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Research Article

ANTIHYPER LIPIDEMIC EFFECT OF POLY HERBAL FORMULATION IN ALBINO RATS USING TRITON-X AND FAT DIET INDUCED HYPERLIPIDEMIC MODELS

Mohd Ahmed Uddin*, Roshan. S, N. L. Mahammed, Mohd Altaf Ahmed Khan

Department of Pharmacology, AZAD College of pharmacy, Moinabad, Telangana-501504

***Corresponding author's Email:** mohammad.lal121@gmail.com

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ABSTRACT

The anti-hyperlipidemic effect of *Polyherbal formulation* (PHF) 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 *Polyherbal formulation* (PHF) (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) , an 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 *Polyherbal formulation* (PHF) possessed significant antihyperlipidemic activity.

Keywords: Triton, fat diet, hyperlipidemia, Polyherbal formulation, 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 [1]. 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. In temperate zones, probably the most commonly grown ornamental species is Hibiscus syriacus, the common garden hibiscus, also known in some areas as the "Rose of Althea" or "Rose of Sharon". *M. oleifera* is a fast-growing, deciduous tree. *Nigella sativa* is an annual flowering plant in the family Ranunculaceae, native to south and southwest Asia. *Nigella sativa* seed is variously called fennel flower, nutmeg flower, black caraway and Roman coriander. The present study was designed to investigate the Anti-hyperlipidemic activity of *polyherbal formulation* in Wistar rats in an attempt to establish traditional use of this Poly herbal formulation [2].

MATERIALS AND METHODS:

Plants Collection: For the present investigation, Poly Herbal Formulation was prepared.

Animals: Wistar albino rats (140-200g) were taken to assess anti-hyperlipidemic activity. All the experimental protocols were approved by Institutional Animal Ethics Committee (IAEC) of Pharmacy College, (No.1516/PO/a/11/CPCSEA).

Phytochemical Screening: The Poly Herbal Formulation may be considered as a bio-synthetic laboratory, not only for the chemical compounds such as carbohydrates, proteins and

lipids that are utilized as food by man but also for a multitude of compounds like glycosides, alkaloids, volatile oils, tannins etc. that exert a physiologic and therapeutic effect.

Hypolipidemic Activity in Normal Rats:

Induction of Hyperlipidemia: Hyperlipidemia was induced in Wistar albino rats by single intraperitoneal injection of freshly prepared solution of Triton-X-100 (100 mg/kg) in physiological saline solution after overnight fasting for 18 h. The animals were divided into four groups of five rats each. The first group was given standard pellet diet, water and orally administered with 5% CMC. The second group was given a single dose of triton administered at a dose of 100mg/kg, i.p. After 72 hours of triton injection, this group received a daily dose of 5% CMC (p.o) for 7 days. The third group was administered a daily dose of Poly herbal formulation 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 [3].

Anti-Hyperlipidemic Activity:

1. Triton X 100 (TR) Induced Hyperlipidemic Model:

Thirty Wister 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 10 hr 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 poly herbal formulation extract (200mg/kg), p.o.,

Group 4: Administered poly herbal formulation extract (400mg/kg), p.o.,

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

On the 8th day, 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 [4].

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 (Seifter & England, 1982). 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 [5].

Thirty wister 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., poly herbal formulation extract (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 poly herbal formulation extract (200mg/kg), p.o., and fed with fd.

group 4: administered poly herbal formulation extract (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.

Statistical Analysis: Results were analyzed by one way ANOVA, followed by Dunnet's test, 'P' value less than 0.05 were taken as significant[6].

DISCUSSION:

Treatment With PHF (200 Mg/kg and 400mg/kg.po,) 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 (Table.1). Triton induced hyperlipidemia in rats is an acute model for the

RESULTS:

Table 1: Effect of PHF 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	PHF (200mg/kg)	101.9±15.37**	99.12±2.76*	41.07±4.61*	41.01±6.62*	19.81±0.55*
IV	PHF (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.

Table 2: Effect of PHF 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	PHF(200mg/kg)	123.0±10.83*	83.16±4.46*	31.00±4.45*	75.41±14.14*	16.59±0.91*
IV	PHF(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.

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 poly herbal formulation administration could be due to an increases catabolism of cholesterol into bile acids.

Administration of poly herbal formulation (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(Table.2). 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 have chosen fat diet which contains 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 *Polyherbal formulation* (PHF) 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 poly herbal formulation (400mg/kg) showed better activity than poly herbal formulation (200mg/kg).

CONCLUSION

The results obtained from the pharmacological screening have led to the conclusions that, poly herbal formulation has significant anti-hyperlipidemic 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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