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Research Article

ANTI-HYPERLIPIDEMIC ACTIVITY OF METHANOLIC EXTRACT OF *RHINACANTHUS NASUTUS*

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ABSTRACT

The anti-hyperlipidemic effect of methanolic extract of whole plant of *Rhinacanthus nasutus* ((RNM) was tested in Triton and fat diet induced hyperlipidemic rat models. Here, Acute hyperlipidemia was induced by administration of single dose of Triton X 100 (400 mg/kg,i.p) and Chronic hyperlipidemia was induced by feeding fat diet for 21 days to rats. Treatment with RNM (200 and 400 mg/kg, p.o) significantly reduced the hyperlipidemia i.e., decreased levels of serum Total Cholesterol, Triglycerides, Low Density Lipoprotein Cholesterol (LDL-C), Very Low Density Lipoprotein Cholesterol (VLDL-C) , and increase of serum High Density Lipoprotein Cholesterol (HDL-C) when compared to vehicle control and standard drug Atorvastatin (10 mg/kg). The results demonstrated that methanolic extract of whole plant of *Rhinacanthus nasutus* possessed significant antihyperlipidemic activity.

Keywords: Triton, fat diet, hyperlipidemia, *Rhinacanthus nasutus*, whole plant, rats, Atrovastatin.

INTRODUCTION

Hyperlipidemia is major risk factor for the atherosclerosis. Other complications are coronary heart disease, ischemic cerebro vascular disease, hypertension, obesity and diabetes mellitus (Type -II). Although many efficacious lipid-lowering synthetic drugs exist, none is effective for all lipoprotein disorders, and all such agents are associated with some adverse effects. Therefore it is a need of the day to search other materials from natural sources that are less toxic, less expensive, which can provide better safety and efficacy on a long term usage. Natural products from plants are a rich source used for centuries to cure various ailments.

Rhinacanthus nasutus (Linn) belongs to Acanthaceae family is a shrub and is well known for its medicinal uses, commonly called as Nagamalli in Telugu. Rhinacanthus nasutus is widely distributed in some parts of the subcontinent India and in the region of Southeast Asia and china. The plant is small slender shrub 1-2 meter height. Various parts of this plant have been used for the treatment of eczema, pulmonary tuberculosis, herpes, hepatitis, diabetes, hypertension, skin diseases, ring worms, cancer, scurvy, leprosy and obesity. The plant leaves, roots and seeds also used as an antidote for snake bites[1-3].

The present study was designed to investigate the Anti-hyperlipidemic activity of methanolic extract of *Rhinacanthus nasutus* whole plant in Wistar rats in an attempt to establish traditional use of this plant.

MATERIALS AND METHODS

Plant material

The fresh whole plant of *Rhinacanthus nasutus Linn* were collected from Thiyagaraja Nagar,Tamil Nadu, India, identified and authenticated by Research officer-Botany, central council for research in Ayurveda & Siddha, Govt. of India.

Preparation of Extract

The whole plant powdered material was subjected to batch extraction in Soxhlet apparatus. The solvent used was Methanol. The powdered material of whole plant of *Rhinacanthus nasutus* was evenly packed in Soxhlet extractor for extraction with solvent. The temperature was maintained on an

electric heating mantle with thermostat control. Appearance of colorless solvent in the siphon tube was taken as the termination of extraction. The extract was then concentrated by distilling the solvent and percentage yield was calculated. Hence forth the Methanolic extract of *Rhinacanthus nasutus* will be called as **RNM**[4].

Test for Phytochemical Analysis

The conventional chemical tests were carried out for the extract of RNM to identify the presence of various chemical constituents [5].

***IN VIVO* STUDIES**

EXPERIMENTAL ANIMALS

Adult Wistar albino rats (150-180 g) of either sex were procured from the laboratory animal house, Hindu College of Pharmacy, Guntur, Andhra Pradesh, India and used in the study. The animals were kept under standard environmental conditions of room temperature (22^0 $\pm 2^0$C), relative humidity (50% ± 5%) and 12 h light and dark cycle. The animals were housed in the colony cages (either three rats or six mice per cage) and provided feed (commercial pellets contain a balanced ration obtained from the Sri Venkateswara Enterprises, Bangalore) and water *ad libitum.* All the animals were acclimatized to the laboratory environment 5 days prior to experiment. The animals were fasted overnight just prior to the experiment but allowed free access to drinking water. All the experiments were carried out in accordance with the guidelines of Institutional Animal Ethics Committee. The study was conducted after obtaining ethical committee clearance from the Institutional Animal Ethics Committee.

ANTI-HYPERLIPIDEMIC ACTIVITY

1. Triton X 100 (TR) induced hyperlipidemic model

Thirty Wistar rats were randomly divided into 5 groups of 6 each. The first group was given standard pellet diet, water and orally administered with 5% CMC. The II,III,IV,V group animals were injected i.p. with 10% aqueous solution of Triton 400mg /kg body weight . After 72 hours of triton injection, the second group received a daily dose of 5% CMC (p.o) for 7 days. The third and fourth group was administered a daily dose of RNM 200 and 400 mg/kg suspended in 5%CMC, p.o., for 7 days, after inducing hyperlipidemia. Fourth group was administered with the standard Atorvastatin 10mg/kg, p.o. for 7 days. Food was withdrawn 10h prior to the blood sampling. The control group animals received the vehicle in the same volume orally.

Group 1: Administered vehicle and served as normal control.

Group 2: Administered Triton X 100 (TR) and served as hyperlipidemic control.

Group 3: Administered RNM (200mg/kg), p.o.,

Group 4: Administered RNM (400mg/kg), p.o.,

Group 5: Administered Atorvastatin (10mg/kg), p.o.

On the 8thday, blood was collected by retero orbital sinus puncture,under mild ether anaesthesia. The collected samples were centrifuged for 15 minutes at 2500rpm. Then serum samples were collected and analyzed for serum Total Cholesterol,Triglycerides, High Density Lipoprotein Cholesterol,Low Density Lipoprotein Cholesterol and Very Density Lipoprotein Cholesterol [6].

Statistical Analysis

Results were analyzed by one way ANOVA, followed by Dunnet's test, 'P' value less than 0.05 were taken as significant.

Table 1: Effect of RNM on serum lipid parameter levels in Triton induced Hyperlipidemic rats

S. No	Groups	Serum Lipid Parameters (mg/dl)				
		Total Cholesterol	Triglycerides	HDL-C	LDL-C	VLDL-C
I	Normal Control (Saline)	84.67±1.28	64.73±8.07	47.27 ±4.62	24.46 ±1.61	12.95±1.61
II	Hyperlipidemic Control	205.7±12.81	117.9 ±7.45	34.98±4.40	147.1±15.1	23.58±1.49
III	RNM (200mg/kg)	101.9±15.37**	99.12±2.76*	41.07±4.61*	41.01±6.62*	19.81±0.55*
IV	RNM (400mg/kg)	96.57±14.16**	89.19±2.80*	43.03±4.66**	36.09±15.01*	17.83±0.56*
V	Atorvastatin (10 mg/kg)	92.27±13.21**	84.32±3.03**	45.10±4.69*	32.44±12.90*	16.86±0.60*

Values are mean ± SEM (n=6).Values are statistically significant at *P<0.05and more significant at **P<0.01 vs hyperlipidemic control using one way ANOVA followed by Dunnet's test.

2. High Fat Diet (FD) induced hyperlipidemic model

Preparation of Feed

Normal animal food pellets were crushed in mortar and pestle to crush into small pieces and then grinded into fine powder in mixer grinder. The other ingredients i.e. cholesterol 2% , Cholic acid 1 % , sucrose 40% , and coconut oil 10% were added in the mixer grinder in an ascending order of their quantity and mixed well. This dried powder was then mixed with same quantity of water every time to make small balls of feed and later this was stored in self sealing plastic covers in refrigerator at 2°C to 8°C. The feed for normal group was prepared similarly by grinding only the normal food pellets and then mixing with water without the other excipients. This preparation of feed was done once in three days for all the animals.

Thirty Wistar rats were randomly divided into five groups of six each. The chronic experimental hyperlipidemia was produced by feeding the above prepared food for 21 days . The rats are then given test plant extracts i.e., RNM (200 and 400 mg/kg, p.o) and Atorvastatin (10 mg/kg, p.o) once daily in the morning orally for 14 consecutive days. During these days, all the groups also received fat diet in the same dose as given earlier. The hyperlipidemic control i.e.,group II animals received the hyperlipidemic diet and the vehicle. The control group animals received the normal laboratory diet and vehicle .

Group 1: Administered vehicle and served as normal control.

Group 2: Fed with fat diet (FD) and served as hyperlipidemic control.

Group 3: Administered RNM (200mg/kg), p.o., and fed with FD.

Group 4: Administered RNM (400mg/kg), p.o., and fed with FD.

Group 5: Administered Atorvastatin (10mg/kg), p.o.,and fed with FD.

On day 15, animals were anaesthetized with Diethyl ether and blood was collected by retro orbital puncture. The blood was subjected to centrifugation for 15 min at 2500 rpm to obtain serum. The collected serum was analyzed for serum Total Cholesterol,Triglycerides, High Density Lipoprotein Cholesterol,Low Density Lipoprotein Cholesterol and Very Low Density Lipoprotein Cholesterol [6,7].

Statistical Analysis

Results were analyzed by one way ANOVA , followed by Dunnet's test , 'P' value less than 0.05 were taken as significant (Table 2).

Table 2: Effect of RNM on serum lipid parameter levels in fat diet induced Hyperlipidemic rats

S. No	Groups	Serum Lipid Parameters (mg/dl)				
		Total Cholesterol	Triglycerides	HDL-C	LDL-C	VLDL-C
I	Normal Control(Saline)	83.84± 1.22	64.07 ± 8.33	48.34 ±4.59	22.69± 5.38	12.81± 1.67
II	Hyperlipidemic Control	187.0±10.85	102.9±5.18	25.05±4.43	141.4±14.04	20.58±1.04
III	RNM(200mg/kg)	123.0±10.83*	83.16±4.46*	31.00±4.45*	75.41±14.14*	16.59±0.91*
IV	RNM(400mg/kg)	107.7±10.74**	76.28±6.76**	36.19±4.67**	56.25± 4.24*	15.23±1.35*
V	Atorvastatin (10 mg/kg)	97.62±10.69**	70.24±4.40*	38.34±4.5*	45.28±14.14*	14.00±0.87*

Values are mean ± SEM (n=6).Values are statistically significant at *P<0.05and more significant at **P<0.01 vs hyperlipidemic control using one way ANOVA followed by Dunnet's test.

RESULTS AND DISCUSSION

The dried and powdered whole plant material of *Rhinacanthus nasutus* was subjected to soxhlet extraction with 95 % methanol and yielded 10 % w/w.

Phytochemical analysis of the plant extract showed different phytoconstituents viz. glycosides, phytosterols, triterpinoids, alkaloids and flavonoids. Several phytoconstituents like glycosides, triterpinoids, Saponins, alkaloids and flavonoids are known to have anti-hyperlipidemic properties.

Treatment with RNM (200 & 400mg/kg, p.o.,) for 7 days successfully prevented the elevation of serum Total Cholesterol, Triglycerides, Low Density Lipoproteins Cholesterol (LDL-C), Very Low Density Lipoproteins Cholesterol (VLDL-C) , and decrease of serum High Density Lipoprotein Cholesterol (HDL-C) in Triton model rats respectively. Triton induced hyperlipidemia in rats is an acute model for the primary

screening of antihyperlipidemic agents. Triton physically alters very low density lipoprotein cholesterol rendering them refractive to the action of lipolytic enzymes of blood and tissues, preventing or delaying their removal from blood and tissues. Hence the antihyperlipidemic effect of *Rhinacanthus nasutus* administration could be due to an increased catabolism of cholesterol into bile acids.

Administration of RNM (200 & 400mg/kg, p.o) for 14 days in fat diet model , successfully prevented the elevation of serum Total Cholesterol, Triglycerides, Low Density Lipoproteins Cholesterol (LDL-C), Very Low Density Lipoproteins Cholesterol (VLDL-C) , and decrease of serum High Density Lipoprotein Cholesterol (HDL-C) in Fat diet model rats respectively. It has been well established that nutrition plays an important role in the etiology of hyperlipidimias and atherosclerosis. Fat diet model is used as a chronic model for induction of hyperlipidemia. In our study we chosen fat diet which contain the common ingredients in our daily food.

Diet containing saturated fatty acids increases the activity of HMG CoA reductase, the rate determining enzyme in cholesterol biosynthesis; this may be due to higher availability of acetyl CoA, which stimulated the cholesterogenesis rate. Moreover, this could be associated with a down regulation in LDL receptors by the cholesterol and saturated fatty acids in the diet, which could also explain the elevation of serum LDL-C levels either by changing hepatic LDLR (LDL-receptor) activity, the LDL-C production rate or both.

LCAT enzyme is involved in the transesterification of cholesterol, the maturation of HDL-C and the flux of cholesterol from cell membranes into HDL. The activity of the enzyme tends to decrease in diet-induced hypercholesterolemia.

The possible mechanism of RNM may involve increase of HDL-C, which is attributed to the mobilization of cholesterol from peripheral cells to the liver by the action of Lecithin Cholesterol O-acyltransferase (LCAT) .The increased HDL-C facilitates the transport of TG or cholesterol from serum to liver by a pathway termed 'reverse cholesterol transport' where it is catabolised and excreted out of the body.

Antihyperlipidemic activity was observed with Atorvastatin (10mg/kg p.o.,), and the RNM (400mg/kg) showed better activity than RNM (200mg/kg).

CONCLUSION

The results obtained from the pharmacological screening have led to the conclusions that, methanolic extract of whole plant of *Rhinacanthus nasutus* has significant antihyperlipidemic activity. Hence it can be exploited as an anti-hyperlipidemic therapeutic agent or adjuvant in existing therapy for the treatment of hyperlipidemia.

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