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EVALUATION OF ANTIHYPERLIPIDEMIC ACTIVITY OF METHANOLIC EXTRACT OF AERIAL PARTS OF *RHINACANTHUS NASUTUS*

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ABSTRACT

The aim of present study was to evaluate the antihyperlipedemic activity of Methanol extract of aerial parts of *Rhinacanthus nasutus* using high fat diet induced hyperlipidemia and Triton X-100 induced hyperlipidemia models in Wister Albino rats. Hyperlipidemia is a disorder of lipid metabolism manifested by elevation of plasma concentrations of the various lipid and lipoprotein functions which is the key risk factor for cardiovascular disorders [CVD]. *Rhinacanthus nasutus* extract was administered at different dose of 250mg/kg, 500mg/kg to hyperlipidemic rats. Atorvastatin is used as reference standard. The statistical analysis were carried out using one way ANOVA followed by Dunnet t-test. The serum total cholesterol, triglyceride, HDL, VLDL, LDL levels were analyzed. Methanoic aerial part extract of *rhinacanthus nasutus*. Exhibited significant [$p<0.05$] effecting reducing the serum cholesterol, lipid levels. The present study clearly demonstrated the anti-hyperlipidemic activity of *Rhinacanthus nasutus* supporting the traditional claim.

KEYWORDS

Hyperlipidemia, *Rhinacanthus nasutus*, High fat diet, Triton X 100 and Atorvastatin.

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INTRODUCTION

Hyperlipidemia is a broad term, also called hyperproteinemia, is a common disorder in development countries and is the key risk factor for cardiovascular disorder (CVD)[1]. It results from abnormalities in lipid metabolism or plasma lipid transport or a disorder in the synthesis and degradation of plasma lipoproteins. The term "dyslipidemia" nowadays in increasingly being used to describe abnormal changes in lipid profile, replacing the old term hyperlipidemia. Hyperlipidemia means abnormal high level of fat in the blood. These fats include cholesterol and

triglycerides. These are important for our bodies to function but when they are high, they can cause heart disease and stroke. *Rhinacanthus nastus* is the shrub and it belongs to the acanthaceae family. And it is well known for its medicinal uses. The entire plant is has been used as an anthelmentic, antiseptic, aphrodiasic, antiparasitic and as anticancer agent. It is also used for treatment of leprosy, eczema, scurvy and dhobi's itch[2]. Leaves, roots and seeds act as an antidote for anake bites. Considering the traditional uses of *Rhinacanthus nasutus* the present study was undertaken to investigate the antihyperlipidemic activity of methanolic extract of leaves of *Rhinacanthus nasutus* against high fat induced hyperlipedemia in rats.

METERIAL AND METHODS

Plant material

Plant material used in this study consisted of the aerial parts of *Rhinacanthus nasutus,* collected and authentified by Prof. K. Madhava Chetty, Ph.D., Research officer - Botany, Tirupathi.

Preparation of extract

The shade dried aerial parts of powdered material was subject to in white cotton wool apparatus. The solvent used was methanol. Equal amount of (200gm) of powdered material was taken in a clean flat bottomed glass container and soaked in 750ml of methanol. The container with its contents were sealed and kept for period of 7 days accompanied by continuous shaking with the shaker the whole mixture then went under a coarse filtration by a piece of a clean, white cotton wool. Evaporation of solvent by using "Rotary evaporation" in a porcelain dish they reduced a gummy concentrate of greenish black. The extract was kept in vacuum dissector for 7 days.

Animals

Wistar male albino rats (150-180 g) were selected for the present study. The animals had free access to standard rat pellet, with water supplied *ad libitum* under the strict hygienic conditions. The experimental protocol was approved by IAEC (Institutional Animal Ethics Committee) of sanzyme pvt.ltd. The study followed all the rules of (CPCSEA) Committee for the Purpose of Control and Supervision of Experiments on Animals.

Chemicals and reagents

Triton X-100 were procured from Unisource chemical pvt. Ltd. and atorvastatin were procured from DR.REDDY'S laboratories (Hyderabad). Total cholesterol, triglyceride, HDL, LDL, and VLDL kits were procured from excel diagnostics pvt. Ltd.(India). They were of analytical grade qualify wistar albino rats were procured from Mahaveer Enterprises, Hyderabad.

Antihyperlipidemic activity

High fat diet induced hyperlipidemia

The systemic administration hyperlipidemia of the surfactant triton x 100 to rats and supportive high fat diet results in elevation of plasma cholesterol and triglycerides. Hyperlipidemia was induced in wister albino rats[3] by single i.p injection of freshly prepared solution of triton x 100 (100 mg/kg) in physiological saline solution after overnight fasting for 18h. And it is supplied with high fat diet[4].

Study design

Wistar albino rats weighing 150-180 gm, were divided in to five groups containing four animals in each group.

Group-1

Normal control (normal saline 10 ml/kg orally) for 7 days.

Group-2

Hyperlipidemic control, three i.v injection of triton x 100 on consecutive days with high fat diet.

Group-3

Hyperlipidemic rats treated with atorvastatin (standard drug) at 10 mg/kg orally for 7 days with high fat diet.

Group-4

Hyperlipidemic rats treated with ethanolic extract of *rhinacanthus nasutus* (ERN) low dose at daily dose of 250 mg/kg orally for 7 days with high fat diet.

Group-5

Hyperlipidemic rats treated with ethanolic extract of *rhinacanthus nasutus* (ERN) high dose at daily dose of 500 mg/kg orally for 7 days with high fat diet.

Triton x-100 induced hyperlipidemia

The wistar rats were randomly divided in to five groups containing six animals. All the groups receives three i.v injection of triton x-100 (100 mg/kg) on consecutive days with supportive high fat diet, stimulatancously with group-2, group-3,

group-4, group-5, except group-1 (normal control) induce hyperlipidemia. Prepared by suspending bulk atorvastatin in aqueous 5 percentage methyl cellulose for 7 days. The group-4 receive ERN at a dose of 250 mg/kg for 7 days and group-5 receives ERN at a dose of 500 mg/kg for 7 days.

Collection of blood samples and serum separation

The rats are anesthetized by either and them blood samples were collected on 0^{th} and 8^{th} day from retro-orbital plexus[4] of rat using micro capillary technique from rats of all the groups, and centrifuged at 3000 rpm for 15 min so as to get serum. The serum is analyzed for total cholesterol, triglycerides and HDL levels using biochemical kits[5].

VLDL Cholesterol = Total Cholesterol – (HDL-Cholesterol +TG/5)

VLDL Cholesterol = TG/5

Statistical analysis

Results are expressed as mean ± S.D. all the results were compared with control subject one way analysis of variance (ANOVA), followed by the dunnet t-test using graph pad prism software 6 version. P values < 0.05 were as considered statistically significant.

RESULTS

In the normal rats the total cholesterol levels as found to be 62.1±2.2. Treatment with triton X 100 caused a significant rise in the levels of cholesterol (159016±4.37, 166.24±2.2, 152.26±2.35 and 163.06±63) in hyperlipidemic control treatment with atorvastatin group the total cholesterol was reduced to 115.2±2.03. The total cholesterol levels of groups treated with extract at dose 250 mg/kg and 500 mg/kg were 135.28±3.6 and 118.7±3.3 respectively and lowering of cholesterol levels. The reduction in cholesterol level extract was significant at ($p<0.05$).

EFFECT OF RHINACANTHUS *NASUTUS* ON TOTAL CHOLESTEROL LEVELS

Effect of *Rhinacanthus nasutus* on serum LDL-C levels

In the normal rats the LDL was found to be 20.24±6.1. Treatment with triton x-100 caused significant rise in the level of LDL (97.52±3.5, 101.16±1.8, 88.87±2.7 and 98.36±6.1) in hyperlipidemic control treatment with atorvastatin group the LDL was reduced to 54.9±1.5. The LDL levels of groups of treated with extract at dose of 250 mg/kg and 500 mg/kg were 70.86±4.1 and 56.09±4.6 respectively and lowering of LDL. The reduction in LDL level by extract was significant at ($p<0.05$).

EFFECT OF *RHINACANTHUS NASUTUS* ON TRIGLYCERIDE LEVELS

In the normal rats the triglycerides was found to be 78.58±3.2. Treatment with triton x-100 caused significant rise in the level of triglycerides (177.2±3.2, 176.57±2.3, 163.27±3.6 and 172.4±4.3) in hyperlipidemic control treatment group with atorvastatin group was reduced to 94.49±2.5. the triglycerides levels of groups of treated with extract at dose of 250 mg/kg and 500 mg/kg were 139.69±6.9 and 109.37±4.1 respectively and lowering of triglyceride was seen to be in dose dependent manner. The reduction in triglyceride s level by extract was significant at ($p<0.05$).

EFFECT OF *RHINACANTHUS NASUTUS* ON TRIGLYCERIDE LEVELS

Effect of *Rhinacanthus nasutus* on serum VLDL-C levels

In the normal rats the VLDL was found to be 14.69±1.8. Treatment with triton x-100 caused significant rise in the level of VLDL (35.43±0.61, 35.31±0.45, 32.65±0.7 and 34.48±0.8) in hyperlipidemic control treatment group with atorvastatin group was reduced to 18.89±0.5. The VLDL levels of groups of treated with extract at dose of 250 mg/kg and 500 mg/kg were 27.92±1.3 and 21.77±0.75 respectively and lowering of VLDL was seen to be in dose dependent manner. The reduction in VLDL level by extract was significant at ($p<0.05$).

Effect of *Rhinacanthus nasutus* on serum HDL-C levels

In the normal rats the HDL was found to be 39.8±2.0. Treatment with triton x-100 caused significant rise in the level of HDL (26.20±1.0, 29.77±1.1, 30.78±0.95 and 30.41±0.95) in

hyperlipidemic control treatment group with atorvastatin group was reduced to 94.49±2.5. The HDL levels of groups of treated with extract at dose of 250 mg/kg and 500 mg/kg were 36.52±0.8 and 40.75±1.72 respectively and lowering of HDL was seen to be in dose dependent manner. The reduction in HDL level by extract was significant at ($p<0.05$).

DISCUSSION

Hyperlipidemia is a one greatest factors contributing to the prevalence and severity of coronary heart diseases[10]. It is a not a disease but a metabolic derangement that can be secondary to many diseases and can contribute to many forms of disease[11]. The change in lipid levels in group number-3 to 5, were comparable with group of hyperlipidemic control (i.e; triton x 100, group-2). The standard group (i.e, atorvastatin group) significantly lowers the serum lipid level ($p <0.001$).

The extract of *Rhinacanthus nasutus* tested in the present study significantly (p, 0.01) prevented hyperlipidemia compared to the hyperlipidemic group. The results presented in this study indicated that the ethanolic extract *Rhinacanthus nasutu* at doses 250 mg/kg and 500 mg/kg treatment decreases the triton X-100 induced increased levels of TC, TG'S, LDL and VLDL. Among these two doses 500 mg/kg decreases the lipid parameters more significantly than 250 mg/kg hence the increased dose is more protective than the lowered dose. Thus the effect has been seen to be in dose dependent manner.

Table No.1: Effect of *Rhinacanthus nasutus* Extract on Serum Total Cholesterol levels

S.No	Groups	Total Cholesterol Levels (mg/dl)	
		0th day	8th day
1	Normal	62.1±2.2	63.13± 2.3
2	Hyperlipidemic	159.16±4.37	158.93±3.0#
3	Standard Atorvostatin 10mg/kg	166.24± 2.2	115.2±2.03*
4	ERN 250 mg/kg	152.26 ±2.35	135.28±3.6*
5	ERN 500 mg/kg	163.06± 6.3	118.7±3.3*

Table No.2: Effect of *Rhinacanthus nasutus* Extract on Serum LDL-C levels

S.No	Groups	LDL-C (mg/dl)	
		0th day	8th day
1	Normal	20.24±2.7	21.84±2.6
2	Hyperlipidemic	97.52±3.5	98.74±3.1#
3	Standard Atorvostatin 10mg/kg	101.16±1.8	54.9±1.5*
4	ERN 250mg/kg	88.87± 2.7	70.86±4.1*
5	ERN 500mg/kg	98.36± 6.1	56.09±4.6*

Table No.3: Effect of *Rhinacanthus nasutus* Extract on Serum triglyceride levels

S.No	Groups	Triglycerides (mg/dl)	
		0th day	8 th day
1	Normal	78.58±3.2	82.42±2.8
2	Hyperlipidemic	177.2±3.2	175.21±5.5#
3	Standard Atorvostatin 10mg/kg	176.57± 2.3	94.49±2.5*
4	ERN 250mg/kg	163.27± 3.6	139.65±6.9*
5	ERN 500mg/kg	172.4± 4.3	109.37±4.1*

Table No.4: Effect of *Rhinacanthus nasutus* Extract on Serum VLDL-C levels

S.No	Groups	VLDL-C (mg/dl)	
		0th day	**8 th day**
1	Normal	14.69±1.8	14.78±2.3
2	Hyperlipidemic	35.43±0.61	35.03±1.1#
3	Standard Atorvostatin 10mg/kg	35.31± 0.45	18.89±0.5*
4	ERN 250mg/kg	32.65± 0.7	27.92±1.3*
5	ERN 500mg/kg	34.48± 0.8	21.77±0.75*

Table No.5: Effect of *Rhinacanthus nasutus* Extract on Serum HDL-C levels

S.No	Groups	HDL-C (mg/dl)	
		0th day	**8th day**
1	Normal	39.8±2.0	38.89±2.7
2	Hyperlipidemic	26.20±1.0	24.90±1.85#
3	Standard Atorvostatin 10mg/kg	29.77 ± 1.1	41.45±0.45*
4	ERN 250mg/kg	30.78 ± 0.95	36.52±0.8*
5	ERN 500mg/kg	30.41± 0.95	40.75±1.72*

EFFECT OF *RHINACANTHUS NASUTUS* ON TOTAL LIPID PROFILES

Lipid profiles obtained on 0th day (Before treatment) and 8th day (After treatment)

S.No	GROUPS	TC		TG		HDL		LDL		VLDL	
		0th day	**8 th day**	**0th day**	**8 th day**	**0th day**	**8 th day**	**0th day**	**8 th day**	**0th day**	**8 th day**
1	Normal	62.1±2.2	63.13±2.3	78.58±3.2	82.42±2.8	39.8±2.0	38.89±2.7	20.24±2.7	21.84±2.6	14.69±1.8	14.78±2.3
2	Hyperlipidemic	159.16±4.37	158.93±3.0#	177.2±3.2	175.21±5.5#	26.20±1.0	24.90±1.85#	97.52±3.5	98.74±3.1#	35.43±0.61	35.03±1.1#
3	Standard Atorvostatin 10 mg/kg	166.24 ± 2.2	115.2±2.03*	176.57 ±2.3	94.49±2.5*	29.77 ± 1.1	41.45±0.45*	101.16 ±1.8	54.9±1.5*	35.31±0.45	18.89±0.5*
4	ERN 250 mg/kg.	152.26 ±2.35	135.28 ±3.6*	163.27 ± 3.6	139.65 ±6.9*	30.78 ± 0.95	36.52±0.8*	88.87±2.7	70.86±4.1*	32.65±0.7	27.92±1.3*
5	ERN 500 mg/kg.	163.06 ± 6.3	118.7±3.3*	172.4±4.3	109.37 ±4.1*	30.41±0.95	40.75±1.72*	98.36±6.1	56.09±4.6*	34.48±0.8	21.77±0.75*

Results are expressed as mean ± SEM; n=6 #P<0.01 vs Normal Control, *p<0.01, vs Hyperlipidemic control.

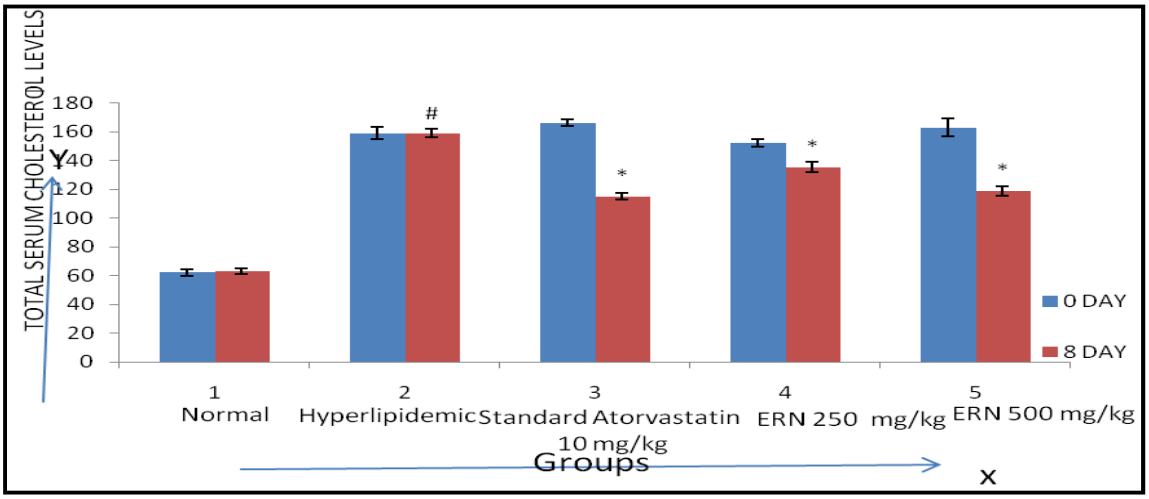

Figure No.1: Effect of *Rhinacanthus nasutus* Extract on Serum total Cholesterol levels# P<0.01 vs Normal Control, *p<0.01, vs Hyperlipidemic control

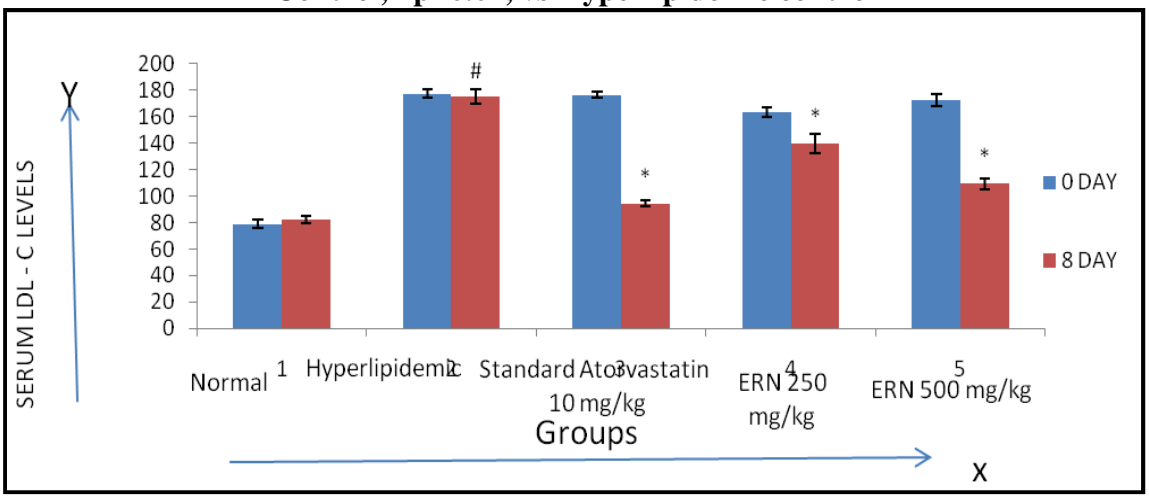

Figure No.2: Effect of *Rhinacanthus nasutus* Extract on serum LDL-C levels #P<0.01 vs normal control, *p<0.01, vs hyperlipidemic control

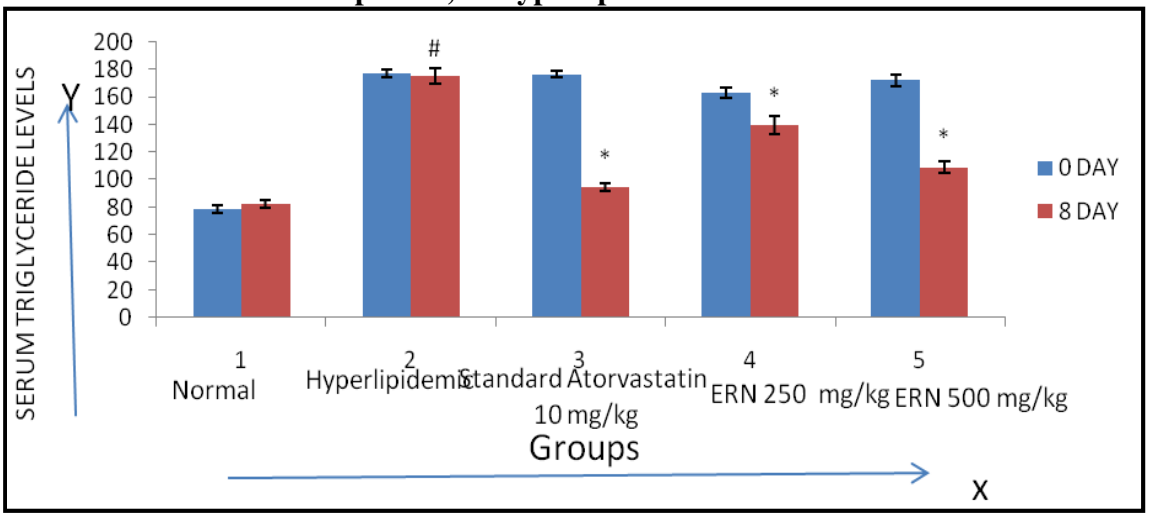

Figure No.3: Effect of *Rhinacanthus nasutus* Extract on Serum Triglyceride levels #P<0.01 vs Normal Control, * p<0.01, vs Hyperlipidemic control

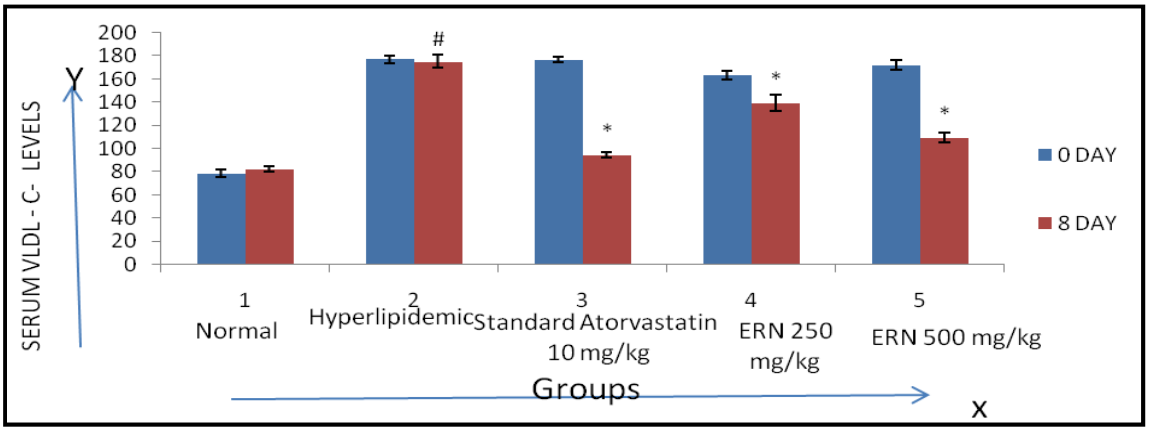

Figure No.4: Effect of *Rhinacanthus nasutus* Extract on Serum VLDL-C levels #P<0.01 vs Normal Control, * p<0.01, vs Hyperlipidemic control

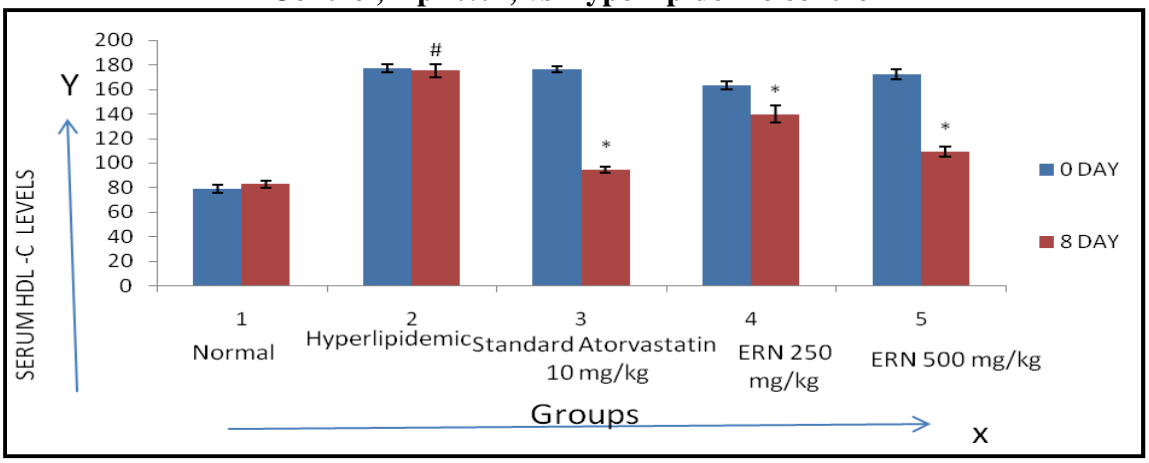

Figure No.5: Effect of *Rhinacanthus nasutus* Extract on Serum HDL-C levels #P<0.01 vs Normal Control, *p<0.01, vs Hyperlipidemic control

CONCLUSION

In conclusion our results suggest that the post treatment with ethanolic extract of *Rhinacanthus nasutus* showed dose dependent antihyperlipidemic activity against triton-X 100 and high fat diet induced hyperlipidemia indicating that naturally occurring plant may be used as starting structures for the potential development of antihyperlipidemic agents.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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