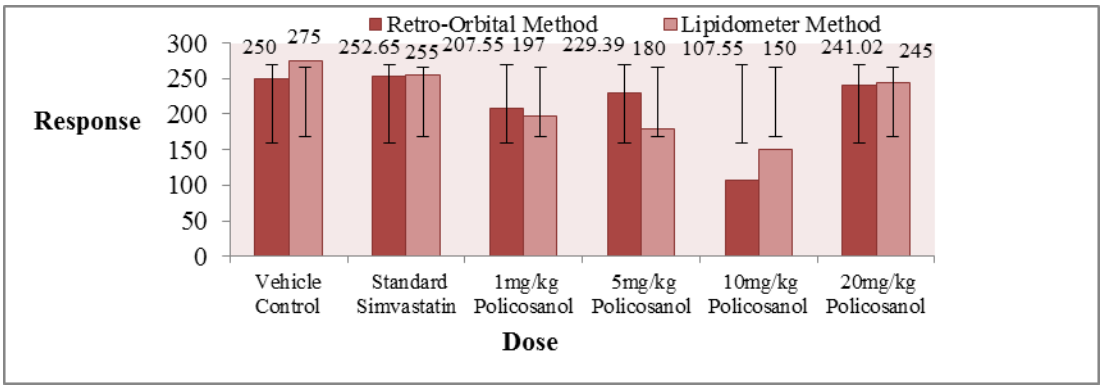


Figure 9: graph between retro-orbital method v/s lipidometer method