



EVALUATION OF ANTI HYPERLIPIDEMIC ACTIVITY IN RODENTS USING LEAF EXTRATS OF *CLERODENDRUM SERRATUM* BY FOOD INDUCED OBESITY IN RATS

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ABSTRACT

The Hypolipemic effect of various extracts of clerodendrum serratum was investigated in rats suffering from high cholesterol, diet induced hyperlipidemia and the phytochemicals in the extracts were analysed. The high-cholesterol diet caused a significant increase in total lipids, total cholesterol (TC), total triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and the atherogenic index, whereas the level of high-density lipoprotein cholesterol (HDL-C) was significantly decreased. The animals were divided into 6 groups where 2 used as test, 1 for standard, 1 for control, 1 for diet induced hyperlipidemic and 1 for the activity of physical exercise. The groups with anti hyperlipidemic medication and without medication by exposing to physical exercise are compared in one aspect and however the group treated with methanolic extract showed better activity than standard and other groups.

KEY WORDS

Anti hyperlipidemic, total cholesterol, LDL, VDL, HDL, VLDL, Atherogenic index.

INTRODUCTION

Hyperlipidemia is a term used to describe several conditions in which high concentrations of lipids exist in the bloodstream and it results from abnormalities in lipid metabolism or plasma lipid transport or a disorder in the synthesis and degradation of plasma lipoproteins [1]. Hypercholesterolemia possess a major problem to many societies as well as health professionals because of the close correlation between cardiovascular diseases and lipid abnormalities [2]. Hypercholesterolemia is the most important factor in the pathogenesis of atherosclerosis. Hyperlipidemia is manifested as hypercholesterolemia and/or hypertriglycerolemia [3]. Hyperlipidemia is associated with risk factors like atherosclerosis, hypertension, Type II Diabetes mellitus, obesity, myocardial infarction, congestive cardiac failure, angina pectoris, gall bladder diseases, degenerative joint diseases, sleep apnea, and

infertility [4]. The blood levels of VLDL (very low-density lipoprotein), LDL (low-density lipoprotein) and TG (triglycerides) increase, whereas the HDL (high density lipoprotein) levels fall in dyslipidemia [5]. CVDs (cardiovascular diseases) that also include CADs (coronary artery diseases) are one of the leading causes of death worldwide, accounting for approximately 17.3 million deaths per year [6]. Dyslipidemia along with hypertension are the principle etiological factors behind development of CVDs [7]. Hypercholesterolemic animals are useful models for studies on cholesterol homeostasis, and drug trials to better understand the relationship between disorders in cholesterol metabolism, atherogenesis as well as possible treatments for the reduction of circulatory cholesterol levels [8].

MATERIALS AND METHODS

Preparation of extracts

The shade dried leaves were coarsely powdered. The powdered material was extracted using methanol (60-700C), for 72 h and successively extracted with methanol and water for 72 h each in a Soxhlet apparatus. The extracts were evaporated under reduced pressure to solid masses and the percentage yield of extracts was found to be 3.7%, 7.5% and 5.6% w/w, respectively.

Experimental Animals

All experimental protocols and procedures were approved by the Institutional Animal Ethics Committee. Male Wister rats, weighing 200–250g, were used throughout the study. The animals were housed in standard laboratory conditions (12-h light/dark cycle, 21 ± 1°C, and relative humidity of 55 ± 5%) with free access to food and water prior to the experiments. After 7 days of acclimatization to laboratory conditions, the animals were randomly assigned to experimental groups, each consisting of 4 rats. Each animal was used only once in the experimental procedures. All experiments were carried out between 9 a.m. and 3 p.m.

Induction of Hyperlipidemia

Method – 1

High cholesterol diet (HCD) comprised the following ingredients: cholesterol 5g, deoxycholic acid 5g, coconut oil 300 ml (300 g), and standard rat chow 700g. Deoxycholic acid was mixed thoroughly with powdered rat chow diet; simultaneously cholesterol was dissolved in 300 ml of warm coconut oil. This oil solution of cholesterol was added slowly into the powdered mixture and thoroughly mixed to obtain soft homogenous cakes. These cakes were daily supplied to rats in each cage in sufficient quantities [9-11]. Body weights of all rats were checked on the day before the start of feeding period and on day 1, 15 & 30 of the treatment period [12].

Method – 2

High cholesterol diet was prepared by mixing cholesterol 2%, Sodium cholate 1% and coconut oil 2% or 30 %, with standard powdered standard animal food. The diet was placed in the cage carefully and was administered for seven days [13].

Collection of Blood and Serum Samples

At the end of the experiment, blood was collected by cardiac puncture from each rat under mild ether anesthesia. The blood samples were used for the estimation of glucose levels and remaining was allowed to clot for 30 min at room temperature and they were centrifuged at 3000rpm for 10 minutes. The serum was used for the study of biochemical parameters [14].

Calculation of Percentage Change in serum parameters:

$$\text{Percentage change (\%)} \text{ on day 30} = \frac{\text{Day 1 serum levels} - \text{Day 30 serum levels}}{\text{Day 1 serum levels}} \times 100$$

$$\text{Percentage change (\%)} \text{ on day 15} = \frac{\text{Day 1 serum levels} - \text{Day 15 serum levels}}{\text{Day 1 serum levels}} \times 100$$

Calculation of Atherogenic Index (AI):

$$\text{Atherogenic Index} = \frac{\text{Total serum cholesterol}}{\text{Total serum HDL-C}}$$

Calculation of Percentage Protection:

$$\text{Protection (\%)} = \frac{\text{AI of Control} - \text{AI of Treated group}}{\text{AI of Control}} \times 100 \quad [15]$$

Determination of Body Weight:

Body weight of the all animals in each group of HFD induced hyperlipidemia method was determined on the 0th, 7th, 14th, 21st, 28th, 35th, 42nd, 49th, and 56th day of the experiment period. Differences in weights were observed [16].

Collection of blood:

Blood was collected before the start of the experiment to determine the baseline serum lipid levels. Next, blood

was collected on 15th day to determine the induction of hyperlipidemia and on 29th day to assess the effect of the test drugs.

Site for Collection of Blood:

Day 0: Tail vein.

Day 15: Tail vein.

Day 29: Cardiac puncture.

Estimation of serum lipid levels:

The blood collected was allowed to clot for approximately 1 hour at room temperature and then centrifuged at 12,000 rpm to obtain the serum. Serum was collected in the Eppendorf tubes and stored at -

20°C until analysis. The serum lipid levels were estimated using the commercial biochemical assay kits. They were analyzed in the Rayto semi auto chemistry analyzer (RT 9600) [17].

Induction of hyperlipidemia by diet induced method and comparison of weights according to groups:

GROUPS	TREATMENT	AVEARGE BODY WEIGHTS (gr)			
		WEEK-1	WEEK-2	WEEK-3	WEEK-4
1	Diet induced hyperlipidemia	204±2.3094	262±2.3094	276.2±1.6384	287.2±4.2158
2	Standard atorvastatin	177±1.5274	167±1.5274	154±2.3094	136±1.1547
3	Test-1 aq. Extract	191±0.5773	183±1.732	174±1.1547	168±2.8867
4	Test-2 meth. Extract	187±1.5274	179±0.5773	165±2.3094	160±1.732
5	Physical Exercise	194±1.1547	188±0.5773	184±2.0816	178±1.1547
6	Control	176±1.1547	181±2.3094	188±4.4014	192±1.732

RESULTS AND DISCUSSIONS:

TABLE-1: Serum lipid profile for diet induced hyperlipidemia

S.NO	Treatment	Total Cholesterol	HDL	LDL	VLDL	TG
1.	Diet induced hyperlipidemia	76	18.6	30.6	30	134

FIGURE-1: Serum lipid profile of group-1 rats

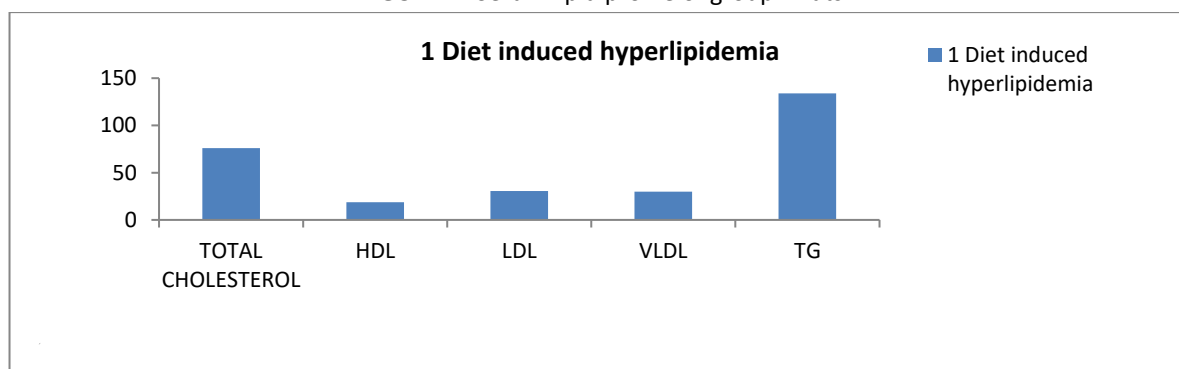


TABLE-2: Serum lipid profile for std. atorvastatin

S.NO	Treatment	Total Cholesterol	HDL	LDL	VLDL	TG
2.	Std. Atorvastatin	62	23.8	38	9	46

FIGURE-2: Serum lipid profile of group-2 rats

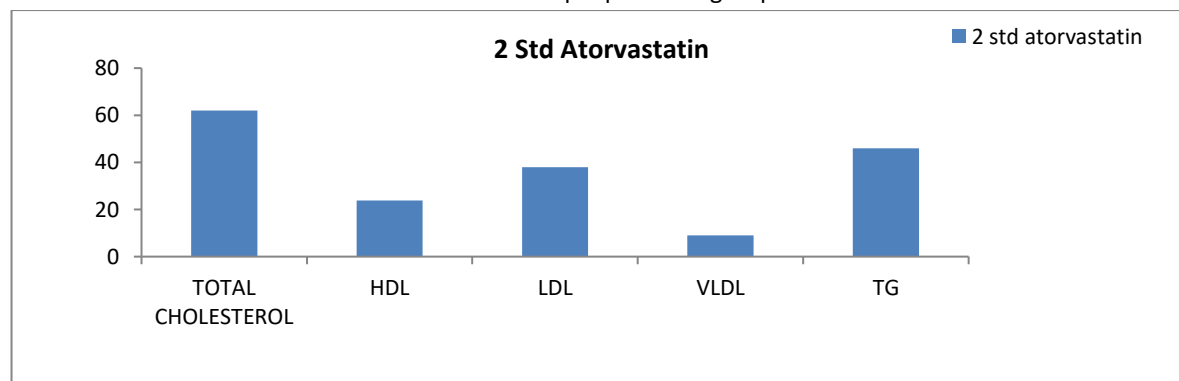
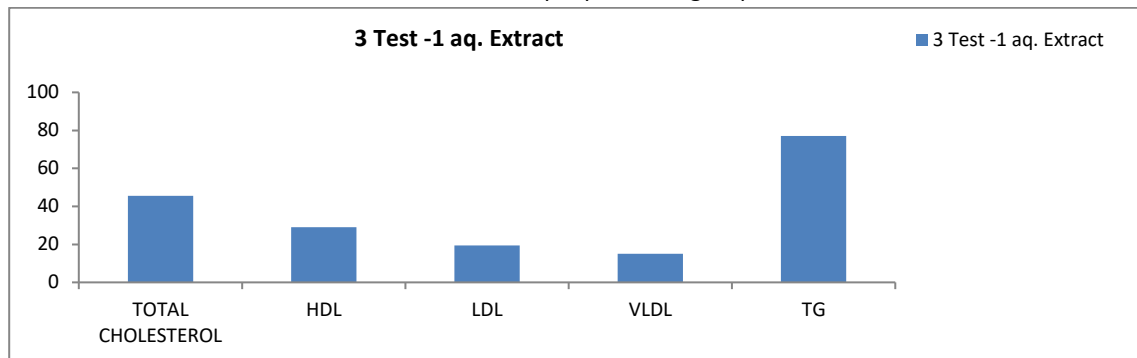
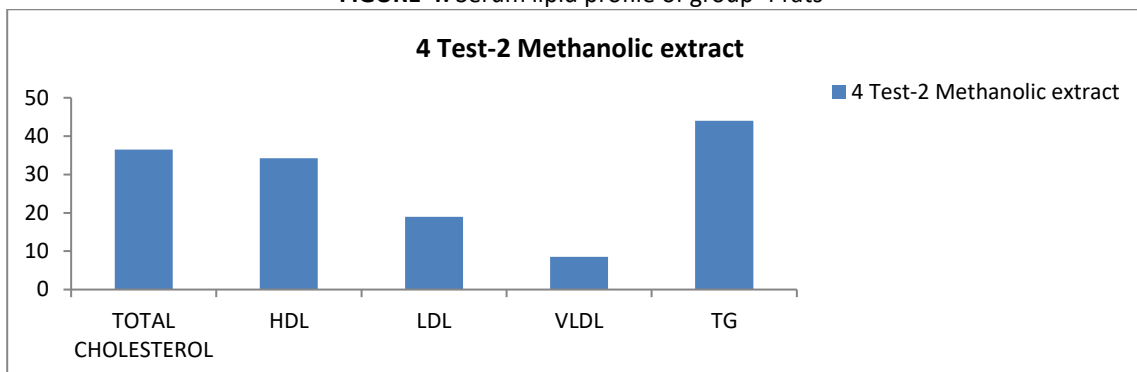


TABLE-3: Serum lipid profile for aq.extract

S.NO	Treatment	Total Cholesterol	HDL	LDL	VLDL	TG
3.	Test-1 aq.extract	45.5	29	19.5	15	77

FIGURE-3: Serum lipid profile of group-3 rats

TABLE-4: Serum lipid profile for Methanolic extract

S.NO	Treatment	Total Cholesterol	HDL	LDL	VLDL	TG
4.	Test-2 Methanolic extract	36.5	34.2	19	8.5	44

FIGURE-4: Serum lipid profile of group-4 rats

TABLE-5: Serum lipid profile for physical exercise

S.NO	Treatment	Total Cholesterol	HDL	LDL	VLDL	TG
5.	Physical exercise	65.25	42.7	36.5	12.75	64.5

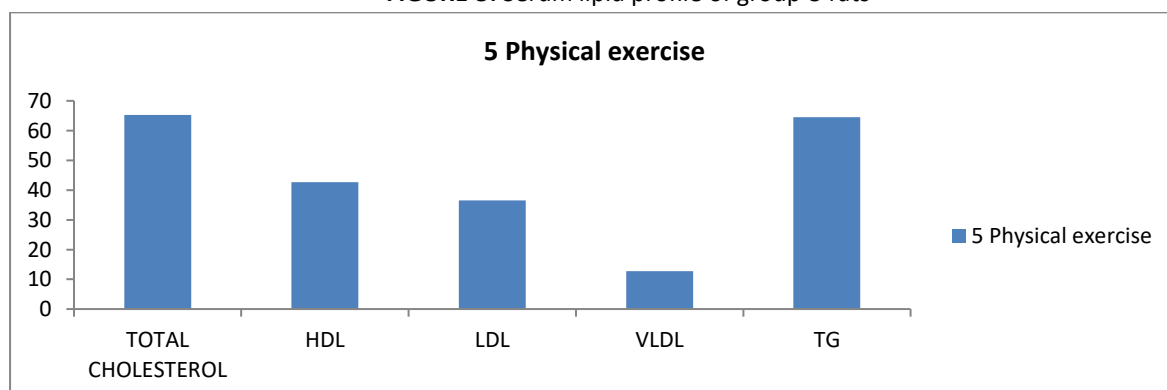
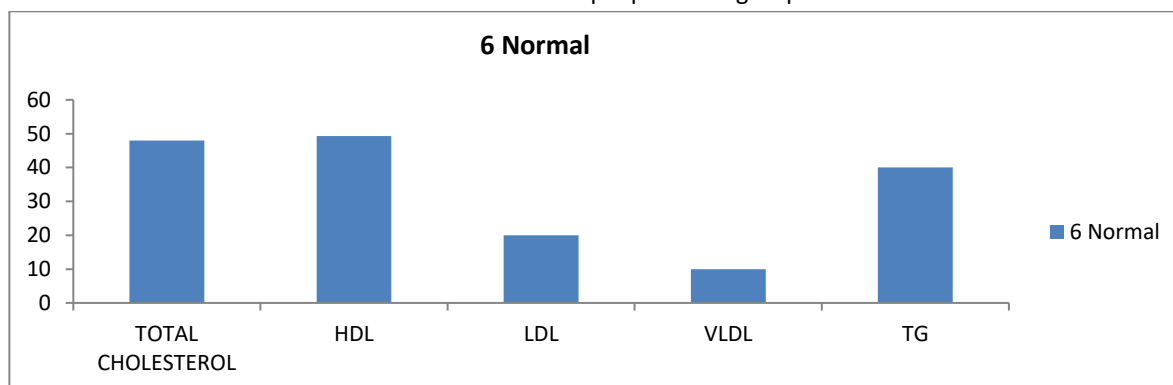
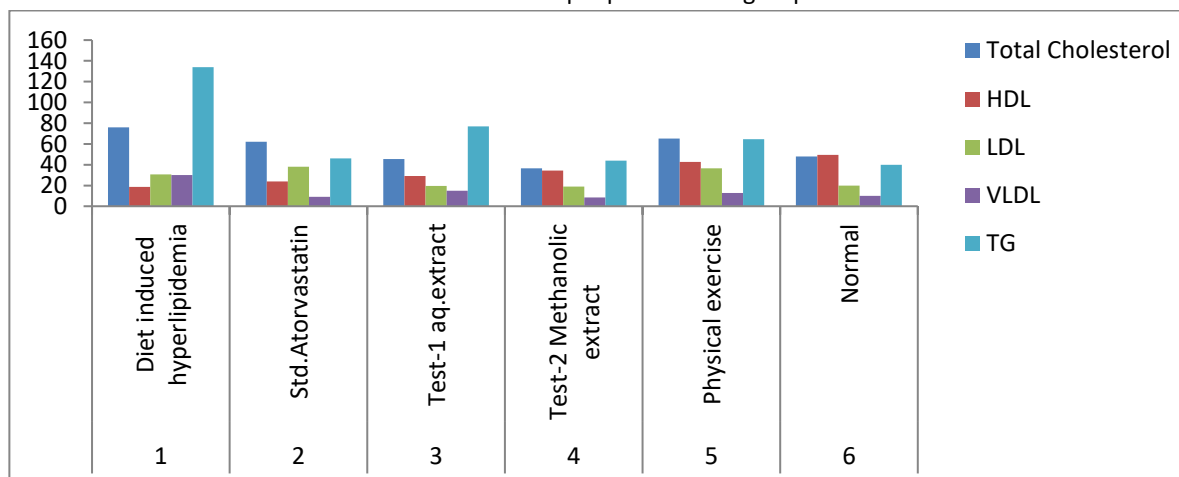
FIGURE-5: Serum lipid profile of group-5 rats


TABLE-6: Serum lipid profile for Normal rats

S.NO	Treatment	Total Cholesterol	HDL	LDL	VLDL	TG
6.	Normal	48	49.3	20	10	40

FIGURE-6: Serum lipid profile of group-6 rats

FIGURE-7: Serum lipid profile of all groups of rats


CONCLUSION

The different groups of rats had undergone the treatment of test and standard drugs for the evaluation of Anti –Hyperlipidemic activity and the Methanolic extract has shown significant anti – hypelipidemic activity than other extracts and standard agents by comparing the parameters like Total cholesterol, HDL, VLDL, LDL and Triglycerides levels.

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