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Hypolipidemic Activity of Methanolic Extract of *Terminalia arjuna* Leaves in Hyperlipidemic Rat Models

D. Bala Sunder Reddy, P. Ravi Kumar, K. Bharavi and U. Venkateswarlu
Department of Pharmacology and Toxicology,
N.T.R. College of Veterinary Science, 521-102, Gannavaram, India

Abstract: Hypolipidemic activity of methanolic extract of *Terminalia arjuna* leaves was evaluated in two hyperlipidemic rat models viz. triton injected and cholesterol and cholic acid fed models. Triton model rats received a single dose of Methanolic Extract of *Terminalia arjuna* Leaves (METAL) orally at 500 mg kg^{-1} b.wt. 2 h prior to triton injection. Cholesterol and cholic acid fed hyperlipidemic rats received METAL at 500 mg kg^{-1} b.wt. orally for 30 days. In triton model hypolipidemic activity of METAL was evaluated by estimating the serum concentrations of total cholesterol and triglycerides before and 18 post treatment hours after triton administration. In cholesterol and cholic acid fed hyperlipidemic rats, hypolipidemic activity was monitored by determining serum concentrations of total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol and VLDL cholesterol on day 0 and 30. Cholic acid and desoxycholic acid in feces were estimated on day 0 and on 30 from the pooled fecal sample collected over 30 days. Serum lipids in general were found to be lowered by METAL in both models. However, METAL administration demonstrated increase in HDL cholesterol levels in cholesterol and cholic acid fed hyperlipidemic rats. Fecal bile acid excretion was found to be enhanced by METAL in cholesterol and cholic acid fed hyperlipidemic rats. The possible mechanism of lipid lowering activity of METAL may be due to rapid excretion of bile acids and/or inhibition of hepatic cholesterol biosynthesis.

Key words: *Terminalia arjuna*, hypolipidemic activity, hyperlipidemia, cholesterol, rats, India

INTRODUCTION

Terminalia arjuna (Roxb.) Wt. and Arn. (Family: Combretaceae) is a large, evergreen tree found throughout the greater part of the Indian peninsula. Bark of *T. arjuna* is a popular ingredient of many ayurvedic formulations used in the treatment of heart ailments. Though many reports are available on the efficiency of *T. arjuna* bark extract in hyperlipidemic states (Tiwari *et al*., 1989; Khanna *et al*., 1996; Ram *et al*., 1997; Shaila *et al*., 1997, 2000), presence of the same activity in its leaves was not extensively reported. It was reported that the leaves of *T. chebula*, another species of *Terminalia* genus possess hypolipidemic activity (Khanna *et al*., 1993). Tannins in *T. arjuna* bark and methanolic extract of *T. chebula* leaves may bind to bile acids in intestines and prevent the enterohepatic recycling of bile acids. Thus increased fecal bile acid excretion is reported in animals that received arjuna bark and methanolic extract of *T. chebula* leaves. Phytochemical analysis of Methanolic Extract of *Terminalia arjuna* Leaves (METAL) revealed high tannin (22.23%) content. Keeping this in view, the present study was designed to screen the hypolipidemic potential of METAL in two hyperlipidemic rat models viz. triton injected and cholesterol and cholic acid fed (diet induced) hyperlipidemic rats.

MATERIALS AND METHODS

Plant extract: METAL (Batch No: PT/05-02330) was procured from Natural Remedies Pvt. Ltd. Bangalore, India. The extract was stored in a vacuum desiccator until use.

Preparation of crude suspension of METAL: A suspension of dried and pulverized METAL in 2% Carboxy Methyl Cellulose (CMC vehicle) in distilled water was prepared daily before dosing.

Animals: Male albino rats of Wistar strain were used in the study. The animals were given standard rodent pellet diet and water *ad libitum* through out the study. The study was conducted in accordance with the internationally accepted principles for laboratory animal use and care as per US guidelines (NIH publication No.85-23, revised in 1985).

Corresponding Author: P. Ravi Kumar, Department of Pharmacology and Toxicology, N.T.R. College of Veterinary Science, 521-102 Gannavaram, India

Experimental procedure

Triton model: Twenty four rats were randomly assigned into four equal groups (viz. group I-IV). Over night fasted rats in groups II, III and IV were injected with 10% aqueous solution of triton WR-1339 (Hi Media Pvt. Ltd., Mumbai) at 400 mg kg^{-1} b.wt. i.p. (Vogel and Vogel, 1997). Group I was injected with normal saline i.p. About 2 h prior to triton injection, rats in group III received METAL at 500 mg kg^{-1} b.wt. orally while the rats in group IV received gemfibrozil (Lopid capsules, Pfizer) orally at 250 mg kg^{-1} b.wt. group II which received triton alone was considered as positive control. Blood was collected from retro-orbital sinus under ether anesthesia just before and 18 post treatment hours after triton administration.

Cholesterol and cholic acid induced hyperlipidemic model: Twenty four rats were randomly assigned into four groups (viz. group I-IV). After 1 week of acclimatization, rats in groups II, III and IV were kept on oral administration of cholesterol at 500 and cholic acid at 50 mg kg^{-1}, suspended in arachis oil given at 10 mL kg^{-1} b.wt. daily for 30 days by means of stomach tube (Arichi *et al*., 1982). Group II, administered with cholesterol and cholic acid in arachis oil served as hyperlipidemic control. Rats in group III and IV received METAL at 500 mg kg^{-1} b.wt. and guggulsterones (Natural Remedies Pvt. Ltd., Bangalore), respectively orally daily for 30 days by means of stomach tube 1 h before administration of cholesterol and cholic acid in arachis oil (Jahromi *et al*., 1992). Blood was collected from retro-orbital sinus under ether anesthesia just before the experiment and on day 30 of the study.

Determination of serum lipoproteins: Serum samples were assayed for total cholesterol, triglycerides and HDL cholesterol using standard enzymatic kits (Bhat Biotech Pvt. Ltd., Bangalore). VLDL and LDL concentrations were determined using Friedwald formula (Friedewald *et al*., 1972).

Determination of bile acids in feces: Rat feces collected over 30 days of study in cholesterol and cholic acid induced hyperlipidemic model were pooled. Cholic acid and desoxy cholic acid (bile acids) levels in feces were estimated employing the method of Mosbach *et al.* (1954).

Statistical analysis: Data were analyzed employing statistical package SPSS/12.0. One-way ANOVA and Dunnett's test at a level of $p<0.05$ were used for statistical analysis of data.

RESULTS

Triton model: Initial concentrations of serum total cholesterol were similar among all the groups and values ranged from 71.8±4.5-75.4±5.1 mg dL^{-1}. In group II (triton positive control) serum total cholesterol concentration was significantly ($p<0.05$) elevated to at 18 h post triton administration. Groups III and IV administered with METAL and gemfibrozil, respectively along with triton showed significant ($p<0.05$) decrease in total cholesterol compared with triton positive control at 18 h after triton administration (Table 1).

Initial concentrations of serum triglycerides were also similar among all the groups and values ranged from 93.2±8.4-103.2±2.5 mg dL^{-1}. In group II (triton positive control) serum triglycerides concentration was significantly ($p<0.05$) elevated at 18 h post triton administration. Groups III and IV administered with METAL and gemfibrozil, respectively along with triton showed significant ($p<0.05$) decrease in triglycerides compared with triton positive control at 18 h after triton administration (Table 1).

Cholesterol and cholic acid induced hyperlipidemic model: The concentrations of serum lipoproteins were similar among all the groups on day 0. Group II (hyperlipidemic control) that received cholesterol and cholic acid in arachis oil along with normal diet for 30 days demonstrated a significant ($p<0.05$) increase in all serum lipoproteins except HDL cholesterol compared to group I. HDL cholesterol concentrations were slightly decreased in group II compared to group I. Groups III and IV administered with METAL and guggulsterones, respectively along with cholesterol and cholic acid in arachis oil for 30 days showed significant ($p<0.05$) decrease in all serum lipoproteins except HDL cholesterol, compared with group II on day 30. Group III showed a significant ($p<0.05$) increase in HDL cholesterol, compared with group II on day 30 (Table 2). Fecal cholic acid and desoxy cholic acid concentrations (μg g^{-1}) indicated a significant ($p<0.05$) decrease in group II compared to

Table 1: Effect of methanolic extract of *Terminalia arjuna* leaves on serum total cholesterol and triglycerides (mg dL^{-1}) levels in triton induced hyperlipidemic rat model

	Serum total cholesterol (mg dL^{-1})		Serum triglycerides (mg dL^{-1})	
Groups	0 h	18 h	0 h	18 h
I	71.8±4.5	73.9±3.30	93.2±8.4	86.7±4.90
II	75.4±5.1	613.0±88.1*	96.6±5.7	1005.4±45.8*
III	74.1±3.4	417.2±12.7**	103.2±2.5	867.8±45.7**
IV	72.8±2.3	208.7±13.1**	101.4±6.8	643.7±50.4**

*Triton group compared with control at $p<0.05$; **Drug treated groups compared with triton group at $p<0.05$

Table 2: Effect of methanolic extract of *Terminalia arjuna* leaves on serum lipoproteins (mg dL^{-1}) and fecal bile acids (µg g^{-1}) levels in cholesterol and cholic acid induced (diet induced) hyperlipidemic rat model

	Group I		Group II		Group III		Group IV	
Parameters	Day 0	Day 30	Day 0	Day 30	Day 0	Day 30	Day 0	Day 30
Total cholesterol[a]	76.7±3.6	73.4±3.8	76.0±3.8	142.6±10.1*	72.5±3.5	93.8±1.6**	70.6±2.0	80.8±2.7**
Triglyceridesa[a]	98.5±5.0	102.1±6.9	102.1±5.5	194.6±9.9*	96.5±3.6	137.2±8.0**	99.2±5.1	132.4±6.6**
HDL cholesterol[a]	43.7±1.6	43.9±1.2	42.7±2.1	37.9±1.4*	40.2±2.6	42.9±1.8	40.4±1.9	44.9±1.9
VLDL cholesterol[a]	19.7±1.0	20.4±1.4	20.4±1.1	38.9±2.0*	19.3±0.7	27.4±1.6**	19.8±1.0	26.4±1.3**
LDL cholesterol[a]	13.3±1.8	12.1±1.8	12.9±1.7	65.7±9.6*	13.0±2.0	23.4±2.0**	11.3±1.3	13.9±1.7**
Fecal cholic acid[b]	78.2±2.9	79.1±3.7	80.1±3.1	56.3±2.8*	81.4±2.7	68.8±3.6	78.6±2.4	81.2±1.6**
Desoxy cholic acid[b]	69.5±2.1	67.8±2.3	71.4±2.8	49.6±2.9*	66.9±3.2	56.1±2.6	69.0±2.8	63.5±2.0**

*Hyperlipidemic group compared with control at $p<0.05$; **Drug treated groups compared with hyperlipidemic group at $p<0.05$; [a]mg dL^{-1} of serum; [b]µg g^{-1} of feces

group I. Groups III and IV demonstrated an increase in fecal bile acids compared to group II whcih however were not significant statistically (Table 2).

DISCUSSION

Hyperlipidaemia indicates the onset of abnormalities in lipid metabolism secondary to the manifestation and progression of atherosclerosis. In addition to diet, use of medicinal plants as a pharmacologic modality in preventing alteration in lipid metabolism has received wide attention from several workers. In the present study on *T. arjuna* leaves, a significant ($p<0.05$) reduction in the levels of cholesterol and triglycerides at 18 h after triton administration was observed in the groups treated with METAL and gemfibrozil when compared with triton only treated group. This indicated that METAL and gemfibrozil effectively antagonized the triton induced hyperlipidemia. A reasonable assessment of a new hypolipidemic agent cannot be made merely by measurement of cholesterol and triglycerides levels and it is of great importance to secure information about the effect of the drug on individual lipoproteins like High Density Lipo protein (HDL), Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL) cholesterol levels (Miller *et al*., 1977; Rossner *et al*., 1978).

Thus in another model of hyperlipideima induced by cholesterol and cholic acid feeding, serum levels of total cholesterol, triglycerides, VLDL and LDL cholesterol were estimated. It was observed that cholesterol, triglycerides, VLDL and LDL cholesterol were significantly ($p<0.05$) elevated and HDL levels were significantly lowered. Rats that received the METAL and guggulsterones along with hyperlipidemic diet for 30 days exhibited a significant ($p<0.05$) decrease in total cholesterol, triglycerides, VLDL and LDL cholesterol when compared to rats that received only hyperlipidemic diet.

Fecal cholic acid and desoxycholic acid values in diet induced hyperlipidemic rat model indicated a significant ($p<0.05$) decrease in their levels in rats receiving hyperlipidemic diet alone when compared to control rats. Rats that received guggulsterones, indicated a significant ($p<0.05$) increase in fecal cholic acid and desoxycholic acid levels when compared to their levels in rats receiving hyperlipidemic diet alone. In rats that received METAL also an increased fecal cholic acid and desoxycholic acid were observed whcih however were statistically not significant.

The hypolipidemic effect of *T. arjuna* bark in hyperlipidemic rat models was reported earlier by Khanna *et al*. (1996), Shaila *et al*. (2000). Similar effects were also reported in rabbit models by Tiwari *et al*. (1989), Ram *et al*. (1997) and Shaila *et al*. (1997). They opined that *T. arjuna* bark extract interfered with the enterohepatic circulation of bile acids and reduced the absorption of dietary cholesterol from small intestine. They also opined that stimulation of plasma Lecithin Cholesterol Acyltransferase (LCAT), hepatic lipases, receptor mediated catabolism of LDL and possible inhibition of HMG Co-A reductase might also be responsible for the hypolipidemic effect of *T. arjuna* bark extract.

CONCLUSION

It is concluded that METAL posses hypolipidemic activity that is almost comparable to that of two established hypolilpidemic agents, viz, gemfibrozil and guggulsterones. The hypolipidemic activity produced by the METAL might have resulted from the multiple actions of various active principles present in the plant. Further studies are required to establish the efficacy of the METAL as a hypolipidemic drug.

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