REPORT

Evaluation of "NARASIMHA" Extract for Hypocholesterolemic Activity in Rats Fed With High Cholesterol Diet

Study No.	:	PST-384/A/13
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Test Facility Management	:	Dr. Pralhad S. Patki

Test Facility	:	Pharmacology Department, R&D Center, The Himalaya Drug Company, Bangalore-562 162, India

STUDY DETAILS

Study Title	:	EVALUATION OF NARASIMHA FORMULATION FOR HYPOCHOLESTEROLEMIC ACTIVITY IN RATS FED WITH HIGH CHOLESTEROL DIET
Test item name/code	:	Narasimha Extract (B.No: NBHD-PF/1308/14-Nisarga BiotechPvt Ltd.) Marketed Product-1 Marketed Product-2
Storage conditions	:	Ambient
Manufactured By	:	Nisarga Biotech & The Himalaya Drug Company, Makali, Bangalore-562 123, INDIA

Test facility	:	Pharmacology Department, R&D Center, The Himalaya Drug Company, Makali, Bangalore-562 162, INDIA.
Sponsor	:	Head- Phytochemistry, R & D Center, The Himalaya Drug Company, Makali, Bangalore-562 162, INDIA.

SAFETY PRECAUTIONS:

Gloves, cap and, face mask were used in addition to protective body garments and shoes to ensure adequate personal health and safety and to avoid inhalation and skin contact with the test item and other chemicals.

INTRODUCTION:

Hypercholesterolemia is a well-recognized risk factor for coronary artery disease. "Atherosclerosis" is the principle underlying cause of coronary heart disease which is the commonest cause of death in industrialized world and of stroke and peripheral vascular disease, which is also a major cause of morbidity and mortality.

Thickening and hardening of arteries and loss of elasticity of the inner arterial wall are among the hallmarks of atherosclerosis. This is a progressive condition which begins in childhood and its clinical manifestations surface in middle and old age. Atherosclerosis is the consequence of three principal biological processes, namely aggregation of smooth muscle cells, macrophages and T-lymphocytes, formation of connective matrix by smooth muscle cells and accumulation, in cells and in the connective tissue surrounding the cells of lipids which are basically in the form of cholesterol esters and free cholesterol. Epidemiological studies have

demonstrated a positive significant relationship between plasma cholesterol concentration, smoking, hypertension, obesity with coronary artery diseases.

Satisfactory treatment of hypercholesterolemia and atherosclerosis by drugs are inadequate/ incomplete in a true sense owing to the adverse effects of drugs available and also their limitation in their beneficial activity.

Objective:

The objective of present set of experiment was to evaluate and compare the effect of "Narasimha" extracts with preparations/ products "Marketed Product-1" and "Marketed Product-2" for hypocholesterolemic activity in rats fed with high cholesterol diet.

Materials and methods:

Test System

Animals	Wistar Rats
Source	Toxicology Department, R&D Center, The Himalaya Drug Company, Makali, Bangalore-562 123, INDIA
No. of animals	48
Sex	Male
Age of treatment	8-10 weeks
Identification	By animal accession number, cage label and picric acid body marking
Justification for selection of test system	Although a variety of experimental animals can be used, rats are preferred because of ease of handling and availability

Justification for route of administration	The oral route is intended route for clinical evaluation hence, its also chosen for preclinical studies
Acclimatization	After veterinary examination for good health and suitability for experiment, the animals were acclimatized for 5 days before treatment.

Test item preparation:

Test drug was prepared freshly as a suspension in demineralized water (DM) just before administration.

Rationale for dose Selection:

Based on the information provided by the sponsor and after extrapolating the human dose to rat dose, the dose of test substance was fixed as 250mg/kg b.wt./day p.o. for all the variants.

Test item administration:

Test drug was administered in the form of oral gavage using intubation catheter.

Procedure:

Hypocholesterolemic activity of Narasimha extract in rats fed with high cholesterol diet:

Forty eight Wistar rats, weighing between 170-190 g were used for the study. The animals were segregated into 6 groups of 8 each based on their body weight. The first and second group served as normal and positive control respectively; the other groups were assigned treatment groups. Group I was given normal diet, while the Groups II-VI

were fed with a high-cholesterol diet throughout the study, which contain 1 % of cholesterol, 0.5 % bile salts (Sodium taurocholate) and 10 % cooking oil (ground nut oil) in normal diet (Cholesterol and Sodium taurocholate were mixed with water to make a slurry then it was added to the mash). After **28 days of induction**, group III to VII were treated as per the details given below.

Group I : Normal control.

Group II : Positive untreated control (HCD)

Group III : Narasimha extract 250 mg/kg p.o. + (HCD)

Group IV : Marketed Product-1, 250 mg/kg p.o. + (HCD)

Group V : Marketed Product-2, 250 mg/kg p.o. + (HCD)

Group VI : Atorvastatin 10 mg/kg p.o. + + (HCD)

All the above treatments were carried out each day under similar constant conditions, as far as possible for 12 weeks (84 days) and blood was collected after **4, 8 and 12** weeks to determine the blood lipid profile. Twenty four hours after the dose administration, the overnight fasted animals were anaesthetized with isofluorane and blood samples were collected by orbital puncture and serum was used for the estimation of total cholesterol, triglycerides, LDL, VLDL and HDL.

Statistical Analysis:

The values were expressed as mean ± SEM. The results were analyzed statistically using One-way ANOVA followed by Dunnet's multiple comparison test to find out the level of significance. The minimum level of significance was fixed at $p<0.05$.

References:

1. Ching YK et al.Consumption of dried fruit of Crataegus pinnatifida (hawthorn) suppresses high- cholesterol diet -induced hypercholesterolemia in rats. *Journal of Functional Foods* 2010; 1:179-186.

2. Tomohiro I, Yukio F. Lowering serum cholesterol level by feeding a 40% ethanol-eluted fraction from HP-20 resin treated with hot water extract of adzuki beans (Vigna angularis) to rats fed a high-fat cholesterol diet. *Nutrition* 2009; 25:318-321

RESULTS:

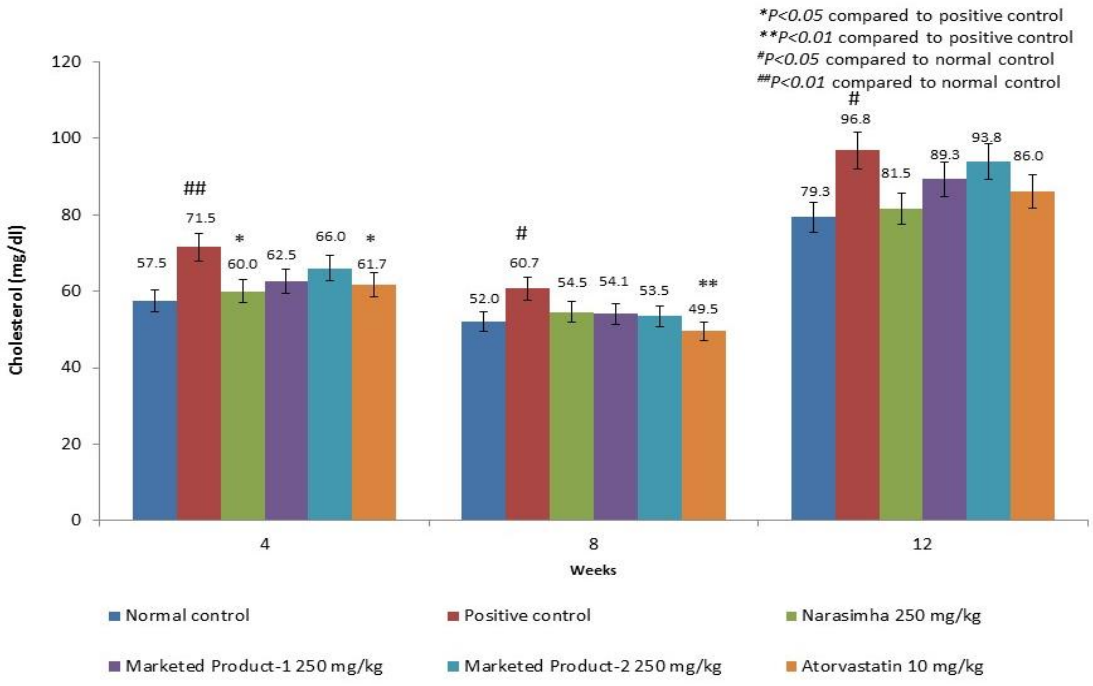

Figure 1. Effect of Narasimha formulation" on serum total cholesterol (TC) in rats fed with high cholesterol diet.

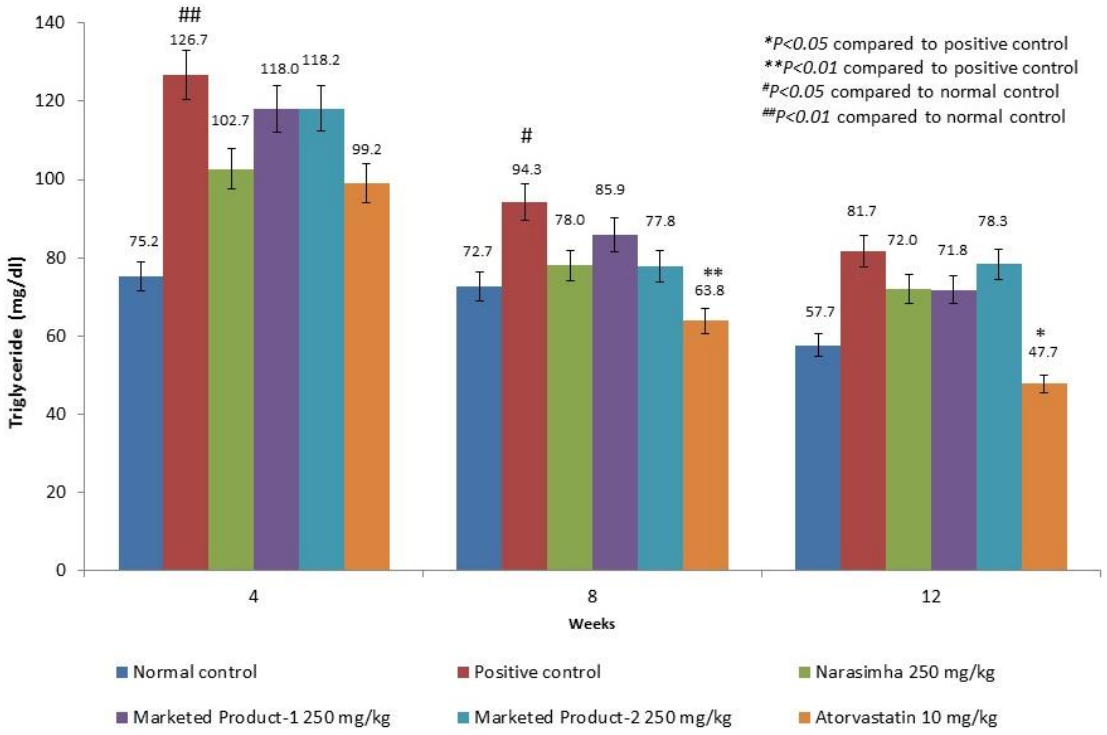

Figure 2. Effect of Narasimha formulation on serum triglyceride (TG) levels in rats fed with high cholesterol diet.

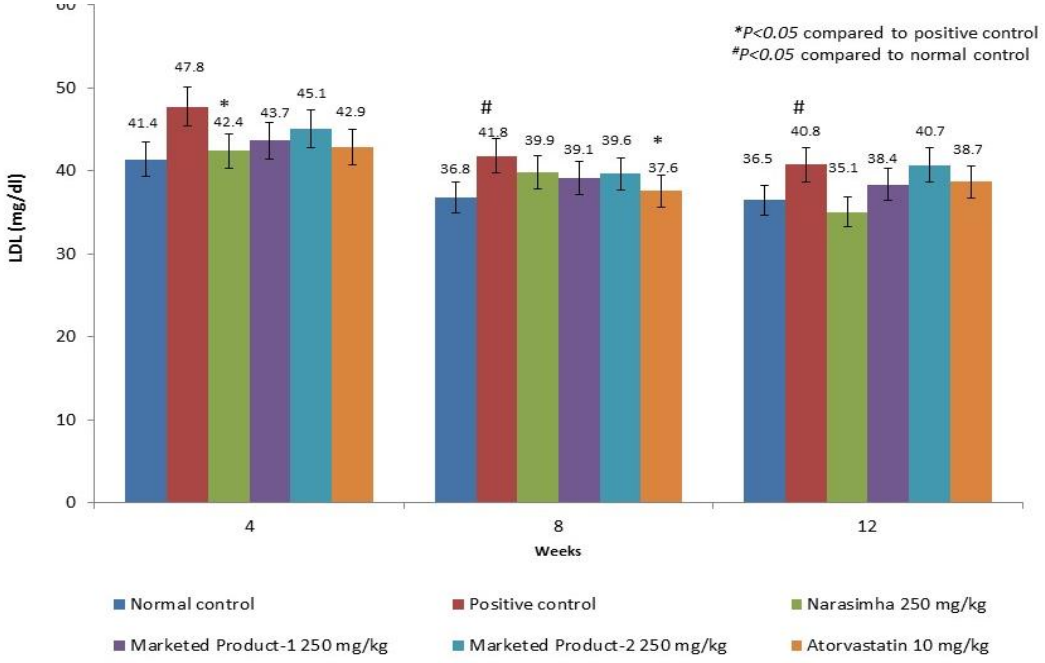

Figure 3. Effect of Narasimha formulation on serum LDL-C levels in rats fed with high cholesterol diet

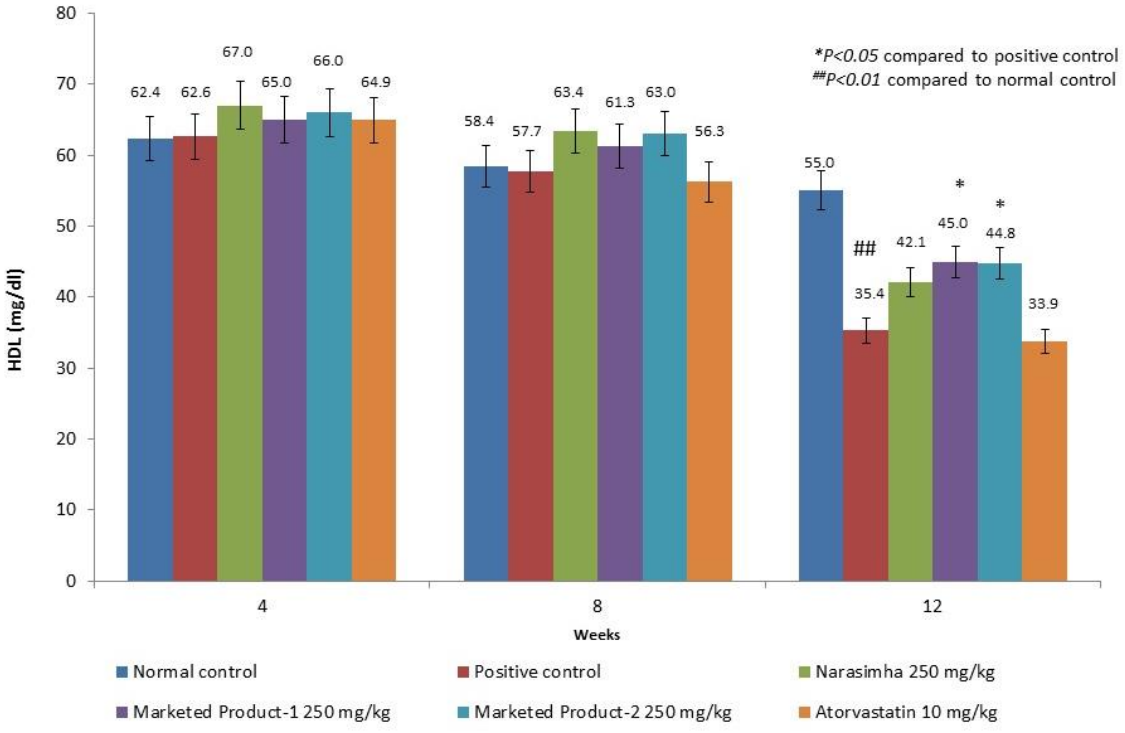

Figure 4. Effect of Narasimha formulation on serum HDL-C levels in rats fed with high cholesterol diet

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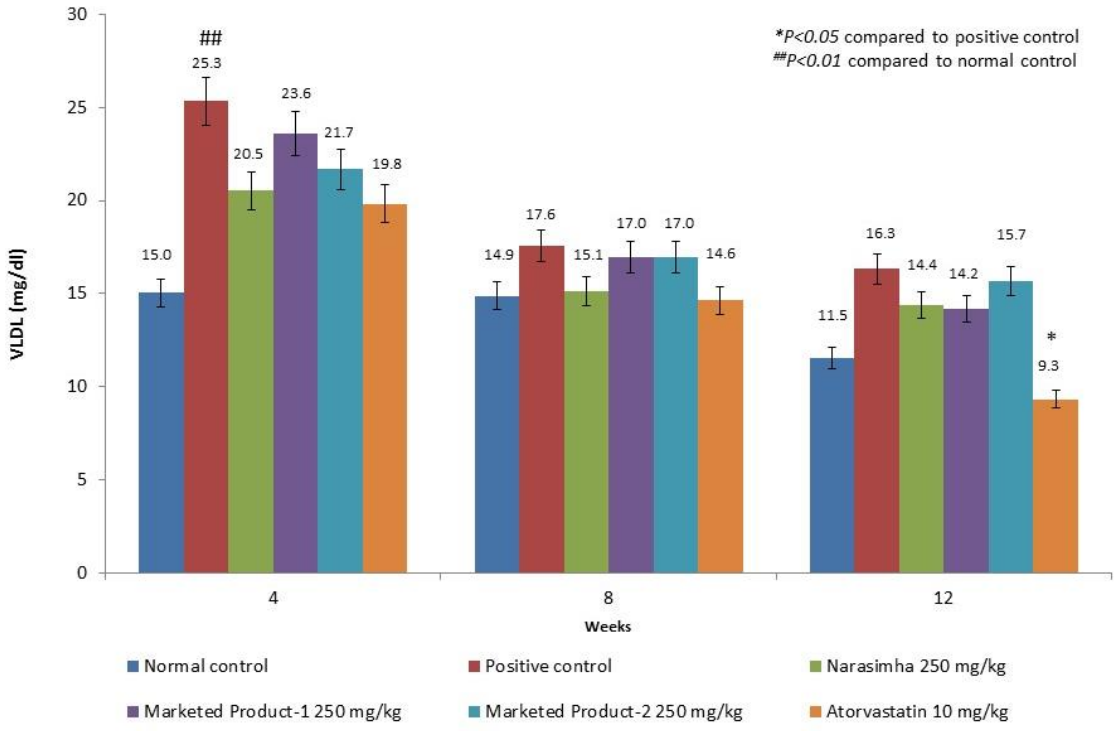

Figure 5. Effect of Narasimha formulation on serum VLDL-C levels in rats fed with high cholesterol diet

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Summary & Conclusion:

- The Hypocholesterolemic activity of Narasimha extract was evaluated for hypocholesterolemic activity in rats fed with High cholesterol diet (HCD). The effect of Narasimha extract was compared with products Marketed Product-1 and Marketed Product-1 (**Figures 1 to 5**).

- Atorvastatin, standard drug used in the management of hypercholesterolemia/ hyperlidemia was used as reference control (10 mg/kg b.wt. p.o/day). All the drugs were administered in the form of gavage using intubation catheter.

- In case of rats fed with high cholesterol diet (HCD) for around 16 weeks, there was a significant increase in serum lipid markers such as total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL) and very low density lipoprotein cholesterol (VLDL) levels, also HDL-C level was significantly decreased.

- Feeding of HCD, and simultaneous treatment of trial drugs to rats at a dose of 250 mg kg b.wt. p.o. per day indicates a sign of reversal of impaired serum lipid parameters. Narasimha extract decreased the serum total cholesterol (TC) at week-4 to a statistically significant level and this trend continued even at week-8 & 12, though it narrowly misses statistical significance, The mean values of serum triglyceride level in Narasimha treated animals are less compared to untreated control. Narasimha extract also decreased the average serum LDL-C and VLDL-C levels, though the decrease in values were not found to be statistically significant. The trial extract of Narasimha also increased the serum levels of HDL-C compared to untreated HCF fed animals (**Figures 1 to 5**).

- From the results of the above experiments, it can be inferred that, all the tested variants decreased the serum markers of hypercholesterolemia but to a different extent. Narasimha extract has showed tendency towards decreasing the serum cholesterol (TC) and triglycerides (TG) levels on all the time point of assessment (**4, 8 & 12 weeks**). The average values of serum LDL-C & VLDL-C in Narasimha treated groups were less than untreated HCD-fed animals. Also, if we consider the average/mean decrease in lipid parameters in all the time points assessment (4, 8 & 12 weeks), the Narasimha extract was found to better compare to other herbal variants tested.

- The above observation was made with selected methodology, dose, mode of administration and time employed.